WORLD'S POULTRY CONGRESS

ABSTRACT BOOK

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OPENING SESSION & PLENARY LECTURES

1000S Leading through change: Lessons from global sustainability efforts in livestock systems.

Kim Stackhouse-Lawson*1 1Colorado State University, Ault, Colorado, United States

As global demand for animal protein accelerates, sustainability has become a defining challenge for agriculture. In this opening keynote, Dr. Kim Stackhouse-Lawson will reflect on more than a decade of leadership advancing sustainability across the livestock sector from corporate transformation at JBS to the development of the U.S. Beef Sustainability Framework through the U.S. Roundtable for Sustainable Beef, and global alignment efforts with the Global Roundtable for Sustainable Beef. Drawing on these experiences, Dr. Stackhouse-Lawson will explore how industry, academia, and producers can meaningfully advance sustainability through data-driven action, transparency, and co-developed research models. She will highlight how these lessons now inform her leadership of Colorado State University's AgNext program, an initiative building trusted science and producer-engaged innovation to support sustainable animal agriculture. This keynote will set the stage for the Congress' sustainability program by framing the key frontiers emerging for poultry and livestock systems and outlining the opportunities ahead for collaboration and impact.

Keywords: sustainability; animal agriculture

Plenary Lectures

1001S How One Health thinking, acting and decision making provide a path to sustainability.

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The objectives of this talk are to define and understand the full scope of One Health, relate examples of needed cross- and trans-disciplinary collaborations to address many of today's challenges and describe how sustainability is enhanced by One Health collaborations.

'What' is One Health? It's a modern day resurrection of an ancient concept of the inextricable interdependence of the environment and all life. While it was zoonotic diseases that first drove the current One Health discussion, many of today's most wicked challenges, from antimicrobial resistance, avian influenza, climate change, disaster preparedness, food safety and security, to plant and soil health, vector-borne and zoonotic diseases, are begging for what we now understand as the trans-disciplinary One Health approach. This talk will describe the full scope of One Health beyond zoonotic diseases. It describes some of the 'systems' challenges that have forced us into silos, shares examples of the critical global need to operationalize One Health at all levels of academia, research, policy and government, and shares examples of collaborative actions to address many of today's most difficult challenges.'So What?' The presentation includes One Health stories that demonstrate how and why it is critical that animal, environmental, and human health workers and social scientists develop collaborative working relationships 'ahead' of public health and food safety emergencies to best 'prevent' and 'respond' to them. It shares examples of why cross-professional and transdisciplinary collaborations are urgently needed. 'Now What?' This presentation will summarize how One Health thinking and decision making can contribute to sustainability for biodiversity, for animal, human, and planet health in general and for the poultry industry in particular. It will relate how One Health can help feed the projected 10 billion people expected to populate the earth before 2050 and how its being understood by the general public, beyond the halls of science, can increase social responsibility not only in the poultry industry but across society. The talk will briefly demonstrate how the One Health approach is now being embraced by many organizations and individuals around the world giving a brief overview of the current global One Health movement. It will outline continuing gaps in implementation and suggest needed actions that those working in the international poultry industry can take to help further One Health thinking, acting and decision making on the path to sustainability.

Keywords: One Health; Transdisciplinary; Antimicrobial resistance; zoonoses; Occupational Health Risks

1002S Advancing Sustainability in Poultry Production to Meet 2050 Protein Demands

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Global demand for poultry products is expected to increase markedly by 2050, driven by population growth, rising incomes, and continued urbanization. Poultry meat and eggs are already among the most widely consumed animal-source proteins due to their affordability, versatility, and favorable nutrient profiles. As consumption expands, the sector faces the dual challenge of scaling production while strengthening environmental stewardship. Because poultry is among the most resource-efficient livestock species, its role in delivering sustainable protein to a growing population is increasingly recognized.

Over the past several decades, remarkable progress in breeding, nutrition, housing design, health management, and processing efficiencies has transformed poultry production systems. Broilers now achieve higher growth rates and improved feed conversion, while layers produce more eggs over longer cycles. These advancements have reduced land and water requirements, lowered energy use, and significantly decreased greenhouse gas emissions per unit of product. Waste reduction and nutrient recycling strategies are further improving environmental outcomes, helping poultry maintain the smallest climate footprint among major meats.

Continued innovation remains essential to meeting future sustainability goals. Precision nutrition and feeding technologies allow diets to be formulated with greater accuracy to support optimal performance while lowering nitrogen and phosphorus excretion. Enhanced welfare management, disease prevention, and biosecurity reduce mortality and improve productivity without expanding resource use. Data-driven monitoring tools help producers optimize housing environments and energy use, while genetic and management improvements support resilience in the face of climate variability. Integrating circular economy principles—such as valorizing manure and byproducts—can further reduce reliance on nonrenewable inputs.

Keywords: Sustainability; Environmental efficiency; Precision nutrition; Protein demand

1003S Behavioral Needs and Welfare Assessment as Cornerstones of Sustainable Poultry Production

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Chicken welfare is of increasing interest in sustainable production of eggs and meat. The majority of meat and egg production in Western countries is driven by economics and still performed under intensive and large scale conditions. Although major changes in housing and management have already been made for the benefit of animal welfare, NGOs and consumers continue stressing its importance and repeatedly ask for further improvement. Most improvements are made on country or regional level, such as banning and moving away from cage housing and moving towards

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production systems with slower-growing broiler chickens and lower stocking densities. These changes take place in the full recognition of some trade-offs with other sustainability aspects (economy, environment) and reflect the need to take the animal into account as an important stakeholder in sustainable production systems.
In animal welfare science, it is widely acknowledged that animal welfare should not only address the basic needs of the animals such as being healthy, good feeding and good housing conditions. Animal welfare today should also consider the extent to which the behavioral needs are met and the resulting emotional state of the animal, including the ability to feel positive emotions.
Current commercial practices in chicken production systems, even in systems with increased welfare requirements, often lack a critical view on the extent to which behavioral needs are met. Routine monitoring of animal welfare indicators is often limited to indicators being easy to collect and addressing some health and housing aspects, such as mortality and footpad dermatitis. The majority of labeling systems focus on resource- and management based indicators of welfare, checking for example enrichment provision or space allowance, but they do not assess the actual welfare on the chickens themselves.
By showing examples of research projects on light conditions and provision of enrichment, it will be shown how behavioral needs can be measured and subsequently taken into account in the design of housing systems. The fact that protocols developed for welfare assessment are lacking feasible measures for important welfare aspects such as behavior and mental state does not mean that existing data collection on animal welfare is not useful. Routinely collected data can provide important information on welfare, as is demonstrated by an example from the broiler production chain. Examples are shown of tools to assess mental states, both positive and negative, and a perspective towards further development for routine application in practice. The value of routine welfare assessment, e.g., for benchmarking and labeling, will be briefly discussed.
Keywords: welfare; housing; management; behaviour; assessment

1004S Genomes to Sustainability: Integrating Genetics, Genomics, Epigenomics, and Bioinformatics to Advance Poultry Health, Welfare, and Global Food Security
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Advances in genetics, genomics, epigenomics, and bioinformatics are transforming our understanding of complex traits that drive sustainable poultry production. Over the past several years, our research has applied high-resolution genome assemblies, functional annotation pipelines, and multi-omic analyses to uncover genetic and regulatory mechanisms underlying economically important traits including production efficiency, disease resilience, and welfare-related traits in poultry. By integrating whole-genome sequencing, transcriptomics, epigenomic profiling, and machine learning–based prediction, we have identified key genetic variants, and regulatory elements associated with resilience to major pathogens, improved feed efficiency, enhanced product quality, and reduced reliance on antimicrobials. Epigenomic discoveries, including DNA methylation and chromatin accessibility signatures, reveal how environmental stress, nutrition, and management shape gene expression and phenotype, connecting molecular biology to animal welfare, food safety, and long-term flock health. These insights support next-generation breeding strategies that accelerate genetic gain while advancing sustainability goals: lowering environmental impact, improving welfare-based selection, strengthening disease resistance, reducing worker exposure to pathogens, and securing affordable protein for a growing global population. This keynote will present how integrative genomic and bioinformatic tools are redefining poultry breeding and enabling a more resilient, productive, and ethically responsible poultry sector worldwide.
Keywords: Genomics; Epigenomics; Bioinformatics; Economically important complex traits; Sustainable poultry production

1005S Decoding Poultry Intelligence: AI-Driven Transformation of Welfare, Sustainability, and Precision Farming
Suresh Neethirajan*1 1Dalhousie University, Dartmouth, Nova Scotia, Canada
The global poultry sector is entering a data-driven era where artificial intelligence and digital twins enable a deeper understanding of animal welfare, productivity, and climate resilience. This presentation highlights recent breakthroughs from the MooAnalytica Research Group at Dalhousie University that demonstrate how intelligent sensing and computational modeling can redefine poultry management systems.
Through advanced natural language processing, bioacoustic data from layers and broilers are decoded to reveal behavioral cues, emotional states, and welfare indicators. Transformer-based deep learning models achieve over 90 percent accuracy in translating poultry vocalizations into measurable welfare metrics, enabling real-time, objective monitoring of bird well-being. Digital twin frameworks further integrate visual, acoustic, and environmental data streams to create dynamic simulations of poultry systems. These digital counterparts model flock behavior, housing microclimate, and physiological responses, supporting precision decision-making and predictive management under varying climatic and production scenarios. Satellite-assisted analytics link farm-level emissions data with carbon modeling platforms to support climate-smart agriculture and verified carbon credit generation. This is complemented by mobile applications such as Cluckify and CluckTalk, which empower producers through intuitive dashboards and participatory welfare reporting. Together, these innovations create an ethical and economically viable blueprint for the future of poultry farming - one that merges welfare science, sustainability, and artificial intelligence into a unified digital ecosystem. The work underscores how technology can enhance empathy, transparency, and efficiency across global poultry production.
Keywords: Artificial Intelligence; Poultry Welfare; Digital Twin; Climate Smart Agriculture; Poultry Production

STUDENT POSTER COMPETITION

Student Poster Competition – Sponsored by Zoetis

5000P Determining factors associated with floor egg levels in Australian cage-free layer flocks
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Floor eggs (FE) or eggs laid outside of the nestbox in cage free egg production systems can contribute to a reported loss of daily egg production of up to 14%. Not only is there a direct economic loss of production but FE can also increase labour demands for manual collection, raise the risk of microbial contamination, and reduce the proportion of marketable eggs

This study aimed to identify and address the management and housing factors associated with FE levels in Australian cage-free egg production systems, with the goal of informing strategies to

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reduce floor eggs.

A cross-sectional survey was conducted across 37 commercial cage-free layer flocks. Informed consent was obtained prior to participation.The survey included 62 questions on housing, space allocation, labour, nest box management, lighting, feeding and litter/enrichment. Responses were entered into REDCap, de-identified, and exported to SPSS for analysis.

Descriptive statistics are reported as mean ± standard error. Associations were assessed using Student's t-tests and ANOVA for categorical variables, with Welch's correction where Levene's test indicated unequal variances. Pearson's correlation evaluated continuous variables. Variables showing notable univariate associations were included in a multivariable linear regression. Significance was set at $p < 0.05$.

Independent t-tests indicated higher FE with litter use ($p = 0.028$), warm lighting ($p = 0.013$), absence of tunnel ventilation (Welch's $p = 0.005$), absence of a solid nest box platform (Welch's $p = 0.007$), and presence of ramps at the nestbox ($p = 0.004$). ANOVA showed operation size differences ($p = 0.004$); lighting control and breed were not significant. Pearson correlations revealed higher FE in smaller flocks ($r = –0.47$, $p = 0.003$), smaller shelters ($r = –0.40$, $p = 0.018$), producers with fewer years involved in the industry ($r = –0.39$, $p = 0.018$), and older hen age at maximum lighting ($r = 0.53$, $p = 0.002$). Backward regression retained flock size ($B = –1.70×10\text{-}4$, $\beta = –0.41$, $p = 0.005$) and ramp presence ($B = 3.31$, $\beta = 0.41$, $p = 0.006$) as significant predictors of higher FE levels. While the presence of tunnel ventilation and solid platforms, and the absence of litter use, were associated with reduced FE, these variables were removed from the regression model. Nevertheless, they remain biologically relevant and may contribute to FE reduction under different management conditions.

From this study FE prevalence is primarily influenced by flock size and nest box ramp presence, with smaller flocks and those with ramps exhibiting higher rates. Eliminated factors may be biologically relevant, highlighting the importance of both flock-level management and nest box design in reducing floor eggs.
Keywords: Floor eggs ; Cage-free egg production; Layers; Flock management; Housing Environment

5001P Black soldier fly larvae (Hermetia illucens) meal as an alternative protein source in organic free range laying hen production: Effects on production performance and egg quality

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This study investigated the effects of feeding black soldier fly larvae (BSFL) meal as a substitute for soybean meal (SBM) on production performance and egg quality of Lohmann Brown laying hens.

A total of 160 18-week-old laying hens were randomly assigned to two dietary treatments (4 pens/treatment, 20 hens/pen, free-range system). The treatments consisted of an SBM-based control diet (SOY) and a BSFL meal-based diet (LAR). Hens in the SOY group received a commercial organic diet containing 13.5% SBM. In the LAR treatment, SBM was completely replaced by BSFL meal. After a two-week adaptation, birds were fed for 14 weeks (20-34 weeks of age). Body weight was recorded at week 20, 25, and 34 weeks of age, and feed intake was measured weekly. Egg production was recorded daily to calculate weekly percentage laying performance. At the end of the experimental period, ten eggs per pen (40 eggs per treatment) were collected for egg quality analysis. Data were analyzed using one-way ANOVA ($p<0.05$).

Feeding BSFL meal in organic diets did not affect hens production performance. During the whole experimental period, no significant differences were observed between treatments in average daily feed intake (SOY=109 vs. LAR=110g/hen/day; $p>0.05$), percentage laying performance (SOY=90.9 vs LAR=89.7%; $p>0.05$), individual egg weight (SOY=57.8 vs. LAR=58.5g/egg; $p>0.05$), or feed conversion ratio (SOY=2.07 vs. LAR=2.11g feed/g egg mass; $p>0.05$).

Egg quality analysis showed that eggs from LAR-fed hens were longer (SOY=54.7 vs. LAR=56.1mm; $p<0.01$), resulting in a significantly lower shape index (SOY=80.2 vs. LAR=78.4; $p<0.01$). However, LAR-fed hens produced numerically heavier eggs (SOY=59.6 vs. LAR=60.9g/egg; $p>0.05$), resulting in higher proportion of marketable egg weight classes M and L (SOY: 15% S, 63% M, 23% L, 0% XL vs. LAR: 0% S, 73% M, 25% L, 3% XL), which is of economic relevance. LAR-eggs showed a significantly lower albumen height, resulting in reduced Haugh Units (SOY=87.4 vs. LAR=82.3, $p<0.01$). Moreover, LAR-eggs contained a significantly higher yolk proportion (SOY=25.7 vs. LAR=27.2%, $p<0.01$), which was associated with a tendency towards lower albumen (SOY=61.4 vs. LAR=60.3, $p<0.1$) and eggshell proportions (SOY=12.9 vs. LAR=12.5, $p<0.1$). Eggshell breaking strength and thickness were not affected by dietary treatment. However, yolk color, as measured by the ROCHE color fan, was significantly lighter in LAR eggs compared to SOY (SOY=7.1 vs. LAR=6.3, $p<0.01$).

In conclusion, replacing SBM with BSFL meal represents a suitable and sustainable alternative protein source for laying hens in organic production systems, maintaining productive performance while inducing only minor changes in egg quality characteristics.
Keywords: Insects; Sustainability

5002P Formulation of Broad-Spectrum Bacteriophage Cocktails for Controlling Poultry-Associated Salmonella Enteritidis

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Objective: The ongoing public health burden of poultry-related salmonellosis and the limitations of traditional chemical sanitizers necessitate the development of effective interventions. This study aimed to evaluate the inhibitory activity of bacteriophages against poultry-associated Salmonella Enteritidis (SE) and to formulate broad-spectrum phage cocktails using the most effective isolates to improve consumer safety and reduce economic losses within the poultry industry.

Experimental Design: Bacteriophages were characterized for host range and lytic activity against poultry-associated SE isolates. Phages showing the strongest inhibitory effects were incorporated into cocktails to broaden strain coverage and strengthen antibacterial activity.

Materials and Methods: Seven SE strains from environmental and diagnostic poultry samples and six phages isolated from sediment, sewage, and livestock wastewater in British Columbia, Canada, were evaluated. Phages were tested for lytic infection activity against SE at 32°C, with samples collected every 10 min for 120 min. Single-step growth curves were generated by enumerating phages with soft agar overlay method to determine burst size and latent period. Phages with high burst sizes and short latent periods were combined in equal ratios (1:1 vol) to create broad-spectrum

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cocktails.
Statistical Analysis: The experiments were independently performed in triplicate. One-way ANOVA with Tukey's HSD (α= 0.05) was used to evaluate differences between phage titer at the end of the burst cycle and initial phage titer using GraphPad Prism, version 9.5.
Results: Phages SE4, SE11, SE13, SE20, and FO1 showed significant increases in titer ($P < 0.05$) and similar latent period (~11 min) during the burst cycle, indicating effective lytic activity. SE20 had the broadest host range (5/7 strains), followed by SE13 (3/7), while SE4, SE11, and FO1 lysed one strain. SE11 exhibited the highest burst size (1,349 PFU/cell), followed by SE4 (320 PFU/cell). FO1 and SE13 had moderate burst sizes (282 and 267 PFU/cell), while SE20 had the lowest (135 PFU/cell) despite its broad host range. Based on performance, SE20, SE11, and SE13 were formulated individually, in pairs, or in combinations into seven phage cocktails.
Conclusions: Phage isolates SE4, SE11, SE13, SE20, and FO1 exhibited significant inhibitory activity against selected SE strains, and seven phage cocktails were formulated with SE20, SE11, and SE13. The formulated phage cocktails will be further evaluated for reducing SE contamination on shell eggs and their effects on egg quality during storage.
Keywords: Bacteriophage; Salmonella Enteritidis; Poultry safety; Host range; Phage cocktail

5003P Impact of cleaning and disinfection on targeted pathogens and bacterial indicators in the egg supply chain in Québec

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Quebec ranks second among Canadian provinces in table egg production after Ontario, accounting for 20% of national output. Currently, 199 producers manage 6 million laying hens, producing about 1.9 billion eggs per year and contributing nearly CAD 313.5 million annually to Quebec's economy. Given its importance and role as a major source of animal protein, producers aim to ensure eggs are free from pathogens, notably Salmonella and E. coli, major threats in this sector. Evaluating cleaning and disinfection (C&D) effectiveness in controlling these pathogens at different production stages is essential. This study assessed the impact of C&D on avian pathogens (APs) and bacterial indicators along the egg supply chain, focusing on laying hen barns (LHBs) and egg production facilities (EPFs). The hypothesis was that characterizing contamination in laying facilities improves control and reduces AP and bacterial indicators presence. Objectives were to: (i) describe targeted APs and bacterial indicators from samples collected along the Quebec laying hen supply chain, and (ii) assess the impact of C&D.
Four EPFs and six corresponding LHBs were studied. Samples were taken from boots, drinkers, feeders, cages, floors, fans, air inlets, droppings (25 g), and dust (25 g) at three time points: i) end of laying cycle in EPFs and corresponding LHBs confining pullets for replacement, ii) after C&D of EPFs, iii) one month after pullet placement in clean EPFs. Analyses included classical Salmonella isolation and enumeration of bacterial indicators (E. coli, Enterobacteriaceae, total mesophilic bacteria). One-way ANOVA and t-Test were used.
Results showed variable Salmonella positivity: 25%, 1.9%, and 30.8% in EPFs before C&D, after C&D, and one month after placement, respectively. C&D reduced bacterial loads by ~2 Log CFU/ml ($p = 0.0006–0.0405$), reflected by decreases in E. coli ($6.64 \times 105 \rightarrow 5.78 \times 104$ CFU/ml on floors), Enterobacteriaceae ($4.70 \times 104 \rightarrow 5.98 \times 102$ CFU/ml on boots, air inlets, fans), and total mesophilic bacteria ($7.19 \times 107 \rightarrow 6.15 \times 105$ CFU/ml on floors, feeders, air inlets, fans).
Recontamination occurred after introduction of 18-week-old pullets: E. coli increased ($5.78 \times 104 \rightarrow 2.26 \times 106$ CFU/ml on floors and boots), Enterobacteriaceae ($5.98 \times 102 \rightarrow 2.64 \times 106$ on floors, feeders, boots, drinkers, air inlets, fans) and total mesophilic bacteria ($6.15 \times 105 \rightarrow 2.59 \times 108$ CFU/ml on the same surfaces). Findings reveal partial C&D effectiveness and rapid recontamination, emphasizing the importance of refining C&D procedures and biosecurity to prevent bacterial persistence and reintroduction within the egg supply chain.
Keywords: egg supply chain, farm, cleaning and disinfection, Salmonella, bacterial indicators
Keywords: egg supply chain

5004P Synergistic Deletion of Methionine Sulfoxide Reductase Gene Clusters in Salmonella Typhimurium Impairs Oxidative Stress Resistance and Intracellular Persistence, Attenuates Systemic Colonization, and Enhances Immunogenicity and Protective Mucosal Immunity in Poultry

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Poultry is a major reservoir of Salmonella Typhimurium, causing human salmonellosis via contaminated products. Antibiotic overuse has led to resistance, threatening public health. To develop an effective live vaccine, we targeted the methionine sulfoxide reductase (msr) genes, which repair oxidized methionine residues (Met-SO) and are vital for survival and virulence of Salmonella. Salmonella encodes five msr genes (msrA, msrB, msrC, msrP, bisC). Using single and combined deletion mutants (ΔmsrA, ΔmsrP, ΔmsrAC, ΔmsrACP, ΔmsrACPB, and ΔmsrACPBbisC), we studied the role of each msr gene in oxidative stress survival, protein oxidation, immune cell survival, colonization in poultry organs, and immune response modulation. Mutant strains exhibited increased protein aggregation, with ΔmsrACPBbisC accumulating the highest levels. Macrophage survival assays revealed msrA, msrC, and bisC are critical for intracellular proliferation, whereas impact of msrP was minimal. Mutants showed impaired cecal colonization and systemic dissemination to the spleen and liver, with severity depending on mutation number. Birds inoculated with msr mutants had altered humoral and cellular immune responses compared to control S. Typhimurium infected birds, underscoring the roles of msr genes in poultry colonization and immune modulation, with implications for live vaccine development. Focusing on immunity, we selected ΔmsrA and ΔmsrAC mutants to evaluate protective efficacy. Birds were immunized, boosted after three weeks, then challenged orally four weeks post-boost. Protective efficacy was assessed by Salmonella colonization in the cecum, spleen, and liver over three weeks post-challenge. The ΔmsrA group showed superior protection compared to ΔmsrAC and controls, clearing the challenge strain effectively. No dissemination to spleen or liver occurred in ΔmsrA birds post-challenge, while ΔmsrAC birds required one week for complete clearance. Control birds showed persistent infection in these organs for three weeks. Cell-mediated immunity measured by lymphocyte transformation tests showed the highest stimulation index in the ΔmsrA group, followed by ΔmsrAC and controls. Humoral immunity, determined via serum IgY and secretory IgA levels, was strongest in ΔmsrA immunized birds, with significantly higher secretory IgA important for muco-

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sal defense. These results indicate ΔmsrA induces robust protective immunity by enhancing cellular and mucosal IgA responses. ΔmsrA is a safe and effective live-attenuated vaccine candidate against Salmonella in poultry, emphasizing the role of mucosal immunity in preventing intestinal colonization and transmission.
Keywords: Salmonella Typhimurium; Methionine sulfoxide reductases; Live attenuated vaccine; Poultry colonization; Immune response

5005P Integrative omics defines metabolic biomarkers and genetic regulatory mechanisms of mortality risk

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Mortality is a key economic and health-related trait in broiler production that critically affects productivity and flock welfare. Its regulation involves complex interactions between multiple genes and environmental factors, making the underlying mechanisms difficult to dissect. To elucidate the genetic and metabolic basis of mortality in white-feathered broilers, we integrated serum metabolomics with multi-omics data from a three-generation broiler population (n=1,277) sharing a uniform genetic background. Comparative analyses revealed that high-mortality birds exhibited a pronounced immunometabolic imbalance, as evidenced by elevated proinflammatory cytokines, stunted growth, and perturbed energy metabolism. Non-targeted metabolomic profiling identified 1,871 metabolites, the majority of which were lipids, among which 150 were differentially accumulated. Through mGWAS of these 150 metabolites, we detected 45,585 metabolite quantitative trait loci that were highly enriched near transcription start sites and in transcriptional regulatory regions, suggesting strong involvement of cis-regulatory elements in metabolic control. To further delineate these regulatory relationships, integration with molecular QTL data from Chicken GTEx revealed extensive overlap between mQTLs and cis-regulatory variants, highlighting tissue-specific genetic effects that were most pronounced in the liver. Despite a highly homogeneous genetic background, the effect sizes of mQTLs differed markedly between the high- and low-mortality groups, indicating context-dependent genetic regulation of metabolism under stress. To substantiate this finding, we compared two linear models with and without the group factor using ANOVA, which revealed varying degrees of metabolic canalization and differential buffering of metabolic variability. Cross-species comparison showed that high-mortality broilers displayed an acute organ failure-like metabolic signature driven by immune–metabolic cascade dysregulation. By integrating LASSO regression and weighted correlation network analysis, we identified 16 mortality-associated signature metabolites, including butyrate and L-cysteine. Fine-mapping and colocalization analyses identified regulatory variants (rs318007359 in MSRA and rs732044877 in PTER) that influence butyrate and L-cysteine levels, which synergistically maintain redox balance through both Nrf2-dependent and Nrf2-independent antioxidant pathways. Collectively, this study establishes a mechanistic immunometabolic framework linking genetic variation, metabolic disruption, and mortality risk, thereby providing novel biomarkers and candidate targets for genetic selection and precision breeding strategies to mitigate mortality in broilers.
Keywords: mortality risk; metabolomics; mGWAS; butyrate; L-cysteine

5006P Evaluation of Lacticaseibacillus casei and berry pomace extracts as synbiotic combination against growth and virulence of Avibacterium paragallinarum

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Avibacterium paragallinarum (AP), the causative agent of infectious coryza, poses a growing health and economic threat to the global poultry industry. This acute or chronic upper respiratory infection affects birds of all ages, resulting in high morbidity and production losses. With increasing restrictions on antibiotic use and trend of organic farming, probiotics, prebiotics, and their combinations (synbiotics) have emerged as promising alternatives for controlling bacterial infections. However, their efficacy against AP remains unexplored.

This study evaluated the antagonistic potential of Lacticaseibacillus casei (LC) and a metabolically engineered LC strain (LC-CLA), designed to overproduce bioactive metabolites specifically conjugated linoleic acid (CLA), against AP. Additionally, the antimicrobial effects of a prebiotic like component, berry pomace extracts (BPE, plant derived phenolic) and its potential synergism with LC or LC-CLA were assessed.

Co-culture assays of AP with LC or LC-CLA, with or without BPE supplementation were conducted to determine time-dependent inhibition. Cell-free culture supernatants (CFCS) derived from LC (CFCS1) or LC-CLA (CFCS2) were evaluated for their antimicrobial activity, alone or in combination with BPE. Growth inhibition assays were performed along with gene expression analyses targeting key genes associated with stress response (recN, rpoB), quorum sensing and virulence (luxS), cell wall integrity and metabolism (ompA, mreC, gltB, purL, metF, nusB, mukB). Statistical significance among treatment groups was determined using one-way or two-way ANOVA.

Both LC and LC-CLA completely eliminated AP within 48 h in co-culture. BPE at 0.2 mg GAE/mL fully suppressed AP growth within 24h, while 0.1 mg GAE/mL significantly reduced bacterial counts by 3.66 log at 24h ($p < 0.05$). CFCS1/CFCS2 inhibited AP growth by ~1.1 log in 48h while achieving complete exclusion by 72h ($p < 0.05$). CFCS combined with BPE further showed enhanced inhibition within 48h by reduction of ~3.5 log compared to control ($p<0.05$). Significant alteration of stress and survival associated genes of AP was observed following only CFCS exposure or in combination with BPE. Furthermore, fluorescence microscopy using SYTO-9 and propidium iodide staining confirmed increased membrane permeability of AP and cell death post-treatment.

These findings demonstrate that probiotic and/or prebiotic interventions can effectively inhibit AP growth and disrupt its stress and virulence mechanisms. Such synbiotic strategies may offer a sustainable and consumer friendly approach to control infectious coryza in poultry production systems.
Keywords: Infectious Coryza; Probiotics; Synbiotics; Berry pomace extracts; Antibiotic alternatives

5007P Gene-ontology analysis of DVB02 field strain of muB Gene of Avian Reovirus (ARV)

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This study aimed to analyze the phylogenetic relationships of Avian Reovirus isolates based on the complete muB gene (M2 segment), which encodes the μB protein, a major outer capsid protein of the virus. This protein is involved in virus entry, membrane

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penetration, and infectivity, so it is under functional and immune pressure. Compared to σC and σB, the μB gene provides a more balanced level of genetic variability, greater phylogenetic stability, reduced immune-selection bias, and improved resolution for evolutionary and epidemiological analyses of avian reovirus. A molecular phylogenetic study was conducted on ARV isolates DVB02 adapted in chicken embryo fibroblast (CEF) cells. The muB gene, encoding the outer capsid protein, was targeted to determine its suitability as a evolutionary marker. The focus was placed on analysis of the muB gene using molecular techniques, followed by phylogenetic comparison with reference isolates.
RNA was extracted from ARV-infected CEF cells using Trizol reagent. Reverse transcription was performed to synthesize cDNA, followed by PCR amplification using muB gene-specific primers. The expected amplicon size was approximately 2031 bp, verified by 1.0% agarose gel electrophoresis. The PCR products were purified, sequenced, and analyzed using the MEGA 11 software. The sequences were aligned with 26 global reference strains retrieved from NCBI GenBank to infer phylogenetic relationships.
Phylogenetic analysis was conducted using the Maximum Likelihood method and Tamura–Nei model in MEGA 11. The tree topology was statistically evaluated using bootstrap analysis with 1,000 replicates to assess the reliability of clustering.
The amplified muB gene sequence showed 98.8% identity with previously reported ARV isolates. Phylogenetic analysis revealed that the Indian ARV isolate (DVB02, 2017) clustered closely with isolates from China (2014–2015), Egypt, and its close resemblance with S1133 live vaccine strain. The isolate was genetically distinct from USA and Taiwan origin ARV strains analyzed in the study. Bootstrap support for major branches exceeded 90%, demonstrating robust clustering and lineage assignment.
The study concludes that the muB gene is a potential molecular marker for ARV gene ontology and phylogenetic analysis. Phylogenetic analysis and clustering of ARV isolate DVB02 strain with isolates of China (2014–2015) and Egypt and its close resemblance with S1133 makes this strain a potential vaccine candidate for control of avian reoviruses across regions.
Keywords: Avian Reovirus; muB gene; , RT-PCR; Phylogenetic analysis; poultry vaccine

5008P Market Characteristics, Trading and Biosecurity Practices of Some Selected Border Rural Live Bird Markets in Ghana: Implications for Avian Transboundary Disease Dissemination

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Rural poultry production is pivotal to food security, livelihood and sociocultural lifestyles of many rural households. However, cross-border trade of live poultry and poultry products at Live Bird Markets (LBMs) has been implicated in the introduction of infectious diseases such as Newcastle Disease (ND) and Avian influenza.
This study investigated the role of market characteristics, trading and biosecurity practices of some selected border rural live bird markets in Ghana on dissemination of avian transboundary diseases.
A cross-sectional semi-quantitative study was conducted using semi-structured questionnaire and observations. The study included a total of 180 rural poultry traders. Questionnaire assessed the demographic information, sources of birds, volume of poultry trades, means of transportation of birds, and handling and biosecurity practices of rural poultry traders in the markets.
The study revealed that, all (100%) traders in Northern Ghana were male, while (73.7%) of traders in Southern Ghana were female. In Northern Ghana, 44.0% of traders purchased birds from less than 5 identified sources including Burkina Faso. Conversely, in Southern Ghana, 67.5% of traders purchased birds from more than 5 identified sources including Togo. Trading activities involved movement from one market to another on a designated market day; every two to four days by 48.0% and 48.7% of traders respectively in Northern Ghana and Southern Ghana. The most common mode of transportation of birds in both locations is motorcycle/tricycle (89.0%) Northern Ghana and (35%) Southern Ghana. The overall biosecurity practices were poor in both locations, (64.0%) Northern Ghana and (58.7%) Southern Ghana and traders have not attended any training or workshop on biosecurity.
The study suggests that the rural poultry trading practices has significant influence on the risk of infectious diseases in local poultry across the border areas. Hence, improved biosecurity, strengthened transboundary disease surveillance and other multidisciplinary approaches is needed in live bird markets at the border towns.
Keywords: Rural Poultry; Cross-Border Trade; Live Bird Markets; Ghana

5009P Detection Of Avian Influenza Virus, H5 And H9 Subtypes In Rural Live Bird Markets At Selected Border Areas In Ghana: Implications For Integrated Surveillance

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With frequent outbreaks of Highly Pathogenic Avian Influenza in Ghana and neighbouring countries in West Africa, there is a need to identify Avian Influenza virus (AIV) subtypes (H5 and H9) from rural poultry at live bird markets in border areas in Ghana.
Through Real-time reverse transcriptase PCR, a total of 770 swab samples from local chicken, guinea fowl, and duck across four border live bird markets were screened for Influenza A virus M gene (Ct<35).
Influenza A prevalence by sample type, location, and species was calculated at a 95% confidence interval (CI) using an exact binomial interval. The overall prevalence of influenza A was 6.4% (CI: 4.7, 8.3). Tracheal swabs recorded 8.8% (CI: 6.2, 12.1), and cloacal swabs, 3.9% (CI: 2.2, 6.3). The prevalence was higher in Southern Ghana, 11.2% (CI: 7.4, 16.0), than in Northern Ghana, 4.4% (CI: 2.8, 6.5) ($p<0.05$). Further subtyping of the M-gene-positive samples confirms the circulation of H5 [4.4% (CI: 2.6, 7.0)] and H9 [2.9% (CI: 1.4, 5.1)] in all four live bird markets from the two locations.
The study highlights the need for transboundary surveillance of H5 and H9 viruses and improved biosecurity practices in border LBMs to reduce maintenance and transmission of AIVs in Ghana.
Keywords: Rural Poultry; Avian Influenza; Live Bird Markets; Transboundary Animal Diseases; West Africa

5010P First characterization of oxytetracycline degradation in poultry using a simulated gizzard and hepatic S9 fractions

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Although oxytetracycline (OTC) has been widely used in poultry since the 1950s, comprehensive studies characterizing its degradation processes and biotransformation within the avian body remain lacking. We recently demonstrated the systemic presence (in plasma) of four OTC metabolites following its oral administration in laying hens. However, the specific organ(s) responsible for this biotransformation remain(s) unknown. The main objective of this study was to determine the respective contribution of the upper gastrointestinal tract and the liver to the biotransformation of OTC following its oral administration in laying hens.

Chicken simulated gizzard conditions were prepared in 50 mL tubes by mixing sterile water, 1 M sodium chloride, and 10 g/L pepsin, and adjusting the pH to 2. After 15 min of pre-incubation, OTC was added and the mixture was incubated at 42 °C with agitation. Samples were collected at 0, 5, 10, 15, 30, 45, 60, and 120 min, and enzymatic activity was stopped by transferring aliquots into acetonitrile. In parallel, a liver S9 fraction was prepared from pooled chicken liver lobes homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl and 2 mM EDTA, followed by centrifugation. The S9 fraction (1 mL) was pre-incubated at 42 °C, then supplemented with 10 mM OTC and an NADH regeneration system; samples were collected at 0, 2, 5, 10, 15, 30, 45, 60, and 90 min and mixed with acetonitrile to stop reactions. All experiments were performed in triplicate, and OTC and its degradation products were identified and quantified using high performance liquid chromatography coupled to mass spectrometry (HPLC–MS/MS).

Results showed a high stability of OTC in both the gizzard simulation and the liver S9 fraction. Despite the high sensitivity of the HPLC-MS/MS method (LLOQ = 0.002 µM), no OTC metabolites were detected. The stability of OTC observed under simulated gizzard conditions is consistent with its molecular structure, which lacks peptide bonds and is therefore not susceptible to pepsin-catalyzed hydrolysis. In the hepatic S9 system, the absence of detectable biotransformation suggests that the 90-minute incubation period may not adequately reflect the longer residence time that occurs during hepatic processing in vivo. Extending the incubation duration would therefore be necessary to more accurately assess potential metabolic conversion.

Overall, these findings indicate that OTC does not undergo substantial degradation in the gizzard environment. Future work should aim to clarify the contribution of hepatic first-pass metabolism to OTC biotransformation and to characterize the antimicrobial properties of any resulting metabolites.

Keywords: oxytetracycline; biotransformation; gizzard simulation; liver S9 fraction

5011P Effects of broiler breeder egg translucency and oviposition time on Salmonella specific maternal antibody transfer

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Raw chicken is a source of Salmonella infections in humans. To reduce Salmonella incidence in broilers, vaccines are administered to breeder flocks to induce Salmonella-specific maternal antibody transfer from hen to chick through the yolk. Egg translucency and oviposition time can influence egg and chick quality, and oviposition time has shown effects on maternal antibody (IgY) levels in the eggs of various wild bird species. The objective was to evaluate how translucency and oviposition time affect Salmonella specific maternal antibody transfer. To do this, eggs were collected from a 35-wk-old commercial broiler breeder flock at 2 oviposition times (A-9am and B-2pm) and held for 3 days before being sorted into 3 translucency scores (T1=low, T2=medium, T3=high). This created 6 experimental groups: T1-A, T1-B, T2-A, T2-B, T3-A, T3-B (N=30 eggs/group). Yolks were collected, weighed, and held at -20oC until further processing. Remaining eggs were incubated, chicks were euthanized at hatch to measure residual yolk weight, the residual yolk and blood plasma was collected, and samples were stored at -20oC until further processing (N=10 chicks/group). Total protein content was extracted from yolk and residual yolk using a chloroform extraction method. Total and Salmonella specific IgY (SAL IgY) levels in yolk, residual yolk, and plasma were measured by ELISA, and the proportion of SAL IgY in total IgY (% SAL IgY) was calculated. Data were analyzed by two-way ANOVA (translucency, oviposition time, and their interactions) using GLM Procedure in SAS. Means were separated using Tukey's HSD (significant at $P \leq 0.05$, trends at $0.05 < P \leq 0.10$). No statistical differences were observed due to translucency for any of the parameters analyzed. SAL IgY levels had a trend by oviposition time for both SAL IgY per mL-and in total-yolk content (P=0.071 and 0.069, respectively). SAL IgY levels were numerically higher in eggs laid in the afternoon (173 µg/mL and 3113 µg total yolk) than in those laid in the morning (126 µg/mL and 2252 µg total yolk). Additionally, yolks from afternoon eggs contained a higher % of SAL IgY (6.05%) than morning eggs (3.58%; P=0.035). There were no differences or trends in SAL IgY from residual yolk (in per mL residual yolk content, total residual yolk content, or % SAL IgY in total IgY) or plasma (in per mL plasma or % SAL IgY in total IgY). Previous research observed that oviposition time can play a role in chick quality, and these results show that oviposition time may also influence pathogen-specific maternal antibody transfer from hen to chick, potentially affecting the chick's disease resistance. However, further research is necessary to understand these interactions.

Keywords: Broiler Breeder; Translucency; Oviposition; Antibodies; Salmonella

5012P In vitro IncB/O-plasmid conjugation in poultry litter between Escherichia coli and Salmonella serovars results in variable decreases in antimicrobial susceptibility

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Plasmids harboring antimicrobial resistance (AMR) genes enable their transfer through conjugation. These genes can be transferred between bacterial pathogens, such as Salmonella, a major zoonotic pathogen affecting poultry farming. To address this public health burden, this study investigates the impact of transferring the IncB/O pH2332-107 plasmid between E. coli and Salmonella serovars Typhimurium (STM) and Enteritidis (SE) on AMR, under subinhibitory concentrations of antimicrobials used in the poultry industry. An in vitro 3 X 6 conjugation assay was conducted with three plasmid recipients - SE P125109, E. coli J62, and STM F98, and one plasmid donor - E. coli H2332; six antimicrobial treatments (T) and 5 replicates. To analyze the phenotypic resistance profile of the bacteria, antibiograms and minimum inhibitory concentration (MIC) tests were conducted. The conjugation assay was conducted on sterile poultry litter with antimicrobials calculated at 1% of the recommended dose for broilers per gram of litter: T1, control; T2, ceftiofur (0.002 mg); T3, gentamicin (0.002 mg); T4, sulfamethoxazole-trimethoprim (0.4+0.08 mg); T5, oxytetracycline (0.32 mg); T6, enrofloxacin (0.012 mg). Additionally, cefalexin MIC tests and antibiograms (amoxicillin-clavulanic acid, ceftiofur, ceftazidime,

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cefuroxime, ampicillin, chloramphenicol, kanamycin, ciprofloxacin, ofloxacin, nalidixic acid, enrofloxacin, streptomycin, tetracycline, gentamicin) were performed. The conjugation frequency was analyzed by two-way ANOVA followed by Tukey's test ($p < 0.05$) - GraphPad Prism® 10.0.0. Conjugation occurred more frequently between E. coli strains. Treatment effects varied: T3 increased SE conjugation frequency, T5 reduced it in STM and E. coli, and T6 decreased it in STM. The plasmid acquisition led to 128-, 64-, and 16-fold increases in cefalexin MICs for STM F98 (from 16 to 2048 µg/mL), SE P125109 (8 to 512 µg/mL) and J62 (16 to 256 µg/mL), respectively; donor MIC was 2048 µg/mL. The antibiogram revealed that the recipients became resistant to the same antimicrobials that the donor was resistant to, except STM, which also acquired resistance to ceftazidime, an antimicrobial to which H2332 remained susceptible. These findings suggest the plasmid harboring AMR determinants can confer elevated resistance levels when interacting with different bacterial genera by mechanisms that are still unknown. We hypothesize that these variable increases in resistance levels might be linked to the secondary resistome, playing a modulating role in AMR gene expression, or to bacterial intrinsic factors, although this requires further investigation.
Keywords: Antimicrobial resistance; Bacterial conjugation; Mobile genetic elements; Secondary Resistome; Antimicrobial Susceptibility Testing

5013P Viperin negatively regulates MAVS-mediated signaling through inhibition of MAVS proteasomal degradation

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Viperin is a highly conserved interferon-stimulated gene with potent antiviral activity against influenza A virus. During IAV infection, interferon signaling strongly upregulates viperin expression as part of the innate immune response. Viperin restricts viral replication by disrupting lipid rafts on the plasma membrane, thereby inhibiting virus budding and release from infected cells. Along with its antiviral role, human viperin suppresses the antiviral immune response by reducing type I interferon production through the MAVS signaling pathway. How exactly viperin mediates this inhibition is unknown. Since no studies have explored the immune modulatory role of viperin in avian species, we used a dual-luciferase assay to test whether duck and chicken viperin inhibit MAVS-mediated IFN-β promoter activity.We found that duck viperin inhibits duck MAVS-mediated signaling in duck cells but not in chicken cells, with certain duck viperin mutants showing enhanced inhibition. Similarly, chicken viperin effectively suppressed MAVS-mediated signaling in chicken cells. We showed that the inhibitory effect was dose- dependent in both avian species. All statistical analysis was completed using GraphPad Prism software one-way ANOVA (* = significant, ns = not significant). Confocal microscopy analysis revealed that chicken viperin does not co-localize with chicken MAVS in DF1 cells. Notably, co-expression of chicken viperin with MAVS altered MAVS expression patterns, mimicking the effect of the proteasome inhibitor MG132, indicating that viperin prevents MAVS degradation required for MAVS-mediated IFN-β signaling. Understanding how chicken and duck viperin suppress MAVS-mediated signaling may reveal a conserved regulatory pathway that is shared across species, including mammals and humans.

Key Words: Immune response, Viperin, MAVS, IFN-β, Proteasomal degradation

Keywords: Immune response; Viperin; MAVS; IFN-β; Proteasomal degradation

5014P Preliminary evaluation of cecal microbiota in a population of probiotic- and additive-supplemented broilers reared under the aerosol transmission induction model of bacterial chondronecrosis with osteomyelitis lameness

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Bacterial chondronecrosis with osteomyelitis (BCO) and its associated lameness is one of the most common and devastating issues the poultry industry constantly faces, both globally and domestically. Leveraging the currently accepted "leaky gut" model of pathogenesis, this study aims to evaluate the cecal community of broilers reared under the aerosol transmission BCO induction model. A trial was conducted using the same induction model for 56d with the following treatments: 1) Positive control (PC) – untreated birds on wire-flooring pens; 2) Negative control (NC) – untreated birds on litter-flooring pens; 3) LOW – birds treated with probiotic E. faecium spray on day-of-hatch (2.0 x 109 CFU/bird); and 4) HIGH – birds treated with LOW spray and combined with probiotic Bacillus amyloliquefaciens inclusion in the diet (500g/ton). Cecal contents were collected from six birds per treatment on d14, d28, d42, and d56 of the experiment, DNA extracted, and sent for 16s V3-V4 amplicon sequencing. Returned sequences were assembled and taxonomically assigned, after which diversity indices were analyzed (including alpha, beta, abundance). No significant effect was found between all treatments and positive/negative control groups in all timepoints, but timepoints are significantly different from each other in both alpha and beta diversity indices ($P < 0.05$). Abundance analysis also shows a high degree of dysbiosis with the phylum Bacillota (Firmicutes) dominating at 95.57% on average across all treatments, followed by Bacteroidota at 5.06% and Pseudomonata at 2.59%. These findings are the first to closely characterize the cecal microbiome in populations of broilers reared under the novel aerosol transmission induction model, offering further insights and possibilities into studies of BCO etiology and pathology.

Keywords: broiler; bco; aerosol transmission; microbiota

5015P Competitive exclusion alternatives can be efficient in promoting intestinal health during SalmonellaHeidelberg infection in chickens

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Salmonella Heidelberg (SH) is a zoonotic pathogen often isolated from chicken products, especially from meat. Competitive exclusion (CE) products, formulated from the commensal microbiota of healthy animals, have been used as an alternative to Salmonella spp. control, and improvement of intestinal health without the need to overuse antimicrobials. We evaluated the effects of CE on the caecal health of broiler chickens during SH infection from 21 days post-infection (DPI).

The CE was prepared from 10 serial subcultures grown under anaerobic and aerobic conditions of caecal feces from adult healthy broilers. One-day-old chicks from the treated groups (A, B, and C) received 100 µL of the respective CE product (anaerobic, aerobic, or combined culture). 24h later, birds from the same groups and the challenged control (group D) received 100 µL of SH inoculum (108 CFU/mL); an unchallenged control group (E, with 12 birds) was used. Moreover, birds were evaluated for cecal colonization, systemic infection, and SH shedding. Five birds from each group were euthanized at 21 dpi, and caecum, liver, splen and cloacal swabs were harvested. After DNA extractionof cecal content, they were subjected to library preparation (FLO-FLG114) and sequenc-

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ing (Nanopore MinION®). Statistics for both Alpha and Beta diversity indices were performed by a paired Kruskal-Wallis test and PERMANOVA ($P < 0.05$), respectively. Differential abundance analysis was performed by means of Linear Effect Size.
In the caecal microbiota of 22-day-old chicks, Group D was enriched in Clostridium (24.58%) and Blautia (22.03%), while Groups A and B displayed more balanced profiles; notably, Group C was characterized by a marked enrichment of Subdoligranulum (60.72%), a SCFA-producing genus. Alpha diversity differed significantly among groups ($P = 0.0412$), with the highest index in the unchallenged control (Group E, 3.44) and the lowest in the challenged control (Group D, 3.02). Beta diversity analysis revealed significant but moderate separation among treatments ($P = 0.002$). Notably, only Group C differed significantly from the challenged control (Group D) in SH counts ($P = 0.00928$). Group C showed less bacterial shedding and cecal colonization and was the only group with birds without systemic infection, revealing the impairment of the mixed group on SH control.
The administration of CE modulated the caecal microbiota in different ways, and not all formulations were effective against SH, which induced dysbiosis characterized by reduced richness; only the combined consortium (combined aerobic/anaerobic - Group C) significantly reduced SH colonization, being associated with Subdoligranulum enrichment and increased intestinal resilience.
Keywords: 16S rRNA; Alpha diversity; Beta diversity; Caecal microbiota; Paratyphoid disease

5016P In vivo characterization of the prophylactic and therapeutic effects of a phytoderivative blend on intestinal Escherichia coli and Lactobacillus spp. loads in laying hens inoculated with an APEC strain

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Antimicrobial resistance (AMR) is a critical issue in human and veterinary medicine. In poultry production, the rise of antimicrobial-resistant bacteria has intensified the search for alternatives to conventional treatments. Among these, phytoderived compounds such as essential oils have gained interest due to their antimicrobial properties. Although used in some livestock systems, their effects in laying hens at peak egg production remain insufficiently described.
This study aimed to evaluate the impact of a mixture of plant derivatives during oral inoculation with an APEC strain on E. coli and Lactobacillus loads in the ileal and cecal microbiota of laying hens. Five experimental groups were established. Group 1 was the negative control, and Group 2 received phytoderivatives only. Groups 3 and 4 received both phytoderivatives (0.5 mL/L in drinking water for seven days) and APEC inoculation, administered either prophylactically (Group 3) or therapeutically (Group 4). Group 5 received APEC inoculation only.
Phytoderivative supplementation for Groups 2 and 3 was provided from arrival until day 0, when hens were orally inoculated with 109 CFU of the APEC strain. Eight hens from Groups 1 and 2 were necropsied at this time. On day 1, Group 4 began phytoderivative supplementation, continuing until day 7. Necropsies were carried out on days 2, 4, 7, and 14 post-inoculation. At each necropsy, ileal and cecal samples were collected for quantitative bacteriology on MRS agar, MacConkey agar, and MacConkey agar containing nalidixic acid. Additional luminal content was stored for later microbiota analyses. Bacterial enumeration targeted the inoculated APEC strain, total E. coli, and lactobacilli to assess shifts in microbial communities.
A modest reduction in E. coli load was noted in the cecum of the therapeutic group. The inoculated strain was readily detected in the ceca of Group 5 but not in birds receiving phytoderivatives, whereas it was detected in ileal samples of Group 3. No changes in lactobacilli populations were observed between groups.
The present study indicates that phytoderivative supplementation, administered either prophylactically or therapeutically, exerted an effect on the infectious APEC strain at the cecal level- a finding of considerable interest. However, this supplementation did not influence the overall cecal lactobacilli populations. A comprehensive characterization of the microbiota composition and diversity will be necessary to further elucidate the microbial shifts associated with phytoderivative administration.
Keywords: Laying Hens; Alternatives; Essential Oils; Escherichia coli; Microbiota

5017P Effect of Dietary Aflatoxin B1 on Cecal Microbiome Composition in Broiler Chickens

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Aflatoxin B1 (AFB1) is a prevalent mycotoxin in poultry diets, and its presence can affect the gut microbiome, as it is a primary target. Disruption of this microbial community by AFB1 leads to a loss of beneficial taxa, promotes opportunistic pathogens, impairs intestinal barrier function and the immune system. The objective of this study is to identify the effect of AFB1 on the gut microbiome of broiler chickens. A study was conducted with a total of 72 Cobb 500 male broiler chicks, which were assigned to the two dietary treatments: 1. Control, and 2. Aflatoxin B1 (0.2mg/kg) with six replicates each for 21 days. Cecal contents were collected on day 21, and relative bacterial abundance was analyzed using Illumina MiSeq sequencing of the V3–V4 region of the 16S rRNA gene. QIIME2 was used for sequence processing, taxonomic assignment, and diversity analyses. The differential in abundance between the two treatments was evaluated using the non-parametric Wilcoxon rank-sum test. The results showed that at the phylum level, the relative abundance of Planctomycetota was decreased significantly by 90%, Nitrospirota_A_437815, and Acidobacteriota were completely eliminated in the AFB1 group ($p < 0.05$). At the family level, Moraxellaceae decreased significantly by 86% in the AFB1 group ($p < 0.05$), and Ruminococcaceae showed a trend of reduced abundance up to 45% in the AFB1 group ($p < 0.1$). Alpha-diversity indices (Shannon, Pielou, Observed Features, Faith's PD, Chao1) did not differ significantly between treatments ($p > 0.05$), but the richness and phylogenetic diversity were consistently numerically lower with AFB1 exposure. Beta-diversity indices showed a divergence in microbial community composition between the control and AFB1 groups, indicating that AFB1 altered overall microbial structure ($p < 0.1$). In conclusion, the results of this study show that AFB1 disrupts the gut microbiome of broiler chickens by altering bacterial taxa and shifting overall community structure. Although alpha diversity remained unchanged, significant reductions in beneficial phyla and families, together with distinct beta-diversity separation, indicate that AFB1 induces dysbiosis by modifying microbial composition rather than overall diversity.
Keywords: Aflatoxin; Gut microbiome; Relative abundance; Diversity index

5018P In ovo feeding of high levels of lysine did not improve embryonic development in broiler chickens

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Broiler chickens are highly susceptible to pathologies immediately after hatching, associated with their immature digestive and immune systems. The in ovo feeding (IOF) procedure was developed in part to improve embryonic development by supplying energy and essential amino acids (EAA), which are the critical nutrients, particularly during late stages. Lysine is one of the most limiting EAA in post-hatch broiler chicken feeds. However, less is known about the lysine requirement of the developing embryo. This study, supported by the AgriFutures Chicken Meat Consortium (PRO-016111), aimed to study the potential of lysine IOF to improve embryonic development. We hypothesized that IOF of lysine would improve embryonic development in a dose-response manner. Three hundred fertile Ross 308 eggs were randomly distributed into six IOF lysine doses (n = 50), T1: control (0.9% saline), T2: 20 mg, T3: 40 mg, T4: 60 mg, T5: 80 mg, and T6: 100 mg per egg. At embryonic day 17.5, 1 mL of the respective treatment was injected into the amniotic fluid. Hatchability, chick quality (deformity, navel score, body weight (BW), and length), and organ weights (n=10; residual yolk, heart, liver, intestine, gizzard and proventriculus) were recorded at hatch. Statistical analyses were conducted using SAS 9.4. Hatchability and chick quality were analysed using PROC LOGISTIC. The BW, length (with egg weight as the covariate), and organ weights (relative to BW) were analysed using PROC GLM. Tukey's test was used for pairwise comparisons ($P < 0.05$). Hatchability was not affected at the levels tested compared to the control, yet the 100 mg/egg dose reduced hatchability compared to the 20 and 40 mg/egg doses (75% vs 92% and 94%; $P = 0.047$). Doses 40, 60, 80 and 100 mg/egg resulted in chicks with deformities and open or leaky navels to those of control and 20 mg/egg groups ($P<0.001$). None of the doses affected BW ($P=0.111$), yet the doses above 60 mg/egg produced shorter chicks than the control ($P<0.001$). No differences in relative organ weights were observed across treatment ($P>0.05$), except for a reduction in relative heart weight in the 100 mg/egg group compared to the 20 mg/egg group ($P = 0.023$). It suggested that IOF of lysine may impact the circulatory system development. which, in turn, may help explain the significant difference on hatchability between these two doses. However, no intention to keep a constant osmolarity or pH was attempted in this research to avoid potential confounding effects between the active principle (Lys) and the solution vehicle. In conclusion, IOF lysine above 40 mg/egg showed signs of toxic effects. This experiment did not test doses lower than 20 mg/egg which warrant further investigation.

Keywords: Lysine; In ovo feeding; Broiler; Embryo; Chick quality

5019P AI-Enhanced Satellite Framework for Precision Mapping of Poultry Carbon Emissions in Canada

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Accurate mapping of atmospheric carbon dioxide over agricultural landscapes is essential for guiding emission mitigation strategies. We present a Spatiotemporal Vision Transformer with Wavelets (ST-ViWT) framework that reconstructs continuous, uncertainty-quantified atmospheric Carbon emission from OCO-2 satellite imagery across poultry-intensive regions of Canada. The model fuses wavelet time-frequency representations with transformer attention over meteorology, vegetation indices, topography, and land cover. The 2024 OCO-2 data, using ST-ViWT attains R-squared = 0.984 and RMSE = 0.468 ppm; 92.3% of gap-filled predictions lie within ±1 ppm. Independent ground validation shows robust generalization (bias = -0.14 ppm; r = 0.928), including faithful reproduction of the late-summer drawdown. Spatial analysis across 14 major poultry regions reveals a moderate positive association between facility density and Carbon emission (r = 0.43); high-density areas exhibit larger seasonal amplitudes (9.57 ppm) and enhanced summer variability. Compared with conventional interpolation and standard machine learning baselines, ST-ViWT yields seamless 0.25° emission surfaces with explicit uncertainties, enabling year-round coverage despite sparse observations. The approach supports integration of satellite constraints with national inventories and precision livestock platforms to benchmark emissions, refine region-specific factors, and verify interventions. Importantly, transformer-based Earth observation enables scalable, transparent, spatially explicit carbon accounting, hotspot prioritization, and policy-relevant mitigation assessment.

Keywords: Greenhouse gas mapping; AI for agriculture; Spatiotemporal modeling; Transformer deep learning; Precision livestock emissions

5020P Evaluating Electron Beam (eBeam) Technology for the Development of a Safe and Effective Campylobacter jejuni Vaccine in Broiler Chickens

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Campylobacter jejuni is a leading cause of foodborne illness worldwide, often linked to poultry consumption. Unfortunately, current strategies to control C.jejuni in poultry have had limited success, and a Campylobacter vaccine could be an effective alternative. Electron Beam (eBeam) technology can be used for producing safe and effective vaccines against poultry pathogens, including Campylobacter. This technology uses electricity as its source, making it environmentally friendly, cost-efficient, and free from chemical or health hazards. It inactivates pathogens by inducing double-stranded DNA breaks that prevent replication while preserving cell membrane integrity and antigenic epitopes that are essential for eliciting strong immune responses. This unique mechanism makes eBeam vaccines as safe as killed vaccines and as effective as live vaccines.

The objective of this study is to determine the possible alterations between freshly cultured and eBeam-treated C. jejuni in terms of membrane integrity, bacterial viability, and protein profiles. To achieve this, a multistrain cocktail containing six different wild strains of C. jejuni was treated with two eBeam doses: 3 kGy and 4 kGy, alongside a formalin control, one of the most used inactivation methods in vaccine development. The cocktail was plated on Campylobacter line agar to assess the vaccine concentration and, after eBeam treatment to check for regrowth of the bacteria. The treated bacterial cocktail was evaluated for membrane integrity (BacLight staining), cell viability (Alamar Blue and BacTiter-Glo assays), and proteomic analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The resulting microbiology analysis data were analyzed by one-way ANOVA followed by Dunnett's post hoc comparison, and for Alamar Blue and BacTiter-Glo assays, two-way ANOVA followed by Tukey's post hoc test was used in GraphPad Prism 10 Software. A P-value <0.05 was considered significant.

BacLight staining showed that both eBeam-treated samples had similar percentage of intact cell membranes compared to the fresh culture, whereas the formalin control showed a high percentage of damaged cells. Metabolic activity assays revealed that eBeam-treated and formalin-killed samples exhibited reduced metabolic activity compared to freshly cultured cells. The protein profiles of eBeam-treated samples showed that most of the proteins

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were similar to those of freshly cultured cells. We also evaluated the effectiveness of this eBeam vaccine in birds and assessed for resuscitation in vivo and detected no viable resurgence.
Our findings support eBeam technology as a viable method for developing safe and effective vaccine against Campylobacter in poultry.
Keywords: Campylobacter jejuni; eBeam; Broiler chickens; Protein profiles; Vaccines

5021P Molecular insights into Myosin oxidation by lipid oxidation products using docking approaches
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The decline in chicken meat quality during storage is mainly caused by lipid peroxidation and the subsequent covalent changes to myofibrillar proteins, especially myosin, which is a key factor in determining the texture, water-holding capacity, and gelation of meat. Although the effects of lipid oxidation on meat quality are well documented, the precise molecular mechanisms of the interaction between reactive aldehydes and myosin remain largely unexplored. This work aimed to investigate the effect of lipid oxidation products (MDA, ONE, tt-DDE, and HNE) on structural changes in myosin using molecular docking. The interaction between myosin and lipid oxidation product was determined using a molecular docking method. The crystal structure of myosin (ID:6YSY) was downloaded from the RSCB protein database (http://www.rcsb.org/pdb). The molecular structure formulas of MDA, ONE, tt-DDE, and HNE were downloaded from the PubChem Molecular Database (https://pubchem.ncbi.nlm.nih.gov/). The CBDOCK and Discovery Studio 2024 were applied to visually observe the binding parameters, such as amino acid residue sites and chemical forces. The preferred binding structure of the myosin-lipid oxidation complex with the lowest energy was exhibited. Molecular docking results showed distinct binding sites and mechanistic hotspots on myosin that facilitate aldehyde-induced modifications and directly affect poultry meat quality. First, we identified that MDA, ONE, and HNE all covalently bind to a specific functional site on myosin (residues A:394-A:400), forming conventional hydrogen bonds and irreversible covalent bonds with residues SER A:395, ASP A:397, and LYS A:400. This provides a molecular-level explanation for irreversible protein cross-linking that directly causes decreased water-holding capacity and tougher texture. Second, we found that tt-DNE works via a different mechanism. In contrast, tt-DDE specifically interacted with a hydrophobic pocket (TYR A:16, TRP A:113, TRP A:131) through Pi-Sigma and Pi-Cation interactions, binding to this pocket and inducing structural distortion due to an unfavorable steric clash, which would severely compromise the gelling capacity that is critical for processed chicken products. These reactions created an unfavorable bump that caused steric clashes, structural changes, and problems with gelling. These results illustrate a synergistic mechanism whereby covalent cross-linking by MDA, ONE, and HNE, in conjunction with structural distortion by tt-DDE, leads to the degradation of chicken meat quality. Understanding these processes will facilitate the design of more effective antioxidant interventions and contribute to improving the quality, stability, and shelf life of poultry products.
Keywords: myosin; lipid oxidation; molecular docking; meat quality

5022P Antimicrobial peptide residue detection in chicken breast muscle tissue
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Antimicrobial peptides (AMPs) are emerging alternatives to antibiotics for poultry production, but reliable residue detection methods to ensure low residual levels remain limited. This study aims to develop a standardized spiking protocol to validate analytical procedures for AMP residue detection in chicken breast muscle. The experimental design involves spiking tissues with a known concentration and processing them through a standardized extraction and cleanup protocol. To validate the spiking protocol, the AMP is spiked at four critical steps of the analytical procedure to allow for the comparison of detection success across different critical points.

Nisin (1 mM in 0.1% trifluoroacetic acid (TFA)) was added at four critical steps, including tissue homogenization, acetone precipitation, TFA-based extraction, and C18 spin-tip cleanup. Each step was tested independently using three biological replicates of breast muscle, resulting in a total of 12 spiking trials. After extraction, samples were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Bruker autoflex MALDI-TOF instrument operated in positive linear mode with α-cyano-4-hydroxycinnamic acid as the matrix. Analyses were performed by the MS Technician in the Department of Chemistry at UBC.

Detection outcomes were evaluated qualitatively by identifying nisin signals in the expected mass range and assessing whether the spectrum exhibited a characteristic nisin peak family within the instrument tolerance. No quantitative peak intensity analysis or statistical testing was performed.

The limit of detection for nisin was determined to be 10 μM. A final sample concentration of 100 μM, achieved by spiking from a 1 mM stock solution, was used for method evaluation. However, nisin was not detected in samples from the first three critical steps, as no characteristic nisin peaks were observed in the mass spectra. Detection occurred only in the samples obtained from the C18 spin-tip cleanup step, indicating that earlier extraction steps may result in substantial peptide loss or matrix interference. Additionally, the commercial nisin contained a high amount of NaCl, which can interfere with MALDI analysis by inhibiting proper matrix crystallization and promoting sodium adduct formation during desorption/ionization.

These results indicate that while MALDI-TOF MS can confirm AMP presence under purified conditions, significant optimization of extraction and cleanup steps is required for reliable AMP detection in complex food matrices. Further protocol refinement on addressing salt interference and improving analyte recovery is necessary before applying this protocol to broader tissue types.
Keywords: Chicken; Antimicrobial peptides; MALDI-TOF MS; Residue detection; Alternatives to antibiotics

5023P Effect of branched-chain amino acids in low-protein diets on performance, skeletal development, gut microbiota, and cellular and humoral immunity in broiler chickens
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The present study investigated the effects of different levels of branched-chain amino acids (BCAA) in low-crude protein (CP) diets on growth performance, skeletal development, ileal and cecal microbiota, and cellular and humoral immunity of broiler chickens. For this purpose, 480 one-day-old broiler chicks of Ross 308 strain were used in 6 treatments and 4 replications. Experimental diets were arranged in a 2 × 3 factorial design with two CP levels and three BCAA levels across starter (d 1–10), grower (d 11–24), and finisher (d 25–42) phases. The CP levels included a standard CP diet (23.0, 21.5, and 19.5%) and a reduced CP diet with a 10%

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decrease (20.7, 19.35, and 17.55%) for the respective phases. The BCAA treatments consisted of standard requirements and diets containing 10 or 20% higher BCAA levels relative to digestible lysine. At the standard level, the valine, isoleucine, and leucine to lysine ratios during the starter period were 76, 67, and 110%, respectively; during the grower period 77, 68, and 110%; and during the finisher period 78, 69, and 110%. The corresponding ratios were increased to 83.6, 73.7, and 121% (starter), 84.7, 74.8, and 121% (grower), and 85.8, 75.9, and 121% (finisher) at the 10% higher level, and to 91.2, 80.4, and 132% (starter), 92.4, 81.6, and 132% (grower), and 93.6, 82.8, and 132% (finisher) at the 20% higher level. Data were analyzed using the MIXED procedure of SAS in a completely randomized design, and mean differences were compared using Duncan's multiple range test at $P<0.05$. Supplementation of BCAA at 10 and 20% above requirements in low-CP diets increased body weight gain and improved feed conversion ratio compared with the low-CP diet without BCAA supplementation ($P<0.05$). Feeding 20% higher BCAA levels in low-CP diets increased tibia weight, breaking strength, and tibial calcium content ($P<0.05$). Moreover, this treatment increased ileal Lactobacillus counts and enhanced the cutaneous basophil hypersensitivity response to phytohemagglutinin at 4 and 12 h post-injection ($P<0.05$). In conclusion, supplementation of BCAA at 20% above recommended levels effectively mitigated the negative effects of CP reduction and improved skeletal integrity, gut microbiota, immune responses, and overall performance of broiler chickens.

Keywords: BCAA; broiler; humoral immunity; skeletal development; immunoglobulin

5024P Utilizing exogenous enzyme technology to enhance the value of alternative feed ingredients produced in Western Canada for poultry

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The use of underutilized, locally available alternative feed ingredients in poultry nutrition can support more circular and sustainable food systems that balance environmental and economic goals. Many of these ingredients are often overlooked primarily due to their high dietary fiber content. Optimizing their use requires precise characterization and strategies to enhance their feeding value. This research aimed to: 1) assess variation in non-starch polysaccharides (NSP) and crude protein (CP) content in Western Canadian ingredients, including short-season corn, soybean meal (SBM), canola meal (CM), cold-pressed canola expellers (CPCE), flaxseed, peas, and soy and oat hulls; 2) evaluate the potential of exogenous carbohydrase enzymes to degrade NSP and improve the dietary fiber bioactivity for poultry health and productivity.

A total of 117 samples were analyzed for NSP and CP. Average NSP and CP contents (% as-is) were 7.2 and 8.0 in corn, 15.3 and 45.1 in SBM, 19.2 and 38.7 in CM, 16.4 and 38.5 in CPCE, 17.7 and 21.5 in flaxseed, 13.0 and 23.9 in peas, 64.2 and 9.5 in soy hulls, and 50.1 and 4.8 in oat hulls. NSP varied most in oat hulls (CV=22.4%) and peas (CV=13%), while CPCE (6.2%) and flaxseed (7.3%) were the most uniform. The results revealed variations among ingredients, in both total NSP content and their constituent sugar composition. Variability in CP content was highest in oat hulls (CV=49%) and peas (CV=18.4%), and lowest in flaxseed and CPCE, with CVs of 5.5% and 6.3%.

Twenty multicarbohydrase preparations, differing in the ratio of xylanase, glucanase, cellulase, invertase, mannanase, and amylase activities, were evaluated in vitro for their ability to hydrolyse NSP in the feed ingredients. The degree of NSP depolymerization was determined based on changes in NSP content, and statistically analyzed using a t-test. Although varied within different enzyme preparations, the greatest NSP hydrolysis occurred in peas (19.6%, $p<0.00$), whereas the soy hulls responded only up to 3.8% ($p=0.24$).

Apparent metabolizable energy (AMEn) for CPCE and peas, determined in laying hens (24 weeks of age), was 2,169 and 2,086 kcal/kg DM, respectively. Enzyme supplementation increased AMEn to 2,420 and 2,185 kcal/kg DM, though differences were not significant.

The observed variability in fiber and CP underscores the need for ingredient-specific nutritional characterization. In vitro enzyme screening showed that NSP hydrolysis efficiency was substrate-dependent, highlighting the potential of targeted carbohydrase strategies to enhance the feeding value of underutilized feedstuffs.

Keywords: alternative feed ingredients; apparent metabolizable energy (AMEn); carbohydrate enzymes; laying hen; non-starch polysaccharides (NSP)

5025P IMPROVING GUT HEALTH AND MITIGATING COCCIDIOSIS IN BROILER CHICKENS THROUGH LACTOBACILLUS ACIDOPHILUS DERIVED POSTBIOTIC SUPPLEMENTATION

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Eimeria spp., the "hidden enemies," in chickens (food producing farm animals) cause coccidiosis, a self-limiting enteric disease of global concern. Ionophore antibiotics help control the infection, but overuse results in resistance, residues, and toxicity. Postbiotics/ Next Generation Probiotics are bioactive, non-viable metabolites from probiotics-offer a promising alternative for improving flock health. In this study, 224-day-old COBB broilers were divided into 7 groups with 4 replicates of 8 birds each (-CONT, +CONT, and infected Lactobacillus acidophilus-derived postbiotics (LAPB) supplemented at 0.2%, 0.4%, 0.6% v/w) for 42 days. On day (D) 24, birds were orally challenged with 10,000 Eimeria oocysts. The effects of LAPB on production, immunity, and gut health were evaluated. Postbiotics were prepared from pure colonies (Kareem et al., 2018, modified). Data were analyzed using one-way ANOVA and Tukey's HSD (SPSS v20). The 2×N methodology yielded high-purity LAPB with metabolite Lauryl/ K2H2 sulfate (anti-coccidiosis) through mass spectrometry. The LAPB fed groups showed higher gross weight gains ($p<0.01$), daily gains ($p<0.05$) and better FCR, feed intake at D42. Higher protein and immune organ index in LAPB fed birds suggested an immune-enhancement activity even under infected conditions. Higher antioxidant capacity, with reduced IL-10 expression and corticosterone indicates reduced inflammation ($p<0.05$) compared in supplemented birds than CONT groups. At 18 dpi, LAPB groups showed higher haemoglobin levels than infected CONT groups, higher humoral immunity for IBDV at 19dpv ($p<0.01$) whereas cell mediated immunity was unaffected with higher in +CONT. Oocyst shedding were significantly reduced by 31.5% in 0.6% LAPB from 6–11 dpi compared to -CONT, with lowest in +CONT, followed by 0.6%, 0.4%, 0.2% LAPB, and -CONT whereas intestinal weight (<3.7% +CONT, <4.6% -CONT). LAPB 0.6% group with higher livability (<6% +CONT, >11.9% -CONT), relative body weight gain (>1.2% +CONT, >11.5% -CONT), villi height (<4.7% +CONT, >17.46% -CONT), Anti-Coccidial Index efficacy (high +CONT: 186.23, medium 0.6% LAPB: 179.24, low -CONT: 155.37), better lesion scores (>5.7% +CONT, <13% -CONT), lower visual score at 6dpi (+ LAPB, ++ +CONT, ++++ -CONT) through blocking and

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competitive exclusion. Inclusion of 0.6% LAPB improved production, immunity, gut integrity and economics with reduced severity of Eimeria infection in broiler chickens. These findings suggest for potential reduction of coccidiostats and antibiotic growth promotors in broiler production. Further, postbiotics can augment the effect of coccidiostats and offers multifaceted gut and immune benefits, helping to tackle Anti-Microbial Resistance worldwide.
Keywords: Eimeria; Coccidiostat; AMR; Postbiotics; Lactobacillus

5026P Digestibility of medium-chain fatty acid-rich oils as alternatives to soybean oil in starter broiler diets

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Dietary fats provide energy and essential fatty acids (FA) in broiler nutrition. Medium-chain fatty acids (MCFA; C8:0–C12:0) are rapidly absorbed and represent an efficient energy source. MCFA-rich oils, particularly those derived from agro-industrial by-products, may offer alternatives to conventional fats while contributing to the circular economy. This study evaluated the effects of MCFA-rich fat sources with different free fatty acid (FFA) levels on ileal FA digestibility in starter broilers. A total of 512 newly hatched female Ross 308 broilers were randomly assigned to four dietary treatments, with eight replicates of 16 birds each. Diets based on wheat and soybean meal were supplemented with 4% of one fat source: black soldier fly oil (BSFO; 36.2% MCFA, 1.5% FFA), palm kernel oil (PKO; 48.8% MCFA, 2.9% FFA), palm kernel fatty acid distillate (PKFAD; 49.5% MCFA, 65.6% FFA), or soybean oil (SO; 61.7% polyunsaturated FA, 0.3% FFA) as control. Ytterbium oxide was included as an inert marker. On day 9, ileal digesta (from Meckel's diverticulum to 1 cm proximal to the ileocecal junction) was collected from 10 birds per replicate (6 replicates per treatment) to determine apparent digestibility coefficients. Data were analyzed by one-way ANOVA in R software, with means compared using Tukey's test ($P \leq 0.05$). Ileal MCFA digestibility was higher in broilers fed MCFA-rich fats (PKO, BSFO, and PKFAD) than in those fed SO ($P < 0.001$). Lauric acid (C12:0) reached approximately 89% digestibility in broilers fed PKO and BSFO, comparable to linoleic acid (C18:2) in SO (88.7%). In contrast, polyunsaturated FA were most efficiently absorbed in SO-fed birds (89.3%) compared with those fed PKO (78.2%), BSFO (72.2%), or PKFAD (73.4%) ($P < 0.002$). Broilers fed PKFAD consistently showed the lowest FA digestibility, with impairment becoming more pronounced with increasing chain length, particularly for palmitic (52.2%) and stearic (42.5%) acids. The elevated FFA content of PKFAD may have reduced the utilization of total FA, MCFA, and monounsaturated FA, suggesting a negative impact of high FFA levels in young broilers. These findings highlight the combined influence of chain length, degree of unsaturation, and FFA content on FA digestibility in young broilers, supporting the inclusion of 4% crude oils rich in MCFA and low in FFA levels (such as PKO and BSFO) as suitable alternatives to conventional oils in starter diets. The efficiency of MCFA utilization was comparable to that of PUFA and clearly superior to long-chain saturated FA such as palmitic and stearic acids. However, when MCFA-rich oils contain very high FFA levels (>60%), both MCFA absorption and overall fat utilization are compromised.
Keywords: Fatty acid digestibility; Medium-chain fatty acids; Lauric acid; Free fatty acids; Broiler chickens

5027P Formulations of different phytogenics as part of bioshuttle programs and as anticoccidial alternatives in broilers

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Coccidiosis is the main disease in the broiler poultry industry. It results in decreases in growth performance, feed efficiency and body weight uniformity. Bioshuttle programs involve the use of vaccination and anticoccidials, but few studies have reported this approach with bioactive natural compounds. By directly targeting Eimeria or by promoting intestinal health in chickens, phytogenics have been proposed as anticoccidial alternatives for broiler production. This study aimed at evaluating the effect of three phytogenic formulations on cocci-vaccinated or Eimeria-challenged broilers. A 35-day trial was conducted using 3,384 Ross 308 males, divided into 8 replicates of 47 birds/pen, subjected to 2 experimental conditions and distributed into 9 experimental groups: 1X dose of a coccidiosis vaccine (1X); 1X+phytogenics (PHY)1; 1X+PHY2; 1X+PHY3; 30X doses of a coccidiosis vaccine (30X); 30X+conventional anticoccidials and antibiotics control; 30X+PHY1; 30X+PHY2; and 30X+PHY3. Formulations were as follows: PHY1 is a dry mixture of cinnamaldehyde, orange and thyme oils; PHY2 is a dry mixture of cinnamaldehyde, eugenol, thyme, star anise and clove oils; PHY3 is a liquid mixture of cinnamaldehyde, thyme and star anise oils. 1X vaccination was administered using Coccivac®-B52 on feed at d1 according to the manufacturer's instructions and the Eimeria challenge was induced at d14 using 30X doses of Coccivac®-B52 in feed. In addition to performance measurements, the effects of the additives on coccidiosis were evaluated by intestinal lesion scores (ILS) and fecal oocyst excretion. Data were analyzed by a mixed model including treatment and barn section as fixed and random effects, and by Kruskal-Wallis tests. ILS were determined on 16 birds/treatment. In 1X vaccinated birds, the formulations had no significant effects on growth performances, on fecal oocyst excretion nor on ILS. However, in 30X challenged birds, PHY2 improved the following parameters compared to 30X controls: d20-d34 feed conversion ratio (FCR) (−3.1%, $P<0.05$), the d20-d34 European Broiler Index (EBI) (+6.7%, $P<0.05$), fecal oocyst excretion (−42%, $P<0.05$) and the Anticoccidial Index (ACI) (+56%, $P<0.05$). PHY2 also tended to improve overall (d0-d34) FCR (−1.7%, $P=0.09$) and overall EBI (+5.5%, $P=0.09$), compared to 30X controls. Overall, the three PHY formulations did not alter the vaccine cycling and one PHY combination showed anticoccidial effects and beneficial results on growth performance in the challenged broilers, suggesting that (i) they are compatible in a bioshuttle program with coccidiosis vaccination and (ii) one formulation could be used as an anticoccidial alternative in broilers.
Keywords: Coccidiosis; Growth performance; Coccidiosis vaccine; Eimeria challenge; Intestinal health

5028P Effects of a natural choline chloride feed additive on performance, egg quality, and fat accumulation of white laying hens.

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The objective of this experiment was to investigate the effects of a

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natural choline alternative (NCA) on the performance, egg quality, fatty liver scoring, and liver fat content of 60 to 72 wk old Hy-line W-36 laying hens. In total, 236 laying hens were allocated at 3 birds per pen across 20 replicate pens for each of the four treatments (initial egg production = 85%; P = 0.96). The dietary treatments were randomly allocated within blocks to include a negative control diet without choline supplementation (NC), NC + full dose choline chloride (CC) at 908g/ton, NC + 1/3 replacement dose (1/3 NCA) at 300 g/ton, and NC + 1/4 replacement dose (1/4 NCA) at 225 g/ton. Hen day egg production (HDEP), egg weight (EW), egg mass (EM), feed intake and feed conversion ratio (FCR) were analyzed every 2 wk to correspond with feed changes. Eggs were collected every 4 wk for egg quality determination. At the end of the experiment all remaining hens were euthanized, and abdominal fat pads were weighed for relative abdominal fat pad weight. Livers were collected from all hens and scored on a fatty liver scale and a sample was analyzed for fat content using an Ankom fat extractor. All performance and egg quality data were analyzed using repeated measures over the 2-week feeding or 4-week egg collection periods, respectively. Data were analyzed using ANOVA in SAS, if $P \leq 0.05$, means were separated using Student's t-test. The HDEP was 83.4, 83.1, 82.8, and 84.7 for the NC, CC 1/3 NCA, and 1/4 NCA hens, respectively. The 1/4 NCA hens had the highest HDEP ($P = 0.12$). Similar responses occurred with EM (53.1, 52.9, 53.0, and 54.0) and FCR (1.925, 1.932, 1.906, and 1.890), for the NC, CC, 1/3 NCA, and 1/4 NCA, respectively. The aforementioned improvements showed a trend, with 1/4 NCA fed hens having the highest EM and lowest FCR. No major or biological differences ($P > 0.05$) were noted in internal egg quality. However, 1/4 NCA hens showed trends of improvement ($P \leq 0.10$) for relative eggshell weight and eggshell thickness. Unexpectedly, there were no differences ($P > 0.05$) in relative fat pad weight, fatty liver scores, or liver fat content across all treatments. The results suggest that choline requirement of laying hens may be lower than previously reported, and the natural choline product might be driving the performance and eggshell quality via other mechanisms.

Keywords: Laying hen; egg production; choline; fatty liver score; fat pad

5029P Effects of xylo-oligosaccharides on growth performance and intestinal health of coccidia and Clostridium perfringens-challenged broilers

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This experiment aimed to evaluate the effects of xylo-oligosaccharides (XOS) on growth performance and intestinal health in broilers under Clostridium perfringens and coccidia. In total, 720 one-day-old Cobb broiler roosters were randomly divided into 8 treatments with 6 replicates of 15 replicate each. The experimental period is 42 days. The 2*4 factor design was used in the experiment, which included 2 types of attack (attack and no attack) and 4 types of XOS additive amount (0, 200, 400and 600 mg/kg). The infected groups received a 20-fold dose of coccidial vaccine orally on 14 days and C.perfringens suspension 1mL (1×109 CFU/mL) on days 18-20. The results show that□the challenge significantly decreased the 21d BW and 1-21d ADG ($P<0.05$) but increased 1-21d and 1-42d F/G, and raised the 22-28d mortality and culling rate ($P<0.05$). The challenge caused a highly significant elevation in intestinal lesion scores($P<0.01$). It significantly decreased the 35d VH and V/C in the jejunum and the 28d V/C and 35d VH in the ileum ($P<0.05$). The CD was notably deeper in the challenged group ($P<0.05$). Furthermore, the challenge led to an extreme significant increase in the levels of DAO in the serum of broilers at both 28d and 35d ($P<0.01$).The challenge significantly reduced the expression level of Claudin-1 at 28d and Nectin at 35d ($P<0.05$). And the expression levels of Mucin-2 and TNF-α were extremely significantly elevated at 35d ($P<0.01$). The challenge significantly reduced the content of SCFAs in the caecum at 28 days ($P<0.05$). The challenge caused a significant decrease in the relative abundance of Tenericutes ($P<0.05$), along with notable reductions in the relative abundances of RuminococcusUCG-014 and Alistipes ($P<0.05$). XOS supplementation tended to reduce 1-21d F/G ($P=0.070$) and mortality ($P<0.073$).XOS groups significantly lowered the lesion scores in the duodenum and ileum of 28d broilers ($P<0.05$). 400 mg/kg XOS significantly enhanced the VH of the ileum at 28d ($P<0.05$). Both 400 and 600 mg/kg XOS led to a significant decrease in DAO levels ($P<0.01$). The addition of XOS significantly upregulated the relative expression levels of Occludin at 28d and Occludin, E-Cadherin, and β-catenin at 35d ($P<0.05$). The XOS groups significantly increased the content of sSCFAs in the caecum at 28d ($P<0.05$). Furthermore, the addition of 400 mg/kg XOS significantly increased in the relative abundance of Faecalibacterium ($P<0.05$). Supplementation with XOS could alleviate the damage to intestinal morphology and intestinal barrier function, as well as the altered cecal flora structure in broilers caused by Coccidia and C.perfringens infections, improve intestinal health and enhance growth performance.

Keywords: xylo-oligosaccharides; broilers; coccidia; Clostridium perfringens; intestinal health

5030P Footpad dermatitis: the role of a carvacrol-based feed additive

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Food pad dermatitis is a multifactorial issue in broiler production, influenced by litter conditions and the quality of droppings. Phodé has developed a feed additive mainly based on carvacrol that improve gut health, enhance growth performance and reduce waste output. The aim of this trial is to evaluate the effect of this dietary supplementation on foot pad dermatitis. This trial was conducted at the NKP Research Farm (Thailand).

336 chicks (Ross 308) were randomly placed in 24 cages (n=14/cage) and assigned to two groups: 12 cages for control group (CTL) feed by a corn-soybean meal diet meeting recommendations for Ross 308, and 12 cages for a treated group (CAR) feed with the same diet with 125 ppm of the Phode feed additive replacing ground corn in the CTL formulation. The birds were bred in 3 phases: starter (d1-d10), grower (d11-d24) and finisher (d25-d35). To increase foot pad dermatitis challenge, the litter amount was reduced from 6 kg to 3.5 kg/m2. Foot pad dermatitis was evaluated as a health indicator using a 0-2 score (0 = healthy footpads; 1 = mild superficial lesions; 2 = severe lesions with haemorrhage and swelling).

Animals were weighted at day 1 and at the end of each feed phases. Feed intake and fed conversion ratio (FCR) were determined for the whole period of production from day 1 to day 35. Data were analyzed using ANOVA in a randomized complete block design (RCBD) using SAS software. P-value inferior to 0.05 was considered significant.

The number of birds showing foot pad dermatitis was significantly reduced by 34% in the CAR group compared to the CTL group, respectively 0.59 ± 0.080 vs 0.79 ± 0.067 (p=0.036).

The starter and grower phase results showed the same trend as those observed at the end of the finisher phase. Feed intake signifi-

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cantly (p=0.018) increased by 2.7% in the CAR group compared to the CTL group, respectively 3.68 ± 0.031 vs 3.58 ± 0.032 kg. The CAR group showed an 8 % higher feed intake compared to the Ross 308 recommendation (2019). Final body weight was also significantly (p=0.002) increased by 3 % in the CAR group compared to the CTL group, respectively 2.59 ± 0.016 vs 2.51 ± 0.019 kg. The CAR group also exceeded the Ross 308 reference by 6%. No significant differences were observed between control and treated groups for FCR.

Dietary supplementation with carvacrol-based additive reduced footpad dermatitis and improved broiler growth performance, suggesting a beneficial impact on animal welfare. The benefice of this carvacrol-based feed additive could be explained by a better digestibility of feed, allowing more dry dropings with less nitrogens for a better litter quality.

Keywords: Broiler; Foot Pad Dermatitis; Carvacrol; Oregano essential oil

5031P Effects of brown seaweed meal on blood glucose, growth, meat quality, and intestinal health of Ross 308 broiler chickens

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The effects of dietary brown seaweed meal on blood glucose, growth performance, feed intake, meat quality, and intestinal lesion scores of Ross 308 broilers were evaluated. The study was a completely randomized design with a 3 (in-hatcher diet) x 2 (grow-out diet) factorial arrangement of treatments. On day 18 of incubation, 792 viable embryos were randomly distributed into nine hatching trays and assigned to one of three in-hatcher feeding treatments: no feed (control), a starter diet and water, and a starter diet containing 2% brown seaweed and water (3 hatching trays/treatment). Diets were provided at hatch until the end of incubation. After hatch, 576 chicks from each treatment were placed into 24 floor pens (24 birds/pen) and fed one of two diets from day 1 to 35 of age: a standard broiler diet (D1) or a standard diet containing 2% brown seaweed (D2). On day 7, 26 and 35, one bird/pen was humanely euthanized to test blood glucose levels, as well as jejunal lesion scores on day 26 and 35, and meat quality analysis on day 35. Data were analyzed using the mixed model procedure in SAS. At day 33, the broilers fed the starter diet containing 2% brown seaweed and water at hatch gained significantly (p=0.02) more weight (2544.86g/bird) than those on the control treatment (2403.54 g/bird). There was no significant effect of post-hatch dietary treatment on day 33 broiler weight. However, feed conversion ratio was higher (p<0.05) in birds fed the 2% brown seaweed diet over the entirety of the growth trial. In-hatcher feeding did not affect (p>0.05) blood glucose or jejunal lesion score. Broilers fed D2 exhibited higher necrotic lesion scores (2.51) on day 35 (p=0.02) as compared with D1 (0.91), with scores ranging from 1 (thin or brittle walls with removable fibri) to 4 (localized necrosis or ulceration). There was no significant difference (p>0.05) among treatments for white striping, muscle pH or cooking loss. There was no significant difference (p>0.05) among the grow-out dietary treatments for L* score. However, broilers fed 2% brown seaweed in the hatcher had significantly darker (p=0.002) breast meat on day 35 (58.28) than the other two treatments, irrespective of post-hatch treatment. There were no significant interactions (p>0.05) between the in-hatcher feeding and grow-out dietary treatments for any of the parameters tested. In conclusion, in-hatcher inclusion of 2% brown seaweed improved early growth. However, long-term feeding throughout the production cycle was linked with reduced feed efficiency and impaired the intestinal integrity of the broiler chickens in this trial. We recommend investigating ingredient processing for future use of this brown seaweed in broiler diets.

Keywords: brown seaweed; broiler chicken; blood glucose; growth; intestinal health

5032P Evaluation of various concentrations of dietary phosphorus in pullet diets from 12-15 weeks of age on response criteria at 18 weeks of age

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An experiment was conducted to determine the non-phytate phosphorous (nPP) requirement of 12 to 15-wk-old laying hen pullets using body weight (BW), mortality corrected feed conversion ratio (FCRm), tibia ash percent (TAP), and tibia ash weight (TAW). The same pullets were transferred back to US industry-based diets and monitored for effects of the reduced nPP feeding from 12 to 15 wk at 18 wk of age. A total of 624 Hy-Line W36 laying hen pullets (12 wk old) were blocked across 48 cages and fed one of eight diets varying in nPP (0.11, 0.14, 0.17, 0.20, 0.23, 0.26, 0.29 and 0.45% nPP and 0.90% Ca). There were six replicate cages of 13 pullets within three-tier A-frame battery cages. Body weight gain (BWG) and mortality corrected feed conversion ratio (FCRm) were calculated over the 12 to 15 wk period. At 15 wk of age, the end of the experimental nPP feeding, 2 pullets per pen were euthanized for fat-free tibia ash. Data were analyzed using JMP Pro 16.0 and means were separated using Student's t-test ($P \leq 0.05$). Regression analysis was not possible due to lack of response to reduced dietary nPP. There were no differences of feeding nPP diets for BWG (188, 177, 179, 172, 164, 169, 168, and 156 g/bird: $P = 0.60$; SEM = 11.1, respectively); FCRm (6.25, 6.57, 6.60, 6.61, 7.71, 6.56, 6.79, and 7.34 g/g: $P = 0.56$: SEM = 0.52, respectively); TAP (54.5, 53.6, 54.7, 55.8, 54.6, 54.5, 55.3, and 54.7%: $P = 0.78$; SEM = 0.87, respectively), or TAW (1,895, 1,920, 2,020, 2,066, 2,008, 2,016, 2,003, and 1,929 g/tibia: $P = 0.22$; SEM = 49.8, respectively). Although no further statistical analysis was possible, it does appear that the values below 0.20% nPP did result in a numeric reduction in tibia ash response. Dietary treatments ended after 15 wk of age, and remaining pullets were maintained in their cages and were phase-fed a corn-soybean meal diet from 15 to 18 wk that contained 0.41% nPP and 2.45% calcium. As expected with the minimal responses directly after low P feeding, there were no carryover effects at 18 wk. Body weights ranged from 1,167 to 1,225 g/bird ($P = 0.23$; SEM = 13.7) and 15 to 18 wk FCRm, TAP, and TAW ranged from 10.13 to 12.02 g/g ($P = 0.93$), 56.0 to 57.8% ($P < 0.05$), and 2,168 to 2,310 ($P = 0.93$), respectively. These data indicate that the nPP requirement for pullets from 12 to 15 wks is lower than current commercial recommendations. Pullets provided adequate nPP after nPP deficiency from 12 to 15 wk of age had time to recover by 18 wk.

Keywords: requirement; tibia ash; non-phytate phosphorus; egg laying pullet; calcium

5033P Performance, Adaptability, and Research Potential of Indigenous Black and Brown Laying Birds under Nigerian Production Systems

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Objective

To evaluate and compare the performance, adaptability, and disease resistance of black and brown indigenous laying birds under Nigerian production conditions, with the aim of generating evidence to inform sustainable poultry development strategies.

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Experimental Design
A comparative field study was conducted on black and brown laying birds across Nigerian smallholder systems. Birds were assessed under extensive and semi-intensive systems using similar local feeds. The focus was on practical performance indicators relevant to rural farming, including productivity, health, and environmental adaptability.
Materials and Methods
Black and brown indigenous layers were sourced from rural farms. Birds were managed under low-input systems with local feeds (grains, insects, kitchen waste). Data were collected over a 12-month laying cycle. Parameters included feed conversion, egg/meat quality, body weight at 20 weeks, disease response, and heat stress behavior. Farmer records and lab diagnostics (e.g., antibody titers) supported data collection.
Statistical Analysis
Descriptive statistics were applied. One-way ANOVA ($p < 0.05$) tested differences between groups in key performance indicators.
Results
Black birds showed significantly better feed efficiency ($p < 0.05$) and higher egg quality—thicker shells and deeper yolk color ($p < 0.05$). While egg output was slightly lower (190–210 eggs/year) than in brown birds, the difference was not significant ($p > 0.05$). Black birds had higher body weight (1.6–1.8 kg) and better meat scores ($p < 0.05$). Disease resistance was markedly higher in black birds, with lower mortality and stronger antibody responses ($p < 0.01$). Heat stress response was superior, with stable production across seasons.
Conclusion
Black laying birds in Nigeria outperform brown ecotypes in disease resistance, feed efficiency, meat and egg quality, and heat resilience—making them ideal for smallholder, low-input systems. However, they remain underutilized and under-researched. Focused genetic improvement and adaptive management could harness their full potential for sustainable, climate-resilient poultry production in Nigeria.
Keywords
Indigenous poultry, Black laying birds, Disease resistance, Climate adaptability, Sustainable poultry production
Keywords: Indigenous poultry; Black laying birds; Disease resistance; Climate adaptability; Sustainable poultry

5034P Application of new reproductive materials in the Hungarian and Slovak Gene Banks
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Conservation of genetic material in poultry nowadays is not only focusing on semen cryopreservation. To enable the preservation of the heterogametic female sex in birds, an alternative approach has been developed, involving the cryopreservation and orthotopic transplantation of day-old ovarian tissue into recipients of the same age. By grafting frozen–thawed ovarian tissue, gonadal chimeric recipients can be produced, which, when artificially inseminated with semen from the donor breed, allow 100% recovery of the original genotype in the F1 generation. The origin of the progeny can be determined by microsatellite marker analysis and phenotype markers.
This method has already been established in several poultry species, our research group was the first to obtain donor-derived progeny through cryopreserved ovarian tissue transplantation in chickens and geese. The Slovak-Hungarian project Interreg HUSK/2302/1.2/018 was successfully awarded, and consequently, the Slovak gene bank will also incorporate this method into its techniques.
To expand the collection of samples stored in the Hungarian and Slovak gene banks, gonadal tissue samples were cryopreserved using the previously established vitrification technique. After the euthanasia of the donor animals, gonads were removed sterile and were immersed in DPBS and FBS solutions on 0°C. Subsequently the tissues were put on acupuncture needles and were treated in two types of vitrification solution (DPBS+20% FBS+7,5% DMSO+7,5% EG, then DPBS+20%FBS+15% DMSO+15% EG+0,5M saccharose) at room temperature. Then the needles were put in cryovials and stored in liquid nitrogen. Since Oravka is the only breed with the status of endangered native chicken in Slovakia, a total of 34 ovary and 58 testis samples were successfully preserved. In the Hungarian gene bank, several gonadal tissue samples from our indigenous chicken breeds are stored, and thanks to this project, gene bank storage could be extended to native waterfowl species: 48 ovarian tissue samples from White Hungarian geese, 93 from White Hungarian ducks, and 55 from Spotted Hungarian ducks were cryopreserved.
Since both the Slovak and Hungarian gene banks are members of the EUGENA network, expanding the stored samples represents a highly important activity for genetic preservation. This method can be used not only for the genetic preservation of indigenous breeds but also for conserving valuable intensive lines in the case of disease outbreaks.

The investigation was supported by Interreg HUSK/2302/1.2/018.
Keywords: gene conservation; poultry gonadal tissue cryopreservation; orthotopic transplantation; Oravka chicken; Hungarian waterfowl breeds

5035P A comparative analysis of egg production and hatching traits in three local chicken ecotypes of Togo
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Local chickens are the most commonly raised poultry breed in the rural areas of Togo, where they play a key role in reducing household poverty and food insecurity. They are referred to as nondescript chicken populations that are adapted to harsh environmental condition including extensive, small-scale, free-range production systems but unknown effective population size. However, their reproductive performance under improved rearing conditions remains poorly documented. This study was designed to evaluate and compare the egg production performance and hatching characteristics of three local chicken ecotypes (Forest, Atakora, and Savannah) under improved rearing conditions at the ITRA/CRAF station in Avétonou. Ninety (90) pullets and eighteen (18) cocks at 20 weeks of age, with 30 pullets and 6 cocks from each ecotype, were used for the study. The birds from each ecotype were divided into three replicate units, each containing 10 pullets and 2 cocks, and were reared separately under identical conditions for 23 weeks. Data collected on laying performance included age at first egg (weeks), body weight at first egg (g), egg production rate (% hen-day), egg weight (g), daily feed intake (g/bird/day), egg mass (g/hen/day), and feed conversion ratio (g feed/g egg). A total of 1,382 eggs laid by hens between 33 and 40 weeks of age were used to evaluate hatching characteristics, including fertility rate (%) and

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hatching rate (%). Data were analyzed using a one-way ANOVA, and means were compared using Tukey's test at a significance level of $p < 0.05$ with R software version 4.3.3. The results revealed significant differences ($p < 0.05$) among the three ecotypes for egg production, egg weight, feed intake, egg mass, feed conversion ratio, and hatching rate. In contrast, age at first egg, body weight at first egg, and fertility rate did not differ significantly ($p > 0.05$). The Atakora ecotype demonstrated significantly ($p < 0.05$) superior performance in egg-laying rate (47.2% hen-day), egg weight (41.2 g), daily feed intake (88.16 g/bird/day), and egg mass (16.29 g/hen/day). Conversely, the Savannah ecotype showed a significantly ($p < 0.05$) lower feed conversion ratio (5.01 g feed/g egg) and a higher hatching rate (85%) compared to the Atakora ecotype. This study suggests that both the Atakora and Savannah local chicken ecotypes possess good potential for egg and chick production, which could be valuable for genetic improvement programs. The Savannah ecotype appears more suitable for selection aimed at chick production due to its superior feed efficiency and higher hatching rate. The Atakora ecotype, with its high laying potential, could be more valuable in breeding programs designed to enhance egg production.

Keywords: Laying performance; local chicken; ecotype; hatching characteristics; Togo

5036P Reproductive Performances in High and Low Water Efficient Female Broiler Breeders under Heat Stressed Conditions

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Global poultry meat and egg productions are facing many challenges, including water scarcity driven by global warming. Recently, two broiler lines were genetically selected for high (HWE) or low (LWE) water efficiency; however, until now their reproductive performances have not been investigated. Therefore, this study aimed, to determine the effect of heat stress (HS) on reproductive performances of HWE and LWE female broiler breeders. Modern Random Bred (MRB), LWE, and HWE hens were placed into battery cages in environmental chambers at 57 weeks of age. Half of each line was assigned to either a HS or thermoneutral (TN) treatment in a 2x2 factorial design. Birds were acclimated for 5 days, then chronic cyclic HS (30°C/8h/day) started at 58 weeks of age in half of the chambers. TN birds were maintained at 18°C. Water consumption was calculated weekly; while body weights were collected prior to HS 1 week after HS, and at the conclusion of the trial. Eggs were collected daily; Egg weight, shell breaking force (kg), Haugh units, and shell thickness (mm) were determined using an Egg Tester Ultimate (ORKA Food Technology). Birds were sampled at 62 weeks of age and hypothalami were isolated and kept at -80°C for molecular analyses (n=6/line/environment). Hypothalamic total proteins were extracted and sent to IDeA National Resource for Quantitative Proteomics, UAMS. Reproductive data were analyzed by Two-Way ANOVA and Tukey multiple comparison test using Graph Pad Prism software (version 10). Data are expressed as mean ± SEM and are considered significant at $P \leq 0.05$. One-way ANOVA showed a significant decrease in HWE egg weight compared to MRB. However, there was no significant difference for shell thickness, egg number, shell-breaking force, and Haugh units between HWE and LWE under both environmental conditions (HS and TN). High-throughput proteomics analysis identified several differentially expressed proteins between lines and environmental conditions. Together, these data indicate that the divergent selection for water efficiency did not deteriorate the reproductive performance; however further investigations are required.

Keywords: Heat Stress; Broiler Breeder; Water Efficiency; Reproductive Performance ; Proteomics

5037P Schizochytrium Algae Powder vs. Expanded Flaxseed: Impacts on Quail Production Performance and Egg ω-3 PUFA Accumulation

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Abstract:

ω-3 polyunsaturated fatty acids (ω-3 PUFA□are essential for humans, and the health benefits of ω-3 PUFA-rich foods are well-documented. Our previous study demonstrated that quail fed diet with addition of Schizochytrium algae powder (SAP) at 3.2% could produce the ω-3 PUFA enriched eggs with 300 mg / 100 g, but results in a laying rate lower than the average of large-scale commercial layer farm. Thus, this study aimed to compare the efficacy of 3.2% SAP to 6% expanded flaxseed (EFLA) on quail laying performance and egg ω-3 PUFA enrichment.

A single-factor experimental design was employed, consisting of three treatments: T1 (control, CON) fed a basal diet, T2 fed diet with 6% EFLA, and T3 fed diet with 3.2% SAP. There were 10 replicates of 50 quails per treatment. The trial was lasted for 8 weeks. To evaluate the fatty acid composition, total egg lipids were extracted using a chloroform-methanol-water system and derivatized into FAMEs via BF3-mediated transesterification. Following isolation with n-hexane, the FAMEs were analyzed by gas chromatography (GC) for quantitative determination of fatty acid profiles. Data were analyzed using one-way analysis of variance (ANOVA) through the general linear model (GLM) procedure in SAS 9.4. Compared to the CON, the results are as follows:

1) Egg weight was significantly increased in the 6% EFLA and 3.2% SAP groups during the periods of 1– 4 weeks, 5– 8 weeks, and 1– 8 weeks ($P < 0.05$) with 1.57%, 1.68%, and 1.73% by 6% EFLA, and 2.96%, 1.53%, and 2.23% by 3.2% SAP respectively.

2) Both 3.2% SAP and 6% EFLA significantly improved the yolk index and Haugh unit of fresh eggs at week 4 ($P < 0.05$).

3) At 14 days, the contents of ALA, DHA, and total ω-3 PUFA in egg ($P < 0.05$) were significantly increased by 3.2% SAP and 6% EFLA with ω-3 PUFA in egg enrichment at 360.58 mg/100 g by 6%EFLA and 333.52 mg/100 g by 3.2% SAP. The ω-3 PUFA of egg yolk was dominated by DHA (70.10%) followed by ALA (29.89%) in 3.2% SAP group, whereas DHA (50.42%) and ALA (49.57%) in 6% EFLA group.

4) 6% EFLA significantly increased the egg alcohol flavor compounds ($P < 0.05$), while 3.2%SAP significantly elevated the alkene and ketone contents ($P < 0.05$).

In conclusion, 3.2% SAP in quail diet could produce the ω-3 PUFA enrichment egg (ω-3 PUFA >300 mg/100 g egg) that was same as 6% EFLA with no negative impact on laying performance. But 3.2% SAP increased the DHA content than 6% EFLA.

Keywords: Quail; production performance; ω-3 PUFA; quail egg; fatty acids

5038P Quantifying Social Rank in Growing Broiler Chickens Using Aggressive Rank Index

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With growing interest in broiler chicken well-being and environ-

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mental enrichment requirements in commercial houses, understanding social rank is crucial for considering how agonistic relationships impact welfare and resource access. Aggressive Rank Index (ARI) is a validated measure of social rank in laying hens. The objectives of this study were to determine whether ARI can be generated in growing broiler chickens and whether these indices differ within a group across time. Birds were individually identified using wing-bands and animal-safe food coloring, then allocated to 6 floor pens (1.83 m by 0.91 m each) for 45 days (n = 5 birds/pen). Aggressive interactions were assessed via 5-minute video recordings every two hours from 04:00 h to 23:00 h on days 1-5, 12, 19, 26, 33, and 40 of life. Ten observational periods totaling 50 min/day yielded 500 minutes of evaluation per bird. Following the methods of Girard et al. (2017), aggressive interactions were classified into three categories: pecks, threats, and fights. ARI was then calculated by dividing the number of aggressive encounters won by the total number of aggressive encounters observed, generating a unique ARI for each bird. Broilers were ranked as low aggression (ARI 0-0.32), medium aggression (ARI 0.33-0.67), high aggression (ARI 0.68-1), or non-participants (birds that did not engage in aggressive interactions). Data were analyzed using RStudio (R version 2025.09.1+401). Fisher's Exact Test was used to analyze the distribution of ARI amongst broilers. To evaluate changes in rank over time, ARI was assessed using a linear mixed model with the main effect of week of life. Over the first 5 days, 23.3% of birds were classified as low aggression, 38.0% as medium aggression, 22.7% as high aggression, and 16.0% as non-participants ($P = 0.01$), demonstrating formation of social rank during the first week of life. Across weeks 1-6 of life, 30.0% of birds were classified as low aggression, 25.6% as medium aggression, 26.1% as high aggression, and 18.3% as non-participants ($P > 0.05$), indicating that while numerical differences existed among categories, social rank did not shift significantly over the grow-out period. There was no effect of week of life on ARI ($P > 0.05$), suggesting that birds may have established social rank during week 1 and these ranks remained relatively stable over time. This pilot study identified ARI as a potential tool for quantifying broiler aggression and identifying differences in social rank throughout the grow-out period in a research setting. Future work should focus on validating this measure in larger broiler groups and in commercial production to determine the impact of rank on bird welfare and resource access.
Keywords: Social Status ; Broiler; Aggressive Rank Index (ARI) ; Dominance ; Welfare

5039P Does one size fit all? Effects of strain and aviary design on roosting behavior of laying hens

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Aviary housing enables hens to perch and roost in elevated spaces, behaviors thought to be important for laying hen welfare. We investigated how hen strain and aviary design influenced nighttime roosting location. We hypothesized that 1) more hens would roost in aviaries and on perches when given easier access to aviary tiers via ramps and flat-topped perches, 2) aviary and perch use would increase over time, and 3) white-feathered hens would roost in aviaries and on perches more than brown-feathered hens. Hy-Line white and brown pullets were reared in 16 groups of 40 (eight/strain) in 3.3 x 3.3 m2 pens with access to the first tier of a three-tiered aviary containing round perches. At 17 weeks of age (woa), pullets gained access to the full aviary, which was modified in half of the pens (four/strain) to include ramps and flat-topped perches. We recorded the number of hens roosting within the aviary (on a perch or the tier flooring) on five consecutive nights per week at 15, 18 and 20 woa. Generalized linear mixed models with binomial distributions were used to test the effects of aviary design, hen age, strain, and their interactions on the total proportion of hens roosting within the aviary and the proportion of hens within the aviary that roosted on a perch. Pen was included as a random effect. Roosting in the aviary was affected by the interactions of age with strain ($p<0.001$) and age with aviary design ($p<0.001$). Nearly all white hens roosted in the aviary throughout the study (mean ± s.e.: 15 woa: 100±0%; 18 woa: 99.9±0.06%; 20 woa: 99.8±1.9%), whereas the proportion of brown hens roosting in the aviary increased over time (15 woa: 56.4±3.3%; 18 woa: 64.7±2.7%; 20 woa: 73.6±2.8%). The proportion of hens roosting in standard aviaries was similar across all ages (15 woa: 74.4±4.5%; 18 woa: 78.9±3.8%; 20 woa: 81.6±3.2%), but increased in modified aviaries from 18 to 20 woa (15 woa: 82.0±3.8%; 18 woa: 85.7±2.9%; 20 woa: 91.8±2.2%). Roosting on perches was also affected by the interactions of age with strain ($p=0.038$; white hens: 46.0±1.6%, 52.5±1.2%, 44.2±1.9% at 15, 18, 20 woa; brown hens: 65.7±2.9%, 65.3±2.7%, 58.7±2.4% at 15, 18, 20 woa) and age with aviary design ($p<0.001$). The proportion of hens roosting on perches was highest at 18 woa in standard aviaries (15 woa: 53.9±3.5%; 18 woa: 64.4±2.4%; 20 woa: 61.1±2.0%) but decreased throughout the study in modified aviaries (15 woa: 57.8±1.8%; 18 woa: 53.4±1.8%; 20 woa: 41.7±1.8%). In summary, the modified aviary design, which improved access to higher tiers, resulted in increased nighttime aviary use. Given that nearly all white hens roosted in the aviary at all ages, these modifications may have most behavioral impact for the brown strain.
Keywords: Aviary; Laying hen; Perching; Pullet; Roosting

5040P Allometric evaluation and bone ash content of locomotor apparatus of broiler chickens under two different environmental enrichment conditions

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The broiler industry's advances in genetics and management have produced faster-growing birds, but this rapid growth has compromised both locomotor health and animal welfare. As welfare concerns rise among consumers and producers, various forms of environmental enrichment (EE) such as perches, have been studied to mitigate these issues.

This study evaluated the effect of two perch designs on leg health in sexed Ross 308 chickens. It was hypothesized that using perches as EE would reduce leg pathologies without affecting bone measurements or ash concentration (AC%), regardless of sex. Four hundred Ross 308 broilers (200 males, 200 females) were randomly assigned to three treatments: Control (MC for males, FC for females), one-level perch (MP, FP), and two-level perch (MPP, FPP), with three replicates per treatment and sex. Weekly weight gain (WWG) and feed conversion rate (FCR) were recorded from the 3rd to 7th week of age (woa). At 7 woa, tibiotarsal–tarsometatarsal joint angles (TJA) were measured. After slaughter, femur (Fm), tibia (Ti), and tarsometatarsus (Mt) from six birds per treatment and sex were collected, measured, weighed, and incinerated to determine AC%.

Data normality was tested using Kolmogorov–Smirnov and Shapiro–Wilk tests. One-way ANOVA followed by Tukey tests were

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applied to normally distributed data, while the Kruskal–Wallis test and Fisher's LSD were used otherwise.
WWG and FCR did not differ significantly among control and EE groups for either sex. In males, MPP birds had higher Fm AC% (38.35 ± 2.05%) than MC (34.56 ± 2.93%) and MP (35.73 ± 2.17%) (p = 0.002) and showed shorter Mt length (8.9 ± 0.43 cm) than MP (9.31 ± 0.33 cm) and MC (9.03 ± 0.33 cm) (p = 0.027). In females, proximal Fm width (1.98 ± 0.19 cm) and Ti length (11.8 ± 0.53 cm) were lower in FPP than in FP (2.18 ± 0.21 cm; 12.42 ± 0.94 cm) and FC (2.15 ± 0.27 cm; 12.34 ± 0.21 cm) (p = 0.027, 0.007). Ti weights were also lower in FPP (24.58 ± 3.34 g) than in FP (27.25 ± 5.38 g) and FC (28.75 ± 2.18 g) (p = 0.039). Left-leg joint angles were significantly lower in FPP (3.87 ± 2.60°) than in FP (5.37 ± 3.13°) and FC (5.10 ± 2.98°) (p = 0.015). No other significant differences were found.
In conclusion, perch-based EE did not affect productive parameters (WWG, FCR) but showed potential benefits for leg health through improved bone mineralization and joint angulation. These results suggest that incorporating perches may improve locomotor welfare in broilers without compromising productivity.
Keywords: broiler welfare; locomotor health; tibiotarsal angulation; environmental enrichment; bone ash

ORAL PRESENTATIONS

Environmental Impact I

2000 Phytase: a historic perspective of technology driving sustainable poultry production
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Phytase is currently ubiquitous across major poultry production resulting in improved use of grain and oil-seed phytate, allowing for the reduced use of inorganic phosphorus (P) in poultry dietary formulation and reducing the amount of P extracted into manure or litter. But if we look back 30 plus years ago, phytases were an unproven technology trying to break into poultry diets. The objective of this presentation is to outline the history of phytase use in poultry diets and to understand how this adaptation has improved the sustainability of poultry production. Phytate is an insoluble form of P that is used by plants to allow for P storage in seeds until germination when P is needed and can be liberated by seed produced phytases. This storage is critical for plant propagation but becomes an issue when these grains and oil-seeds are fed to non-ruminant animals that lack the pre-gastric fermentation to efficiently utilize the P from the phytate. Poultry nutritionists have traditionally managed this issue by the addition of inorganic P to drive bird performance and welfare, resulting in an increase in P excreted from the backend of the birds. This increased excreted P has built up around long-term poultry production areas resulting in environmental impacts and the need to increase excreta management when applied to croplands and fields. Although the science of phytase use in poultry has been demonstrated for some time, commercial adoption did not occur in a widespread basis until the 1990's. Early phytases were effective at degrading phytate and releasing P for use by the bird, but over time additional efficiencies have been captured to increase the value of phytase to poultry production. Using current scientific values for P liberation, it is possible to reduce total dietary P concentrations by approximately 25% with the use of phytase. Not only does the use of phytase reduce overall P output, but it also allows for more efficient use of poultry manure without additional transportation costs (economic and environmental costs). Genetic progress and feed efficiency have traditionally been used to demonstrate the sustainability of poultry production, but the adoption and use of phytase enzymes are also a great example of technology driving the sustainability of poultry production.

Key words – poultry, sustainability, phytase, phytate, technology

2001 Towards Sustainable Organic Broiler Production: Impacts of dietary use of Algae and Legume Proteins
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Sustainability in broiler production is linked to feed-related environmental impacts, particularly carbon footprint and nitrogen emissions.
Soy protein has a high climate impact, particularly when the effect of land-use change (LUC) is considered, making strategies to reduce soy protein important for sustainable broiler production. Previous studies show improved digestibility and nitrogen retention in broilers fed microalgae compared with soy, indicating potential reductions in NH3- and N-emissions. Therefore, replacing soy protein with a mix of microalgae and pea protein in organic broiler diets to reduce environmental impact was investigated, with focus on maintaining growth performance, yield, and animal welfare.
540 broilers were distributed between nine pens of 60 birds and allocated one of three diets: (1) soy-based control, (2) based on the microalgae Scenedesmus sp. and pea protein, and (3) pea protein-based diet. The broilers were kept under practical organic production conditions and weighed on day 8, 24, 29, 36, 43, and 47. Growth performance, footpad and plumage conditions, carcass and meat quality were evaluated. For each treatment, average climate impact per kg live chicken was calculated using ESGreen-Tool, which aggregates effects of farm activity and GFLI data for conventional feed ingredients, on greenhouse gas (GHG) emissions applying international GHG guidelines, with the output being kg CO2e emissions/kg live bird.
For statistical analysis the software program R was used. Linear and logistic regression models were used for growth performance and welfare parameters.
Group 2 tended ($p<0.10$) to display greater growth performance and a significantly higher live weight (1.8 kg LW) on day 47 than group 1 ($p=0.039$, 1.7 kg LW). The growth rate for group 2 was improved ($p=0.020$, 53.2 g/day) from day 36 to 43, compared with group 1 (46.2 g/day). The carbon footprint was 4.22 and 3.33 kg CO2e/kg live chicken for groups 1 and 2, respectively. The plumage condition was improved for group 2 ($p<0.0001$, 0.33 LSmean) compared to group 1 (0.65 LSmean).
Group 2 had a higher breast meat yield than group 1 (30% vs.29%, $p=0.025$). There was a consumer preference ($p=0.01$) for group 2 rather than group 1. The climate impact decreased by 21% kg CO2e/kg broiler, when including microalgae-pea as main protein source compared with a soy-based diet (including LUC), it also improved animal welfare and breast meat yield.
In conclusion, a combination of microalgae and pea protein is a promising alternative to soy protein in sustainable organic broiler production concerning climate impact, animal welfare, and product quality.
Keywords: Microalgae, pea protein, organic broiler, climate, welfare.
Keywords: Microalgae; Pea protein; Organic broiler; Climate; Wel-

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2002 Effect of different levels of dietary energy on growth, gut ph, carcass, and bone traits of ross 308 broiler chickens

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This study was designed to evaluate the effect of different levels of energy-protein ratios on the growth, gut pH, carcass, and bone traits of Ross 308 broilers in an 8-week trial in high-temperature housing conditions. Four hundred and twenty (420) day-old Ross 308 broilers were allocated to five dietary treatments of different energy levels with six replications in a Completely Randomized Design. Each treatment had 84 birds with 14 birds per replicate. The treatments were T1, 2,900 kcal/kg; T2, 2,975 kcal/kg; T3, 3,050 kcal/kg; T4, 3,125 kcal/kg; and T5, 3,175 kcal/kg. The data collected include growth performance, gastrointestinal pH, organ and bone traits. The results showed a significant effect (P<0.05) of treatments on live weight, gain, intake, and FCR except liveability on d 14, 28, 42, and 56, indicating birds fed high-energy diet (3,125 kcal/kg) performed better compared to those on other treatments. Gizzard pH of birds fed diets containing 2,975 and 3,125 kcal/kg was higher (P<0.05) compared to those fed 2,825, 2900, and 3,049 kcal/kg. Gizzard weight was highest (P<0.05) on d 56 for birds fed diets of 2900, 2975, 3050, and 3,125 kcal/kg. Femur diameter was highest (P<0.05) for birds fed diets comprising 2975, 3050, and 3175 kcal/kg compared to those on 2900 and 3125 kcal/kg on d 24. On d 56. Birds fed a lower energy diet of 2900 and 2975 kcal/kg recorded greater tibia and femur weight. Overall, the study showed that the 3,175 kcal/kg diet enhanced growth performance, particularly live weight, gain, and FCRc in broilers.

Keywords: Broilers; Dietary energy; Bone traits; Gut pH; high housing temperature

2003 Production performance and energetic efficiency of brown layers in a cage free system

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The objective of this study was to characterize production and energetic patterns among commercial brown layer strains (Bovan, Hy-line, and Tetra), for hen-day egg production (HDEP, %), egg mass (EM, g/b/d), production ME (MEp, kcal/b/d), and energy cost (kcal/kg EM) across single cycle cage free conditions. Data from the 40th North Carolina Layer Performance and Management Test were used to model three strains (B1-3). Each strain was reared in identical UEP Guideline compliant cage-free pens (2 replicate pens/strain; 60 birds/pen). Strains were fed common rearing and production diets (5 and 8 phases, respectively). Bi-weekly HDEP, EM, feed intake (g/h/d), and body weight (BW, g) were recorded during rearing, with additional BW transfer (17 wk), 69, 73, and 93 wk. Strain-specific Gompertz models were fitted to estimate weekly BW for energy partitioning, with all models having an $R2 \geq 89$. Maintenance ME (kcal/b/d) was calculated using weekly BW0.75 times a weighted temperature adjustment, and MEp was determined by difference. Energy cost was determined as kcal/kg of egg produced ([MEp/EM] x 1000). For HDEP, EM, MEp, and energy cost, a repeated measures GLM evaluating the linear and quadratic effects of week, strain, and week by strain in JMP 18. An AR(1) covariance structure was applied to week, to account for the covariance in successive time points. Non-significant interactions ($\alpha = 0.05$) were removed. Peak HDEP of 85.80, 91.70, and 93.65% was achieved for B1-3 at 33, 37, 33 weeks, with an approximately linear decline post peak ($P < 0.05$). Differences ($P < 0.05$) were noted among strains, with B2 and B3 maintaining higher average HDEP than B1 (84.6 and 82.9 vs 75.1%, respectively). For EM, a quadratic response ($P < 0.05$) was observed for week, and differences between strains ($P < 0.05$). Strain B2 had the highest average EM (54.4 g/b/d) and B1 had the lowest average EM (48.1 g/b/d), whereas B3 EM (52.5 g/b/d) was similar to both B2 and B1. A quadratic response ($P < 0.05$) for week was also observed in MEp, along with strain differences ($P < 0.05$). Strain B2 allocated the most ME to production (134 kcal/b/d), higher than B1 and B3 (122 and 115 kcal/b/d, respectively). Energy cost had a quadratic response for week ($P < 0.05$) only, indicating a shared efficiency trajectory. However, mean energy cost differed ($P < 0.05$), with B3 having the lowest cost (2223 kcal/kg egg) compared with B1 and B2 (>2450 kcal/kg egg). Collectively, the brown strains displayed distinct production profiles and energy use patterns, with B3 demonstrating the most favorable energetic efficiency relative to its output, while B1 produced fewer eggs with greater ME required per unit of egg mass.

Keywords: brown layers; egg production; cage free; metabolizable energy; energetic efficiency

2004 Evaluating white layer strains production and energetic performance in a cage free system

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The objective was to characterize divergence in production patterns and energetic efficiency among commercial white layer strains (Babcock, Hy-Line W36, LSL-Lite, Tetra, and Dekalb), when fed and managed identically. Hen-day egg production (HDEP, %), egg mass (EM, g/b/d), production ME (MEp, kcal/b/d), and energy cost (kcal/kg EM), for strains were evaluated under a standardized cage free system (UEP guidelines). Data were obtained from the 40th North Carolina Layer Performance and Management Test, where strains W1-5 were housed in identical cage free pens (2 replicate pens, 60 birds/pen). Common rearing and production diets (5 and 8 phases, respectively) were fed accoring to body weight (BW, g) and production. Bi-weekly HDEP, EM, feed intake, and BW were recorded, with additional BW at 17, 69, 73, and 93 wk. Weekly production BW were estimated using strain-specific Gompertz functions ($R2 \geq 90$). Maintenance ME (kcal/b/d) was calculated from BW0.75 and a temperature-adjusted constant, with MEp determined by difference. Energy cost (kcal/kg egg) was calculated as [MEp/EM] × 1000. Repeated-measures GLM (JMP 18; $\alpha = 0.05$) included linear and quadratic effects of week, strain, and strain by week, with AR(1) covariance structures for weeks. For HDEP, a week x strain interaction ($P < 0.05$) was observed, indicating that strains differed in both timing and magnitude of peak production. Peak HDEP occurred at 90.4% at 33 wk for W1 and at 96.6, 92.1, 97.8, and 97.8% at 29 wk for W2–W5, respectively. Similarly for EM, a week x strain interaction ($P < 0.05$) was observed, in accordance with the rise in production and separation among W1-5. The greatest average EM was observed for W4, W5, and W2 (58.6, 57.3, and 57.1 g/b/d, respectively), whereas W1 and W3 averaged substantially lower EM (48.7 and 46.7 g/b/d, respectively). For MEp, there was no interaction between week and strain ($P > 0.05$), however a quadratic response for week ($P < 0.05$) and difference among strains ($P < 0.05$) was observed. Efficient strains (W4, W2) partitioned more ME toward production (138.8 and 130.8 kcal/b/d), while lower-producing strains (W3, W1) showed reduced MEp (106.5 and 116.5 kcal/b/d). Energy cost also differed among strains ($P < 0.05$), although the effect of strain shifted according to week due to the week x strain interaction ($P < 0.05$). Nonetheless, W1 consistently had higher energy cost compared with the other strains across weeks. Overall, white egg layer strains

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showed considerable variability in production level, EM trajectory, and energy conversion efficiency. Even under identical diets and management, genetic strain strongly influenced the magnitude and timing of production and the energetic cost required to sustain it.
Keywords: white layers; egg production; cage free; metabolizable energy; energetic efficiency

2005 Interactive Effects of Feeding Frequency and Light Duration on Hematology, Lipid Profiles, and Heat Stress in Ross 308 Broilers

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Hematological and lipid profiles are critical indicators of poultry health, reflecting responses to nutritional and environmental stressors. In tropical broiler production, optimizing feeding and lighting regimens is essential to mitigate heat stress and high metabolic demands. This study investigated the interactive effects of feeding frequency and light duration on hematology, lipid profiles, and stress responses in Ross 308 broilers. A total of 600 Ross 308 chicks were assigned randomly to twenty-four floor pens in a 2 × 3 factorial design in a CRD with feeding frequency set at either twice daily (2FT) or four times daily (4FT), and light duration maintained at 16, 20, or 24 h (treatments: 2FT_16H, 2FT_20H, 2FT_24H, 4FT_16H, 4FT_20H, and 4FT_24H). Each treatment was replicated four times with 25 birds per pen (1.5 x 1.5 m = 2.25 square meter), resulting in 11 birds per square meter. Water was provided ad libitum, while feed was provided either twice or four times daily depending on the treatment. Although light intensity was not measured, white LED lighting was consistently used during the experimental period. Interactions ($P < 0.05$) were observed between feeding frequency and light duration for key hematological indices, including white blood cells (WBC), hemoglobin (HGB), and hematocrit (HCT). Birds under 4FT_24H exhibited higher WBC counts (231.48 vs. 210.11), HGB (10.76 vs. 8.98), and HCT (38.58 vs. 28.45%) compared with 2FT_16H. Feeding frequency and lighting duration also interacted to influence stress indicators, lymphocytes (LYM), heterophils (HET), and HET-:LYM, whereas no significant interactions or main effects were detected for lipid profiles (total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein cholesterol). Specifically, 2FT_16H birds showed reduced LYM (8.18 vs. 11.15%), elevated HET counts (74.68 vs. 70.48%), and higher HET:LYM (8.91 vs. 6.35) compared with 4FT_24H. In conclusion, frequent feeding (4FT) improved immune-related markers (WBC, HGB, LYMP%), while reduced feeding frequency (2FT) elevated stress indicators (HET%, HET: LYM). Extended light duration (24H) further enhanced WBC counts and mitigated stress responses. The study demonstrates that feeding frequency and light duration interactively modulate broiler physiology, providing a viable management strategy to enhance resilience in tropical production systems.
Keywords: Broiler chickens; feeding frequency; light duration; hematology; stress indicators

2006 Re-evaluating THI thresholds in broilers for better heat stress assessment

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Heat stress remains an ongoing concern in poultry farming. Accurate heat stress assessment is essential to start interventions and mitigate welfare and performance losses. The temperature-humidity index (THI) combines ambient temperature and relative humidity as stress indicator, but current guidelines are often outdated for modern broiler strains and do not account for rapid weight gain or farm-specific factors, underestimating heat stress onset and severity.

This study evaluated the impact of animal- and farm-related factors on heat stress responses. Data were collected during 3 summers (2022-2024) in two semi-commercial Belgian broiler houses on the Experimental Poultry Centre (Geel, Belgium), comprising 4441 animal (Ross 308) observations and 894 pen observations including data from day 21 on. Individual data included body temperature and respiration rate (visually counting the animal's breaths for 20 seconds), while pen-level data contained feed intake and panting prevalence (% of birds displaying panting behaviour). Observations were modelled against THI at the time of observation and against the average THI at the day of observation, respectively (assessed by continuous logging equipment and calculated by: THI = 0.8 x T + ((RH/100) x T - 14.3)) + 46.4) using linear broken-line models. Body weight (BW, g), stocking density and housing type were tested as covariates based on AIC and error metrics.

The breakpoint for respiration rate occurred at 79.6 THI, which decreased with 0.2 units per 100 g BW increase ($p<0.001$), thus a breakpoint for individual weight can be calculated by: 79.6 – (0.002 x BW). For body temperature, the breakpoint was 77.3 THI and decreased by 0.11 units per 100 g BW increase ($p<0.001$), which means that a breakpoint for individual weight can be calculated by: 77.3 - (0.0011 × BW). Respiration rate increased by 5 breaths/min per THI before the breakpoint and 20 breaths/min per THI after the breakpoint. Body temperature increased by 0.03 °C with every increase in THI before the breakpoint and 0.18 °C per THI after the breakpoint.

Panting prevalence and average daily feed intake were evaluated using stocking density and housing facility as covariates. Panting prevalence increased by 4% per THI before the breakpoint (74.9 THI), whereafter the increase was 9% per THI. Daily feed intake declined linearly (4 g/day per THI unit). These results demonstrate nonlinear responses to THI. Distinct, weight-dependent breakpoints occurred between 77.3 and 79.6 THI, representing a set of critical thresholds beyond which animal indices deteriorate more rapidly. Overall, these findings highlight that static THI guidelines underestimate the heat stress onset and severity in broiler production.
Keywords: broilers; temperature-humidity; broken line models; heat stress; respiration rate

2007 Effect of fasting on growth, gut histomorphological and microbial responses of heat-stressed broiler chickens

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Heat stress (HS) occurs when animals are unable to regulate their body temperature and produce or absorb more heat than they can dissipate. In Bangladesh, a severe and prolonged "heatwave" was observed during the summer of 2024, with temperatures reaching nearly 43 degree C, leading to a sudden drop in productivity and high mortality among fast-growing commercial broilers, ultimately causing significant financial losses for farmers. This problem is particularly acute in poultry, as they are unable to regulate their body temperature due to excess metabolic heat, the absence of sweat glands, and highly insulated feather covering. The current study aimed to optimize fasting duration to mitigate the HS in broilers raised under hot and humid conditions for 5 weeks. A total of 500 broiler day-old chicks were allocated into five treatment groups: T0 = Non-fasted controlled temperature (25-26 degree C), T1= Non-fasted heat stressed (30-38 degree C), T2 = 6 hours fasted heat stressed, T3 = 8 hours fasted heat stressed, and T4 = 10 hours fasted heat stressed. The temperature, both in controlled and open-sided houses, was variable at different points of a day.

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The Completely Randomized Design was used. The temperature-humidity index (84-89) clearly showed that the birds faced the challenge of extreme HS. As expected, the T0 group showed better growth (2168.25 g/b) and feed conversion ratio (FCR;1.49); however, among the heat-stressed birds, T3 exhibited the significantly highest growth (1921.14 g/b) and FCR (1.60). Fasting of heat-stressed broilers achieved higher dressing yield, breast meat, and liver yields but significantly lowered abdominal fat and birds' mortality. Fasting for 8 and 10 h significantly improved breast meat pH, water-holding capacity and reduced cooking loss. It also enhanced the color quality of broiler breast meat. Serum glucose (158.59 mg/dl), AST (178.25 U/L), ALT (8.1 U/L), and Heterophil-Lymphocyte ratio (0.39) were significantly lower, while packed cell volume was higher in T3 (30.67 mm). Villi height, width, and crypt depth of gut segments also improved significantly in T3. Total bacterial and coliform counts in cecum contents decreased notably with longer fasting periods. Fasting improved the length, width, and weight of the spleen, bursa, or thymus, which had regressed in heat-stressed birds. The benefit-cost analysis indicated higher profitability in T3. Based on these findings, it can be concluded that an 8 h fasting is an effective approach to mitigate the HS under the hot and humid climatic conditions of Bangladesh.
Keywords: Commercial poultry; Heat stress; Feed restriction; Fast-growing; Gut health

2008 Influence of Genetic Background on Heat Stress Signatures and Thermal Resilience Pattern in Meat type Chickens

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Increased thermal stress in tropics necessitates the identification and characterization of region-specific poultry breeds with inherent tolerance to ensure sustainable production under changing climatic conditions. The present study evaluated the adaptability of three chicken genotypes with different growth rates —slow-growing, CARIDEBENDRA (CDR), medium-growing, CARIBRODHANRAJA (CBD) and fast-growing VENCOBB 430Y (COBB)—to elucidate their thermotolerance mechanisms and potential option for mitigating the impacts of climate change. Chickens (60 per strain) were distributed as three study groups and reared under standard managemental conditions till 33 days. On day 35, birds were subjected to short-term cyclic heat stress (HS) in a controlled psychrometric chamber (34.5 ± 1°C; 75 ± 5% RH; THI > 90) for 7 days, 6 h/day, and samples were collected on days 1, 3, 5 and 7 of HS exposure. Corticosterone concentrations and heterophil-to-lymphocyte ratios were higher (P=0.000) in COBB, intermediate in CBD, and lowest in CDR, with all strains showing a progressive rise under heat exposure. The attenuated response in CDR reflects reduced HPA axis activation and enhanced stress resilience. Hormonal profile showed a triiodothyronine suppression (p=0.000) under HS, though CDR and CBD maintained comparatively higher levels. Thyroxine showed temporal (p = 0.000) and strain–time interactions, indicating an initial compensatory hyperthyroid phase followed by its suppression with prolonged exposure. Plasma HSP70 concentrations differed (p=0.014) among strains, being highest in CDR, followed by CBD and lowest in COBB, reflecting rapid activation of cellular defense mechanisms. Although insignificant, HSP90 was relatively higher in CDR and CBD compared with COBB. HS reduced (p=0.000) antioxidant capacity in all strains, indicating oxidative imbalance; however, CBD and CDR maintained higher antioxidant, suggesting superior oxidative stress resilience and partial recovery by the end of exposure. Upregulation (p=0.000) of HSP70 and GPX1 genes was observed, particularly in CBD and CDR, indicating early activation of chaperone-mediated protection and antioxidant defense. In contrast, COBB exhibited delayed and transient responses, consistent with lower thermotolerance. Collectively, CDR, followed by CBD, demonstrated superior thermotolerance through moderated endocrine activation, stable immune responses, and enhanced molecular resilience. These findings highlight the potential of these genotypes as valuable genetic resources for breeding programs aimed at improving heat tolerance and ensuring sustainable poultry production under tropical and climate-stressed conditions.
Keywords: Broiler genotypes; Heat stress; thermo-tolerance; endocrine responses; HSP70

2009 Comparative study of the reproductive performance of African and European guinea fowl reared under a litter-based system in tropical climates

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Summary

The guinea fowl, although very popular, has lower reproductive performance than the hen. The development of high-performance strains such as Galor and the use of artificial insemination in cages have made it possible to intensify its production. However, in the face of new animal welfare regulations gradually prohibiting the use of cages on farms, it is becoming urgent to find breeding alternatives that maintain a satisfactory level of performance. This study aimed to compare the reproductive performance of African local and European guinea fowl (Galor) reared on the ground in West African conditions.

A total of 270 guinea fowl, including 150 of Galor strain (120 females and 30 males) and 120 of African local strain (60 females and 60 males) aged 25 weeks, were divided into three treatments with five replicates each: T1 (Local ♂ × Local ♀), T2 (Galor ♂ × Galor ♀) and T3 (Local ♂ × Galor ♀). A sex ratio of 1 male for 2 females was applied. Each repetition was raised on litter in a 6 square meter parquet floor equipped with a nesting box, a drinking trough and two feeders. Feeding and watering were provided manually. During the 40 weeks of the trial, feed consumption, weight growth, average egg weight, and laying rate were measured weekly. Eggs laid each week were collected, stored, and then incubated under standard conditions over the weekend to determine fertility and hatching rates. Data were subjected to one-way analysis of variance followed by a Tukey post-test for comparison of means. The results are expressed as an average ± SEM. The results indicate that food consumption and weight growth were significantly lower ($p < 0.001$) in the T1 treatment. The laying rate and egg weight of Galor females (T2 and T3 treatments) were significantly higher ($p < 0.001$). Fertility was detected in hatching eggs from T1, T2 and T3 treatments from 26, 36 and 28 weeks of age, respectively. In the last 30 weeks of the trial, fertility was significantly higher ($p < 0.001$) with the eggs from the cross with the local males (68.3% in T1 and 65.7% in T3) than with the Galor males (22.4%). The hatching rate was higher ($p < 0.05$) in T3 (81.6%) than in T1 (74.4%) and T2 (75.4%).

In conclusion, Galor guinea fowl have a high egg-laying potential, but limited fertility in litter-based rearing. The use of local males significantly improves the fertility of Galor females.

Keywords: African local guinea fowl, Galor guinea fowl, Litter-based rearing, Fertility, Hatchability

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Keywords: African local guinea fowl; Galor guinea fowl; Litter-based rearing; Fertility; Hatchability

2010 Trade-off between foot pad health and ammonia emissions in commercial broiler production

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In Flanders, poultry producers face a dual challenge. On one hand, they are legally required to reduce ammonia emissions from poultry houses; on the other hand, retailers increasingly demand production methods that enhance animal welfare. This study compares performance, environmental and animal welfare indicators of a slow-growing low stocking density broiler concept against the conventional broiler production concept.

The experiment was conducted in a semi-commercial research facility comprising eight pens of 112.5 m2 each. The experimental groups were a conventional fast-growing strain (Ross 308) at high density (42 kg/m2; 2360 birds per pent) (CH), versus a slower-growing strain (Hubbard Redbro) at lower density (30 kg/m2; 1700 birds per pen) (SL). Both groups were each flock setup on the same dates in four pens, and this was repeated during four consecutive flocks. CH broilers were slaughtered at 40 days of age, and SL broilers at 49 days. Thinning (25%) was performed on day 33 (CH) and day 40 (SL).

All pens were identical in layout and provided with alfalfa bales and perches for environmental enrichment. Data on mortality, feed intake, and water intake were collected daily. Footpad lesions were scored on day 32 and 39 (CH), and on day 39 and 48 (SL). Ammonia concentrations were continuously monitored using Fourier Transform Infrared Spectroscopy (FTIR). The ventilation rate was measured with calibrated measuring fans.

Although the slaughter weights of CH birds (2620±70 grams) and SL birds (2680±98 grams) were comparable, significant differences were observed in performance parameters. CH birds exhibited higher daily growth rates (64.4±1.8 grams/animal/day vs. 53.8±2.0 grams/animal/day, resp.), daily feed intakes (97.1±1.7 grams/animal/day vs. 92.7±4.0 grams/animal/day, resp.) and daily water intakes (173.6±5.8 ml/animal/day vs. 159.8±6.2 ml/animal/day, resp.) than SL birds. In terms of environmental impact, ammonia emissions were significantly lower in the CH bird pens (4487±1477 grams/day) than in the SL bird pens (12509±2241 grams/day). Litter quality was superior in the SL pens as reflected by dry matter content (69±3 % vs. 59±5 %, resp.), moisture score (6.19±0.48 vs. 4.92±0.67, resp.) and friability scores (5.28±0.53 vs. 4.33±0.60, resp.). Consequently, footpad lesion scores were markedly lower for SL birds (0.82±0.20) than in CH birds (1.82±0.75). This study showed an improved footpad health but also increased ammonia emissions associated with slower-growing broiler strains at lower stocking densities. The findings highlight the complex interaction and trade-off between welfare-oriented production strategies and their environmental implications within commercial broiler production systems.

Keywords: Slow-growing; Stocking density; Ammonia; Environmental Impact; footpad

General Health/Disease/One Health I

2011 The Gut as the Central Hub of Disease Resistance in Poultry: Integrating Nutrition, Microbiota, and Immunity

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Modern poultry production faces a convergence of challenges, including evolving pathogens, antimicrobial resistance, and the global mandate to reduce antibiotic use. Recent research suggests that successful interventions, ranging from dietary additives to vaccines, share a common biological focal point: the gastrointestinal tract. Rather than a simple digestive organ, the gut serves as a central hub integrating nutrition, microbial ecology, and immune regulation. As the host's largest immune organ and primary environmental interface, the intestinal tract employs the microbiota to shape disease resistance via competitive exclusion, immune modulation, and the production of bioactive metabolites. These processes maintain epithelial barrier integrity and regulate inflammatory signaling, which are critical for defending against major pathogens such as Salmonella, Campylobacter, Clostridium perfringens, Avian Influenza Viruses, and Eimeria. Nutrition acts as the primary driver of this network; dietary fibers, probiotics, and plant-derived bioactives are increasingly used to bolster gut resilience and reduce pathogen colonization. This presentation provides an integrative overview of the gut as the control point for poultry disease resistance. Key focuses include the nutritional modulation of mucosal immunity, emerging microbiome-based interventions, and the synergy between gut health and traditional biosecurity. By framing the intestine as a unifying biological framework, this talk highlights the necessity of multidisciplinary strategies to ensure a resilient and sustainable supply of animal protein.

2012 Transmission Study Testing Hvt-H5 Vaccines Against Highly Pathogenic Avian Influenza (HPAI) H5N1 Virus (Clade 2.3.4.4b), 24- weeks Post Vaccination

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This study is the second of four transmission experiments assessing long-term protection provided by vaccination of laying hens under field conditions against highly pathogenic avian influenza (HPAI) H5N1 (clade 2.3.4.4b). The primary objective is to evaluate whether vaccination reduces virus transmission (R<1).

Previously, vaccination with VECTORMUNE® AI or VAXXITEK HVT+IBD+H5 provided protection at 8 weeks post-vaccination. This study investigates protection at 24 weeks, including the effects of a Volvac® B.E.S.T. AI+ND booster at 12 weeks after VAXXITEK HVT+IBD+H5 vaccination. Chickens reared under field conditions were transported to high-containment facilities at peak egg production and challenged with HPAI H5N1. Four groups were studied: (1) non-vaccinated controls, (2) VECTORMUNE® AI-vaccinated, (3) VAXXITEK HVT+IBD+H5-vaccinated, and (4) VAXXITEK HVT+IBD+H5 plus booster.

All control chickens were infected (R=15.4). Transmission was lower in vaccinated groups (VECTORMUNE® AI: R=1.0 or 1.9; VAXXITEK HVT+IBD+H5: R=7.9 or 2.8), with only the booster group achieving R<1 (R=0.6 or 0.3). Mortality was 100% in controls but reduced to 10% in groups 2 and 3, with none in the booster group. Virus shedding was lower in vaccinated chickens. All vaccinated groups developed antibodies, and VECTORMUNE® AI showed significant T-cell proliferation and T-cell activation at 7 dpi.

Vaccination reduced transmission, mortality, and virus shedding. Only the booster group achieved R<1, though confidence intervals were broad. Further analysis of field data and additional transmission studies are needed to confirm vaccine effectiveness over the full production cycle and its potential to prevent sustained transmission.

Keywords: Highly Pathogenic Avian Influenza (HPAI) H5N1; Vaccina-

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tion; Transmission; Laying hens; Booster immunity

2013 MOLECULAR EPIDEMIOLOGY OF INFECTIOUS BURSAL DISEASE VIRUS IN EUROPE IN 2021-2025

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Infectious bursal disease virus (IBDV) is a globally distributed pathogen with well-established implications for the poultry industry, due not only to acute disease but also to the consequences of the resulting immunosuppression. IBDV features a double stranded, bi-segmented RNA genome that is highly prone to mutation and reassortment, which in turn may lead to significant phenotypic differences. Recent diagnostic advancements, particularly the adoption of phylogenetic classification systems considering both genome segments, prompted the execution of several epidemiological surveys, allowing a better understanding of the actual variability between IBDV strains circulating around the world.

The present study focuses on the IBDV situation in Europe, reporting the results of molecular diagnostic activities conducted in 10 countries between 2021 and June 2025. Over this period, a total of 2147 pooled bursal samples, each representing a different flock, were analyzed using two RT-PCRs, allowing the characterization of both the VP2 and VP1 genes of the detected IBDV strains. Vaccines strains were found in 838 samples (39.0%), whilst 716 samples (33.3%) tested negative and 18 (0.9%) were positive but could not be sequenced. The remaining 575 samples (26.8%) were positive for field strains: specifically, 538 belonged to genotype A3B1 (Very virulent/classical reassortants), 32 to A9B1 (Portuguese), 3 to A0B1 (Serotype 2), 1 to A3B2 (Very virulent) and 1 to AxB1 (Unknown genotype). The largely predominant field type was represented by the so-called A3B1 North-Western European reassortants (508 detections): such strains, which have been associated to subclinical IBD with severe immunosuppression, were found in all the investigated countries, spreading at an alarming rate despite emerging only around 2017. Other relevant field types, such as a separate A3B1 clade and the A9B1 IBDVs, were respectively found only in Italy and Portugal, demonstrating a more restricted circulation, which was further suppressed by the entrance of the North-Western European reassortants in said countries.

Even if based on convenience sampling, these results point to a stark increase in infectious pressure in Europe and, when considered together with recent epidemiological evidence from other regions of the continent, they suggest that a shift toward subclinical forms might be occurring. The present data thus demand for attentive monitoring and control measures, aimed not only at protecting against clinical forms but also at suppressing viral circulation.

Keywords: Infectious bursal disease virus; Europe; immunosuppression; molecular epidemiology; reassortment

2014 In Ovo Model for Avian Hepatitis E Virus in Layer Chickens

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Avian hepatitis E virus (aHEV) is the causative agent for big liver and spleen (BLS) disease. Commercial broiler breeders and layer hens are susceptible to infection, however clinical presentation varies. Clinical signs of disease include decreased egg production ranging between 10-40% in layer hens and 5-20% in broiler breeders. Increased mortalities are also observed, along with higher consumption of feed and water. Hepatosplenomegaly is also a common sign of viral infection. Lack of experimental models has limited research on aHEV pathogenesis. In this study, an in ovo inoculation method was used to establish an aHEV model using a commercial layer breed and Specific-Pathogen Free (SPF) layer hens. Embryonated eggs (SPF n = 36, commercial layer breed n = 44) were intravenously inoculated with infectious liver homogenate at day nine of incubation. Control birds (SPF n = 10, commercial layer breed n = 10) received phosphate buffer solution. Day 14 embryos and hatched chicks were culled at varying time points for viral detection and disease presentation. Liver and spleen were collected during post-mortems for virus detection by PCR. Additional liver and leg bone were collected in 10% formalin for histopathology. Body weights of chicks were collected every two days and organ/ body weight ratios were calculated at dissection time points. Blood was collected from two-week-old chicks for detection of anti-aHEV antibodies via indirect ELISA. Data were analysed by Shapiro-Wilke normality test and two-way ANOVA with a 0.05 significance level using GraphPad Prism software. Viral RNA was detected at day 14 of incubation, however lack of viral specific antibodies at two weeks post hatch was noted. Gross lesions and discolouration of liver were observed in infected groups. Fluctuations in body weight and hepatosplenomegaly was not significant at any time point ($P > 0.05$). Microscopic lesions were observed in majority of infected liver samples, predominantly lymphocytic infiltration in hepatocytes and the presence of globular leukocytes and plasma cells. Thinner extracellular matrix and lacunae surrounding osteocytes, in addition to accelerated disintegration phases were noted in infected metatarsus and tibia samples. Breed-specific differences were observed. This study demonstrated that aHEV infection produced via in ovo intravenous inoculation was able to generate viral replication in tissue and stimulate clinical signs of disease in embryos and chicks.

Keywords: Histology; Embryos; Intravenous Inoculation ; Disease; Specific-Pathogen Free

2015 Tissue tropism of Australian infectious bronchitis virus strains has shifted from the kidneys to predominantly the upper respiratory tract

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The tissue tropism of the historical and contemporary Australian isolates of infectious bronchitis virus (IBV) was evaluated at the histological and molecular levels. A total of 195 2-week-old SPF chickens were randomly assigned to seven groups. Fifteen chickens were allocated to Group 1, and 20 birds were assigned to each of Groups 2 to 7. Chickens in Group 1 (negative control) were inoculated with sterile medium. Chickens in Groups 2 to 7 (infected groups) were inoculated with 6 different IBV strains of various isolation years (1962 to 2013) and genotypes. On day 3 post inoculation (3 DPI), 10 additional naïve SPF birds were introduced into each of Groups 2 to 7 to serve as in-contact birds. Ciliostasis of the tracheal sections were evaluated. Sections of trachea and kidneys were fixed in formalin for histological examination. Viral genome concentrations were quantified by RT-qPCR using ch-GAPDH as a reference gene.

The results were compared using two-sided Fisher's exact tests, analyses of variance (ANOVAs) and Tukey's multiple comparisons tests, Kruskal-Wallis and correcting for multiple comparisons using False Discovery Rate, depending on the data.

Ciliostasis was significantly higher in the groups infected with strain N1/03. Replication in both the conjunctiva and upper trachea yielded high IBV genome copy numbers across all strains used in this study, while replication in mid and lower trachea was more

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variable, with some strains showing low replication rates. Replication in the kidneys was high only in chickens infected with strain N1/62. Replication in the caecal tonsils was also highly variable and strain dependent. All the strains showed active replication in the proventricular tissue at 5 days post-challenge, but none exhibited histological changes or macroscopic lesions.
This study demonstrates that more recent recombinant IBVs (all isolated during the 21st century) have acquired stronger tropism and greater virulence for the respiratory tract, characterised by more persistent replication in the trachea and more severe lesions in the lower respiratory tract. It confirms that IBV produce early replication in the upper respiratory tract, which declines rapidly after one week of infection. In contrast, tissues such as the caecal tonsils and kidneys exhibited a delayed replication, increasing from 5 to 9 DPI. It also demonstrates the capacity of multiple IBV strains to replicate to significant levels in the proventriculus and kidneys, even though they do not cause detectable proventriculitis or nephritis, respectively. However, the immunological reasons behind these differences remain unknown and may be the subject of future investigations.
Keywords: infectious bronchitis virus; pathogenicity; Vaccines; virulence; Tissue tropism

2016 Inclusion body hepatitis: From outbreak to control (2022-2025)

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Background
Inclusion Body Hepatitis (IBH) emerged in Israel as a major cause of mortality in broiler farms during 2022, with a substentiall increase in diagnosed cases compared with the previous years. The rapid spread and associated economic losses prompted the implementation of a vaccination program in broiler-breeder flocks aimed at reducing vertical and early horizontal transmission.
Objective
To describe the epidemiological course and molecular characteristics of IBH outbreaks during 2022–2025 and to evaluate the outcome of the vaccination strategy applied to the broiler-breeder flocks.
Materials and Methods
A retrospective-prospective study was performed on all confirmed IBH cases diagnosed between 2022 and 2025 at the Poultry Health Laboratory affiliated with the Israeli Egg and Poultry board. Diagnosis of suspected cases was made by real-time PCR targeting the Fowl Adenovirus (FAdV) polymerase gene, with subsequent sequencing and phylogenetic analysis of the hexon gene. Epidemiological data, including, age at diagnosis, mortality rate, season, and geographic distribution, were compiled and statistically analyzed using t-tests, ANOVA, and chi-square (X^2) tests at a significance level of $\alpha = 0.05$..
Results
IBH incidence surged in 2022 and 2023, with 124 and 150 confirmed cases, respectively representing a 5.5- to 6.8-fold increase compared with 2021. Following the introduction of the bivalent inactivated FAdV-8b + 11 vaccine in breeder flocks, the incidence declined by 44 % in 2024, and by 90 % overall up to and including Q3-2025 to 14 cases. Molecular typing showed progressive replacement of FAdV-D (24 % to < 5 %) by FAdV-E (> 95 % by 2024). The mean age at diagnosis increased from 2.5 ± 1.1 weeks (2015–2022) to 3.4 ± 1.1 weeks (2023–2025; $P < 0.001$), and mean mortality declined from 15.7 % to ≈ 10 % ($P < 0.05$). Seasonality persisted, with ≈ 70 % of detections in Q1–Q2.
Conclusions
The IBH epidemic evolved from widespread multi-serotype outbreaks to partial endemic stabilization following breeder vaccination. The marked reduction in case numbers, later onset age, and lower mortality rates indicate effective maternal immunity and reduced early-life transmission. Continued surveillance and breeder vaccination remain essential to maintain control and prevent serotype re-emergence.
Keywords: Inclusion Body Hepatitis; Fowl Adenovirus; broiler; epidemiology; vaccination

2017 Lessons learned from the One Health management of the 2021 avian influenza outbreak in Senegal

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Objective
The study aimed to draw key lessons from the 2021 HPAI outbreak through a twofold approach: (i) assessing the economic losses suffered by small-scale poultry; and (ii) evaluating the intersectoral management of the epidemic. The data was collected between June and August 2021.
Description of the problem
The 2021 outbreak of highly pathogenic avian influenza (HPAI) in Senegal's Saint-louis region exposed the fragility of socio-economic and health systems responsible for zoonosis management in environment characterized by intense interactions between wildlife, domestic animals and human populations. The proximity of wetlands hosting migratory birds makes this area a hotspot for the zoonotic diseases emergence and spread.
Materials and methods
The research combined two complementary components. The first consisted of a socio-economic survey of 57 family-run poultry farms located around the Djoudj, Langue de Barbarie and Guembeul parks to estimate production and income losses. The second component employed a semi-quantitative knowledge, attitudes and practices (KAP) survey involving 42 professionals from the animal health, human health and environmental sectors to assess collaborative management and information sharing. The data was collected between June and August 2021. SPSS software was used for data analysis.
Results
Results revealed a 72% decline in turnover among family poultry farms, with estimated losses of ranging from 5 to 7 million FCFA and high poultry mortality rates. Women, who constitute the majority of smallholder poultry producers (81.5%), were disproportionately affected with average losses estimated at 118 685 FCFA, underscoring their socioeconomic vulnerability and limited access to biosecurity training. Despite high theoretical knowledge of HPAI (90%), institutional coordination and prevention mechanisms were insufficient, revealing persistence weaknesses in intersectoral communication and operational cohesion.
Conclusions
These findings highlight three key lessons: the need to strengthen biosecurity capacities among woman poultry famers, to institutionalize One Health governance at local level, and to enhance the economic resilience of family farms health crises epidemics. The Saint Louis experience demonstrates that the successful implementation of One Health depends as much on technical competence as on operational cohesion between sectors.
Keywords: Avian influenza; One Health; Zoonoses; Economic resilience; Outbreak management

2018 Comparison of Vaccine Takes for Combinations of Mass and GA08 Type IBV Vaccines Applied in a

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Commercial Hatchery

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Measuring IBV vaccine take, defined as the number of samples positive for IBV and mean Ct value of those positives 5-7 days post-vaccination, using qPCR has become commonplace in the broiler sector of the poultry industry. Measuring vaccine take can be used to evaluate application methods in the hatchery, compare hatcheries and their personnel within a company or complex, or compare vaccines to each other. To this end, a commercial broiler integrator utilized vaccine take analysis to compare different combinations of 3 different Mass type, 2 different GA08 type, and 2 different NDV/IBV (Mass type IBV) combination vaccines from different manufacturers. Each Mass type IBV vaccine was used in combination with each GA08 type IBV vaccine and applied via coarse spray; each NDV/IBV vaccine was applied in combination with one manufacturer's GA08 type IBV while only 1 NDV/IBV vaccine was applied in combination with the other manufacturer's GA08 type vaccine and were applied via gel applicator. Each vaccine combination was used for 2 weeks in the same hatchery, all samples were collected from chicks 6-7 days post-vaccination during the 2-week trial period for each vaccine combination, and 15 samples from 6 different farms were collected. Descriptive statistics were calculated for each set of vaccine takes by farm, including mean Ct value, standard error of the mean, and percent positive. Data showed that both GA08 type IBV vaccines infected well, with each being greater than 92% positive for all samples tested, one vaccine had a slightly lower average mean Ct value (higher viral load) across farms than the other, and combining with any of the Mass or NDV/IBV vaccines did not influence positivity or load. Conversely, all Mass type IBV vaccines infected well with one GA08 vaccine (greater than 91% positive) but were much more variable when paired with the other GA08 type vaccine (41%, 63%, 83%, and 95% positive, respectively). Additionally, average mean Ct values across farms were higher (less viral load) for 3 of the 4 Mass type IBV vaccines when paired with this GA08 type vaccine. Taken together, the data shows the value and importance of using vaccine takes as a tool to evaluate IBV vaccine combinations in a program, as it appears not all vaccines are completely compatible.

Keywords: Infectious Bronchitis Virus; Hatchery vaccination; Massachusetts; GA08; Vaccine take

2019 Phylogenetic analysis of over 1,000 IBDVs from North American chicken flocks since 2009 and how today's commercial killed vaccines protect against IBDVs in each major cluster

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While the adoption of bursal derived vaccines dramatically improved progeny protection against Del-E and other similar variant IBDVs, by the late 1990s novel variants like AL2 and T1 had emerged that demonstrated the ability to still infect progeny containing high antibody levels at an early age. Since 2009 our diagnostic lab has sequenced the variable region of VP2 recovered from over 1,000 US flocks. This presentation will show one phylogenetic tree containing all these IBDV sequences. Results will show that the IBDVs fall into 6 general clusters: 1) Classic—16.6%, 2) AL2—28.9% (318N, 321-E), 3) Group-6—25.6% (322E), 4) Del-E prototype—12% (254S, 323-E), Del-E subtype—9% (254N, 323-E) and Variant Other—7.9%. The Classic cluster is made up mostly of the Faragher insert contained in most rHVT-IBD vaccines. The Group-6 cluster, named after the many IBDVs sharing the same restriction enzyme pattern, contains the most extensive branching. The Del-E prototype cluster represents about 10% of all US sequences. Over half of the Del-E subtype cluster containing the mutation 254N also contain 299S—making them 98% identical to the China variant type that has spread to much of Southeast Asia, Western Canada (BC/Del-E) and, most recently, Middle East. The Variant Other cluster notably includes viruses from Pennsylvania that are also most prevalent in Eastern Canada and named after the South Africa variant 05SA8. The presentation will highlight the selection of a dozen IBDVs—including one that is 100% identical to BC/Del-E—from these various clusters for vaccination/challenge studies. A focus will be made on mutations in the 4 hydrophilic peaks and how today's commercial killed vaccines cross protect against them.

Keywords: IBDV; VP2; AL2; Group 6; BC/DEL-E

2020 Propagation of classical and variant infectious bursal disease virus (IBDV) in Vero cell line

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Infectious bursal disease virus (IBDV) causes immunosuppression in chickens, predisposing them to secondary infections. In Canada, the highly immunosuppressive variant IBDV (varIBDV) SK09 is the predominant strain circulating in the broiler chicken industry. The objectives of this study were to propagate varIBDV SK09, classical IBDV strains D-78 and ST-12 in Vero cell line and to study bursal immune cells by flow cytometry following oral infection of broiler chickens with varIBDV SK09, classical IBDV D-78 and ST-12. At seven days of age, one group was orally challenged with varIBDV SK09, while the second, third, and fourth groups received classical IBDV strains D-78, ST-12, and saline, respectively. Flow cytometry was used to analyze B cells, T cell subsets, monocytes, and macrophages in the Bursa of Fabricius (BF) at 5-, 12-, and 29-days post-infection (DPI). Flow cytometry analysis of the BF revealed significantly elevated T cell populations (at 5, 12, and 29 DPI) and monocyte/macrophages (at 5 DPI) in the varIBDV SK09 group compared to classical strains ST-12, D-78, and control groups ($P < 0.05$). B cell and IgM positive B cell depletion in the BF was significant (5, 12, 29 DPI) in the varIBDV SK09 group compared to classical strains ST-12, D-78, and control group ($P < 0.05$). VarIBDV SK09 induced marked B-cell depletion, > 90% at 5 DPI (12 days of age), > 85% at 12 DPI (19 days of age), and > 20% by 29 DPI (36 days of age). VarIBDV SK09 depletes B cells early as 5 DPI and B cells never reach to original levels following infection. Therefore, the immunosuppression caused by the circulating varIBDV SK09 represents a significant challenge for the Canadian poultry industry in enhancing disease control while ensuring food safety and human health. The Vero cell line might be helpful in studying pathogenesis of varIBDV SK09.

Keywords: IBDV; Bursa of Fabricius; B cells; Vero cells; Broiler chicken

2021 From Assessment to Action: How an Online Tool to Assess Avian Influenza Can Improve HPAI Preparedness in Poultry Farms

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This study presents and evaluates FarmRisk, a digital self-assessment tool for poultry farms that identifies and ranks biosecurity gaps relevant to highly pathogenic avian influenza (HPAI). It demonstrates how an expert-validated scoring system and aggregated user data reveal farm-specific vulnerabilities and support compliance with the risk assessment and biosecurity duties required under the EU Animal Health Law (AHL) (Regulation (EU) 2016/429).

Europe is experiencing another HPAI epidemic in 2025, with several hundred confirmed outbreaks in commercial poultry. In Germany, 66 outbreaks in poultry farms in October-November 2025 resulted in 241,000 culled birds. These events cause severe animal-welfare impacts, economic losses and trade restrictions, underscoring the need for proactive prevention and structured, documented farm-level biosecurity assessment. The FarmRisk tools for poultry, pigs and cattle provide a practical and standardised means for farms to meet the biosecurity and risk-assessment obligations defined in the AHL.

A catalogue of 100 risk-factor-based questions was developed through literature review and expert consultation, covering six domains: farm premises; farm organization; hygiene processes; clean–unclean interface; operational management; and cleaning/disinfection/rodent control. Answers were coded by compliance level (0–1). Weights for all questions and domains were established through a two-stage Delphi process involving 17 experts. The tool has been available since 2018, is free of charge, and provides anonymous online use. It calculates a farm-specific biosecurity score, assigns traffic-light categories (<33%, 33–66%, >66%), and generates a PDF report with a prioritised list of recommendations. Aggregated metadata allow comparative analyses.

Since launch, the tool has been used in 2,385 poultry holdings across Germany. Overall, 40% of farms achieved green scores, 48% yellow and 12% red. Lowest compliance occurred at the clean–unclean interface and in operational hygiene, while structural and personnel-related measures scored highest. Variability in metadata confirms that the tool identifies farm-specific weaknesses and supports targeted improvements.

FarmRisk provides a validated, standardised approach for HPAI-related biosecurity assessment and supports compliance with the EU Animal Health Law. Its relevance has increased because QS Qualität und Sicherheit GmbH has mandated its use for all poultry, cattle and pig farms in Germany, and compensation funds may reduce or deny indemnities when required biosecurity standards are not met. The tool strengthens preparedness and resilience across poultry production systems.

Keywords: biosecurity; Animal Health Law ; avian influenza; risk assessment; Delphi method

Genetics, Genomics & Epigenomics I

2022 Kick Off Speaker: 75 years of breeding broilers for rapid growth and against related 'defects'

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Since the 1940s, higher growth rate has been the main broiler-breeding objective, being essential for efficient meat production. Breeding for more rapid growth has been very successful, with broilers growing faster every year, continuously reaching higher body weights at earlier ages. Over the years, several growth-related 'defects' have emerged, most notably were (1) excessive fat deposition, (2) leg problems, (3) ascites.

Due to their association with elevated growth rate, initially each defect was considered a biological limit to selection for further higher growth rate, and growth-reducing diets had been suggested to mitigate these defects, at the cost of reduced efficiency. However, our experimental-selection studies showed that the tendencies to develop these defects have been heritable, and genetically they are independent of growth rate. Thus, identification of the genotypes prone to develop each defect facilitated selection against them while continuing successful selection for rapid growth rate, and for other important traits.

In recent years, new defects, breast muscle myopathies, have emerged: White Striping (WS, in 2007), Wooden Breast (WB, 2011), and Spaghetti Meat (SM, 2015). As with the earlier growth-related defects, the use of growth-reducing management (lower-density diets) and slow-growing breeds have been suggested to reduce the prevalence and severity (P&S) of the breast muscle myopathies, while breeding companies have amended their programs with selection against them. Indeed, WS had gone and WB is currently found only among males reared rapidly to high BW (>3.5 kg). The P&S of SM have not been declining yet, apparently due to later emergence and to different biological nature. Whereas P&S of WB clearly increase with higher body weight and breast yield and rarely found in females, P&S of SM are higher in females than in males, and within-sex they are lowly associated with BW and breast size. Moreover, SM cannot be measured or assessed on live broilers, and the SM observed in processed carcasses reflect the effects of defeathering. We suggest that aggressive defeathering used as a tool to better phenotype the inherent tendency of broilers to develop SM, will lead to a successful genetic reduction in SM.

The continuous genetic elevation in growth rate and BW of broilers has been accompanied with higher levels of internal heat. Hot ambience hinders the dissipation of this heat, forcing contemporary broilers to minimize feed intake and consequently reduce actual growth and efficiency. This "defect" is being mitigated by sophisticated cooling, but due to its cost, genetic mitigation by reducing or elimination feather coverage is suggested as sustainable future mitigation.

Keywords: growth rate; fast-growing broilers; breast muscle myopathies; susceptibility to heat; feather coverage

2023 A Chicken Graph Pangenome Integrating T2T Assemblies Illuminates Causal Structural Variants for Growth Traits

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Chickens were the first livestock species with a reference genome, yet any single linear assembly cannot capture species-wide diversity. Telomere-to-telomere (T2T) assemblies and graph pangenomes now enable more complete representation of genomic and structural variation, but linking graph-resolved variants to complex traits remains challenging.

We de novo assembled six chicken breeds, including HQLA (Lingnan Yellow Chicken Line A03), a founder of our Advanced Intercross Line (AIL; HQLA × Huxu Beard). The AIL comprises 1,186 F16 birds with ~7× resequencing and 75 recorded traits (growth, carcass, feed efficiency, blood biochemistry, feather traits), and 305 F18 birds with ~15× resequencing and multi-tissue transcriptomes, with key phenotypes of body weight and shank circumference. De novo and public assemblies were integrated with Minigraph-Cactus on the GRCg7b coordinate backbone to build a chicken graph pangenome, and population-scale genotyping on the graph was performed using PanGenie. Colocalization analysis of structural variants (SVs) with eQTL/sQTL signals (via PP4) was used to

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prioritize shared causal variants.
We generated six high-quality genomes (1.0–1.1 Gb); the HQLA assembly is gap-free (T2T), with 16.21% repeats and >97% BUSCO completeness. The graph added 149 Mb of non-reference sequence and comprised 70,238,857 nodes, 89,684,682 edges, 15.3 M SNPs, 2.4 M indels and ~68 k SVs. F18 variants called on the graph were imputed into F16 to enable SV-based GWAS. These analyses revealed leading SV signals for body weight and shank circumference, which frequently colocalized with eQTL/sQTL peaks. Regulatory annotation showed that candidate SVs overlap tissue-relevant enhancers, promoters and CTCF/TAD features. Our results demonstrate that integrating T2T assemblies, a graph pangenome and graph-based SV genotyping enables precise dissection of the regulatory architecture of growth traits in chickens, and highlight the value of graph references for discovering causal SVs and accelerating genomic selection in breeding.
Keywords: Chicken; Telomere-to-telomere; Pangenome; Structural variation; Molecular QTL

2024 A three-dimensional cis-regulatory map of duck genome provides new insights into precision breeding
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Objective
This study constructed a comprehensive three-dimensional cis-regulatory map of the duck genome. The epigenomic panorama could precisely locate the genetic variations and potential genetic mechanisms regulating the formation of economic traits during duck domestication and artificial selection, thus contributing to precision duck breeding.
Experimental design
This study generated 384 genome-wide epigenomic sequencing data from 24 major duck tissues. The RNA-seq was used to profile gene transcription levels ,and ATAC-seq was used to analyze chromatin accessibility. Histone modifications have been used to predict different types of potential regulatory elements such as enhancers and promoters, including H3K27Me3, H3K4Me1, H3K27ac and H3K4me3. Hi-C experiments were used to studied the higher-order chromatin structure and link distal enhancers to their target genes.
Materials and methods
The experimental ducks were obtained from Beijing Nankou Duck Breeding Technology Co., Ltd., and fresh tissues were collected for epigenomic sequencing. The reproductive tissues were obtained from 24-week-old Pekin ducks, which represents the sexual maturity stage, while the other tissues were obtained from 6-week-old Pekin ducks, which represents the early rapid development stage.
Statistical analysis
After quality filtering, the most chromatin open regions were detected in retinal, the most promoters(chromatin open regions with H3K4Me3 enriched) were annotated in hypothalamus, and the most enhancers(chromatin open regions with H3K27ac enriched) were annotated in ovary. The number of promoters for tissue-specific expressed genes was significantly lower than the number of enhancers($P<1e-09$).
Results
This study divided the duck genome into 15 chromatin states, a total of 140,023 promoters and 268,064 enhancers were annotated, representing 29.2% of the genome's total length. 0.97 million enhancer-promoter interaction pairs were detected in 24 tissues, with the highest predicted numbers in liver and muscle tissues. The conservation rates of the annotated promoters and enhancers in the duck genome compared to the chicken genome were 42.18% and 20.72%, respectively.
Conclusions
This study constructed the most comprehensive map of regulatory element interactions in the duck genome, significantly improving the functional annotation level of poultry genomes. This map will make a significant contribution to basic research on avian evolution, breeding, and ecology.
Keywords: Duck; Regulatory elements; Genetics; Variation; Breeding

2025 A Chicken Developmental Genotype-Tissue Expression Atlas for context-specific regulatory genomics
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Although population-based molecular quantitative trait loci (molQTL) mapping provides a powerful framework for elucidating how non-coding genome-wide association study (GWAS) variants regulate intermediate molecular phenotypes to influence complex traits, gene regulation is profoundly context-dependent and exhibits dynamic changes. The functional Chicken GTEx atlas, being restricted to adult tissues, constrains insights into the genetic architecture of complex traits and their manifestation during developmental phases. Here we presented a chicken developmental Genotype-Tissue Expression (dGTEx) projects, which span 14,638 bulk-RNA-seq samples from 16 tissues of 942 birds (16× whole-genome coverage) spanning three developmental stages with balanced sex ratio, together with 96 single-nucleus RNA-seq datasets resolving 116 cell types. We comprehensively characterize cis- and trans-regulatory genetic associations for gene expression, demonstrating that this atlas enables greater detection of cis- and trans-eQTL signals than previous studies, with non-primary cis-eQTL and primary trans-eQTL signal discovery remaining unsaturated despite saturation in eGene discovery. Analysis reveals substantial regulatory allelic heterogeneity, observing that cis-eQTLs with broader tissue sharing exhibit smaller effect sizes, higher minor allele frequencies (MAF), and stronger enhancer enrichment. The atlas systematically characterize the pleiotropic effects of these functional variants, explicitly assessing their context-dependent behavior across tissues, developmental stages, sexes, and cell types, while elucidating regulatory patterns mediated by transcription factors (TFs), developmental hormones, co-expression networks, and cell-type fractions. Finally, we highlight this atlas's utility in interpreting GWAS loci for 42 developmental traits and enabling cross-species comparisons of developmental gene regulation underlying complex traits.
Keywords: Developmental GTEx (dGTEx); eQTLs; Context-dependent; Traits

2026 Comprehensive analysis of 122 guinea fowl genomes across three continents delineates their domestication and evolutionary patterns with special reference to India
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The present study was undertaken to investigate the genomic diversity, population structure, and selection signatures of Indian guinea fowl (Numida meleagris) in comparison to global domestic and wild populations, using whole genome sequencing (WGS) data and advanced bioinformatics tools. The analysis included 130 individuals from three distinct groups: Indian guinea fowl (n = 6), domestic guinea fowl (n = 89), and wild guinea fowl (n = 35), hypothesized to be genetically divergent due to geographical and evolutionary isolation. Efficient variant calling and quality control in GATK and PLINK programs resulted in over 8 million (8,622,463) high-quality (LD-independent) SNPs with an average genotyping rate of 98.89%. Principal component analysis (PCA), ADMIXTURE, and TREEMIX delineated distinct clusters, indi-

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cating clear population stratification. The minor allele frequency (MAF) distribution showed unique genomic patterns among different population, suggestive of localized adaptation. Selection signature analysis within and across populations identified key genes associated with adaptation in Indian guinea fowl. The intra-population iHS analysis revealed genes such as BCL11B, IL18, SPX, TRPV4, ACSS2, ME3, and STAU2 under recent positive selection, related to immune regulation, thermotolerance, and metabolic adaptation. Cross-population approaches (XP-EHH) identified additional selective sweeps encompassing genes like IGDCC4, ROBO2, HSPA5, TGM4, and CDH13. In total, 63 candidate genes were found to be under selection across intra- and inter-population analyses. These genes play important roles in immune response, oxidative stress, heat tolerance, feed efficiency, pigmentation, and reproductive physiology. The identified genomic regions provide valuable insights into the adaptive evolution of Indian guinea fowl and may serve as potential targets for genomic selection in future breeding strategies aimed at enhancing climate resilience in poultry.

Keywords: Guinea fowl; Selection signature; WGS; Thermotolerance; Immune response

2027 Landscape genomic and epigenomic approaches reveal signatures of climate adaptation in local chicken breeds

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Since the introduction of Gallus gallus domesticus to the European continent, the species has differentiated into numerous indigenous breeds. While local chicken breeds exhibit a wide array of morphological phenotypes resulting from human-driven isolation and selection, researchers have hypothesized that these breeds also adapted to their environment in terms of both climate and breeding practices. As climate change threatens poultry production, these local breeds are putatively valuable animal genetic and epigenetic resources to adapt to the future environmental constraints. In this study we propose a multi-dimensional approach to identify the underlying genes and methylation patterns contributing to environmental adaptation. A total of 455 individuals across 25 breeds spanning six countries were genotyped for a total of 45,212 SNPs. Twenty climatic variables were extracted from the WorldClim database for each breed's location of origin. Landscape genomics approaches, redundancy analysis (RDA), latent factor mixed model (LFMM) and gradient forest (GF), were used to reveal Genomic-Environment Association (GEA). To account for the genetic structure, we computed genomic admixture patterns which we added as a covariate within the RDA model while population structure is estimated simultaneously within the LFMM approach, and is not yet possible to include in the GF analysis. We identified 1,126 putatively adaptive SNPs with RDA (p-value < 0.05), 68 with LFMM (q-value < 0.05), and 1,335 with GF (top 5% most influential SNPs). Gene ontology and enrichment analyses performed using STRING identified genes related to regulation of biological process, nervous system development and circulatory system development enriched in both RDA and GF approaches (q-value < 0.05). A total of 35 overlapping genes were identified by all three techniques, eight of which (YAP1, ZNF704, DNMT3B, WWOX, GLIS1, RPRD1B, AUTS2, TSHZ2) are involved in the regulation of transcription by RNA polymerase II (GO:0006357), highlighting a potential role of transcriptional regulation in climate adaptation. RRBS data produced across 12 of the 25 breeds identified ~3.5 million CpG sites. Analyses are ongoing to perform CpG quality control, identify potential breed-specific CpG sites and conduct environment-methylation associations. Together, this integrative framework highlights the value of local breeds to identify climate-associated adaptive markers and to guide future breeding strategies toward climate-resilient chickens. Funding: GEroNIMO (H2020 GA No 101000236)

Keywords: Landscape Genomics; Adaptation ; Local Breeds; Methylation; Epigentics

2028 Whole-Genome Analysis of Population Structure and Endogenous Retroviruses in Taiwan Commercial Red- and Black-Feathered Chickens

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Taiwan country chickens are valued in poultry production due to their meat flavor, strong adaptability, slower growth rate, and robust physique, often exhibiting high genetic diversity and unique phenotypic traits. These characteristics make them important both for the poultry industry and for genetic resource conservation. In Taiwan, the main commercial country chickens are the red- and black-feathered breeds, which account for more than 85% of country chicken market (about 110 million birds per year) and hold significant economic and cultural importance. However, the population genetic structure and genomic characteristics of these chickens remain poorly characterized. In this study, we performed whole-genome sequencing (WGS) of Taiwan commercial red- and black-feathered country chickens to investigate their population genetic structure and the diversity of endogenous retroviruses (ERVs). Blood samples were collected from 80 red-feathered and 59 black-feathered chickens for DNA extraction. Principal component analysis (PCA) and unrooted phylogenetic trees revealed clustering by breed (PC1: 2.84%; PC2: 1.50%), with further sub-clustering within breeds according to farm of origin, indicating differences in breeding directions among lines and providing an initial overview of the population genetic structure of Taiwan commercial country chickens. Endogenous retroviruses are ancient viral sequences integrated into host genomes that may influence genome evolution, gene regulation, and host phenotypic traits, making them important for understanding genetic diversity and potential phenotypic variation. Using the same WGS data, we applied a k-mer-based approach (k = 23) to identify potential ERV insertions. The top ten ERVs with the highest insertion coverage showed differential distribution among populations, providing a preliminary overview of ERV diversity in Taiwan commercial country chickens. Further analysis of key endogenous retroviruses revealed differences in viral coverage between the red- and black-feathered breeds, suggesting breed-specific patterns, while some insertion sites and corresponding genes were shared across both breeds. Additionally, breed-specific insertion sites and genes were identified, highlighting the integration patterns of endogenous retroviruses in Taiwan commercial country chickens and providing a foundation for future studies on their effects on genome stability and potential phenotypic traits.

Keywords: Taiwan commercial country chicken; Population genetic structure; Endogenous retrovirus; ERV; Whole-genome sequencing

2029 Phenotyping and CL8X Genetic Information Coding System for traditional chicken populations: the case of the Western highlands of Cameroon

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This study seeks to establish a standardised protocol for documenting and sharing phenotypic diversity in local indigenous chicken

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populations from the Western Highlands of Cameroon, addressing the challenges posed by significant genetic diversity and varied traits among traditional poultry. The study examined phenotypic diversity in conventional chicken populations in the Menoua and Noun Divisions (localities), sampling 830 adult chickens from 257 households. Chickens were photographed, assigned unique IDs, and assessed for traits such as plumage colour and morphological features. Key genetic loci and significant genes associated with plumage, comb type, and colouration were analysed to classify distinct morphotypes. A CL8X coding system was created to standardise trait categorisation. The student's t-test was used to compare sexes across the live-weight categories (Heavy, Medium, Light). The Chi-square test was used to assess relationships between locality (Menoua and Noun) and feathering distribution phenotypes (Naked neck, Normal, Frizzle, Feathered shank, Crested) at the 5% significance level. Indigenous chicken populations showed notable variability in feathering phenotypes. "Normal" feathering was most prevalent, accounting for 66% in Menoua and 62% in Noun. Other types included feathered shanks (15% and 13%), with crested (3% and 8%), frizzle (0.7% and 2%), and naked neck types (2.4% and 2.7%) being less common. The chi-square confirmed an association between feathering distribution and locality (p-value = 0.004). Regardless of locality, phenotype, or live weight category, the t-test revealed sexual dimorphism in mean live weight ($p < 0.05$). Ten critical genes affecting feathering traits were identified, including six known and three novel to this population. Significant genes were characterised, including those for "Normal" feathering (N), vulture hook (VH), and others affecting specific traits. The study also identified primary morphotypes driven by MC1R and TYR loci, with wild (E*N, 29.6%) being the most prevalent plumage colour, followed by dominant wheaten (E*WH, 23.2%) and light brown (E*B, 17.6%). In total, 125 morphotypes were documented using the CL8X system, of which 65.6% were from Menoua, yielding a diversity rate of 15.08%. The findings emphasise unique gene mutations, particularly in Menoua. This research highlights the role of factorial genetics and phenotyping in understanding the traits of indigenous chickens. The standardised protocol will document phenotypic data for mutant genes, aiding conservation and improvement efforts. Integrating phenotyping with molecular analysis will enhance the accuracy of the CL8X coding system for identifying traits in other livestock species.

Keywords: Phenotype diversity; mutant genes; coding; identification; indigenous chicken

2030 Genetic analysis of growth and production traits in Dahlem Red chicken using REML Animal model

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A comprehensive analysis of growth and production traits was carried out to assess the direct additive genetic, maternal effects, estimated breeding value (EBV), genetic parameters and genetic trends using REML animal model with generation and hatch as fixed effects in Dahlem Red (PD-3) chicken population. The data collected over three generations on juvenile body weights (10,036 chicks) at 4 (BW4) and 6 (BW6) weeks of age; shank length at 4 (SL4) and 6 (SL6) weeks; adult body weight (2,190 hens) at 16 (BW16), 20 (BW20) and 40 (BW40) weeks of age; age at sexual maturity (ASM); egg weights at 24 (EW24), 28 (28EW), 40 (40EW); egg production at 40 weeks of age (EP40); egg mass at 40 weeks of age (EM40) were utilized in the study. Dahelm Red is a female parent line of Gramapriya chicken variety selected for higher egg mass based on the Osborn index. Generation and hatch had significant ($P \leq 0.01$) effect on body weights and shank length. ASM, EW28, EW40, EP40 and EM40 were significantly ($P \leq 0.01$) influenced by generation and hatch except EW24. The average LSM for ASM, EW40, EP40 and EM40 were 154.18 ± 0.28 d, 55.43 ± 0.10 g, 95.52 ± 0.51 eggs and 5.29±0.26 kg, respectively. The model with additive direct genetic and residual variance components was the best for ASM, EP40 and EM40. For EW40 model with direct additive, maternal, their interaction and residual was the best fit model. The maternal influence was not observed for ASM, EP40 and EM40 as maternal effects ceases with age. The heritability (h2) estimates were low to high in magnitude for all the growth traits. Heritability estimates for ASM, EW40, EP40 and EM40 were 0.28 ± 0.05, 0.56 ± 0.10, 0.13 ± 0.04 and 0.11 ± 0.04, respectively. ASM showed a negative genetic and phenotypic correlation with EP40 and EM40, respectively. The correlations between egg production and egg weights at different ages was negative with varying magnitudes. The egg mass had a direct positive association with egg production and egg weight. The EBV for EW40, EP40 and EM40 were 0.68 g, 4.47 eggs and 0.18 kg, respectively. EM40, the primary trait of selection had positive linear trend over the generations along with EP40 and EW40, the associated traits. The genetic trend of ASM showed a decreasing trend in the desired direction. The direct response in EM40 was 0.85 kg per generation and correlated response in EP40 was 2.02 eggs in three generations of selection for higher egg mass.

The study concludes that Dahlem Red population has required genetic variability in both growth and production traits. The EBV trends for primary and associated traits showed positive linear response in the desired direction.

Keywords: Dahlem Red; Breeding Value; Heritability; REML; Egg Mass

2031 Development of Meat-Type Quail Through Pure Breeding Within Quail Genotypes of Bangladesh

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This study aimed to develop meat-type quail through selective breeding based on 6-week body weight (BW), while maintaining accurate pedigree records across four quail genotypes. A total of 1,427-day-old chicks from Black (Bl)(n=321), Brown (Br)(n=355), Dhakai (Dh) (n=388), and White (Wh)(n=363) quails were hatched to produce the 14th generation (G14). Birds received balanced diets during brooding (27% CP, 3000 kcal ME/kg) and laying (24% CP, 3000 kcal ME/kg). Progenies were wing-banded and reared separately by genotype. Selection was performed at 6 weeks based on BW to produce the next generation. Dhakai quail showed significantly ($p<0.001$) higher egg weight (11.39±0.09 g) and day-old chick weight (7.19±0.068 g) than other genotypes. Chick weight ratio was significantly highest ($p<0.05$) in Wh (63.31±0.76%) compared to Bl (63.12±0.76%), Br (61.18±0.76%), and Dh (60.18±0.76%). At the 5th week, Dh had the highest BW (152.40±1.73 g), followed by Wh (143.30±1.73 g), Bl (137.65±1.73 g), and Br (129.55±1.73 g). Egg production from 11–20 weeks was significantly higher ($p<0.001$) in Wh (52.93±0.33) followed by Dh (52.50±0.33), Bl (50.41±0.50), and Br (50.39±0.51). Although hen-day egg production did not differ significantly($p>0.05$), Wh numerically scored highest (83.39±0.51%). FCR (0–5 weeks) was significantly better ($p<0.05$) in Dh (2.65±0.04) than in Bl (2.96±0.04), Wh (2.89±0.04), and Br (3.12±0.04). Dh exhibited superior weekly growth rate. Dressing percentage and crude protein content at 6 weeks did not differ

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among genotypes. Expected 6-week BW gains in males were 9.49, 3.48, 2.84, and 2.71 g in Dh, Br, Wh, and Bl, and in females were 10.89, 5.30, 3.17, and 2.26 g, respectively. Dh showed the highest selection differentials: males 21.04 g and females 23.99 g. Dhakai quail demonstrated superior growth, BW, FCR, and selection response, indicating strong potential for developing meat-type quail. Continued selection is recommended to achieve target body weight.

Keywords: Quail genotypes ; Pure breeding; Selection response; Genetic progress; Performance evaluation

2032 Genotypic variations in reproductive and developmental traits of breeder guinea fowl

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Fertility and hatchability are major constraints in guinea fowl production, limiting reproductive efficiency and farm profitability. This study evaluated the influence of guinea fowl breeder genotypes on embryonic development, fertility, hatchability and keets quality. Three genotypes were considered: Local × Local (L×L), Galor × Galor (G×G), and Local × Galor (L×G). A total of 2,418 hatching eggs: 839 from L×L, 824 from G×G and 755 from L×G breeder hens aged 67 weeks were incubated in a Petersime® Visio incubator at 37.7°C and 60% relative humidity with automatic turning. The parameters measured included the pH at setting, days 2 and 6 of incubation, and weights of albumen, yolk, and amniotic fluid at the 10th to 22of incubation at 3-day intervals (5 samples per treatment, for a total of 25), and fresh and dry embryo weights, fertility, hatchability, and day-old chick quality. General Linear Model Minitab software was used for data analysis. Means were compared to Tukey's post-test if significant. Means were considered significant if $p < 0.05$. The results revealed significant effects of genotype on several reproductive and developmental traits. L×L eggs had lower egg, albumen, and yolk weights ($P < 0.05$) and smaller embryos than G×G and L×G eggs, which were comparable. Shell thickness, Haugh unit, and water loss during incubation did not differ ($P>0.05$), and albumen and yolk pH remained stable regardless of genotype. Embryonic organ weights (heart, liver, gizzard) were comparable, except for a lighter gizzard in G×G embryos at internal perforation and a smaller heart in L×L chicks at hatching ($P<0.05$). L×L embryos also had lower fresh and dry weights at the end of incubation, suggesting reduced yolk nutrient utilization. Fertility was significantly higher ($P<0.05$) in L×L (70.04%) and L×G (65.52%) genotypes than in G×G genotypes (11.67%). Hatching and embryonic mortality rates were similar between genotypes ($P>0.05$). However, L×G eggs hatched earlier and more synchronously ($P<0.05$), with a shorter external perforation time and a more pronounced hatching peak, while G×G eggs hatched later and less synchronously. At hatching, the weight and quality of day-old chicks were lowest in the L×L genotypes ($P<0.05$), but similar between the G×G and L×G genotypes. In conclusion, the Local × Galor genotype exhibited intermediate but favorable reproductive and developmental performance, combining the adaptability of local birds with the productivity of the Galor line. This cross appears promising for improving fertility, hatching rates, and quality of guinea fowl in sustainable guinea fowl farming programs.

Keywords: Genotype; Guinea fowl egg; Incubation, fertility; Embryonic development

Gut Microbiome and Health I

2033 Pitfalls, opportunities and progress in applied microbiome research in broiler chickens

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The intestinal microbiome is essential for broiler health, physiology, immunity and consequently performance. During the short life span of broilers, an age-dependent increase in taxonomic diversity occurs in the intestinal tract. There is a a transition from a compositionally simple community towards a taxonomically diverse ecosystem. In the caeca, there are multiple maturation stages, with stage I (up to day 6) being dominated by facultative anaerobes including Escherichia-Shigella, Enterococcus, and Clostridium, reflecting the immature, oxygen-rich gut environment immediately post-hatch. Most of these bacteria are taken up from the environment in which the animals are hatched and housed, and multiple opportunistic pathogens are colonizing the gut, potentially causing intestinal and systemic diseases. These include septicemic disorders caused by bacteria that translocate from the gut to internal organs, including colibacillosis and bacterial chondronecrosis with osteomyelitis. Further microbiota maturation stages represent transitional phases with a rise in strict anaerobic short-chain fatty acid producers, while the functionally mature last stage is characterized by obligate anaerobes including Faecalibacterium and Subdoligranulum. Due to the sterile hatching conditions (lack of maternal microbiota transfer) and widespread antimicrobial usage during the life span of broilers in the field, the maturation of the microbiota is delayed or compromised, and often does not reach full maturation. This is a critical problem as microbiota maturation is correlated with disease resistance. Multiple strategies can be used to speed up microbiota development and prevent or restore dysbiotic shifts, induced by pathogens (eg. Eimeria, viruses, bacterial pathogens) or toxic feed components (eg. mycotoxins). As an example, early feeding has shown to cause a significantly faster microbiota maturation, reaching the microbial community profiles characteristic of later stages at younger ages compared with those that had been feed deprived for up to 24 hours (hatching window). Also novel management strategies such as on-farm hatching is a potential method to ensure rapid microbiota maturation, as well as administration of competitive exclusion cultures or probiotics. Many feed additives are marketed to stabilize the microbiota but often focus on anti-microbial properties and less on promoting beneficial microbes. For some however, data show clear effects on the taxonomic and functional microbiota composition in experimental settings, but field data are often lacking because of poor standardization (and antimicrobial use as bias). While scientific literature contains enough data on microbiota composition shifts in relation to health, and data of effects of feed additives on microbial composition and disease resistance are numerous, extrapolation to the field is difficult. The lecture will focus on problems with current data on the relationship between microbiota and health, but also discuss future needs and opportunities in microbiome research.

2034 Early-life colonization by hen-derived gut bacteria shapes the intestinal microbiota, host gene expression and gut histology of chicks

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In natural conditions, newly hatched chicks acquire gut bacteria from the mother hen. However, commercial hatcheries raise chicks in the absence of adults, affecting microbiome establishment and immune deficiency. Here we examined how early exposure to host-adapted versus non-host-adapted gut microbiota influences bacterial colonization and intestinal characteristics in newly hatched chicks.

Chicks were exposed immediately after hatch to cecal content

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from either an adult hen (poultry-adapted microbiota), an adult mouse (foreign microbiota), a mixture of both, or PBS (control). After seven days, gut colonization was characterized by 16S rRNA sequencing, and the phylogenetic distribution of colonizers was analyzed at the order level. Cecum and bursa tissues were analyzed by RNA-seq, and histological sections of cecum and jejunum were stained to assess acidic mucin content. Statistical differences were analyzed using one-way ANOVA ($p \leq 0.05$).
53% of hen-derived bacterial taxa successfully colonized chicks, whereas only 1% of mouse-derived taxa did, demonstrating strong host specificity. Phylogenetic analysis revealed colonization by diverse hen-derived taxa including Bacteroidales, Clostridiales, and Lactobacillales. Exposure to hen microbiota reshaped the cecal community, while Bacteroidales was absent in controls it became dominant (56%) in adult hen cecum treated chicks, and Clostridiales decreased from 86% to 37%. RNA-seq of cecum and bursa tissues showed immune modulation, including upregulation of genes involved in neutrophil degranulation, Toll-like receptor signaling, and the polymeric immunoglobulin receptor (pIgR). Histological analysis revealed increased acidic mucin production in the cecum and jejunum of chicks exposed to hen or mixed microbiota.
Together, these findings show that host-adapted gut bacteria establish a diverse and functional microbiome that shapes intestinal environment and enhances mucosal immune fitness. This work highlights the potential of microbiome-based strategies to strengthen chick health under commercial growth conditions.
Keywords: chick microbiota; hen-derived bacteria; gut colonization; intestinal histology; poultry health

2035 A single early exposure to adult microbiota drives sustained increases in propionate and gut maturation across five weeks in broiler chicks

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In nature, vertical transmission of symbiotic bacteria occurs mainly through direct contact between adults and offspring. In commercial settings, however, chicks are reared in the absence of adults. Previous work showed that this practice delays cecum microbiota maturation and disrupts its function. We hypothesized that the disruption to microbiota maturation due to growing chicks without adults, also affects other communities along the digestive tract and for the full 5-week broiler growth cycle.
To emulate vertical transmission from hens, newly hatched chicks (n=42) were exposed to adult cecal content (input), while controls received PBS (n=42). Every week some animals were sacrificed, and microbial colonization of cecum content and mucosa, jejunum, and crop was evaluated by 16S rDNA sequencing. Body weight and cecal short-chain fatty acids (SCFA) were also measured. Differences in microbial composition, distance from the input, and abundance were assessed using Bray–Curtis dissimilarity (PERMANOVA) and ANCOM-BC2, while SCFA and body weights were compared using Wilcoxon rank-sum tests.
The cecal community was extensively altered by treatment, with persistent differences from controls ($P<0.05$) throughout the five weeks. Distance to input analysis ($P<0.05$) showed that the microbiota of treated chicks was more similar to the input, indicating accelerated maturation compared to the control. Cecum content showed the largest shift (23 treatment-enriched and 103 control-enriched ASVs), followed by the cecum mucosa (30 treatment- enriched and 101 control-enriched ASVs), with modest effects in the crop (5 ASVs; all treatment-enriched) and jejunum (2 treatment-enriched, 2 control-enriched ASVs). Of note, treated chicks had lower weights during the first two weeks of life ($P < 0.05$) but managed to catch up with control chicks later on. Finally, propionate concentrations were markedly higher in treated chicks ($P < 0.05$ or better in 4 of 5 weeks), along with total SCFA levels ($P < 0.05$ or better in 3 of 5 weeks).
To conclude, raising chicks without adults mainly affects the cecal microbiota. However, the jejunum and crop communities were also affected. Treatment was also accompanied by higher SCFA levels, suggesting differences in fiber utilization between the groups. Importantly, differences persisted for the full 5 weeks, indicating that the practice of growing chicks in the absence of adults affects broilers through the full growth cycle. Notably, These results provide new insight into which bacterial groups can successfully colonize each intestinal region as well as stage of development, providing a basis for developing targeted probiotics to improve chick development and health.
Keywords: Cecal microbiota; Broiler chicks; Gut maturation; Short-chain fatty acids (SCFA); Gut colonization

2036 Detection of bacterial strains with high potential for colonization of the chickens' caeca

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To isolate caecal bacterial species from organically raised chickens and evaluate their ability to colonize the caeca and modulate the microbiota of newly hatched chicks.

Caecal contents from eight organic laying hens were used to isolate bacteria under selective anaerobic conditions. Overall, 190 isolates were obtained and sent for sequencing; 13 failed, leaving 177 successfully sequenced isolates. Eight strains were selected for in vivo testing—Enterococcus faecalis, Exiguobacterium arabatum, L. garvieae, L. saerimneri, L. salivarius, L. agilis, L. reuteri and Pediococcus pentosaceus—based on two complementary criteria: (i) representation of distinct taxa recovered from organic-hen caeca and (ii) documented probiotic potential in the literature (gut colonization capacity, antimicrobial activity, and/or host-beneficial immunomodulatory or performance-associated effects in poultry). Ross broiler chicks were allocated to ten groups (10 birds/group), receiving either a single strain, a consortium of all eight strains, or saline (control). Inocula were administered twice (200 µL; 1010 CFU/mL) by gavage on days 1 and 2. On day 8, caecal contents were collected to quantify colonization by strain-specific qPCR (gBlocks standards) and to profile the caecal microbiota by 16S rRNA V4 sequencing. qPCR counts and alpha-diversity (Observed species, Chao, Shannon, Simpson) were compared by one-way ANOVA with Tukey's test, and beta-diversity (Jaccard, Bray–Curtis) by AMOVA.

In single-strain groups, L. saerimneri showed the highest persistence (9.3×103 gene copies/µL; $p<0.001$ vs control), followed by P. pentosaceus (4.1×103; $p<0.001$), L. reuteri (8.1×101; $p<0.001$) and E. faecalis (1.2×101; $p=0.002$), while all remained below 1 copy/µL in controls. L. agilis was not detected, and L. salivarius persisted only at very low abundance when given alone but reached 5.7×102 copies/µL in the consortium ($p\leq0.001$). The consortium showed limited per-strain persistence, consistent with the reduced individual dose. Chicks receiving L. agilis or L. saerimneri had greater richness than controls (Observed species $p=0.0489$ and $p=0.0103$; Chao $p=0.0055$ and $p=0.0075$), whereas P. pentosaceus increased diversity by the Simpson index ($p=0.0298$)

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without affecting Shannon. Jaccard AMOVA showed significant shifts in composition in chicks receiving L. agilis, P. pentosaceus, L. saerimneri and the consortium ($p \leq 0.001$), whereas Bray–Curtis revealed a marked effect only for P. pentosaceus ($p \leq 0.001$).

Colonization capacity varies across strains. L. saerimneri persisted while increasing richness. L. agilis and P. pentosaceus also induced distinct community changes, supporting further investigation as host-adapted probiotic candidates for poultry.

Keywords: Poultry; Microbiota; Probiotics; Colonization; Caeca

2037 Breeder age and farm environment shape performance and gut microbiota in progeny broilers

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The gut microbiome of breeder hens plays a key role in reproductive success, egg quality, and progeny development. Its composition is shaped by host genetics, age, diet, and management. Maternal interventions can influence offspring microbiota and intestinal development, but the impact of breeder farm environment and age on progeny outcomes remains unclear.

This study compared the progeny performance from two breeder farms with matched genetics and ages but differing management and productivity (Farm A: high-performing; Farm B: low-performing). Breeder caecal droppings were collected at seven time points (13 - 57 weeks). Progenies were sourced from flocks at pre-peak (P3), peak (P4), post-peak (P5), and decline (P6) phases. In each cycle, 4,920 Ross-308 chicks were randomly assigned to 12 pens (410 birds/pen, 6 pens/group), reared under commercial conditions on a maize-wheat diet, and processed at day 39/40. Weekly faecal samples were collected. Generalised linear mixed models, accounting for breeder age and farm, were applied to performance data including European Poultry Efficiency Factor (EPEF) and Feed Conversion Ratio (FCR). Microbiome profiling used Illumina sequencing of 16S and ITS rRNA amplicons, processed via Sickle, SPAdes, VSEARCH, and QIIME2. Alpha/beta diversity analyses were conducted in R using phyloseq and vegan, with ANOVA and PERMANOVA.

Both breeder farm and age significantly affected EPEF ($p < 0.05$) and corrected 2-kg FCR ($p < 0.01$), with progenies from younger flocks and Farm B generally performing better. Breeder farm and age influenced breeder gut microbial diversity (PERMANOVA, R2 = 51%), while progeny diversity was shaped only by breeder age (R2 = 8%). Younger flocks in Farm A showed higher richness and diversity that declined with age; Farm B flocks maintained more stable and later higher evenness. Across P3 - P5, richness was similar, but Shannon and Simpson indices revealed modest yet significant differences in evenness, with Farm B birds showing greater stability. Key taxonomic drivers included Bacteroides and Synergistes in breeders, and Ruminococcaceae and Lactobacillus in progenies.

These findings show that breeder environment and age have lasting, stage-dependent effects on progeny performance and gut microbiota. The breeder flock is a critical leverage point for improving broiler efficiency. Management strategies that support microbiome stability at the breeder level may enhance productivity across generations. Future work integrating functional metagenomics and longitudinal performance data will help clarify mechanisms behind these transgenerational effects.

Keywords: Broiler; Breeder microbiota; Gut microbiome; Metagenomics; Breeder

2038 Cross-sectional and temporal effect of extreme heat stress on the gut microbiota of broilers

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Global climate change is increasingly impacting the livestock industry, leading to economic losses and threatening food security. Poultry are particularly vulnerable to heat stress due to the absence of sweat glands. Broilers have a thermoneutral zone at 19–22°C; temperatures above 34°C induce severe heat stress, altering behavioral, physiological, biochemical, and microbiological parameters. This study aimed to test the effects of extreme heat stress (EHS) on different sections of the gut at various duration. At 28 days, 252 broilers were equally assigned to control (CON) and EHS groups, housed in 18 cages per treatment (7 birds/cage). From days 29–35, the EHS room temperature was increased at a rate of 3.5°C/h to 35°C, maintained for 3 hours, and then reduced to 21°C over 1 hour (8 hours of EHS). Jejunal and cecal samples were collected from six randomly selected birds per treatment at 0, 4, and 8 hours on days 1 and 7 of EHS for metataxonomic analysis. On day 1, EHS significantly reduced the observed amplicon sequence variants (ASVs) in the jejunal samples compared to CON ($p < 0.05$). On day 7, cecal ASVs were higher ($p < 0.001$), whereas jejunal ASVs remained unchanged. Other alpha diversity indices were unaffected. The Bray–Curtis and Jaccard indices, as determined by PERMANOVA, revealed significant differences in the structure of jejunal and cecal microbiota (day 1: $p = 0.001$, $R = 0.38$; day 7: $p = 0.001$, $R = 0.67$), confirming segment-specific community differentiation. Furthermore, the cecal community structure was significantly affected by EHS ($p < 0.05$) at day 7. At the phylum level, Bacillota dominated both jejunum and cecum, with jejunum showing higher relative abundance. Genus-level analysis revealed a jejunal dominance of Lactobacillus and Limosilactobacillus, characterized by lower diversity, while the cecum harbored Bacteroides fragilis, Romboutsia hominis, Enterococcus cecorum, and Blautia hominis. Typical small-intestinal lactic acid bacteria, Lactobacillus johnsonii and Limosilactobacillus reuteri, dominated the jejunum. LEfSe analysis identified EHS–responsive taxa as potential biomarkers. EHS jejunum showed Limosilactobacillus enrichment. In cecum-EHS, Romboutsia ilealis, Desulfosporosinus acidiphilus, Eubacterium coprostanoligenes, Aristaeella lactis, and A. hokkaidoensis were enriched on day 1; Blautia glucerasea, B. faecicola, Lachnoclostridium edouardi, Agathobaculum desmolans, and Limosilactobacillus urinaemulieris on day 7. Overall, the results revealed that the jejunal microbiota is more prone to dysbiosis by acute EHS, while the cecal microbiota was disturbed due to prolonged stress.

Keywords: extreme heat stress; broilers; gut microbiota; microbial markers; dysbiosis

2039 In ovo Administration of Probiotic and Postbiotic Compounds in Broiler Chickens: Impact on Intestinal Morphology and Gut Microbiota

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In ovo technique is an effective early-intervention strategy in poul-

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try, allowing substances to be delivered directly into the egg before hatching. This study evaluated the effects of in ovo administration of two compounds with probiotic and postbiotic properties.
A total of 150 embryonated eggs were randomly assigned to three treatment groups (n = 50). On day 17.5 of incubation, 0.05 mL of the following preparations, diluted in Marek's disease vaccine, was injected manually into the amniotic fluid: Group P, 1 × 10^5 CFU of a probiotic blend (FSG68/22); Group T, 50 CFU of a freeze-dried, tyndallized intestinal extract from healthy adult chickens (Italian patent n. 102021000016055) plus the same probiotic; Group C, vaccine alone (control). Eggs were returned to hatching baskets after injection. After hatching, three groups of 45 chickens were obtained, each divided into 3 replicates (15 birds/replicate), basing on previously published studies and statistical considerations. Animals were reared for 42 days in the same facility, under identical environmental and feeding conditions. Zootechnical parameters (body weight and feed intake) were monitored. After slaughter, intestinal samples (5 birds/replicate) were collected for histopathology. Meconium samples were obtained immediately after hatching, and faecal samples at 42 days of age. Microbiota composition was assessed by 16S rRNA sequencing.
Cardinal data were analysed using parametric tests when normally distributed (Shapiro–Wilk, ANOVA with Holm–Šidák, paired t-test) and non-parametric methods otherwise. Ordinal data were evaluated with Kruskal–Wallis and Dunn's tests, with significance set at $p < 0.05$. Sequencing reads were quality-checked, assigned to OTUs, and compared across samples based on their observed frequencies.
Hatching rate was not affected by the treatment (97.2% C; 98.1% P; 97.8% T). Meconium microbiota was dominated by the Firmicutes, mainly Staphylococcaceae and Lactobacillaceae. Alpha diversity increased in faecal microbiota, with Firmicutes, Actinobacteriota, and Proteobacteria prevailing. Group T showed the highest alpha diversity. Lactobacillus, Weisiella, and Corynebacterium were the most representative genera in Groups C, P, and T, respectively. Groups P and T showed improved feed conversion ratio ($p = 0.0134$), higher weight gain ($p < 0.0001$), and increased villus height in the duodenum, ileum, and caecum ($p < 0.01$). These results confirm the safety and effectiveness of in ovo administration and suggest its potential to enhance gut health and modulate early-life intestinal microbiota in poultry, despite the limited sample size.
Keywords: In ovo; Probiotics; Postbiotics; Microbiota; intestinal morphology

2040 Comparing sequencing of full-length 16S rRNA and the V4 hypervariable region for characterizing the chicken caecal microbiota

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To compare full-length 16S rRNA sequencing with V4 hypervariable region sequencing for caecal microbiota characterization in chickens, and to assess the impact of different bioinformatic methodologies on taxonomic resolution and diversity metrics.
Caecal samples from 30 broiler chickens were collected at 7 and 42 days of age. At hatch, chicks received a caecal microbiota transplant (CMT) by oral gavage consisting of either caecal contents from Brown Lohmann organic hens, caecal contents from conventionally raised Ross 308 broilers, or sterile saline (control).
DNA from caecal content and donor inocula was extracted and sequenced by PacBio (full-length 16S) and Illumina MiSeq (V4 region). Reads were processed using three methodologies: V4-RDP (V4, OTU clustering and classification with the RDP database), 16S-RDP (full-length, OTU clustering and classification with the RDP database) and 16S-Athena (full-length, ASV clustering and classification with the Athena database).
Alpha-diversity (Observed richness, Chao, Shannon, Simpson) and beta-diversity (Jaccard, Yue & Clayton) were computed. Group and method effects were evaluated by one- and two-way ANOVA with post-hoc tests. Pearson correlations quantified agreement between methodologies for alpha-diversity indices and genus-level relative abundances.
The 16S-Athena method classified more reads at the genus level (73.2%), followed by the 16S-RDP (55.7%) and the V4-RDP (44.6%) approaches. Overall, 16S-Athena was particularly effective at identifying Bacteroidales, 16S-RDP provided better resolution for Firmicutes. Alpha-diversity estimates differed significantly between methods ($p \leq 0.001$): There was a strong correlation between the 16S-Athena and V4-RDP for Chao index ($r=0694$, $p \leq 0.001$) and Observed species richness ($r=0.7$, $p \leq 0.001$), but neither were correlated with the 16S-RDP. Additionally, there was no correlation between any of the methods for the Simpson or Shannon indices. Beta-diversity analyses consistently differentiated microbiota structures between CMT groups regardless of the analysis approach.
Full-length 16S rRNA sequencing provides higher taxonomic resolution than V4 sequencing, and all three approaches captured compositional differences in the caecal microbiota of chickens. The choice of bioinformatic methodology substantially affects taxonomic assignments and richness estimates, underscoring the need for methodological transparency and caution when comparing poultry microbiome studies.
Keywords: chicken caecal microbiota; full-length 16S rRNA; V4 region; PacBio; Illumina

2041 Fecal Microbial Biomarkers as Indicators of Intestinal Health and Productivity in Laying Hens

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Recent advances in sequencing technologies and metagenomic data analysis have enabled high-resolution characterization of the intestinal microbiota in animals. This progress highlights the need for standardized methodologies that translate microbial information into actionable field tools. A key focus is the identification of microbial biomarkers capable of reflecting intestinal health or dysfunction while predicting productive performance. The objective of the present study was to identify microbial biomarkers in the fecal microbiota of commercial laying hens that serve as indicators of intestinal health and productivity, and that can be implemented in field-level monitoring by producers.
A total of 48 fecal samples (8 per flock) were collected from six flocks (two per region) belonging to commercial egg-producing companies across three regions of Colombia. Samples were preserved in sterile tubes with absolute ethanol and subjected to Illumina sequencing of the 16S rRNA gene (V3–V4 regions). Health and production data were obtained through structured interviews with farm personnel. Sequencing reads were processed to infer amplicon sequence variants (ASVs) and analyzed using the phyloseq package in R (RStudio). Regarding the statistical tests used, we assessed community structure and diversity, and then applied Spearman correlations and LEfSe (Linear Discriminant Analysis Effect Size), using a Kruskal–Wallis test ($\alpha = 0.05$) and an LDA score cutoff of 4.0 to identify taxa significantly enriched according

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to productive status. At the community level, Bacillota was the predominant phylum, with Lactobacillus as the most abundant genus across all samples. The second most abundant fraction varied among Bacteroidota and Proteobacteria. Enrichment and correlation analyses revealed that the genera Akkermansia, Oscillibacter, Butyricicoccus, and Ruminococcus, as well as the family Prevotellaceae, were consistently associated with superior productive indicators. Conversely, Escherichia/Shigella and Megasphaera were linked to less efficient flocks, suggesting a microbial profile unfavorable to intestinal health and performance. Distinct positive and negative microbial biomarkers associated with productivity were identified in laying hens. While these findings warrant further validation, they demonstrate that despite the high taxonomic variability often observed across different systems, specific common taxonomic groups persist and could serve as productivity markers. This supports a novel analytical approach based on profiling specific fecal microbiota biomarkers as a practical field-applicable tool to describe flock status and support management and nutritional decisions.

Keywords: poultry; productivity; eubiosis; health; laying hens

2042 Antibiotic-Induced Gut Microbiota Depletion on Production Performance, Health, and Nitrogen Metabolism in Laying Hens during the Late Laying Period

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The gut microbiota plays a key role in host nutrition, digestion, immune regulation, and metabolism in poultry. This study aimed to investigate the effects of gut microbiota depletion and recovery on production performance, health, and nitrogen metabolism in laying hens. A total of 48 69-week-old Lohmann Pink laying hens were randomly assigned to two groups with 6 replicates of 4 hens: control group (CONT, sterile water) and ABX group (A composite antibiotic solution: 5 mg/ml vancomycin, 10 mg/ml neomycin sulfate, 10 mg/ml metronidazole, 10 mg/ml ampicillin, and 0.1 mg/ml amphotericin B prepared in sterile water), all hens were administered by oral gavage twice daily for 14 days. The trial lasted 6 weeks. Egg weight was significantly reduced in ABX group during recovery phase ($P < 0.05$). The ABX group significantly reduced apparent digestibility of crude protein in the microbiota depletion phase and increased apparent digestibility of crude protein and AAs during the recovery phase ($P < 0.05$). In the liver gene expression of AKT1, S6K, IGF-2 was upregulation in the ABX during recovery phase ($P < 0.05$). The ABX group had higher serum levels of free amino acids (Arg and Trp) ($P < 0.05$), and lower pro-inflammatory cytokines (IL-1β, IL-6, LPS) ($P < 0.05$), reproductive hormones (E2, AMH, FSH, Leptin) ($P < 0.05$) and growth hormone (IGF-1, IGF-2, T3, T4) ($P < 0.05$), but these levels gradually recovered. No significant differences were observed in intestinal lesion score or morphology between the two groups. And IL-1β gene expression was upregulated in the Jejunum, while AvBD1 gene expression was downregulated in the ileum ($P < 0.05$). Feces microbial alpha diversity (Pielou_e, Shannon, Simpson) was significantly decreased in the ABX group ($P < 0.05$). Beta diversity differed significantly between the two groups. At the phylum level, the relative abundances of Bacillota and Bacteroidota differed significantly between groups, while genus-level analysis showed marked alterations in dominant taxa, including Enterococcus and Lactobacillus ($P < 0.05$). LEfSe analysis identified (LDA score > 4, $P < 0.05$) Escherichia-Shigella was enriched in the ABX group, whereas Enterococcus, Lactobacillus, and Gallibacterium were abundant in the control group. These differential taxa were significantly associated with serum inflammatory cytokines, apparent nutrient digestibility, and serum free amino acids ($P < 0.05$). In summary, gut microbiota depletion and recovery reduced average egg weight in late-laying hens and affected the apparent digestibility of nutrients and serum free amino acids, providing theoretical support for further investigations into the contribution of gut microbiota to host nitrogen metabolism.

Keywords: gut Microbiota depletion; antibiotic; production performance; nitrogen metabolism; laying hens

Innovation and Technology I

2043 Innovation and Technology in Poultry Production: A Vision of the Future

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Researchers at the Georgia Tech Research Institute have developed advanced technologies for the poultry industry for over 50 years under a state apparatus called the Agricultural Technology Research Program. These technologies span the entire production chain from farm to fork and include a breadth of technologies from sensors to integrated systems. In addition to R&D, this program also supports technical assist, education and outreach. We will highlight the program itself and how it has uniquely set GTRI as a technology leader in “outside-the-box” thinking as related to the poultry industry. As part of this, results from recent field-tests for an automated cone-loading system and an automated deboning system will be highlighted in addition to operation of a poultry house robot assistant. Special emphasis will be given to describe how new advances in artificial intelligence are playing a role in these systems and how they are implemented

2044 Poultry Innovation Through Design: The Case Study of Hydra Peck

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This abstract investigates the hypothesis that integrating structured design methodologies and stakeholder participation significantly enhances innovation outcomes in poultry feeding technology. While rigorous scientific methods dominate discussions on innovation, the crucial role of design methodologies often remains undervalued and overlooked in academic literature and industry discourse. Recognizing this critical gap, “Hydra Peck” illustrates how integrating design thinking, participatory methodologies, and diverse stakeholder perspectives can revolutionize technological advancements within the poultry industry.

Today, poultry farmers face three major challenges: rising feed prices, pressure to reduce carbon emissions, and the need to improve animal welfare. Hydra Peck, an innovative wet-feeding system for broilers, addresses all three. Wet feed unfolded through literature and industry research as having a positive impact on body weight gain, feed intake and gastrointestinal tract development of broilers. These, along with a lower environmental burden of feed production, and a diminution of chick mortality, lead to a higher profitability for the farmer. Yet despite all these advantages, technical challenges were standing in the way of the adoption of wet feed.

Through a series of workshops and prototype refinements, the Hydra Peck development process incorporated various design approaches and involved multiple stakeholders including poultry farmers, industry representatives, and researchers. RIO, Dutch acronym for Reflexive Interactive Design, is the main structured and iterative design methodology which paved the way to our novel

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feeding technology. RIO encourages iterations through exchange with key actors, and reconciles new solutions to functionalities in the final design concept with long-term needs and requirements of the stakeholders.
Hydra Peck, outcome of the design process, mixes wet and dry ingredients into a fresh porridge-like feed and reliably provides it through the barn. Our system designs away the technical complications of providing wet feed through preventing clogging, spoilage, and litter problems while keeping feed fresh and hygienic through automated cleaning. Preliminary stakeholder feedback reinforced high satisfaction levels, and highlighted how inclusive participation enhanced technology acceptance and practical usability.
This case study illustrates that neglecting the integral role of design in favor of purely scientific approaches limits potential technological advancements. An effective and impactful innovation is not a one-person job, but is a multi-perspective initiative where many opinions and needs should be taken into consideration to obtain an optimal final outcome.
Keywords: design; broilers; wet feed; innovation; feeding system

2045 Beyond the barn: Free-range activity of dual-purpose chickens

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Understanding to which extend chickens actually make use of outdoor areas is important for meeting breed-specific needs. This question is increasingly important in the context of free-range husbandry systems, which allows the animals to exhibit a broader range of natural behaviors and thus improve welfare. This study aims to investigate the free-range activity of 6 crossbreeds, focusing on differences based on the impact of the genetic background on free-range behavior. Crossbreeds were layer- and meat-type dual-purpose crossbreeds, originating from roosters of local breeds (Bielefelder BIE, Altsteirer ALT and Ramelsloher RAM), and commercial layer (White Rock WR) and broiler (Ranger RG) parental hens. All animals were hatched, reared, and kept on site, with hens being assigned to six separate pens based on genotype. Each pen initially housed 32 chickens, with a stocking density of 2.1 animals/m2. Starting from week 10 of life, each pen had access to a free-range with a covered veranda as a transition area from 08:00 to 22:00, adjusted according to daylight hours. The behavior of the chickens was continuously recorded via video. In a 24h time slot, every exit towards the outdoor and every entry into the barn was manually recorded and timestamped of all 192 hens in week 26 at peak of lay. The response variable was the number of chickens outside per time, normalized to group size. Crossbreed and time block were used as explanatory variables. Due to non-linear temporal free-range patterns and breeds-specific characteristics, a generalized additive model was applied. Significant differences were found between crossbreeds. Around noon, most of the animals of the crossbreed ALT x RG were stayed inside in the barn ($p \leq 0.001$). At the same time, most of the animals of the crossbreed BIE x RG were outside in the free-range ($p \leq 0.001$). In the afternoon, most of the animals of the crossbreed RAM x RG were outside in the free-range, significantly exceeding all other crossbreeds ($p \leq 0.001$). Crossbreeds showed distinct temporal free-range pattern, indicating genetic variation in free-range behavior. Being aware of free-range behavior, i.e. the temporal motivation to access, pophole openings or feeding times can be adjusted to further promote natural behaviors on a breed-specific base. The hole study highlights the importance of a match between breed and husbandry system including management procedures aiming to promote animal welfare.
Keywords: alternative husbandry; animal welfare; behavior; generalized additive model; outdoor pattern

2046 Formulation and Evaluation of a Phyto-Biotic Spray for the Shelf-Life Extension of Table Eggs

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India's currently 3rd largest egg producer globally, producing 138.38 billion eggs. However, the Indian egg industry faces a significant annual post harvest loss of 8.87% during farm to fork channels. Climate crisis along with improper infrastructure like roads, packaging, collection etc. leads to significant annual egg loss of 8.87% during farm to fork channels. To ameliorate these losses by increasing egg shelf life, we have developed six phyto-biotic spray formulations. It was assessed based on parameters like degrees of washability, pH (5.57 to 5.87), evaporation times (30 to 55 minutes), drying times (1.31 to 3.37 minutes), film thickness (0.03 mm and 0.12 mm) etc. Subsequent analyses revealed that formulation F5 exhibited optimal quality for providing an effective coating on eggs, for enhancing their storage capacity. To evaluate the effect of selected Phyto biotic spray on egg quality parameter under different storage period and temperature conditions, 640 chicken eggs were collected from the farm and stored at various temperature, as T1- Control (28°C), T2- Refrigerated (2-8°C), T3- Spray on egg (storage-28°C) & T4- Spray on egg (storage- 2-8°C). Various external and internal qualities, microbial load parameters of eggs were investigated at specific intervals of 0, 6, 12, 18, 24, 30, 36 and 42nd days. Results exhibiting significant ($P<0.01$) differences between the treatment groups where both storage temperature and the spray application on eggshells influenced weight loss in eggs over time (at 42 days: 15.58% for T1, 6.99% for T2, 8.87% for T3, and 4.52% for T4). Untreated eggs at 28°C (T1) saw a marked increase in air cell diameter from 16.48 mm to 33.68 mm over 42 days. In contrast, sprayed eggs at 2–8°C (T4) showed the best preservation, with a significantly lower air cell diameter of 25.44 mm ($P<0.001$), demonstrating superior quality retention. Non-sprayed eggs at 28°C exhibited a notable increase in albumen pH, yolk pH and whole egg pH ($P<0.001$), indicating enhanced all pH changes at higher temperatures. In contrast, sprayed eggs at 2-8 °C maintained stable albumen pH, yolk pH and whole egg pH levels throughout the storage period. As storage duration progressed, E. coli counts escalated sharply in T1, reaching 4.50 ± 0.04 by Day 42, whereas T2 and T3 recorded counts of 2.71 ± 0.05 and 3.38 ± 0.04, respectively, indicating that lower temperatures effectively inhibited bacterial growth compared to T1. All above results demonstrated that using a Phyto biotic spray as a coating effectively maintained egg quality even at elevated temperatures (28°C) for up to 30 days, while at 2-8°C, their storage period is extended to more than 42nd day (60 days).
Keywords: Post Harvets loss ; safety; egg spoilage ; storage; Shelf life

2047 Global Poultry Feed Industry: Present Status, Future Prospect and Challenges with Special Reference to Bangladesh

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Feed plays a significant role in the global food industry, enabling economic production of animal proteins throughout the world. The

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global feed industry produces around 1 billion 245 thousand tonnes of compound feed annually, supporting commercial production in over 130 countries and employing more than 0.25 million people. Out of this, 44% is poultry feed, 28% percent is pig feed, 13% is dairy feed, 8 % is other ruminant feed and lastly 4% is aquaculture feed , The global animal feed market will reach USD 588.50 billion in 2025, up from around 602.76 billion in 2030 having the annual growth rate (CAGR) of 4.3% until 2034. With an estimated 8,000 large-scale feed plants and extensive on-farm mixing, commercial feed manufacturing generates over USD 400 billion in annual turnover. As demand for animal protein rises- driven by population growth, urbanization, advances in production and feeding technologies, sustainability and environmental concerns, increasing awareness of animal health and welfare, economic prosperity and higher purchasing power-so does the need for accessible, safe and high quality feed. According to the United Nations Food and Agriculture Organization (FAO) estimates that by 2050, the demand for food will grow by 60 % and that between 2010 and 2050 production of animal is expected to grow by around 1.7% per year with meat production projected to rise by nearly 70% and dairy by 55%. On regional basis, Asia-Pacific is the largest producer with 475 million metric tonnes (MMT) followed by Europe with 261 MMT, North -America with 259 MMT, Latin America with 200MMT, Africa with 51 MMT, the Middle East with 35 MMT and Oceania with 10 MMT. On a national basis, China is the World's largest feed producer with approximately 262 MMT, followed by USA with 238 MMT, Brazil with 83 MMT, India with 52 MMT, Mexico with 40 MMT, Spain with 36 MMT, Russia with 35 MMT, Vietnam with 24 MMT, Japan & Turkey with 23 MMT respectively, which approximately 64 % of the world compound feed production. The feed industry in Bangladesh, supplies 6.57 million tonnes of feed to the 44 crore livestock population, including poultry. The amount of investment in this sector is about 28, 000 crore BDT which supply 85% of the country's poultry and acqua feed need. Though global poultry feed market offers significant potentialities, but several challenges like volatility in raw materials prices, Russia-Ukraine war, competition for resources and environment, regulatory and compliance issues. Based on the reviewing of existing status, prospect and challenges, this paper will highlight the major directions to overcome present crisis of poultry feed industry in global as well as national perspective.

Keywords: Global Feed Industry; Present status ; Prospect; Challenges; Special

2048 A machine learning approach for authentication of laying hen housing systems based on egg quality parameters

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Eggs originating from outdoor housing systems, such as organic and free-range production, are often sold at a higher price than conventional eggs. This price difference creates an incentive for potential fraud, highlighting the need for reliable and cost-effective authentication methods. In this study, we evaluated whether internal and external egg quality parameters could be used to classify eggs according to housing system (indoor vs. free range) using supervised machine learning. In 2019, a total of 33,216 eggs were collected from 76 commercial farms across Belgium. Egg quality parameters were measured, including whole egg weight, dynamic stiffness, shell deformation, breaking force, albumen height, Haugh unit, shell thickness, yolk color, and cuticle thickness.

Classification models were developed using a tree-based pipeline optimization tool (TPOT) to optimize supervised machine learning pipelines. The models were trained on a subset of our dataset, only containing the brown laying hen data (n = 23986). The white laying hens were never housed in an outdoor system and therefore not useful to train our outdoor/indoor classification model. The best model trained with all quality traits was an XGBClassifier, which achieved an overall testing accuracy of 76.6%. Yolk color had the highest feature importance. Therefore, a second model trained using only yolk color as a feature, implemented with an ExtraTreesClassifier, reached an accuracy of 74.1%. Although the full model performed slightly better in terms of overall accuracy, the yolk-only model showed the lowest false positive rate for outdoor eggs (7% vs. 13%).

The overall accuracy of the models was moderate and the best predictor was yolk color, Its potential as screening tool has to be nuanced. Yolk color is highly influenced by feed and a possible bias with housing system could be suggested as dietary recommendations can vary according to management practices.

Egg quality parameters seem to be robust and little affected by housing system. The use of machine learning on quality traits needs to be reconsidered as tool for discriminating the origin of eggs. Since the model was trained exclusively on Belgian eggs and no white hens were included in the dataset, additional data such as diet, breed and origin, is needed to confirm potential other parameters.

Keywords: egg quality; machine learning; authentication ; housing system ; TPOT

2049 Biogas production from co-digestion of poultry droppings through dry-anerobic digestion technology

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The study aimed to promote biogas production from poultry droppings (PD) through anaerobic dry-digestion, replace industrial chemical fertilizers with dried bio-fertilizer, and enhance environmental safety by disposing of droppings and agricultural waste (AW) e.g. Cabbage, tomato, and other agricultural crops residue. The study involved ten weeks of dry anaerobic digestion of poultry droppings and agricultural waste, co- digested with cow dung and urine, with 300 kg of waste for three treatment group T1= (Poultry Droppings+ Cow Dung + Urine), T2= (Poultry Droppings + Agricultural Waste+ Urine), T3= (Poultry Droppings + Agricultural Waste + Cow Dung) digested for 49 days. Temperature control is crucial during feedstock preparation, as it enhances microbial activity and accelerates the anaerobic digestion process. The study reveals significant disparities in moisture content, total solids, volatile solids, ash content, and pH levels between PD and AW. Result shows that PD exhibit higher moisture content 72.78% and lower total solids 7.34% than AW 68.39% and 17.77%, respectively. Notably, PD demonstrates a higher volatile solid content 89.16% compared to AW 85.27%. The C/N ratios for PD 5.42 and AW 31.42 highlight distinct carbon-nitrogen compositions. Weekly biogas output across three treatments T3 consistently producing higher biogas, with significant differences noted from the 4th week onwards. Treatment 3 also exhibits the highest weekly average volume of biogas production was 7.35 cubic meters. Biogas composition analysis indicates significant differences in methane (CH4) and carbon dioxide (CO2) percentages among treatments and T3 consistently leading in methane production. pH monitoring reveals fluctuations, impacting methanogenic activity. The nutrient content of anaerobic digestion residues varies among treatments, with T2

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showing significantly higher potassium content 0.53 g/kg and T3 demonstrating the highest values for total nitrogen, phosphorus, and calcium. Thus, it can be said that the application of T3 (Poultry Droppings + Agricultural Waste + Cow Dung) at the field level is the most effective therapy when compared to other treatments.
Keywords: Biogas; anerobic fermentation; waste management; dry-digestion; renewable energy

2050 Screen of Salmonella responses to host associated metabolites reveals that propionate reduces iron bioavailability

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Salmonella is one of the most important foodborne pathogens affecting poultry and human health. Contaminated flocks result in substantial economic losses and remain a major source of zoonotic transmission. Understanding how Salmonella senses its environment might result in the development of new anti-bacterial strategies.

Thus, the immediate transcriptional responses of Salmonella enterica serovar Typhimurium (Salmonella) to 19 host-associated metabolites were characterized by RNAseq. Bacteria were exposed for 10 minutes in defined minimal conditions to the 19 metabolites. This short exposure design minimized indirect effects and revealed which compounds trigger direct transcriptional responses and what these responses are. Among the tested molecules were short chain fatty acids (SCFAs), amino acids, sugars, and other molecules. Non-parametric package 'NOISeq' was used for analysis and to determine significance. Growth in the presence of physiological concentrations of these molecules was also measured. Finally, a pipeline was developed which identifies differentially expressed metabolic pathways even when specific genes are not statistically modulated.

This approach successfully reduced background processes enabling the identification of compound specific gene changes. Exposure to 10 of the compounds resulted in only 1-4 differentially expressed genes (DEGs(. This included transcriptional changes of up to 150-fold. Modulated genes encode import systems or enzymes for the integration of compounds into metabolism. Of note, while some responses were not previously known, many of these DEGs were expected from Escherichia coli data, implying many specific responses are transcription factor mediated. Exposure to seven compounds resulted in no specific responses, implying Salmonella does not sense these compounds, at least in tested conditions. Interestingly, Salmonella specifically down regulated SPI-2 in response to 5-oxoproline exposure, which was the only compound to significantly affect Salmonella growth, implying SPI-2 inhibition might be part of a stress response. Finally, in response to propionate exposure Salmonella activated enterobactin biosynthesis implying propionate might inhibit iron bioavailability.

To conclude, this work lays the framework for analysing specific bacterial responses to environmental signals. Of special interest is the possibility that propionate, a SCFA that is also studied as a feed additive, effects iron bioavailability in the gut, as this might represent a new strategy in combating gut pathogens.
Keywords: Salmonella; poultry; propionate; RNAseq; gut conditions

2051 Occurrence of fumonisins detected in 2021–2024 harvested corn from different countries in Africa, the Americas, Asia, and Europe

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Fumonisins are mycotoxins commonly found on agricultural crops that can negatively affect livestock. The aim of the present study was to determine the occurrence and concentrations of fumonisins B1 and B2 (FB1 + FB2) in corn harvested from 2021 to 2024 across 20 countries spanning Africa, the Americas, Asia, and Europe (Algeria, Argentina, Austria, Brazil, Colombia, Costa Rica, Croatia, Ecuador, Greece, Guatemala, India, Italy, Mexico, Peru, Poland, Serbia, South Africa, South Korea, Thailand, and USA). Corn samples were analyzed using LC-MS/MS triple quadrupole system (Agilent 6460 series) with a multi-mycotoxin method (Farkas et al., 2017). Results are expressed in µg/kg (parts per billion, ppb) relative to a feed with a moisture content of 12%, and descriptive statistical analysis was performed. The results showed that between 2021 and 2024, fumonisins were detected in 80–90% of corn samples. The average fumonisin concentrations were 2,797 ppb, 2,181, 2,120 and 1,291 ppb in 2021–2024, respectively. Fumonisin concentrations varied widely each year. For example, in 2021 concentrations ranged from a minimum of 40 ppb to a maximum of 28,739 ppb. Similarly broad ranges were observed in 2022 (22–28,035 ppb), 2023 (40–15,056 ppb) and 2024 (10–22,220 ppb). Overall, fumonisin prevalence increased globally from 2021 to 2024 even as average concentrations declined. Nevertheless, these yearly average concentrations still exceeded the recently established 1,000 ppb reference point for fumonisins in poultry and pig feed (EFSA Scientific Opinion, 2022). The large variation in fumonisin concentrations underscores the need for regular animal feed analysis. Finally, the co-occurrence of multiple mycotoxins can have significant implications for food and feed safety, as combined exposure to multiple mycotoxins can lead to synergistic toxic effects, increasing human and animal health risks.
Keywords: corn; total fumonisins; LC-MS/MS; co-occurrence; FB1 + FB2

2052 A Virtual Bird Finite Element Model for Understanding Keel Bone Damage in Laying Hens

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Commercial laying hens live at risk of damaging their keel bone during their lives, which is a large welfare problem in the egg production industry.

This damage consists of both keel bone deviations and keel bone fractures, and the exact mechanisms of this damage remain largely unknown due to the limits of observation.

Digital twins of humans have been utilized to surpass similar limitations, and in principle the same can be done for a laying hen. Thus, a finite element digital twin of a laying hen (hereafter referred to as a virtual bird) is created to analyze keel stresses during dynamic events.

In this study, the virtual bird was created by means of CT image segmentation and corresponding mesh definition via the Simpleware ScanIP preprocessor, alongside assignment of simulation parameters via the ABAQUS finite element environment. Simulations were then ran on the ABAQUS explicit solver. Simulated reaction forces of the bird's impact with a rigid plate are validated against empirical results from a pendulum impact experiment with a deceased laying hen conducted in a laboratory setting. Agreement between these two datasets supports the assertion that the virtual bird presented in this study can be used to ascertain key phenomena of a laying hen during impact.

Further simulation configurations were ran against a perch, with parameters such as impact site, presence of an egg, and presence of an ossification front, modeled with a discrete stiffness change around the caudal tip of the keel.

Results of these simulations indicate high compressive stresses on

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the outer edge of the keel associated with the contact of the keel with the perch.
Additionally, the model shows high tensile stresses (more generally indicative of material failure) on the inner edge of the keel, associated with the inertial effects of the impact.
In most impact simulations, both the inclusion of the ossification front and the egg serve as an exaggerating factor of these tensile stresses, indicating a potential point of failure.
In all simulations, the point of connection of the ribcage and cranial front of the keel bone experiences significant tensile stress, suggesting an alternate failure mode for dynamic impact events.
This virtual bird demonstrates the applicability of the finite element method to further the understanding of keel bone damage in laying hens, and presents future opportunities to study the phenomena.
Keywords: keel bone damage; digital twin; Virtual Bird; Simulation; Stress Analysis

2053 Deep Learning-Based Estimation of Carcass Traits in Poultry Using CT Images

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Carcass composition is a pivotal indicator for the evaluation of poultry production. CT scanning technology, a non-destructive testing method that has wide application in human disease detection, has seen limited application in the analysis of poultry carcass composition and lacks corresponding data analysis methodologies. This study developed a CT scan-based method for determining carcass composition. The study employs a two-stage strategy of "classification followed by segmentation," using the Pekin duck as the research subject. Firstly, an automatic slice selection method based on vertebral target detection is proposed. This aims to address the issue of numerous invalid slices in CT scans. The study adjusted the window width and levels in order to highlight vertebral features, and constructed a vertebral dataset. Subsequently, a range of models were employed for analysis, culminating in an evaluation of their performance. Following a thorough evaluation of the available methodologies, the RT-DETR model was selected for the task of localising the longest vertebra in the coronal plane. This model has been shown to possess optimal vertebral target detection capabilities. This methodology is employed to identify effective slice sequences containing breast muscle, sebum, and liver in cross-sectional CT images. Subsequently, the lightweight ShuffleNetV2 network was introduced to accelerate CT slice screening, significantly enhancing both efficiency and accuracy in slice selection. The integration of a Multi-Scale Detail Augmentation (MSDA) module, in conjunction with deep separable convolutions and lightweight upsampling structures, has resulted in the development of an enhanced UNet++ model, designated as MSDA_UNet++. This enhancement leads to an improvement in segmentation accuracy whilst concomitantly reducing computational complexity. The findings demonstrate that the ShuffleNetV2 classification model attains an accuracy of 99.26% on the test set, thereby facilitating expeditious and precise identification of containing slices. The MSDA_UNet++ segmentation model achieves an IoU of 94.54% and a Dice coefficient of 97.18% in segmentation tasks, outperforming other mainstream models in both accuracy and efficiency, while reducing the number of parameters and computational cost by 89.89% and 84.72%, respectively. The findings demonstrate the efficacy of the proposed methodology in this study, which exhibits high precision and stability in determining poultry carcass composition. This provides a novel technical instrument for automated live phenotyping and breeding research in poultry.
Keywords: Carcass Traits; CT Image; Deep Learning; Precision Estimation

Nutrition: Amino Acids and Protein I

2054 Nutritional strategies and biological limits of nitrogen utilization efficiency in poultry

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Given global population growth, increasing poultry production, climate change, and the limited and uneven distribution of arable land and other natural resources, improving protein and nitrogen utilization efficiency (NUE; nitrogen retention relative to nitrogen intake) in poultry is essential for the sustainable supply of high-quality food. This presentation provides an overview of major factors influencing NUE in poultry production, with a particular focus on nutritional aspects, including protein feed evaluation, feed additives, and animal-related factors.
In addition to the concentration of essential amino acids in feed protein, their digestibility at the terminal ileum can vary substantially among feed ingredients. Standardized prececal digestibility has therefore become a key trait in feed formulation. The methodological details of digestibility assays strongly influence results, and some degree of harmonization of assay conditions has been achieved in broiler chicken studies. However, systematic comparisons remain scarce, and available evidence suggests that amino acid digestibility values determined in broiler chickens may differ from those in laying hens and other poultry species. Variability in raw material digestibility remains a challenge, particularly for developing rapid yet sufficiently precise prediction methods for feed producers. Feed enzymes can improve amino acid digestibility, although responses are not always consistent. Furthermore, novel or unconventional feed resources require comprehensive evaluation beyond digestibility in order to identify regionally appropriate solutions for adequate amino acid supply.
Avoiding excess amino acid intake is the most effective strategy to improve NUE. This requires precise knowledge of digestible amino acid requirements and their influencing factors. In growing poultry, continuous genetic progress alters growth performance traits, necessitating regular reassessment of nutrient requirements. Such efforts can be supported by modeling approaches that integrate biological responses with the economic framework of production systems. Feeding strategies with more phases may help better match supply to changing requirements. Ultimately, variation in post-absorptive amino acid metabolism may be more relevant to NUE than variation in amino acid digestibility. Achieving an optimal pattern of digestible amino acids can be facilitated by incorporating free amino acids into the feed formulation, complementing the targeted combination of protein sources. In recent years, research has extended to metabolic interactions among amino acids, between amino acids and metabolizable energy, and to the role of nonessential amino acids, particularly in the context of low-protein diets. With an emphasis on such diets, the emerging field of gut microbiota-mediated effects on NUE is increasingly being considered, alongside gut health.
Overall, increasing NUE in broiler chickens from the current practical levels of approximately 40-50% to potentially 70% or more appears biologically feasible, and the necessary nutritional tools are largely available. The extent to which such improvements can be realized in practice will depend on resource availability, economic feasibility, and constraints within production systems.

2055 Performance, nutrient utilization, and starch-to-protein disappearance rate in broilers fed field peas and faba beans as alternatives to soybean meal

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This study tested whether field peas (FP), faba beans (FB), or their combination can partially or fully replace soybean meal (SBM) in broiler diets without compromising performance, carcass yield, or nutrient utilization. Six dietary treatments progressively substituted SBM with FP, FB, or both across four phases. In the starter phase (0–10 d), SBM was reduced by 40%, 55%, or 70% using 12% or 17% FP or FB, or 12% FP + 12% FB. In the grower phase (10–24 d), SBM was reduced by 66% or 100% using 17% or 25% of a single legume, or 12.5% each of FP and FB. In the finisher (24–35 d) and withdrawal (35–42 d) phases, SBM was fully replaced with 25% FP, or 25% FB, or 12.5% of each. Each treatment had 10 replicates of six straight-run Ross 308 chicks per cage. Growth performance was recorded, and nutrient utilization was assessed by determining apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), the AME:gross energy (GE) ratio, nitrogen retention, and water intake. Protein and starch digestibility were measured at the distal jejunum and distal ileum; starch-to-protein (S:P) disappearance rates and ratios were calculated for each segment. Data were analysed by one-way ANOVA with cage sex ratio as a covariate, and means were separated by Tukey's test ($P < 0.05$). The results showed that partial replacement of SBM (<55% starter, <66% grower) did not influence final BW and FCR compared to the control treatment ($P > 0.05$). However, complete replacement of SBM from the grower phase reduced BW, increased FCR, and reduced breast meat yield, especially with FP diets ($P < 0.05$). AME, AMEn, and AME:GE ratio was not affected by dietary treatments ($P > 0.05$). Diets containing FP and/or FB increased nitrogen retention and excreta dry matter and reduced water intake ($P < 0.05$). Protein and starch digestibility were not affected ($P > 0.05$), but FP and FB increased the S:P disappearance rate ratio in the distal jejunum and ileum compared to the control group ($P < 0.05$). In conclusion, our findings suggest that FP and FB can partially replace SBM in broiler diets without adverse effects on growth, carcass yield, or nutrient utilization, but complete replacement from the grower phase onward impairs performance. The lower dietary crude protein content, coupled with higher starch levels and the resulting alterations in S:P disappearance dynamics, may explain the observed responses to FP and FB inclusion.
Keywords: Broiler chicken; Field peas; Faba beans; Soybean meal alternative; Nutrient digestibility

2056 Impact of Transition to 100% Organic Feed With or Without Additional Protein and Fiber on Brown Laying Hen Performance and Egg Quality

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Objective
This study aimed to evaluate the effects of transitioning laying hens from 95% to 100% organic feed, with or without supplementation of protein and fiber sources, on zootechnical performance, egg quality, and feed efficiency. The test was conducted in experimental conditions focused on the feed effect in small, enriched cage housing and well-controlled environment.
Experimental Design
A total of 288 Bovans Brown hens were allocated to four dietary treatments (9 replicates of 8 hens per group in cage): G1: 95% organic (control), G2: 100% organic, G3: 100% organic + protein (2% brewer's yeast), G4: 100% organic + fiber (2% pea hulls and 2% oats). The trial lasted 11 weeks (from 22 to 32 weeks of age), with weekly measurements of performance, consumption, and egg quality.
Materials and Methods
Feeds were formulated to meet nutritional requirements, with variations in crude fiber and protein sources. Performance parameters included egg production rate, egg mass, feed intake, feed conversion ratio (FCR), body weight and downgraded eggs. Egg quality was assessed at 22, 27, and 32 weeks. Statistical analyses included ANOVA and GLM models depending on variance homogeneity.
Statistical Analysis
One-way ANOVA and GLM were used to assess treatment effects. Significance was set at $P < 0.05$.
Results
Transitioning to 100% organic feed (G2 and G3) did not significantly impair overall egg production compared to the 95% control (G1). However, the G4 group (fiber supplementation) showed significantly lower body weight, egg mass, and feed intake and higher FCR from week 26 onward. Eggshell weight and its proportion, Haugh unit, yolk color were also reduced in G4. Conversely, G3 (protein supplementation) maintained egg production while the intake and FCR were higher than G1 and G2 but showed improved yolk color. No significant differences were observed in downgraded eggs and fecal dry matter across treatments.
Conclusion
While 100% organic feed is feasible without compromising performance in the condition of this trial (well controlled environment, cage housing, small number of birds), excessive fiber inclusion (as in G4) may negatively affect productivity and egg quality. Additional protein supplementation (G3) appears better beneficial in maintaining performance and enhancing yolk pigmentation. Consequently, it is important to properly manage the maximum fiber incorporated in the 100% organic feed formulation.
Keywords: Protein sources; laying hens; Fiber sources; Organic; egg production

2057 Egg Quality and Nutritional Profiling of Guinea Fowl Varieties: A Comparative Study

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Guinea fowl egg is noted for its thicker shell, higher yolk content, higher vitamins, minerals and extended shelf life compared to chicken. This study investigated the egg quality traits, proximate composition and amino acid profile of eggs from four varieties of guinea fowl namely White, Lavender, Pearl and Mixed available at Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh. A total of 200 guinea fowls (50 from each variety) were reared on floor under intensive management system. The birds were fed a layer diet (ME=2900 kcal/kg and CP= 17%). At peak production stage, 80 eggs (20 per variety) were collected to evaluate external and internal egg quality, proximate composition and amino acid profile. For amino acid analysis, eggs were freeze-dried at –55 °C using a freeze dryer (Christ, Germany), hydrolysed, filtered and pre-column derivatized prior to quantification using Ultra Performance Liquid Chromatography (Waters, USA). Data were analysed using one-way ANOVA by Statistical Analysis System (SAS) (version 9.4 M7, 2022). Egg quality traits revealed no significant differences ($p > 0.05$) among the varieties in terms of egg weight, albumen index, yolk color, yolk index, albumen percentage, yolk percentage, shell percentage and shell thickness. In addition, the values of the Haugh Unit of all varieties were above 79 and that qualifies all eggs as Grade AA indicating a high level of internal quality. Similarly, there was also no significant variation ($p > 0.05$) among varieties as shown by proximate analysis. Moisture percentage was 66.75 % (Mixed) to 68.82 % (Lavender) whereas

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the crude protein content was 12.46 % (Lavender) to 14.18 % (Pearl). Ether extract was 5.6%-7.13% and and ash content ranged from 1.950% to 3.383%. Conversely, the profile of amino acids was bearing a highly significant difference ($p<0.0001$) among all varieties according to all 17 measured amino acids. Pearl variety had the highest values of both essential (e.g. lysine 6.173 ug/g, valine 5.456 ug/g, leucine 5.053 ug/g, methionine 2.121 ug/g) and non-essential amino acids. In conclusion, although the overall external, internal quality and proximate composition were not different among varieties, the Pearl variety has numerically enriched crude protein and significantly higher amino acid profile, which makes it a potential candidate of functional food developments and genetic modification schemes in guinea fowl as the production system.

Keywords: guinea fowl; egg quality; amino acid profile; proximate; UPLC

2058 HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS FED VARYING LEVELS OF FALSE YAM (ICACINA TRICHANTHA)MEAL

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This study evaluated the haematological and serum biochemical indices of broiler chickens fed diets containing varying inclusion levels of sun-dried false yam meal (SFYM) as a partial replacement for maize. A total of 150-day-old broiler chicks were randomly assigned to five dietary treatments of 0, 4, 8, 12 and 16% SFYM inclusion levels designated as T1, T2, T3, T4 and T5 respectively in a completely randomized design (CRD) with three replicates of ten birds per treatment. Data on haematological indices showed that increasing SFYM levels led to a significant ($P<0.05$) decrease in packed cell volume (PCV), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and percentage heterophil, whilst the white blood cell (WBC) increased. Percentage eosinophil and lymphocytes were significantly ($P<0.05$) higher in broiler chickens maintained in T2 and T5 respectively compared to other treatment groups. The serum biochemical indices revealed significant ($P<0.05$) reduction in total protein, glucose, alkaline phoshpatase (ALP), and aspartate transaminase (AST) of broiler chickens as the inclusion levels of SFYM increased, while the concentration of albumin, urea and creatinine increased. These findings suggest that SFYM can be used to substitute maize partially up to 16% level without any negative impact on broiler chicken health.

Keywords: Broiler chickens; False yam; Haematology; Processing; Serum

2059 Full-fat black soldier fly larvae grown on food waste streams supports normal growth, nutrient digestibility, and meat quality in broiler chickens

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This study evaluated the effects of varying inclusion levels of food waste reared full-fat black soldier fly larvae (Hermetia illucens; BSFL) on broiler performance, nutrient digestibility, and meat quality. A 42-day feeding trial was conducted using 192 Ross 308 male broilers allocated to 24 pens and assigned to four dietary treatments (0%, 3%, 6%, and 9% BSFL; 6 pens per treatment, 8 birds per pen). Control diet was a wheat-soybean meal-based formula where soybean meal was partially replaced by full-fat BSFL in experimental diets. Experimental diets were iso-caloric, iso-nitrogenous, and iso-amino acidic, formulated to meet or exceed nutrient recommendations by Aviagen for the breed. Birds had ad libitum access to feed and water throughout the trial. Diets were provided in three phases: starter (days 1–10), grower (11–28), and finisher (29–42), meeting the breeder's nutrient recommendations. The BSFL experimental diets were fed during the grower and finisher phases (32 days). Growth performance was recorded fortnightly. On day 42, three birds per pen were randomly sampled for ileal digesta collection, and one bird per pen for breast meat quality assessment, including pH, colour, water-holding capacity, cooking loss, shear force, and proximate composition. Data were analysed using one-way ANOVA with Tukey's post-hoc test to quantify differences ($P < 0.05$).

There were no significant effects of BSFL inclusion on growth performance. Ileal digestibility of crude protein, dry matter, and organic matter was also unaffected. However, crude fat digestibility increased significantly with BSFL inclusion ($p = 0.035$) compared to the control diet (66.4%), reaching 77.9%, 75.8% and 73.8% at 6%, 9% and 3% BSFL inclusion, respectively. Meat quality parameters were not influenced by BSFL inclusion, and moisture, fat, and ash contents of breast meat remained unchanged. In contrast, breast muscle protein content increased significantly ($p = 0.011$), from 19.2% in the control group to 22.2% at the 9% inclusion level.

In conclusion, the main findings confirm that full-fat BSFL is a promising alternative protein source in broiler diets, with no adverse effects on growth performance, ileal nutrient digestibility, or meat quality. Inclusion of full-fat BSFL enhanced fat digestibility and increased breast meat protein content. Based on the findings of the current trial, full-fat BSFL produced from food-waste substrates can be included at up to 9%, replacing around 30% of soybean meal while maintaining broiler performance and meat nutritional value, supporting more sustainable feed strategies. Further research is required to investigate higher inclusion rates of BSFL to replace soybean meal.

Keywords: Broiler chicken; Black soldier fly larvae; Growth performance; Meat quality; Ileal digestibility

2060 Processing of mixed crops of wheat and faba beans for broiler feed

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Wheat-faba bean mixed crops (WFBMC) offer a lot of benefits in crop rotation systems. To use mixed crops for poultry feeds, processing techniques, like toasting, expansion or extrusion, were tested for their ability to reduce anti-nutritional factors (vicine and convicine) and for their impact on protein digestibility. One batch of WFBMC was toasted at 90°C for 20 min, while a different batch was toasted at 110°C for 10 min. Toasting did not result in reduced levels of vicine or convicine, and changes in protein solubility were minimal (55.9% for the non-treated mixed crop, vs. 57.3% for 90°C, 20 min vs. 54.5% for 110°C, 10 min). Different batches from the same WFBMC harvest were expanded at 18 bar and 100-105°C (EXP1), at 22 bar and 110°C (EXP2), or at 25.5 bar and 121°C (EXP3), or extruded at 30 bar and 130°C (EXT). Vicine levels decreased compared to non-treated WFBMC by 15 to 21%, whereas convicine levels were not affected. Protein solubility was highest for EXP3 (96.7%) compared to the other conditions (92.7% for non-treated mixed crop; 90.9% for EXP1; 91.1% for EXP2; 94.3% for EXT).

Effects on apparent ileal crude protein digestibility coefficient (AICPdc) in broilers were determined using 6 replicates of 3 broil-

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ers each. Ileal content was pooled within replicate before analysis. AICPdc was affected by the toasting conditions, with 100°C, 10 min resulting in a significantly lower AICPdc (74.4% vs. 77.6% for the control (mixed crop not toasted), with toasting at 90°C for 20 min resulting in a significantly higher AICPdc (79.2%). The poorer results with higher toasting temperatures might be explained by protein denaturation or Maillard reactions that are known to render nutrients unavailable for digestibility.
Effects of expansion or extrusion on AICPdc in broilers were assessed as described before. Interestingly, AICPdc for all the expanded conditions was significantly higher (73.4% for EXP1; 75.2% for EXP2; 74.9% for EXP3) than for the control (66.8%; standard feed with soybean meal) and the extrusion conditions (68.4%). Similar to the toasting conditions, higher temperatures as in extrusion did not result in the highest protein digestibility. Expansion as a processing technique is a better option to increase protein digestibility of WFBMC for broilers.
In conclusion, heat treatment of WFBMC had different effects on the levels of vicine and convicine, depending on the conditions. However, effects on the levels of vicine and convicine cannot be linked to higher protein digestibility coefficients. For the latter, it are the processing conditions that will have an impact on protein digestibility. These mixed crops can be perfectly used in broiler diets as protein source.
Keywords: wheat; faba bean; broiler; processing; digestibility

2061 Interactive influence of feedstuff fiber level and fat type on standardized digestibility, independent of the morphology of digestive organs or cecal content pH in broiler chickens

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In previous studies, we reported that the fat type used in the assay diets had a marginal effect on standardized ileal amino acid digestibility (SIAAD) of feedstuffs, whereas the effect of the feedstuff was more pronounced. In the current experiment, we investigated the role of feedstuff fiber level interacting with fat type on SIAAD, the morphology of the digestive organs, and cecal content pH.
A total of 260 male broiler chicks were assigned to 52 metabolism cages with six treatments (3×2), each with six replicates, to examine the effect of fat type (soybean oil or lard) on the SIAAD in three feedstuffs with different fiber profiles (corn, wheat, or wheat bran). Additionally, two nitrogen-free diets with the two fat types were used to measure the basal endogenous amino acid flow. All birds received a basal corn-soybean meal diet from d 0 to 16, followed by experimental diets from d 16 to 21. On d 21, all birds were euthanized, and digesta were collected from the distal ileum to determine the SIAAD. The duodenum, jejunum, ileum, gizzard, and pancreas were collected to measure their relative lengths and weights. Cecal content was also collected for pH measurement. Data were analyzed using two-way ANOVA, and means were separated using Tukey's HSD. Significant feedstuffs İfat type interaction ($P < 0.05$) was observed for all indispensable amino acids (AA) except His, Val, and Trp, whereas Cys, Glu, Gly, Pro, and Ser were the exceptions among dispensable AA ($P < 0.05$). Generally, corn had the highest digestibility regardless of fat type. In contrast, wheat assay diets with lard had higher AA digestibility ($P < 0.05$). Birds that received the wheat bran assay diet had the highest relative organ weights ($P < 0.05$), irrespective of fat type. Birds receiving assay diets with soybean oil had longer jejunum ($P < 0.05$). The cecal content pH was in the order corn > wheat bran > wheat ($P < 0.01$). In conclusion, the interaction of fiber level and fat type influenced AA digestibility, but the effects on the morphology of digestive organs and the cecal content pH were influenced by feedstuff alone. Therefore, it is important to consider the fat type used in assay diets when determining the SIAAD of feedstuffs with significantly different fiber profiles.
Keywords: Standardized ileal digestibility; feedstuff; fat type; morphology; lard

2062 Impact of soybean meal replacement with non-bound amino acids and local feed ingredients on growth and digestibility in broiler diets based on wheat, sorghum or barley

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Soybean meal (SBM) is the primary protein source in Australian broiler diets but relies heavily on imports, raising sustainability and cost concerns. Replacing SBM with local high-protein feed ingredients and non-bound amino acids (NBAA) is a potential approach. This study aimed to evaluate the effects of replacing SBM with a combination of NBAA and local ingredients in broiler diets based on wheat, sorghum, or barley on growth performance and nutrient digestibility.
The experiment followed a 3 × 3 factorial arrangement with grain types (wheat, sorghum and barley) and SBM inclusion levels (normal, medium and low). Normal SBM inclusion levels were 250 and 210 g/kg in the starter (day 0-14), grower (day 15-28) phases. Medium and low SBM levels represented reductions of 25% and 50%, 50% and 75% in the two phases. SBM reduction was achieved by various inclusion of NBAA, canola products, and field peas, without controlling crude protein levels. Each treatment included eight replicate floor pens with 20 off-sex male Ross 308 chicks. On day 28, three birds per pen were euthanized, digesta from distal jejunum and ileum were collected to determine apparent digestibility coefficients of starch and protein (N). Data were analysed by two-way ANOVA, with statistical significance set at $P < 0.05$.
An interaction between grain type and SBM inclusion level affected BW at d 28, where low SBM inclusion significantly reduced BW in the sorghum and barley-based diets, but not in the wheat-based diets. Birds fed barley-based diets had lower FCR compared to those fed wheat or sorghum-based diets. Reducing SBM inclusion reduced FI and increased FCR over the 0-28 d period. Reduced apparent starch digestibility in both intestinal segments, and reduced apparent N digestibility in distal jejunum of birds fed sorghum-based diets. SBM reduction decreased starch digestibility but increased N digestibility. Medium and low SBM inclusion reduced apparent starch digestibility in both intestinal segments, while low SBM inclusion increased apparent jejunal N digestibility compared to normal or medium SBM diets. Birds fed low SBM diets showed increased apparent ileal N digestibility compared to those fed medium SBM diets.
These results suggest that replacing SBM with NBAA and local ingredients can maintain nutrient utilisation in wheat and barley-based diets. Sorghum-based diets were more sensitive to SBM reduction, highlighting the need to consider grain-specific nutrient interactions in low SBM broiler diets. The findings suggest a feasible way to reduce SBM in broiler diets, supporting sustainable poultry production in Australia.

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Keywords: broiler chickens; soybean meal; alternative ingredients; non-bound amino acids; grain type

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2063 Advancing in vitro models for young broilers: A full gastrointestinal tract simulation to evaluate alternative protein ingredients

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Soybean meal (SBM) is the predominant protein source in broiler diets due to its high protein content, complete amino acid profile, and economic viability. However, sustainability of SBM use in European animal feed is increasingly questioned, increasing pressure to identify alternative protein sources, particularly for starter broilers whose immature gastrointestinal tract (GIT) limits protein digestion efficiency.

Although in vitro models are encouraged to reduce animal experiments in line with the three R principle, existing models are often insufficient, as they typically assess either upper GIT digestion or lower GIT fermentation, rarely simulating the full GIT from proventriculus to caeca or accounting for the young broiler physiology. To address this, we developed a comprehensive in vitro digestion model mimicking the entire GIT of starter broilers, enabling simultaneous evaluation of protein digestion kinetics and microbial fermentation.

Pea was assessed as a partial replacement for SBM through complementary in vitro and in vivo experiments using isonitrogenous and isoenergetic diets containing SBM or 10%, 20%, or 30% pea. In vivo, 240 starter broilers (0–10 days) were allocated to four treatments, with six replicate pens per treatment (10 birds per pen). Samples were pooled per pen. Body weight gain, ileal crude protein digestibility, and cecal microbiota and fermentation products, e.g., short-chain fatty acids (SCFA) and ammonia, were assessed. In vitro digestion kinetics (10 replicates per diet) and fermentation (12 replicates per diet) were evaluated. Statistical analyses were performed using ANOVA with Tukey post hoc tests for parametric data and Kruskal–Wallis tests with Dunn post hoc tests for non-parametric data ($P < 0.05$). Non-linear multiple regression modelling was used to compare in vitro digestion kinetics.

In vitro results showed comparable protein digestion kinetics for SBM and 10% pea diets, whereas 20% and 30% pea diets exhibited reduced digestion extent. These findings were confirmed in vivo: 10% pea inclusion did not affect performance or ileal CP digestibility (approximately 60%), while higher inclusion levels reduced CP digestibility (approximately 55%) and body weight gain. Although 20–30% pea diets altered gas production kinetics during fermentation, no differences in SCFA concentrations were observed. Overall, these results demonstrate the value of physiologically relevant in vitro models and indicate that pea inclusion up to 10% is feasible in starter broiler diets without compromising performance. Moreover, the developed in vitro model provides a robust tool for pre-screening novel feed ingredients in young broilers, reducing the immediate need for in vivo testing.

Keywords: Broiler nutrition; in vitro gastrointestinal model; protein digestion; cecal fermentation ; pea protein

2064 Predicting apparent ileal protein digestibility in broiler chickens using a dynamic mechanistic model

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Rising global demand for poultry makes precision feeding key to improving resource use, sustainability, and performance. For poultry, this requires knowledge of where and how much nutrients are digested along the gastrointestinal tract. Mechanistic models are mathematical representations of a system's behaviour, integrating the underlying physical, biological and chemical mechanisms, and thus may be useful for predicting nutrient digestibility in poultry. Therefore, the objective of this research was to develop a protein digestion kinetic model framework, as a first step towards creating a precision formulation decision support tool for poultry. A mechanistic model of protein digestion in broilers was developed based on prior work in swine and a model for transient plug flow reactors (PFRs) by the Kitchin Research Group at Carnegie Mellon University. Crude protein (CP) intake was modelled as small consecutive boluses. Separate pools for complete mixing were used to represent the crop and proventriculus/gizzard. To more accurately represent the flow of digesta through the small intestine, PFRs were used with separate pools for undigestible (UDP), slowly-digestible (SDP) and rapidly-digestible (RDP) protein fractions. Degradation of SDP to RDP was calculated using the quantity of protein (g) and the fractional degradation rate (/min). Inflows come from previous GIT pools for all protein fractions, an endogenous protein source for the UDP fraction, and SDP fraction for the RDP pool. Outflows include digesta passage and absorbed protein. A behaviour analysis was conducted to preliminarily evaluate the behaviour and digestibility predictions of the model by incrementally adjusting the driving variables - CP %, feed intake (g/kg) and proportions of UDP, SDP and RDP fractions. The structure was successfully represented in the coding language Python, combining both the traditional pools of instantaneous mixing and less conventional PFRs. The preliminary behaviour analysis indicates correct behaviour of the model. Apparent ileal digestibility increases when CP % or feed intake is increased. Variations in the proportion of UDP, SDP and RDP fractions show that apparent ileal digestibility corresponds to the size of the UDP fraction. The presented model is one of the first digestion models that incorporates both instantaneous mixing pools and PFRs. Future work includes rigorous evaluation of the model and representation of other nutrients. This work contributes to the development of precision feeding decision support tools for poultry, and the resulting potential to optimize animal performance.

Keywords: Apparent ileal protein digestibility ; Mechanistic modelling ; Plug flow reactor ; Broiler; Digestion

Nutrition: Feed Additives I

2065 The strategic importance of feed additives in promoting health, performance and sustainability in broiler production

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The global phase-out of antimicrobial growth promoters (AGPs) has fundamentally reshaped nutritional strategies in the poultry industry, creating an urgent need for effective, safe, and sustainable alternatives. This transition is driven by several converging forces: rising global demand for poultry products, increasingly antibiotic restrictions, evolving regulatory frameworks that favor environmentally responsible production, and the growing threat of poultry disease outbreaks. While the reduction of in-feed antibiotic use is an essential step in combating antimicrobial resistance, the withdrawal of AGPs poses challenges to producers, including increased susceptibility to enteric dysbiosis, compromised gut integrity, and reduced growth performance. It is now well-established that no single "magic bullet" can replicate the broad-spectrum benefits of AGPs. An integrated approach is required, including optimized biosecurity, sound management practices, and targeted nutritional strategies. With this approach, it is very important to strategically use functional feed additives. A diverse range of additives, including organic acids, essential oils, enzymes, organic minerals, and probiotics, have been widely recognized as promising alternatives

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to in-feed antibiotics, with complementary mechanisms to support gut health, modulate the immune response, and improve nutrient utilization. In summary, functional feed additives represent a cornerstone of modern, responsible broiler production. When applied as part of a well-designed, integrated program, they offer a scientifically sound pathway to improving feed utilization efficiency, sustaining performance, and safeguarding bird health — ensuring the long-term safety, sustainability, and economic viability of the broiler industry in a world beyond antimicrobials.
Keywords: Feed additives, antimicrobial alternatives, broiler production, gut health, poultry nutrition

2066 Comparative evaluation of the wheat bran and soyhull alone or in combination with stimbiotic on growth performance, short-chain fatty acids profile, and relative expression of pectoralis protein synthesis and degradation genes in broiler chickens

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Stimbiotic (STB) (xylanase and xylo-oligosaccharide) supplementation, along with wheat bran (WB) or soyhull (SH) as fiber sources, may modulate fermentation patterns, promote the proliferation of beneficial microbiota, and enhance growth performance. This 35-day study compared WB and SH, alone or in combination with STB, or STB supplementation alone, on growth performance, cecal short-chain fatty acid (SCFA) , and relative mRNA expression of pectoralis protein metabolism in broilers. For soyhull inclusion, diets were formulated with low-protein soybean meal (LPSBM). The LPSBM was produced by adding SH to the standard soybean meal (SSBM) at a 56 g/kg rate to make an LPSBM of 440g/kg crude protein. A total of 924 day-old male broiler chicks were allocated to six treatments in a randomized complete block design: 1) SSBM; 2) SSBM + 0.1 g/kg STB; 3) SSBM + 50 g/kg WB; 4) SSBM + 50g/kg WB + 0.1g/kg STB; 5) LPSBM diet; and 6) LPSBM + 0.1g/kg STB. Birds and feed were weighed on days 0, 10, 28, and 35. On day 35, cecal content and pectoralis muscle were analyzed for SCFA and protein metabolism genes. The data were analyzed using one-way ANOVA using JMP Pro 18. During the starter phase, there was a significant increase in weight gain (WG) ($P < 0.05$) in birds receiving SSBM + WB + STB compared to those receiving SSBM alone. A trend ($P = 0.069$) was observed with feed intake (FI), where birds receiving both WB and STB had higher feed intake compared to SSBM and LPSBM + STB treatments. No significant treatment effects were observed in the growth performance for the grower and finisher phases. There were no significant treatment effects for protein synthesis (4EBP1 and mTOR) and degradation (FBXO9 and MYOG) genes on day 35. There was no significant effect of treatments on acetate, butyrate, and propionate; however, the acetate: propionate ratio was significantly higher in SSBM + WB compared to SSBM + WB + STB or SSBM treatments. In conclusion, WB, along with STB, improved starter phase WG in broilers, but these effects did not persist during the grower and finisher phases. There were no significant effects of treatments on genes for muscle protein metabolism or on total cecal SCFA concentrations. Wheat bran inclusion did, however, increase the acetate: propionate ratio, indicating a shift in cecal fermentation patterns. Together, stimbiotic-fiber combinations can enhance early growth and modulate microbial fermentation; however, their long-term effects on growth and muscle protein metabolism appear to be modest.
Keywords: Wheat bran; soy hull; stimbiotic; growth performance; SCFA

2067 Dietary strategies to enhance the growth performance in broilers fed with high-canola diets

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Reduced feed intake observed with high canola-based diets could be attributed to low pellet durability index and the presence of glucosinolates. In addition, the lower digestibility of methionine in these diets may contribute to poor growth performance in broiler. Therefore, this study aimed to evaluate different nutrient strategies to improve feed intake and mitigate compromised performance associated with diets containing high levels of canola meal (CM). It was hypothesized that these dietary interventions would enhance broiler performance primarily through improvements in feed intake. A total of 252 Ross 308 male birds were used in 7 dietary treatments, which included a wheat-soybean meal-based control diet and six other treatments with varies dietary strategies such as inclusions of pellet binder (10 g/kg), glutamate (10 g/kg), potassium iodide (25 mg/kg), sodium metabisulphite (2.50 g/kg), 10% higher digestible methionine, or faba beans (150 g/kg) in diets containing 140 and 180 g/kg of CM during the grower and finisher phases, respectively. Diets were formulated to be iso-energetic with similar digestible essential amino acid levels. Dietary treatment was offered to six replicates of six birds per cage from 9 to 35 days post-hatch. Data were subjected to one-way ANOVA using JMP Pro 17 software. In the overall period (9-35d), high CM-based diets with dietary strategies did not significantly affect body weight gain ($P = 0.175$), feed intake ($P = 0.119$) and mortality ($P = 0.624$). However, the feed conversion ratio (FCR) was significantly improved ($P < 0.001$). For instance, with faba beans improved FCR to 1.353 g/g, followed by sodium metabisulphite to 1.372 g/g, potassium iodide to 1.375 g/g, higher digestible methionine to 1.380 g/g and glutamate to 1.392 g/g. But there was no significant difference in FCR between the pellet binder and control (1.400 versus 1.433 g/g). For carcass traits, there was a significant dietary impact on Pectoralis major ($P < 0.001$), Pectoralis minor ($P = 0.012$), leg quarters ($P = 0.029$), and abdominal fat pad ($P = 0.014$) weights. Dietary treatments significantly affect full gizzard weight ($P = 0.030$), empty gizzard weight ($P = 0.011$), and pH in the gizzard ($P = 0.008$). In this study, FCR was related to gizzard characteristics, with a quadratic relationship observed between FCR and gizzard pH ($r = 0.457$; $P = 0.012$). Additionally, FCR was negatively correlated with empty gizzard weight ($r = -0.371$; $P = 0.017$). These findings suggest that gizzard pH and gizzard development play a role in feed efficiency. In conclusion, applying faba beans, sodium metabisulphite, and potassium iodide in high CM-based diets generated better feed efficiency compared to the control diet.
Keywords: Canola meal; Growth; Faba bean; Sodium metabisulphite; Potassium iodide

2068 Growth performance and carcass characteristics of broiler chickens fed diets supplemented with varying combination of ginger, black pepper and thyme

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There is increase in consumer demand for poultry products free from antibiotic residues. Feed producers are adopting different nutritional strategies to replace antibiotics with organic feed additives. A forty-two-day study was conducted to determine the effect of varying combination of ginger, black pepper and thyme in the diets of broiler chickens on growth performance and carcass characteristics. A total of 200 unsexed day-old broiler chicks (Cobb 500) were used for this experiment. Starter and finisher basal

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diets were formulated such that the blends were added at 2g/kg of Thyme, 5g/kg of Black pepper and 5g/kg of Ginger to the basal diet at different combinations making five treatments, i.e T1 (basal diet only), T2 (basal diet +1g/5kg oxytetracycline), T3 (Ginger + Black pepper), T4 (Black pepper + Thyme) and T5 (Ginger + Thyme). The birds were assigned to the five treatments, replicated 4 times and the experiment was divided into starter phase (day 0-21) and finisher phase (day 22-42). Data were collected on live weight, daily feed intake while feed conversion ratio (FCR), average weight gain (AWG) and average final weight (AFW) were calculated for both phases. At day 42, two birds per replicate were picked whose weight (2.2-2.4kg) were close to average replicate weight for carcass analysis. Data were subjected to one-way ANOVA in a completely randomized design using general linear model procedure of the SAS 9.1.3 software package for windows. At the starter phase, AFW and AWG of birds in T3 were ($P < 0.05$) higher than birds in T4 and T5 but similar to birds in T1 and T2. The FCR recorded for birds in T3 and T5 were statistically ($P < 0.05$) lower compared with other treatment groups. Percentage mortality was significantly higher in T1 while other treatments were similar. Finisher phase, AFW of birds in T3 was higher ($P < 0.05$) compared with other treatment groups. AWG of birds in T1 and T3 were higher ($P < 0.05$) than other treatments. The FCR of birds in T1 and T3 were statistically lower than other treatment groups. The average feed intake of birds in T2 was higher ($P < 0.05$) compared with other treatments. The diets significantly ($P < 0.05$) influenced the drumstick and head with birds in T1 and T3 having significantly higher values for drumstick and head respectively. The lung, heart, liver, and proventiculus were significantly ($P < 0.05$) influenced by the diets such that the values obtained for lung, heart and proventiculus were higher for birds in T3 while T4 and T5 recorded the least values for liver. It was concluded that the combination of ginger and black pepper had the best synergistic effect on broiler chickens' growth performance.

Keywords: Black pepper; carcass; ginger; growth performance; thyme

2069 Effects of manihot esculenta leaf meal on growth performance, hematological and serum biochemical values of broiler chickens (Cobb500)

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This experiment investigated the effect of dietary Manihot esculenta leaf meal (MELM) on growth performances of Cobb 500 broilers. A total of 300 day-old chicks were assigned into three dietary treatment groups of basal diet with 0% (ME0), 2.5% (ME2.5) and 5% MELM (ME5), having 5 replicates of 20 birds each. All the diets had similar levels of crude protein and metabolisable energy and met the requirement of the bird according to their stage of development. During the experimental period, feed intake (FI), live weight, and feed conversion ratio (FCR) were recorded weekly. At 6 wk of age, blood samples were collected from 15 birds per treatment for hematological and biochemical analysis. Carcass quality was also determined. Results showed that feed intake (FI) was higher ($p<0.05$) in the chickens of ME5 group compared to other treatments. Birds in ME5 group had a higher feed conversion ratio than the birds of the other treatments whose value were similar. Average live weights and weight gain of birds in the ME0 and ME2.5 groups were similar and statistically higher ($P<0.05$) than those of ME5. Carcass weight of ME2.5 group was higher ($p<0.05$) than those of ME0 and ME5. The weights of liver, heart and intestine showed no significant difference among across the treatments. The weights of abdominal fat in the birds of ME2.5 and ME5 groups were lower compared to the control group. Total cholesterol, triglyceride and uric acids were decreased significantly ($p < 0.05$) in birds fed 2.5% and 5% of MELM. Red blood cells, packed cell volume and lymphocytes, hematocrit, hemoglobin increased with the increase ($p < 0.05$) in manihot esculenta level. In conclusion, manihot esculenta leaf meal (MELM) can be used up to 2.5% into the diets of broiler chickens without any deleterious effect on the performance and health of the birds

Keywords: Manihot esculenta; broiler chickens; growth performances; hematology; serum biochemical

2070 Enhancing broiler performance with a novel combination of PhytoComplex and target-release butyrates

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Background and objective

Modern poultry production faces increasing challenges related to gut health, nutrient utilization, and sustainable performance. The objective of this study was to evaluate the efficacy of a novel feed additive, a unique combination of plant-derived bioactive compounds (PhytoComplexes) and target-release butyrates, in improving the zootechnical performance of broilers.

Experimental design

A total of 2,040 Ross 708 broilers were allocated to five dietary treatments over a 42-day period: control, 200 g/t PhytoComplex 1 (Glycericia glabra, products of Artemisa vulgar and Satureja hortensis), 250 g/t PhytoComplex 2 (Rubus fruticosus), 500 g/t PhytoComplex 1+2, and PhytoComplex 1+2 with target-release butyrates (Rubus fruticosus, Glycericia glabra, products from Artemisa vulgar and Satureja hortensis, sodium and calcium butyrates; Presan® Evolve [PE]).

Material and methods

Birds were housed under commercial-like conditions on re-used litter and fed a three-phase diet containing DDGS to induce mild gut inflammation. Performance parameters including body weight (BW), feed intake, feed conversion ratio (FCR), and mortality were recorded for the overall study period.

Statistical analysis

One-way ANOVA was used to test for main treatment effects. The pen was considered as experimental unit. P-values < 0.05 were regarded as significantly different.

Results

All PhytoComplex treatments showed similar final BW compared to the control, with PE yielding the numerical highest gain (+46 g) ($p > 0.1$). Feed intake was significantly reduced ($p < 0.05$) across all PhytoComplex groups compared to the control. FCR was significantly improved in all treatments compared to the control, with PE achieving the lowest adjusted FCR (1.673 vs. 1.802 in control, adjusted to 2.5 kg BW). Reduced FCR can be translated into lower feed costs per kg of live weight, the main driver of profitability in broiler product, resulting in PE being a cost effective solution. Although gut health was not measured, earlier in vitro work showed that PhytoComplex 1 reduced LPS induced IL-6 production in human macrophages, which may explain the improved performance results observed. Currently the PhytoComplexes are produced on a large scale to accommodate commercial use.

Conclusions

These results demonstrate that combining PhytoComplex with target-release butyrates synergistically enhances broiler performance mainly through feed efficiency. This is the result of the specific ingredients supporting gut health and nutrient absorption.

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This offers a promising solution for sustainable and health-oriented poultry production.
Keywords: PhytoComplex; Target-release butyrates; Performance; Broilers; Feed Additive

2071 Standardized Citrus Extract: a natural strategy to improve gut health, welfare, and performance of broiler chickens

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After successfully meeting the challenge of productivity, the poultry sector now faces a new challenge: maintaining these achievements while ensuring animal health and welfare, to meet an increasingly strong societal demand. Feed additives offer a promising way to address this challenge. This study evaluated the effect of a Standardized Citrus extract, known for its positive effects on gut health, to assess whether these benefits translate into improved performance, welfare, and carcass traits in broiler chickens
756 one-day-old male Ross 308 chicks were randomly allocated to two groups: a control group (CTL) fed a standard diet and a citrus-supplemented group receiving 250 g/T of a Standardized Citrus Extract (SCE, Nor-Spice AB®, Nor-Feed SAS), with 14 and 13 replicates of 28 birds, respectively. Growth performance was recorded weekly until day 35, as well as carcass traits from 10 birds randomly selected per group. Welfare indicators (footpad dermatitis, litter quality, gait score, and hock burn) were evaluated at day 35 using the Welfare Quality® protocol. Gut health was assessed through caecal short-chain fatty acids (SCFA) analysis (20 birds/group), jejunal permeability by transepithelial electrical resistance (TEER), and blinded histopathological examination of jejunal samples (10 samples randomly selected per group). Statistical analyses were performed using Student's t-test or Mann–Whitney U test (welfare), with significance set at $p < 0.05$.
Results showed that SCE dietary supplementation improved broiler chickens' performance, i.e., final bodyweight (2364.34g Vs 2201.83g). and EEI (461.24 Vs 426.31) compared to the CTL group. The carcass weight was also significantly higher in the SCE group (1639.6 g Vs 1562.8 g) with a comparable carcass yield (70.6 % Vs 69.1%). In addition, the fat percentage was lower in the SCE group (1.08 g Vs 1.36 g/100 g). Regarding broiler chickens' welfare and gut health parameters, Footpad Dermatitis (FPD) and gate score were also lower in birds supplemented with SCE. The SCFA measurement showed a lower concentration of iso-butyric acid (0.08 vs 0.06 mM/g), iso-valeric acid (0.11 Vs 0.07 mM/g), and total putrefactive SCFA (0.19 Vs 0.13 mM/g) in the SCE group. The differences in gut permeability, measured as TEER value, indicate that using citrus extract may help lower the risk of gut inflammation, which was confirmed by histopathological observation.
This study suggests potential benefits of using SCE as feed additive. Indeed, SCE supplementation improved gut health, which was correlated with better performance, carcass traits, and welfare.
Keywords: Citrus extract; broiler chickens; growth performance; welfare; gut health

2072 Effect of dietary functional feed additives on broiler meat composition, fatty acid profile and oxidative stability

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Traditional food ingredients have increased greater attention due to their potential to generate functional foods and nutraceuticals. An experiment was conducted to evaluate the dietary effects of Linum usitatissimum (Flaxseed), Trigonella foenum-graecum (Fenugreek), and Moringa oleifera leaf (Moringa, MOL) added in broiler feed with seven different combinations. The objectives of this study was to investigate growth performance, carcass quality, oxidative stability, cholesterol content, and fatty acid composition of broiler. A total of three hundred thirty-six-day (336) old mixed sex Cobb 500 broiler chicks were split into 7 dietary treatments for five weeks, with 4 replications having 12 chicks per replication. The diets of the treatments were formulated from the basal feed as follows: T1 - Control diet; T2 - Flaxseed 0.5% + MOL 1%; T3 - Flaxseed 1% + MOL 1%; T4 - Flaxseed 1.5% + MOL 1%; T5 - Fenugreek 0.5% + MOL 1%; T6 - Fenugreek 1% + MOL 1%; T7 - Fenugreek 1.5% + MOL 1%. The birds were distributed following Completely Randomized Design (CRD) in each pen, and the data were analyzed using the SPSS IBM statistical package 27. The analytical measurements were done in triplicates, and the results were presented as the average of 3 analyses ± the standard deviation (SD). Feed and fresh water were offered ad libitum feed intake and free access to water throughout the period (35 d rearing). At the age of 35 d, eighty-four (84) birds were randomly selected per treatment, 3 per replicate in each pen, and were individually weighed. The final body weight gain was similar in all groups in 35 days with significantly ($p < 0.05$) reduced feed intake in T4 than control. In comparison to the control group (34.92 mg/100g), the cholesterol content of thigh meat dropped significantly ($p < 0.05$) in groups T3, T4, T6, and T7 with the values of 26.31, 26.07, 26.50, and 26.24 mg/100g meat respectively. Following the third week of preservation, the additives groups' thiobarbituric acid-reactive substances (TBARS) assessments for breast and thigh meat were the significantly lowest ($p <0.05$). In breast meat, the omega 3 fatty acids were increased in T4 (Flaxseed 1.5% + MOL 1%) and T7 (Fenugreek 1.5% + MOL 1%) groups with the value of 2.61 and 2.41 g/100 meat than control 1.35 g/100g, alongside significantly increased total polyunsaturated fatty acids. In the experiment gut health data was not examined. Overall, the findings show that diets containing up to 1.5% Flaxseed and 1.5% Fenugreek with combination of 1% Moringa enhanced the omega-3 fatty acids and meat composition of broiler meat with lowering lipid oxidation.
Keywords: Flaxseed; Fenugreek; Moringa; fatty acids; oxidation

2073 Zootechnical additives associated with antimicrobials: effects on immune response in broiler chickens

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Antibiotic growth promoters (AGPs) are commonly used in poultry farming to enhance growth and health, but concerns about antibiotic resistance have driven the search for alternatives. This study evaluated the effects of zootechnical additives in combination with AGPs on leukocyte differential activity and phagocytic activity. A total of 1400 one-day-old Cobb 500 male chicks were assigned to a completely randomized design with seven treatments and eight replicates of 25 birds per unit. The treatments included a basal diet without AGPs, a basal diet with AGPs, and AGPs combined with different zootechnical additives: prebiotics (fructooligosaccharides, galactooligosaccharides, beta-glucans, and mannan-oligosaccharides), probiotics (Bacillus subtilis LFU160), essential oils (cashew nut shell liquid), and organic acids (butyric acid glycerides). The variables were analyzed using the non-parametric Kruskal–Wallis test at the 5% significance level, which was carried out using the PROC NPAR1WAY procedure. When significant effects ($p < 0.05$) were detected, the means were compared using Dunn's test, using SAS® statistical software. Highest values for percentage and frequency of phagocytic of monocytes in broilers at 6 days of age, were observed in the AGP + essential oil (13.9% and 5.8, respectively) and AGP + butyric acid (16.0% and 7.5, respectively) treatments ($p < 0.001$). In terms of the phagocytic heterophils percentagem and na frequency, highest values found in the AGP + butyric acid (10.7% and 4.8, respectively) ($p = 0.0014$ and $p = 0.0173$, respectively). At 21 days of age, the highest percentagem and frequency of phagocytic monocytes was found in the treatment without AGPs (0.8% and 187.9, respectively) ($p = 0.0008$ and $p = 0.0048$, respectively). Highest heterophil percentage was found in the AGP + prebiotics 1 and 2 treatment (3.2%) ($p = 0.0004$). The highest occurrence frequencies of phagocytic heterophils at 21 days of age were observed in the AGP + prebiotics 1 and 2 (564.1) and AGP + prebiotic 1 (529.0) treatments, which differed only from the AGP + organic acid (285.7) treatment, which showed the lowest value ($p = 0.0097$). Regarding the phagocytosis index, the highest values were observed in the AGP + prebiotics 1 and 2 (442.6) and AGP + prebiotic 1 (400.1) treatments, which differed significantly only from the treatment without AGPs (250.5) ($p = 0.0048$). In conclusion, zootechnical additives can be used alongside AGPs to enhance broiler immune function, particularly during early growth stages with promising results. These findings provide valuable insights for poultry producers seeking to optimize feed strategies while reducing the reliance on antibiotics.
Keywords: antibiotic growth promoters; organic acid; essential oil; prebiotic; probiotic

2074 Dietary Synergy of Vitamin C and Zinc–Manganese-Amino Acid Complex Enhances Growth and Meat Quality of Heat-Stressed Broilers

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This study investigated the effects of vitamin C (VC) and zinc-manganese-amino acid complex (ZMAC) supplementation on growth performance, carcass traits and breast meat quality of broilers exposed to chronic heat stress. A total of 240 Lohmann Meat broilers were randomly allocated into four dietary treatments with six replicate pens of ten birds each. The dietary treatments were CONT(control); VC(basal diet with 200 mg VC); ZMAC(basal diet with 50 mg of ZMAC); and VC+ZMAC(basal diet with 200 mg VC and 50 mg of ZMAC). Birds were reared under chronic heat stress conditions (30–33°C) for 28 days. All data were subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Package for Social Science (SPSS, version 23). Dietary supplementation with VC, ZMAC, and their combination significantly improved body weight gain and feed conversion ratio during both the starter and grower phases ($P < 0.05$). Although dietary treatments did not influence protein intake, specific growth rate, or growth efficiency, protein efficiency ratio was significantly increased during the growing phase ($P < 0.05$). The VC+ZMAC treatment resulted in a significantly higher dressing percentage ($P < 0.05$), while other carcass traits were not affected. Broilers fed VC, ZMAC and VC+ZMAC diets showed a marked reduction in the incidence of severe breast muscle myopathies, including spaghetti meat (SM), wooden breast (WB), and white striping (WS), compared to the control group ($P < 0.05$). The breast meat crude protein content was significantly higher in the ZMAC and VC+ZMAC supplemented group compared to control ($P < 0.05$). Dietary VC significantly increased the tryptophane and valine contents, while ZMAC and VC+ZMAC increased the lysine, tryptophan threonine and total essential amino acid (SEAA) contents of broiler breast meat ($P < 0.05$). In conclusion, supplementation with VC, ZMAC and their combination improves growth performance, and breast meat quality of broilers under chronic heat stress. Further studies are recommended to evaluate different dose levels and combinations of these supplements for optimizing broiler performance under stress conditions.
Keywords: Broilers; chronic heat stress; vitamin C; Zinc–Manganese-Amino Acid Complex; breast meat quality

2075 DIETARY BENZOIC ACID AND ESSENTIAL OILS ENHANCE HATCHABILITY AND CHICK QUALITY IN BREEDER HENS

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Restrictions regarding growth promoting antibiotics in livestock has driven the search for alternatives able to maintain hatch quality. Therefore, the present study aimed to assess the effects of different inclusion rates of Lumigard TCB, a compound of benzoic acid with thymol and carvacrol (LG) in broiler breeder hen's diets. A total of 192 Ross AP95 breeder hens were randomly distributed in one of four dietary treatments – NC: Corn and soybean-meal based diet; PC: NC+40ppm virginiamycin; LG250: NC+250ppm LG; LG500: NC+500ppm LG. Hens were acquired at 20 wks of age and distributed in 12 pens per group, four hens per pen, and were fed experimental diets from 23 up to 65 weeks of age. 40 AP95 roosters were also individually allotted and received a common diet. Feed and lightning regimes were done according to the lineage manual. At 43 and 53 weeks of age, fresh semen was collected, and hens were inseminated with 0,5 µL of pooled semen. Eggs were collected for a week after each insemination and kept at 16oC until set at a single-stage incubator for 21 days. At each hatch, non-hatched eggs were analyzed for embryo mortality and classified into early (1-7d), mid (8-14d) and late (15-21d) death, as well as infertile and pipped eggs. Fertility, hatchability and hatch of fertile rates were assessed. Chicks were weighted and measured at hatch, and 25 chicks of each sex per group were inspected for dehydration, unhealed navels or red hocks. Chick measures were analyzed with ANOVA, with means separated by Tukey's test, while hatch and chick quality rates were analyzed using Chi2 test with significance set at 0.05. Despite no differences in fertility rates amongst groups in both hatches, eggs from hens fed LG500 diets showed higher hatch of fertile than NC at 53 wks of age (94.0 vs 80.7%, $P<0.001$). No differences were observed in embryo mortality from 43-week-old hens between groups, but eggs incubated

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from hens fed LG showed a lower incidence of pipped eggs than eggs from hens fed NC at 53 wks of age. Chicks from 43-wk-old hens fed LG250 showed longer lengths than other groups (19 vs 18.7cm PC,P<0.001) and chicks from hens fed experimental diets showed lower dehydration than chicks hatched from hens fed NC diet. At 53 wks of age, chicks from LG500 hens showed lower weight among groups (P<0.05), while chicks from LG250 hens had the longest length overall (19.3 vs 18.8cm PC, P<0.001). Results suggest that LG can be a safe alternative for antimicrobial usage in breeder hens, allowing maintenance of hatchability rates and low embryo mortality and also increase chick length, which highly correlates with posthatch performance.

Keywords: Chick quality; embryonic mortality; Incubation; Offspring; Phytogenic

Reproduction & Breeders I

2076 Sixty years of divergent genetic selection, advancements and setbacks in reproductive efficiency

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Over the last sixty years, the poultry industry has transitioned from local small scale production systems to a heavily integrated and concentrated global industry. As part of this transformation, advances in intensive divergent genetic selection played a pivotal role with layers capable of producing over 500 eggs per cycle, and broilers reaching market weight within 5 weeks. However, achieving these milestones did not come without costs as we continue pushing the physiological limits of animals. For layers, the drastic increase in egg production was achieved by advancing the age at first egg, increasing peak production and significantly extending laying cycles. Of course, adjustments in management and nutrition also followed. However, egg output is ultimately linked to resource availability and utilization, especially calcium for egg-shell formation. This became apparent with the increased incidence of calcium deficiency and osteoporosis. Interestingly, our recent data suggest that while pushing for extended laying cycle, breeding companies have also selected layers for improved calcium and bone metabolism, thus partly mitigating these negative effects. Furthermore, we also reported that advancing sexual maturation was associated with faster growth trajectory as pullets reach their metabolic threshold earlier and we proposed that this metabolic trigger initiates from adipose accumulation. On the broiler side, intensive selection focused on increasing the rate of lean tissue (breast muscle) accumulation. However, this has come at the expense of the reproductive capacity of breeders who display poor egg laying and abysmal fertility. On the female side, we propose that as for layers, egg production is dependent on resource availability, which are limited under feed restriction programs resulting in extremely lean body composition, especially around the peripubertal stage. On the male side, it has been well documented that intensive genetic selection for growth resulted in behavioral issues for broiler breeder roosters with ineffective mating, while more recently focus has shifted to semen quality. It is worth mentioning that whether layers or broilers, modern genetic selection programs are based on the concept of heterosis, which requires to maintain multiple pure lines for cross breeding. Unfortunately, as the industry consolidates and integrates, the overall genetic diversity has been on the decline and lower producing lines are discontinued rather than preserved as a backup for potential population collapse. This is in part due to the cost and space required to maintain breeding populations and, despite advances in genetic preservation through cryopreservation, gonadal transplantation and germ cell manipulation, no clear strategy has yet been established. Thus, to ensure the future of the poultry industry, we propose that more emphasis should be put in incorporating physiological parameters in breeding programs, adjusting management practices, and ensuring genetic diversity remains accessible through preservation.

2077 Motile sperm separation by a migration–gravity sedimentation method: impact on sperm function and freezability

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Objective

Sperm heterogeneity between/within male(s) is of importance for adaptation of competitive and dynamic fertilization environment. In vitro sperm storage in poultry remains constrained due to a poor cryotolerance. Motile sperm selection devices have developed for human assisted reproductive technology. MIGLIS® (Menicon Life Science, Japan) is a sorting device based on a migration–gravity sedimentation principle, and has been also shown to enhance semen quality in domestic animals. This study is a first demonstration that MIGLIS method improves poultry semen cryotolerance via two differential mechanisms.

Materials and Methods

Pooled semen collected from multiple Rhode Island Reds was washed by Tes–NaCl buffer + 5 mM Ca2+, and separated into good sperm (G sperm) and poor sperm (P sperm), using MIGLIS method. Exp. 1: G sperm recovery and motility were examined. Their ability to penetrate into the inner perivitelline layer (IPVL) was assessed based on the number of holes formed on IPVL after co-incubation with sperm. Acrosomal responsiveness was assessed by FITC-PNA labeling after stimulating acrosomal reaction by IPVL lysate. Bacterial contamination was assessed by measuring CFU at 24 h post–incubation on an agar–plate. Exp. 2: ATP and lactic acid contents were quantified in G and P sperm. Mitochondrial membrane potential (ΔΨm), and reactive oxygen species generation (ROS) were also assessed. Exp. 3: Post–thaw viability and motility were examined after cryopreservation. Multiple comparisons were conducted using one– or two–way ANOVA, followed by Dunnett test or Tukey's HSD test. Pairwise comparisons were performed using t–test or Mann–Whitney U test.

Results

Exp. 1: G sperm recovery elevated as an increase in the deposited sperm number; the recovery rate was about 10%. SMAS analysis showed higher VCL, VAP, and ALH in G sperm than control (96.7 vs. 82.8 μm/sec; 35.8 vs. 30.8 μm/sec; 2.5 vs. 2.0 μm). Although no difference was seen in IPVL penetrability and acrosomal responsiveness between them (67.4 vs. 70.0 holes; 32.3 vs. 34.7%), lower CFU was observed in G sperm than control and P sperm (13 vs. 40 and 35 CFU/2 × 108 cells). Exp. 2: ATP and lactic acid contents were higher in G sperm than P sperm, while no difference was detected in ΔΨm and ROS. Exp. 3: Higher cryosurvival was observed in G sperm than control and P sperm (80.4 vs. 62.1 and 60.2%). G sperm showed higher motile % (69.1 vs. 60.8%), VSL (9.9 vs. 7.8 μm/sec), VCL (31.1 vs. 26.8 μm/sec), and VAP (12.3 vs. 9.4 μm/sec) than control.

Conclusion

Migration–gravity sedimentation–based separation enables poultry semen to elevate cryotolerance by alleviating bacterial contamination and selectively collecting sperm with high metabolic potential.

Keywords: Sperm; Cryopreservation; Sperm separation; Energy metabolism; Bacterial load

2078 Optimizing glycerol removal from cryopreserved chicken semen using a miniaturized sucrose-Percoll system

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Glycerol serves as an effective cryoprotectant for chicken sperm preservation, providing essential protection against freeze-thaw damage. However, this creates a fundamental dilemma: the same glycerol that preserves sperm viability exhibits toxicity in the female reproductive tract, reducing fertilization success and limiting artificial insemination efficiency in poultry production. We recently developed a sucrose-Percoll (SP) solution to remove glycerol from post-thaw sperm through synergistic osmotic buffering and density gradient separation. However, the current protocol (500 μL semen + 2 mL SP in 15 mL centrifuge tubes) demands considerable reagent volumes and extended processing times, constraining commercial scalability. This study aims to optimize the protocol through miniaturization. Semen from 10 Taiwan Native Chicken roosters (45-50 weeks) was collected, diluted, and cryopreserved with 11% glycerol following controlled-rate cooling and storage procedures (Lin et al., 2024). Frozen straws were thawed at 4°C for 3 minutes. Four semen-to-SP ratios were evaluated in 1.7 mL microcentrifuge tubes: 250+250 μL, 250+500 μL, 500+250 μL, and 500+500 μL. Following centrifugation at 800 g for 15 minutes at 4°C, pellets were resuspended in 200 μL Lake 7.1 diluent. Quality assessment included total motility, progressive motility, membrane integrity, recovery rate, and residual glycerol concentration. Two-way ANOVA showed semen volume significantly affected motility, membrane integrity, and residual glycerol, likely due to cell concentration-dependent effects on centrifugation dynamics and osmotic balance. SP volume significantly impacted only membrane integrity. Recovery rates remained consistent across treatments, confirming protocol reliability. One-way ANOVA demonstrated comparable outcomes for motility, membrane integrity, and recovery parameters. However, 250 μL semen + 500 μL SP achieved significantly lower residual glycerol. This 1:2 ratio is crucial for optimal osmotic regulation, where sucrose maintains osmotic equilibrium by preventing excessive water influx during glycerol efflux and stabilizes membranes during centrifugation. The optimized protocol delivers 75% reagent reduction and shortened processing time. Microcentrifuge tube compatibility with standard equipment enables seamless scalability from research to industrial programs. This streamlined single-step approach offers a practical alternative to conventional serial dilution-centrifugation protocols. Current fertility trials are assessing fertilization improvements, with potential to establish this as a breakthrough for post-thaw sperm processing in poultry production.
Keywords: Sperm cryopreservation; Glycerol removal; Sucrose-Percoll; Protocol miniaturization; Insemination

2079 Isolation and in vitro culture of pheasant primordial germ cells and evaluation of chicken–pheasant hybrids as sterile surrogate hosts for avian genetic conservation

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Pheasants (Phasianus colchicus) are of major economic and ecological importance, notably for game production and biodiversity restoration. However, traditional conservation methods relying on semen cryopreservation cannot preserve the female genome. This study aimed to develop a biotechnology-based approach using primordial germ cells (PGCs) to ensure complete genetic preservation of both sexes.
We sought (i) to isolate circulating PGCs from pheasant embryos, (ii) to develop in vitro culture conditions using combinations of growth and differentiation factors, and (iii) to evaluate the potential of chicken–pheasant hybrids as sterile hosts for exogenous PGC transplantation. PGCs were collected at different embryonic times, identified by DAZL immunostaining, and cultured in eight media formulations. Viability was assessed microscopically over time. Hybrid embryos were produced by artificial insemination of pheasant hens with semen from X33 roosters. Comparisons of cell viability across media were performed by one-way ANOVA. Hatchability and growth data were expressed as mean ± SD.
The optimal collection time for pheasant PGCs was 67 h of incubation. Several media supported PGC survival, although none yet allowed sustained proliferation. Among tested formulations, those containing basic fibroblast growth factor (bFGF) and stem cell factor (SCF) yielded the best viability. Hybridization attempts produced hatchability rates of 65 % and 7 % in two trials, suggesting immunological incompatibility between species. Hybrid males and females showed no difference in body weight at 10 weeks compared with pure pheasants.
Our results demonstrate the feasibility of isolating and maintaining pheasant PGCs in vitro, though further optimization is required for efficient amplification. Chicken–pheasant hybrids show promise as potential sterile hosts for PGC transplantation, offering a novel route to restore Gallus gallus and Phasianus germlines from cryopreserved cells. As pheasants are a seasonal species, the sterility of these hybrids will be verified at the start of 2026, and the results will be presented at the Congress.
Keywords: pheasant, primordial germ cells, hybridization, germline conservation, avian biotechnology
Acknowledgement: we thank our partner Gibo'Vendée for providing pheasants and valuable support.
Keywords: pheasant; primordial germ cells; hybridization; germline conservation; avian biotechnology

2080 Donor–host compatibility in transgenic PGC transplantation: toward standardized protocols for avian genetic resource conservation

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The conservation and restoration of poultry genetic resources rely increasingly on biotechnologies using primordial germ cells (PGCs). In Gallus gallus, these precursors of gametes can be isolated from embryonic blood, cultured, and cryopreserved to regenerate complete genotypes. This study, conducted within the European H2020 project Cryostore , aime to evaluate donor–host compatibility during the transplantation of transgenic PGCs expressing green fluorescent protein (PGCs-GFP) from the INRAE X33 line into different recipient genetic backgrounds in order to evaluate their germline transmission rate after their transplantation in the surrogate hosts with different genetic background.
PGCs-GFP were transplanted into three types of host embryos: broiler, layer, and Bresse. Developmental follow-up was performed at embryonic stages E8, E10, E14, E16, E18, post-hatch day 7, and up to 25–30 weeks of age. Gonadal colonization, cell survival, and germline development dynamics of PGCs-GFP were analysed by fluorescence microscopy and molecular assays. To determine the level of donor-derived germline contribution, quantitative analysis of the GFP transgene was performed in embryonic gonads and semen from germline chimeras by real-time PCR. Based on these results, roosters were selected for mating with potential hen germline chimeras to evaluate germline transmission and genotype restoration rates through progeny genotyping. Hatchability rates and PGCs-GFP colonization levels across host types were analysed using one-way ANOVA. The preliminary data indicate significant variation in hatchability between host lines, suggesting differential sensitivity to the transplantation procedure. Early observations also reveal variable levels of gonadal colonization by PGCs-GFP among recipient genotypes, potentially linked to embryonic devel-

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opment kinetics. These findings represent an essential step toward establishing a standardized PGC-based protocol for avian line regeneration. Identifying the most compatible host line will enhance the efficiency of genotype restoration and reduce losses associated with inter-strain variability. This work contributes to long-term strategies for safeguarding avian biodiversity and ensuring the resilience of Gallus gallus genetic resources against future health crises. The complete set of experimental results will be available for presentation at the World's Poultry Congress in August 2026.
Keywords: Gallus gallus; primordial germ cells; transgenic chicken; germline chimera; donor–host compatibility

2081 Optimizing the selection and genetic improvement in Indian White Leghorn strain of chicken using Bayesian inference

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Indian White Leghorn strains of chicken are being crossed with suitable strains to develop high egg producing variety catering to the needs of rural population and small poultry farmers. This study was undertaken to optimize the selection and genetic improvement program in one such strain, IWH (selected for higher egg numbers up to 64 weeks), using genetic estimates obtained through Bayesian inference. The data size comprised of 2,510 birds sired by 140 males and 500 females spanning four generations (2020-2024). Genetic parameters were estimated for almost all the major layer economic traits viz., egg number, egg weight, egg shell and internal egg quality traits by utilizing BLUP animal models incorporating direct additive, direct maternal and maternal permanent environmental effect in different combinations and using BLUPF90 family of programs. Posterior genetic variance and heritability of each trait was estimated based on the best fitted model and subsequently, posterior genetic correlations were deduced. The posterior direct additive heritability mean (h2additive) for ASM, egg numbers up to 24, 28, 32, 40, 52, 64 and 72 weeks was 0.18±0.04, 0.25±0.11, 0.30±0.09, 0.20±0.09, 0.17±0.09, 0.17 ± 0.06, 0.09 ± 0.05 and 0.06 ± 0.04 respectively whereas for egg weights at 28, 40 and 52 weeks, the estimates were 0.32±0.09, 0.29±0.09 and 0.55 ± 0.06 respectively. h2additive estimates for egg shell strength, egg shell weight and egg shell thickness were 0.24±0.14, 0.36±0.18 and 0.32±0.17 respectively whereas for internal egg quality traits like egg width, egg length, albumin length, albumin width, yolk diameter, haugh unit, albumin weight and yolk weight, the corresponding estimates were 0.35±0.12, 0.19±0.09, 0.19±0.11, 0.35±0.12, 0.24±0.10, 0.22±0.11, 0.35±0.11 and 0.15±0.09 respectively. All the traits except later egg number traits (i.e. egg production up to 64 (EP64) and 72 weeks (EP72)) showed medium to high heritability estimates. The reduced genetic variability in the selection trait (EP64) may be on account of intense selection over the years. Based on the findings, an appropriate selection index comprising of egg number, egg weight, egg shell and internal egg quality traits could be devised for genetic improvement and more sustainable production in the rural context.

Keywords: White Leghorn, Bayesian, Selection, Breeding, India
Keywords: White Leghorn; Bayesian; Selection; Breeding; India

2082 Application of stem cells in poultry breeding and cultured chicken meat production

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Objectives:
The pluripotent stem cells from commercially valuable poultry breeds; Silkie (SK) or Barred Plymouth Rock (BPR) have been used as donors and transplanted into recipient embryos of White Leghorn (WL) which is highly prolific breeds to generate germline chimeras. These germline chimeras after sexual maturity were mated for efficient production of donor derived offspring for stem cell breeding. The early embryos (blastoderm) containing stem cells in area pellucida has been ex-ovo cultured to proceed organ generation for the cultured meat production.

Materials and Methods:
The stem cells as donors were obtained from blastoderms of SK and BPR. About 700 of the donor cells were microinjected into each recipient of blastoderms from WL. Prior to injection of the donor cells into recipient, surgical removal of the recipient stem cell and UV irradiation was perforemed to improve chimeric rate. These chimeric embryos were ex-ovo cultured to obtaine chimeric hatchlight. The blastoderms containing stem cells were ex-ovo cultured for cell proliferation to generated organs suitable for cultured chicken meat.

Results and Conclusions:
When the chimeric manipulation have been perforemed, chimeric embryos in 25% (50 chimeras hatched in 200 of treated embryos) in SK donor, or 35% (70 chimeras ahatched in 200 of treated embryos) in BPR have been hatched. When these chimeras have been sexually matured, 25 ot of 50 (50%) or 49 out of 70 (70%) in SK or BPR generated pure SK or BPR offspring, respectively. These results suggested that our strategies of recipients's stem cell cell removal/inactivation statistically improved ($P<0.05$) the chimeric production as compared to the ratio of non-trastment of recipient's stem cell removal (15% in chimeric production rate). When the stem cells or embryos were cultured, three-dimensional structures such as breast meat, thigh meat, liver, and heart were regenerated. In case of generated breast meat, the meat was localized from the base of wing to showlder and the meat has little fat and whitish color in macroscopical observation
These results suggested that our novel stem cell culture strategies and technologies should lead to stem cell breeding and cultured meat production.
Keywords: stem cell; germline chimera; culture meat

2083 Oviposition performance and reproductive behaviour of endangered native chicken breeds: a baseline study

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Local chicken breeds are a largely untapped genetic reservoir, offering unique traits in productive performance, although their reproductive traits remain virtually unexplored. In Piedmont (northwest Italy), three endangered breeds are involved in national conservation programs: Bionda Piemontese (BP), Bianca di Saluzzo (BS) and Millefiori Piemontese (MP). Little information is available for the first two, while no data are available for MP, being considered extinct in the '90s and only recently rediscovered. To fully characterise these breeds, the first-year oviposition performance and reproductive behaviours of 176 birds (10 females and 1 male/pen; 6 replicates for BP and BS, 4 replicates for MP) were studied. Specifically, eggs were collected and weighted daily, and video recordings were conducted on each pen for 2 consecutive days in spring, summer and autumn for a total of 3 video recordings per day (2 h at sunrise, 2 h at midday, 2 h before sunset). Following a time-sampling technique (1' of frequency recording

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every 10'), crowing (CR), waltzing (WA), chasing (CH), tidbitting (TI) and number of approaching hens (AH), wing flapping (WF), solicitation (SO) forced mounts (FM), voluntary mounts (VM), and brooding (BR) were assessed. Behavioural frequencies were analysed by generalized linear mixed model with breed, season and time of day as fixed factors and their interactions considered. Statistical significance was set at $p<0.05$. The earliest breed to reach sexual maturity was BP (125 d), followed by BS (131 d) and MP (160 d). The number of eggs laid during the first oviposition cycle was 170 for BP, 144 for BS and 120 for MP, with mean weights of 61.9 g for BP, 62.2 g for BS and 59.8 g for MP. Breed influenced all behaviours except for SO, FM and BR, with BP generally showing higher frequencies than BS and MP. Seasonal effects were detected for WA, CH, AH, SO, VM and BR, with increased activity in spring and autumn. Time of day affected CR, SO, FM and VM, resulting higher during daytime ($p<0.001$, $p<0.001$, $p=0.027$, $p<0.0001$, respectively). Significant breed × hour of day interactions were found for CR (BP was highest in the morning, BS and MP showed delayed patterns; $p=0.027$) and WF (higher activity at noon by BP than BS and MP; $p=0.016$). No differences were found regarding TI. Overall, BP showed earlier sexual maturity, higher egg production and more intense behavioural activities across most contexts, while MP displayed delayed maturity and lower activity levels. These results provide baseline data for conservation programs and suggest that genetic divergence among breeds extends to behavioural traits, underscoring the importance of preserving the unique diversity of local chicken breeds.

Keywords: Local chicken breeds; Italian poultry biodiversity; Reproductive behavior; Oviposition performance

2084 RNA-seq Reveals Impact of Ovulation-Insemination Intervals on Fertility and Key Regulatory Genes in Laying Hens

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Introduction Poultry sperm can be stored and remain viable for days to weeks after a single artificial insemination (AI) or natural mating, allowing producing fertile eggs over an extended period. The oviduct is an important microenvironment for the temporary storage of sperm. The effort to understand the effect of oviduct gene expression on sperm function and reveal the mechanism may help to improve the fertility. The present study firstly investigated the impact of AI-Oviposition intervals on fertility and secondly identify the key genes involved in regulating the fertilization process using RNA-seq.

Materials and methods Hens were allocated into six experimental groups (n = 50), for which the AI was performed at 30min, 2h, 5h, 8h, 17h, and 23h post oviposition. Eggs were collected for 18 days to calculate the fertility to evaluate the influence of different AI-Oviposition interval on sperm storage. The uterovaginal junction (UVJ) of the female reproductive tract of four experimental groups with statistically significant differences in fertility from the previous experiment were selected for subsequent analysis (30min, 5h, 8h, and 23h group). The function of the deferentially expressed genes between these time points were analyzed using GO and KEGG pathway enrichment analyses.

Results All groups exhibited a decline in fertility over time. The fertility of 23h group was significantly lower than other four groups ($P < 0.01$). The 5h and 8h groups exhibited the highest fertility. Given that a small number of differentially expressed genes were identified in the comparison between the 5h and 8h samples, we decided to merge the 5h and 8h samples for subsequent bioinformatics analysis (5-8h group). In the comparison between 30 min and 5-8h, there were 111 up-regulated DEGs and 97 down-regulated DEGs. When comparing 23h with 5-8h group, 272 up-regulated DEGs and 141 down-regulated DEGs were identified. In the 5-8h group, multiple pathways related to oxygen transport, antioxidant response, extracellular matrix remodeling, and steroid hormone synthesis were significantly up-regulated, collectively creating a microenvironment conducive to sperm survival and storage. Conversely, these pathways were generally down-regulated in the 23h group, which may contribute to reproductive tract dysfunction and reduced sperm viability.

Conclusions This study identified the 5-8h post-oviposition as the optimal insemination window and revealed the key genes and pathways underlying this. Future research should further investigate the functions of these key genes and their specific mechanisms in sperm-female reproductive tract interactions.

Keywords chicken, artificial insemination, fertility, uterovaginal junction, RNA-seq

Keywords: chicken; artificial insemination; fertility; uterovaginal junction; RNA-seq

2085 The Association of the Prolactin (PRL) Gene with Egg Productivity of Indigenous Kedu Chickens

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This study aimed to identify point mutations in the prolactin (PRL) gene and evaluate their potential association with egg productivity in indigenous Kedu chickens. Two hundred female Kedu chickens were observed from 150 days of age until the end of the 180-day laying period. Blood samples were collected for DNA extraction, and the PRL gene was amplified using Polymerase Chain Reaction (PCR). PCR products were visualized by electrophoresis and sequenced to detect the point mutations. Genotype and allele frequencies, as well as heterozygosity, were analyzed using MEGA 12 and DnaSP v6 software. A single nucleotide polymorphism (SNP) was identified at position 454 (C > T) in the PRL gene. The genotype frequencies were 0.10 (CC), 0.65 (CT), and 0.25 (TT), with allele frequencies of 0.43 (C) and 0.57 (T), respectively. The observed heterozygosity (Ho = 0.49) indicates moderate genetic diversity within the population. Based on individual egg production records from 150 days of age, hens with the CT genotype had the highest mean egg production (86 ± 7.9 eggs/180 d), followed by TT (78 ± 6.3 eggs) and CC (62 ± 5.5 eggs). These results suggest that the PRL T allele is favorably associated with improved laying performance in Kedu chickens. These findings can be applied to develop marker-assisted selection strategies to identify superior laying hens and enhance the genetic improvement programs of indigenous Kedu chickens.

Keywords: Electrophoresis; Kedu chicken; point mutation; Polymerase Chain Reaction ; Prolactin Gene

2086 Effect of feather colour genotype on the reproductive performance of guinea fowl eggs hatched in Ghana

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This study examined the effect of feather colour genotype on the reproductive indicators of guinea fowls. Three hundred and fifty-seven (357) Guinea fowl eggs laid by Pearl (136), Black (111) and Lavender (110) feather coloured fowls aged between 37 and 52 weeks were used in the study. The study employed a 2 x 3 factorial in a randomized complete block design with two hatches (blocks) and three feather colour genotypes. The results revealed that pearl (69.34%) and lavender (66.84%) feather coloured genotypes recorded significantly ($p<0.05$) higher fertility compared to the black

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genotype (54.36%). Feather colour genotype had significant effect ($p<0.05$) on hatchability with the pearl feather colour genotype recording the highest (51.56%). The study showed no significant ($p>0.05$) effect of feather colour on hatch out body weight of guinea keets. It was observed that eggs from black and pearl feather colour genotypes were heavier ($p<0.05$) than lavender coloured genotype. Egg shape index category had no significant ($p>0.05$) influence on fertility and hatchability of eggs. It is concluded that pearl feather colour genotypes demonstrated superior reproductive performance than lavender genotype followed by black feather colour genotype. The utilization of the black feather colour genotype for genetic improvement of fertility and hatchability is necessary to prevent this genotype from being endangered and loss of diversity among Guinea fowls population in Ghana.
Keywords: Feather colour; Guinea fowl; Genotype; Fertility; Hatchability

Welfare and Behavior I

2087 The other "Impact Factor": Getting welfare research into practice

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A major goal of most applied scientists, especially animal welfare scientists, is to make a difference in the world. One of the most effective ways to do this as a scientist is to have one's research results directly applied to agricultural practices. There are several ways to promote your research for application, including participation in industry or professional committees that develop animal care guidelines and working directly with industry partners on research projects. Getting you and your students out of the lab and onto the farm allows you to understand the real-world research gaps and to ask highly relevant research questions. Additionally, building relationships with industry partners, including farming organizations, farmers and stock people, can support your research program in several ways. Access to commercial settings – farms, transport, processing- is often necessary to produce research results with external validity. Although field studies can be less controlled and often quite challenging to perform, they also offer opportunities to forge a connection with the people responsible for farming practices, and they allow you to tailor your research with application in mind. It is also critical to develop a relationship built on trust and mutual respect and to be prepared to learn more from your industry partners than they do from you. Finally, science-industry partnerships provide opportunities to transfer research results directly to end users and offer your students ideal professional development experiences, allowing them to learn how to communicate their research findings to a variety of audiences and opening doors to careers in industry.

2088 Use of covered outdoor area (veranda) by BL 1 star broilers

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Since early 2023, Dutch supermarkets only sell fresh chicken products certified under the Better Life 1 Star (BL 1 star) label. A key requirement is that broilers have access to a covered outdoor area (veranda) from 21 days of age. Although the veranda must cover at least 20% of the house floor area, little is known about its actual use by slow-growing broilers. This study monitored veranda use during two seasons (spring and autumn) on six commercial BL 1 star farms, comprising 16 houses (total 12 flocks) with an average of 13,800 Hubbard JA757 broilers. Verandas covered 27% of the total floor area. Observations were conducted weekly from day 21 until slaughter, for five weeks per flock. On each observation day, the number and behaviour of broilers in one veranda section (4–5 m) were recorded every 30 minutes between 10:30 and 15:30 and extrapolated to the total veranda. Behaviours were classified as active (e.g., walking, scratching) or inactive (e.g. sitting, standing). Pop hole threshold height, weather conditions, and spatial preferences within the veranda were also recorded. The data were analysed using REML variance components analysis. Veranda use increased with age: from 3% in week 4 to approx. 10% in weeks 7 and 8 ($p = 0.001$). Usage varied widely between farms and houses at farms (2–18%). Resting behaviour in the veranda rose from 56% in week 4 to 70% in week 8 ($p < 0.001$). Broilers preferred zones near solid boundaries: 46% were near the house wall, 35% near the wire mesh, and 19% in the middle ($p = 0.04$). Pop hole height influenced use: when ≤10 cm, 9.7% of broilers used the veranda on average, compared to 4.9% when >10 cm ($p = 0.019$). During both periods, average outdoor temperature was 12.8°C. No consistent association was found between temperature and veranda use. However, wind affected younger broilers (≤5 weeks): at wind force 1–2, 6% were present in the veranda, versus 1% at wind force ≥3 ($p = 0.002$). In conclusion, under average outdoor conditions, veranda use was lower than expected based on available floor area. Younger broilers made limited use of the space, though occupancy increased with age. Low pop hole thresholds and protection from wind appear important in promoting veranda use, especially in younger broilers.
Keywords: broilers; BLk 1 star; veranda; behavior

2089 Effects of black soldier fly and mealworm meal inclusion on leg health and fluctuating asymmetry in slow-growing broilers during the starter phase

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Insects are increasingly explored as sustainable alternative protein sources in poultry nutrition, yet their effects during the starter phase remain unclear. This study investigated the impact of dietary inclusion of insect meals on leg health (gait score and tibial growth plate) and fluctuating asymmetry (FA) in Hubbard Redbro broilers. Two independent 10-day experiments were conducted using black soldier fly (BSF) or mealworm (MW) larvae meals. In each trial, 147 one-day-old chicks were allocated to seven dietary treatments (0, 2.5, 5, 7.5, 10, 12.5, and 15% insect meal inclusion), with three replicate pens per treatment and seven birds per pen. Gait scoring was performed on all birds on day 9. On day 10, birds were slaughtered, and six bilateral morphological traits were measured from the carcasses. The right tibias were then collected, and the thickness of the growth plate zones (articular, proliferative, and hypertrophic) and cortical bone were measured. Data were analyzed using linear and mixed models in R, with significance set at $p < 0.05$. Higher gait scores indicate poorer locomotion. Gait scores were higher at 12.5% MW inclusion (1.11; $p = 0.021$) compared to 2.5% (0.90), while BSF inclusion had no significant effect. Tibial hypertrophic zone thickness was higher at 12.5% MW compared to 0% ($p = 0.041$), other histological traits remain unaffected. The hypertrophic zone reflects differences in chondrocyte maturation and growth dynamics and may be associated with increased longitudinal growth, without necessarily indicating improved bone strength. No effects on growth plate and cortical bone thickness were detected for BSF ($p > 0.05$). Body weight was included as a covariate in the analysis of gait score and tibial hypertrophic zone, showing a significant positive effect for both ($p < 0.001$), indicating that heavier birds tended to have higher gait scores and thicker hypertrophic zones. The composite FA index was significantly lower at 12.5% MW inclusion compared to all other levels (all $p \leq$

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0.003), indicating greater developmental stability. Overall, 12.5% mealworm inclusion increased gait scores and hypertrophic zone thickness, but values remained low, indicating no welfare impairment at this early stage. The reduced FA at this inclusion level indicates a more stable skeletal development. No effects were observed for BSF, highlighting species-specific responses to insect meals.
Keywords: animal welfare; bone health; developmental instability; poultry

2090 Effect of black soldier fly larvae dehydration and provisioning method on growth performance, gut development and active behaviour of fast and slower-growing broiler

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Commercially housed broilers frequently experience limited environmental stimulation and various leg-related problems, compromising their welfare. The use of black soldier fly larvae (BSFL) has been identified as a potential enrichment strategy. Scattering dehydrated BSFL could serve as a more practical enrichment alternative since live BSFL are labour-intensive and require strict environmental conditions. This study comprises two trials: the first assesses broiler behavioural responses to live and dehydrated BSFL, while the second determines the effects of BSFL scattering on the litter on growth performance, gut development, and activity of fast and slower-growing broilers. All procedures received ethical approval from Nottingham Trent University (ARES1826455). In trial 1, four pens containing four chicks (Ross 308, 18 days old) were used in a crossover design to compare live and dehydrated BSFL. Birds received 10 g of each BSFL type in cafeteria-style troughs alongside a basal diet for 2 days, and consumption, time spent, and activity were recorded for 10 min. In trial 2, 320 day-old male broilers (160 Ross 308; 160 Hubbard JA757) were allocated to 32 pens (10 birds/pen) and offered 15 g/day dehydrated BSFL either in troughs (CTRL) or scattered on litter (SCAT) alongside a standard broiler diet. Body weight and feed intake were recorded weekly, gut morphometry was sampled from two birds per pen at each stage, and focal bird behaviour was observed every 30 s for 5 min after BSFL delivery on days 8, 15, 22, 29, 36, and 42. Trial 1 data were analysed by one-way ANOVA, and trial 2 by two-way ANOVA with Fisher's LSD ($P = 0.05$) and Bonferroni adjustment in RStudio. The result of trial 1 showed that the broilers ate significantly more live BSFL per minute (0.923g/min) compared to dead BSFL (0.41g/min), with no significant difference in activity stimulated by both ($p > 0.05$). Trial 2 showed that the effect of the insect provisioning method on growth performance and gut development was insignificant. Scattering BSFL on the litter significantly increased total activity ($p = 0.018$) in the fast-growing broiler strains, while there was no significant difference in total activity in the slower-growing broilers, regardless of provisioning method ($p > 0.05$). Broilers showed stronger attraction and preference for live larvae compared to dehydrated larvae. Using dehydrated BSFL offers lower costs and eliminates the challenges associated with transporting and maintaining live BSFL. This study shows that scattering dehydrated BSFL on the litter promotes active behaviour in fast-growing broilers, without adversely affecting growth performance and gut development, offering a more practical alternative to live BSFL.
Keywords: Broiler chicken; Black soldier fly larvae; Enrichment; Growth; Activity

2091 Acoustic response of laying hens to the enrichment with live larvae of black soldier fly (Hermetia illucens)

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Vocal communication reflects animals motivational and emotional states and has the potential to be used as a non-invasive welfare indicator, where Enrichments, including feeding or foraging stimuli, can modulate arousal and social interactions. Thus, the present study evaluated the acoustic response of laying hens receiving live larvae of Hermetia illucens (HI). Along 5 weeks (36-40 weeks of age), 1,800 laying hens of two genotypes (Brown and White) in an experimental aviary were divided in two groups and supplemented with live HI live larvae (10% daily feed intake) once a day (4 pens) or not (4 pens). Audio recordings were collected once a week at the bird level in all pens and segmented into "before", "during (20 minutes)" and "after (20 minutes)" larvae administration periods (unit of analysis: 5-second non-overlapping windows). A set of 55 acoustic features was extracted and used to train several machine learning classifiers (Support Vector Machine, Random Forest, K-Nearest Neighbors, Artificial Neural Network, Logistic Regression, Decision Tree, and Gradient Boosting) to distinguish the acoustic response between enriched and not enriched pens. The models achieved high accuracies for the during and after administration periods (97.4% and 93.2%, respectively), demonstrating that HI enrichment induces a highly discriminable acoustic response. Based on feature importance analysis, the most discriminative features were the MFCC coefficients (7-8 out of 10), followed by formant-based features (F4 mean, Formant dispersal), i.e., the groups differed primarily in the timbre and spectral energy distribution of vocalization. Independent samples t-tests and Cohen's d analysis confirmed these robust differences: 8/10 and 9/10 of the top features were statistically significant ($P < 0.05$) in the during and after periods, respectively. Most of the top features showed large effect sizes ($|d| > 0.8$), confirming a strong group separation. The acoustic pattern of the enriched group was characterized by higher MFCCs and elevated/dispersed formants (e.g., higher F4 mean), consistently with positive arousal or heightened feeding motivation. In conclusion, the acoustic response of laying hens to the larvae enrichment is characterized by a distinct signature detectable by machine learning models with high fidelity both during and after the administration. These findings highlight the potential of acoustic analysis as a robust, non-invasive proxy for monitoring the immediate and residual emotional state of the birds. Acknowledgements: Agritech National Research Center European Union–NextGenerationEU – D.D. 1032 17/06/2022, CN00000022; EU Horizon Europe Project 101136346 EUPAHW.
Keywords: Vocalization; Welfare; Emotional states ; Aviary; Laying hens

2092 Impact of plastic huts and metal ramps as environmental enrichment on behavior in Ross 308 broilers to 32 d of age

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Environmental enrichment increases housing complexity, allowing birds to express a broader range of behaviors than those in unenriched environments. However, the effect depends on factors like type, quantity, and combination of enrichments. This study assessed the effects of plastic huts and metal ramps on Ross 308 broiler behavior up to 32 days of age. A single trial included 7,256 broilers housed in eight rooms (9.75 × 6.50 m) at a final estimat-

ed stocking density of 31 kg/m2. Four rooms were enriched with a plastic hut and metal ramp (E), and four served as unenriched controls (C). At 29 days, a designated area in each room was recorded continuously for 24 hours using ceiling-mounted infrared cameras. The field of view included the metal ramp but excluded the hut. Videos were analyzed using instantaneous scan sampling at 20-minute intervals based on an ethogram. Data in the litter area were analyzed as the average percentage of time birds spent performing specific behaviors using a completely randomized design in a one-way factorial arrangement; normality was tested, and log + 1 transformed as needed. Significance was set at $P \leq 0.05$. Behavioral expression on top and beneath the metal ramp was quantified as the average percentage of time birds spent performing each behavior in those locations ± SEM. Birds were considered on top of or beneath the ramp if more than half of their body was observed on top or beneath it. The percentage of time birds spent wing and leg stretching in the litter was higher during the photoperiod (photo: 06:00–00:00) in birds with enrichment access compared to controls (E: 0.22% vs C: 0.13%; P=0.03; SEM=0.023). No significant differences were observed for the average percentage of time spent at the feeder or drinker, resting, standing, walking, running, preening, dust bathing, wing flapping, ruffling, head scratching, environmental pecking, object pecking, gentle pecking, or performing unknown behaviors in the litter during the photoperiod. Similarly, during the scotoperiod (scoto: 00:00–06:00), no treatment effects were detected for any of these behaviors in the litter, including wing and leg stretching. For the metal ramp, birds spent the highest percentage of time resting underneath (photo: 62.25±3.335%; scoto: 98.16±0.851%), followed by resting on top (photo: 14.67±0.952%; scoto: 0.30±0.305%). Standing was the second most common behavior, slightly higher on top (photo: 2.94±0.826%; scoto: 0.22±0.216%) than underneath (photo: 1.90±0.832%; scoto: 1.02±0.677%) the metal ramp. Overall, birds actively used the enrichments, which allowed them to expand their behavioral repertoire and led to a small increase in wing and leg stretching, both indicating improved welfare.
Keywords: broiler management; broiler welfare; broiler behavior; behavioral repertoire; broiler production

2093 Providing pecking blocks to laying hens in enriched colonies: foraging and dust bathing at the scratch area

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Pecking blocks (PB) are a form of edible enrichment for laying hens designed to accommodate foraging, which is considered to be a behavioural need. Anecdotal evidence suggests that PB may also stimulate dust bathing behaviours. Previous studies have been conducted on the effects of PB as foraging enrichments in non-cage systems, but their effectiveness in enriched colony (EC) systems is unknown. Foraging opportunities in EC systems are often limited to scratch mats, so PB could provide an additional, biologically relevant material to meet hens' needs. We tested whether providing PB in EC next to the scratch mats affects foraging and dust bathing behaviours at the scratch area. We used a 2x2x3 factorial design in which treatment (pecking blocks (TRT) or no pecking blocks (CONT)), genetic strain (796 Dekalb White (DW) and 796 Bovans Brown (BB)), and EC style (30-bird (N=12), 32-bird (N=16), and 60-bird (N=12)) were the main effects. TRT were provided PB continuously from 18-70 weeks of age (WoA). Videos of the scratch area in each EC were recorded throughout one day from lights-on until lights-off five times throughout the trial. Videos were scanned at 10-minute intervals, and the number of hens exhibiting foraging behaviours (pecking and scratching) directed at the PB and scratch mats were counted, as well as the number of hens dust bathing on the scratch mats. Generalized linear mixed models tested the fixed effects of strain, treatment, and EC style on the average proportion of hens per cage seen foraging or dust bathing throughout the day. There was an interaction between treatment and strain on foraging behaviour (P=0.004). A higher proportion of hens (expressed as a percentage) were observed foraging in TRT groups of both strains (1.99% in DW and 1.62% in BB), and the lowest average proportion was seen in BB CONT (0.57%). The highest proportion of hens foraging was seen in the 30-bird EC (1.63%) compared to the 30- and 60-bird styles (P<0.001). There was no effect of PB treatment on the proportion of dust bathing observed (P=0.263). There was an interaction between EC style and strain, in which BB housed in the 32-bird EC had the highest average proportion of hens dust bathing (0.89%), and DW housed in the 30-bird EC had the lowest (0.35%). In conclusion, pecking blocks were shown to be effective at encouraging foraging behaviour but not dust bathing in enriched colony systems.
Keywords: Laying hen; Pecking blocks; Enriched cages; Foraging; Dust bathing

2094 The cost of change: How gaining and losing enrichment affects feather cover and mortality in pullets and laying hens

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During feather pecking (FP), when laying hens peck at and destroy the feather cover of conspecifics. FP can lead to skin injury, cannibalism, and death, making it a global animal welfare issue. One management strategy to mitigate FP is the provision of enrichment, such as pecking blocks (PBs), which redirect pecking away from other birds. However, enrichment practices vary between farm and birds may receive enrichment during the rearing, and/ or laying, both, or neither. Mammalian research shows that gaining or losing enrichment can act as a stressor, potentially leading to behaviour problems.

Six rearing farms (in total 29 pens) and 6 laying farms (in total 13 pens) in Ontario (5000-15,000 birds per farm, 2000-3000 per pen) participated in a longitudinal study assessing the effects of PBs on feather cover and mortality. The 4 experimental treatments included birds receiving PBs during rearing (YN), during laying only (NY), during neither (NN) and during both periods (YY). Simultaneously, 3 types of PBs differing in composition and shape were provided: A: mixed grains, B: mixed grains and molasses, and C: mineral based, at a ratio of 1 per 1000 birds. Feather scores (0 [best feather cover] - 2 [worst feather cover]) were assessed at 12, 18, 30, 50, and 65 WOA. Mortality data were collected daily by the farmer. At the end of the rearing period (18 WOA) feather cover was perfect (all score=0) in pens with and without PBs. During laying feather cover and mortality was analyzed via multinomial generalized linear mixed model with fixed effects of age, treatment, and strain and a random effect of pen within farm. Feather scores revealed a significant effect of PB- treatment-age combinations (p<0.0001). At 65 WOA, YY birds had the highest probability of maintaining perfect feather cover (22.7%), whereas YN birds had the lowest (7.6%), even lower than birds that never received enrichment (NN) (11.5%), suggesting that removal of enrichment may exacerbate FP more than never having it. Mortality was low overall and increased with age (p=0.0005) but there was no effect of PB enrichment (p=0.5912). Mortality rates during 30-50 WOA (1.4 ± 0.06798%) and 50-65 WOA (1.5

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± 0.06798%) were higher than at 19-30 WOA (0.4 ± 0.06798%) (P=0.0114 and P=0.0093 respectively).
Consistent Pbs provision throughout rearing and laying did not affect mortality but yielded best feather cover outcomes. In contrast, flocks that experienced changes in enrichment, gaining or losing PBs, showed increased feather damage, indicating that instability in enrichment provision may negatively impact bird welfare.
Keywords: Feather Pecking; Enrichment; Pecking Blocks ; Feather Cover; Mortality

2095 Refinement of a validated, novel laser enrichment device for broilers: How does a 6-minute laser 2 times daily exposure affect behavior?
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Modern broiler production systems prioritize rapid growth and efficiency; however, these advancements are often accompanied by reduced physical activity, compromised leg health, and limited behavioral repertoire as the birds age. Previous work showed laser enrichment enhanced broiler locomotor activity without compromising key welfare indicators. The current study evaluated broiler behavior when exposed to a 6-min, twice-daily laser enrichment over a 7-wk grow-out period. A total of 1,320 mixed-sex Ross 708 chicks were placed in floor pens and randomly allocated to either laser enrichment or control treatments (20 pens/treatment). Laser devices projected 5 moving red points across 2 adjacent pens and operated twice daily for 6-min periods (05:30 and 17:30). Eighty focal birds (5 per pen; 8 pens/treatment) were randomly selected for behavioral observations. Bird home pen behavior (active, inactive, at feeder, and at drinker), walking distance, pen-wide movement, and laser following were collected from video recordings during laser-on periods from d 0 to 8, and then weekly (d 9, 16, 23, 30, 37, and 42). All data were analyzed using PROC GLIMMIX. Bird home pen behavior, walking distance, and pen-wide movement data had the fixed effects of treatment (laser vs control), day (0-8), or week (1-7), and their interaction. Laser following data reported the main effect of day/week because data were only collected from laser-enriched pens. Significance was denoted at $P \leq 0.05$. Bird home pen behavior did not differ by interaction, treatment or d during d 0-8 ($P > 0.05$). Treatment x wk affected inactivity over wk 1-7, while active, at feeder and at drinker remained similar across treatments. Laser-enriched birds were more inactive wk 2 and 6 (25.2% and 22.4%) and had 25.6% lower inactivity levels wk 3 than control ($P = 0.01$). Treatment x d affected walking distance ($P = 0.03$), with laser-enriched birds walking farther on d 2 and 4 (105.4 cm and 79.7 cm, respectively; $P = 0.03$). Walking distance also differed by wk ($P = 0.01$), with birds walking 30.3-47.0 cm farther on wk 1 than wk 2, 4, 6-7. Pen-wide movement did not differ by treatment x d ($P = 0.28$) but differed by treatment x wk ($P < 0.0001$). Laser-enriched birds had 6.6% more movement in wk 1, whereas control birds had 6.3-10.9% more movement in wk 6-7. When considering treatment, laser-enriched birds increased their movement by 4.0% during d 0-8 ($P = 0.01$). Laser following did not vary by d ($P = 0.21$) but differed by wk ($P = 0.004$), with wk 3 showing 2.4-4.0% more following than wk 1, 2, and 4-7. In conclusion, laser enrichment had age-dependent effects on broiler activity but did not reduce feeder or drinker use during enrichment periods.
Keywords: Broiler; Environmental enrichment; Pen-wide movement; Laser following; Walking distance

2096 Laser enrichment improves broiler immune cell profiles without inducing fear or stress
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Advances in broiler genetics and production systems have accelerated growth and efficiency but have reduced voluntary activity, raising concerns about welfare. Previous research using laser enrichment has shown promise in increasing bird activity and supporting welfare; however, the laser impact on bird stress and immune cell presence requires further investigation. Therefore, the objectives were to evaluate laser enrichment on broiler behavior, stress, and immune cell presence. Day-old, mixed-sex Ross 708 broilers were placed in floor pens and randomly assigned to either laser enrichment treatment or non-enriched control (n = 1,320 birds, 20 pens/treatment) for 7-wk. Laser devices projected 5 moving red-light dots across paired pens twice daily for 6-min periods (05:30 and 17:30) throughout the trial. A novel object test (latency to approach and total number of birds approaching within 3-min) was conducted on all birds, and a tonic immobility (TI) test was performed on 1 random bird per pen on wk 1 and 6 (n=40). Blood was collected from 1 random bird per pen on d 7, 10, and 49 for flow cytometry and corticosterone assays. Novel object and TI data were analyzed using the PROC GLIMMIX procedure (SAS 9.4) with fixed effects of treatment, week, and their interaction. Flow cytometric and corticosterone data were analyzed using PROC MIXED with fixed effects of treatment, day, and their interaction. Significance was denoted at $P \leq 0.05$. Novel object test was unaffected by treatment x wk interaction ($P \geq 0.05$). Latency to approach did not differ by treatment or wk ($P \geq 0.05$). However, more birds approached the novel object in the laser enrichment group than control (1.85 vs 1.40) and in wk 1 than wk 6 (1.95 vs 1.30; $P \leq 0.05$). TI did not differ by treatment x wk interaction or treatment ($P \geq 0.05$), but increased duration by 158.3 s in wk 6 than wk 1 broilers ($P < 0.0001$). Laser enrichment altered immune cell populations on d7, with enriched birds exhibiting higher monocyte/macrophage+, Bu-1+ B cells, CD3+ T cells, and CD3+CD4+ helper T cell populations ($P \leq 0.0001$). Laser enrichment maintained elevated CD3+ and CD3+CD4+ cells and showed greater CD3+CD8α+ and reduced Bu-1+ cells compared to controls by d 10 ($P \leq 0.05$). At d 49, Bu-1+ and CD3+CD8α+ population differences had resolved. Laser-enriched birds continued to show elevated CD3+ and CD3+CD4+ cells, and higher monocyte/macrophage+ cells ($P \leq 0.05$). Corticosterone level decreased by 40.4-42.6% from d 7 and 10 to d 49 ($P < 0.0001$) but was not impacted by treatment x d interaction or treatment ($P \geq 0.05$). In conclusion, findings suggest laser enrichment may promote broiler immune development without inducing fear or negative stress responses.
Keywords: Broiler; Corticosterone; Environmental enrichment; Immune cells; Tonic immobility

2097 Abnormal and Enrichment-Related Behaviors in Broiler Breeders During Feed Restriction.
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Feed restriction is a common practice in broiler breeder (BB) rearing to control growth and improve reproductive performance; however, it is also a welfare concern as it results in hunger and frustration. Frustration can lead to abnormal behaviors (AB), which may be mitigated by environmental enrichment (EE). This study aimed to 1) examine if abnormal behavior performed by BB during feed restriction differs between on- and off-feed days, and 2) determine if engagement with EE differed between on- and off-feed days.
A total of 168 BB, 84 pullets (Ross 308 AP), and 84 cockerels (Ross 344) were housed in 14 pens (2.23 m2; 12 birds/pen). Two enrichments, one pecking stone (PS) and one bag of shavings (SB), were added to each pen on d40. Feed restriction via a 4-3 feed regimen started on week 5. The frequency of abnormal and EE

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usage behaviors was analyzed twice a week (one day off-feed and one day on-feed) on weeks 6, 8, 10, 12, and 14. Data were analyzed by two-way ANOVA for the effects of sex and feeding status (PROC GLIMMIX). During on-feed days, cockerels performed more repetitive pecking at feeder (RPF) (32.74, 15.17, on- and off-feed, respectively; $P < 0.01$), and repetitive pecking at waterline (RPW) (3.88, 2.34; P=0.04), and less repetitive pecking at other objects (RPO) (0.41, 5.34; P=0.03), than on off-feed days. Pullets had higher RPW (3.97, 2.06, P=0.04), and lower RPO (1.39, 6.82; P=0.03) and feather licking (8.61, 14.94; P=0.04) on on-feed days than on off-feed days. The time of day of RPF, RPW, and RPO performance differed with access to feed, but feather licking and pecking did not. Regarding the EE engagements, pecking at the PS and SH was highest with cockerels during off-feed days (5.63, 15.43, for PS and SH, respectively), then by pullets during off-feed days (3.71, 12.57), followed by pullets on-feed days (1.97, 5.76), and cockerels on-feed days (1.57, 5.31; P=0.03 and P=0.02, respectively). Regardless of feed access, cockerels sat on SB more than pullets (4.26, 3.91, on and off, respectively), and pullets on-feed (1.91) sat on SB more than pullets off-feed (0.71, $P<0.01$). Birds sat on both PS (0.60) and pecked at while standing on the PS and SB more during on-feed days (0.45 and 1.91, respectively) than off-feed days (1.31, 1.66, and 3.67, $P < 0.01$, $P < 0.01$, $P < 0.01$, respectively). Standing on the SH was higher during on-feed days (4.10), compared to off-feed days (2.61; $P < 0.01$). Cockerels engaged with the EE more than pullets. RPF and RPW were performed more during on-feed days, while RPO was performed more during off-feed days. Both cockerels and pullets pecked more at the EE during off-feed days. Access to feed influenced both the type of AB performed and EE usage.

Keywords: Welfare; Environmental Enrichment; Repetitive Pecking ; Stereotypic Behavior; Pullets

Elanco Animal Health Symposium: The role of live vaccines to prevent Salmonella in poultry production

The experience with live Salmonella vaccines in Europe
Filip Van Immerseel*1 1Ghent University, Merelbeke, Belgium

Extending Salmonella protection during the laying period
Doris H. Mueller-Doblies*1 1Elanco Austria GmbH, Wolfsgraben, Austria

An approach to control Salmonella gallinarum
Pablo Chacana*1 1INTA Argentina, Buenos Aires, Argentina

International Poultry Welfare Alliance Welfarelab2050 Sprint: Science

Elena Myhre, IPWA Staff
Shawna Weimer, IPWA Board Member
Richard Griffiths, IPWA Board Member
Andy Vance, Executive Director of IPWA
Katy Tarrant, Chair of IPWA
Karen Christensen, IPWA Sub-Committee Chair

Nor-Feed Symposium: Three nutritional drivers to sustain productivity in extended laying cycles of laying hens

Dr. Michael Elliot
Dr. María del Pilar Castañeda Serrano, DVM, PhD
Dr. Anne-Sophie Valable, PhD
Dr. Paul Engler, PhD

Environmental Impact II: Sustainability

2098 Evidence-based decision making for sustainable poultry industries: The Canadian example
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The sustainability of animal agriculture has moved to the fore of contemporary food systems discourse. As a result, increasing expectations from supply chain partners and consumers with respect to sustainability measurement, management and reporting, as well as regulatory and market access considerations present both opportunities and challenges for the feather flock industries.

Application of standardized methods and use of high quality data are foundational to effective sustainability measurement and management efforts. This presentation will describe the current state of art in sustainability assessment, providing examples from sustainability research and management initiatives in the Canadian industry egg. This includes the development of baseline industry models, assessment of green technology options for egg farmers, and the creation of a farm-level sustainability measurement and management platform. Key opportunities for sustainability impact mitigation, in particular with respect to achieve net zero greenhouse gas emissions status by mid century, will be identified and discussed.

2099 Utilisation of Outdoor Ranges by Laying Hens: A Field-Based Evaluation of Animal- and Design-Related Indicators in Austrian Organic Systems
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The effective use of outdoor ranges is a key indicator of animal welfare in organic laying hen husbandry systems. However, objective methods to assess range use remain limited. This study aimed to test a field-based approach for objectively evaluating the utilisation of outdoor ranges by commercial organic laying hens in Austria, integrating both animal- and environment-based indicators. We hypothesized that eroded pasture areas and natural cover, as indirect indicators of range use, are significantly associated with the direct indicator of range use, specifically the presence of chickens on the range.

Seventeen organic farms with a standard flock size of 3,000 hens per group were assessed. Range use and structure were quantified using drone imagery (Camera: DJI: FC3411, 5472x3078 pixels, 300m AGL). Animal distribution (chicken count=CC), frequency of use (grass sward removed=GR and extensively used=EU), and range structures (natural=NC and artificial cover=AC) were annotated using COCO Annotator, an open-source image annotation platform. Using state-of-the-art algorithms (HerdNet for object detection, UNet++ for semantic segmentation), a total of 17 labelled images were prepared for training, with 13 used for training, two for validation, and two for testing. A precision of 83% was achieved for the automatic detection of hens (83% of the chicken identified by the model were chickens according to ground truth-imagery). For the segmentation of GR, we report an Intersection over Union (IoU) of 71.6%, while IoUs for AC reached 57.4% and 53.8% for natural cover. For investigating the relationship between AC, CC, EU, GR and NC, ground truth images were used. A Pearson Correlation test of the annotated images confirmed that CC correlates significantly with GR ($r=0.853$, $p<0.05$) and NC ($r=0.939$, $p<0.05$), while no correlation was found between CC, AC and EU.

Although additional real-world annotated images are needed to further improve model accuracy, our findings demonstrate the po-

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tential of AI-supported methods for assessing range use and inspite of hens occluded by natural cover (not visible on drone imagry), CC, GR and NC correlate significantly, as hypothesized.
Keywords: free-range design; organic egg production; behaviour; quality insurance ; machine learning

2100 Pathways Toward Net-Zero Poultry Production Through Biochar Derived from Poultry Litter

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Objective: Poultry manure management remains a sustainability challenge due to nutrient losses and greenhouse gas emissions. Converting poultry litter to biochar via pyrolysis can retain carbon and nutrients while reducing emissions. This study evaluated the effect of pyrolysis temperature on biochar yield, composition, and physicochemical properties to identify trade-offs between carbon stability and agronomic relevance.
Experimental Design and Methods: Poultry litter was subjected to slow pyrolysis at temperatures from 300 to 600 °C under identical heating conditions. Pyrolysis was conducted at a heating rate of 5 °C/min with a 1-h residence time. No statistical analysis was performed; results are based on observed trends across temperatures. Biochars were characterized using proximate and ultimate analyses, pH, electrical conductivity (EC), Brunauer–Emmett–Teller (BET) surface area, pore size distribution, Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and X-ray diffraction (XRD).
Results: Biochar yield decreased from 47.75% at 300 °C to 30.1% at 600 °C, while carbon content increased from 42.33% to 68.85%. Volatile matter declined from 52% to 18%, and fixed carbon increased from 35% to 64%. Atomic hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios decreased with temperature, indicating increasing aromaticity and thermal stability. Biochar pH and EC increased with temperature, reflecting greater alkalinity and mineral concentration. BET analysis showed relatively low surface areas, particularly at lower temperatures. FTIR analysis revealed a wider variety of oxygen-containing functional groups in low-temperature biochars, whereas higher temperatures promoted functional group loss and formation of aromatic C=C structures. The presence of diverse functional groups at lower temperatures suggests greater potential for nutrient retention and adsorption-related applications.
Conclusions: Pyrolysis temperature determined the most suitable application of poultry litter biochar. Low-temperature biochars (300–400 °C), characterized by lower carbon condensation and a wider range of oxygen-containing functional groups, are better suited for agricultural applications, including nutrient release and contaminant adsorption. In contrast, high-temperature biochars (≥500 °C) exhibited increased aromaticity, reduced H/C and O/C ratios, and greater thermal stability, making them more appropriate for long-term carbon sequestration. These findings demonstrate that poultry litter pyrolysis can be tailored to produce biochars optimized for specific environmental applications while reducing manure-related waste and emissions.
Keywords: Poultry litter; Biochar; Pyrolysis; Carbon sequestration; Adsorption

2101 Supplementing broiler diets reduced in energy and amino acids with a combination of lysolecithin, synthetic emulsifier, and monoglycerides improves performance, profitability, and sustainability

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Environmental footprint of broiler production drives nutritional strategies focusing on sustainability alongside growth performance and cost reduction. Supplementing a combination of lysolecithin, synthetic emulsifier, and monoglycerides (LEX) to broiler diets reduced in AME and amino acids was investigated to determine its effect on performance, ileal digestibility of nutrients, profitability, and environmental sustainability compared to a higher density control diet. A total of 780 one-day-old, male Ross 308 broiler chickens were randomly distributed to 3 dietary programs for 42 days: a control basal diet (PC); PC reformulated to reduce AME and digestible amino acids 70 kcal/kg and 1.5% respectively (NC); and NC + 500 mg/kg of LEX (NC+LEX). Each group consisted of 20 pens with 13 birds each. Birds were fed diets containing phytase (500 FTU/kg) and xylanase. Performance parameters were recorded per feeding phase and for the overall trial. On day 28th, 1 bird per pen (20 birds per group) was randomly selected to determine the ileal digestibility of crude protein, starch, fat, and energy. To assess the profitability of LEX supplementation, the income over feed cost (IOFC) for each treatment was calculated. The environmental sustainability impact of feed (Climate change, Water use, and Land Use), for each treatment per kg of liveweight produced at farm gate, was also calculated, considering LCA values for each feed ingredient, feed intake, and final bodyweight. Performance and digestibility data were analyzed by one-way ANOVA (GLM), with means separated using Tukey's HSD. Birds from PC and NC+LEX, compared to birds from NC showed lower FI (PC, 5194 vs. NC, 5333 vs. NC+LEX, 5161 g; $p<0.001$), higher BWG (PC, 3317 vs. NC, 3165 vs. NC+LEX, 3278 g; $p=0.001$), better FCR (PC, 1.568 vs. NC, 1.686 vs. NC+LEX, 1.575; $p<0.001$), and higher ileal fat digestibility (PC, 91.39 vs. NC, 87.70 vs. NC+LEX, 92.52 %; $p<0.001$). The IOFC of NC+LEX treatment was 16 and 177 €/1000 birds higher than PC and NC, respectively. The environmental sustainability impact per kg of liveweight produced at farm gate of NC+LEX treatment was 5% and 6.4% lower for climate change, 6% and 6.5% lower for water use, and 1.4% and 6.7% lower for land use, compared to PC and NC treatments, respectively. In conclusion, the study shows that adding LEX to broiler diets reduced in AME and amino acids improved growth performance, fat digestibility, profitability, and environmental sustainability. LEX supplementation did not impair growth performance compared to broilers fed more expensive and less sustainable diets, higher in AME and amino acid content, resulting in feed cost reduction, increased profitability, and improved environmental sustainability.
Keywords: Broiler; Sustainability; Performance; Digestibility; Profitability

2102 Low protein diet strategy with 1000 FTU phytase/kg diet improves environmental impacts without compromising broiler performance

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This study evaluated the zootechnical performance and environmental impacts of broilers fed low crude protein (CP) diets supplemented with phytase, compared to a standard diet, using a life cycle assessment (LCA) approach. A total of 1,320 Ross 308 chicks were randomly assigned to five treatments (11 replicates of 24 birds). The experimental dietary treatments consisted of 1) a positive high CP diet control (PC) with average SID Lys:CP of 5.5 % and based on Ross 308 (2022); 2) a negative low CP diet control (NC1) with average SID Lys:CP of 6.0% (i.e 1.5 to 2.0% points

CP reduction between PC and NC1); 3) NC2, similar to NC1 with lower calcium (-0.1 pt), available phosphorus (-0.3 pt), and amino acids (-3 to -5 pts in ratio to Lys); 4) and 5) NC2 with addition of either 500 (NC2 + 500 FTU/kg) or 1000 FTU/kg phytase (NC2 + 1000 FTU/kg). All low CP diets had 50–75 kcal/kg less metabolizable energy, following Cappelaere et al. (2022). Performance data (body weight (BW) gain, feed intake (FI) and calculated feed conversion ratio (FCR)) were analyzed using ANOVA (GLM) with Tukey's HSD post hoc test ($P<0.05$). LCA was conducted from cradle to feed mill and farm gate using feed, performance and farm data, with GFLI v2.2 database (2024) and following FAO LEAP (2020) and PEFCR guidelines. Seven EF impact categories were assessed. Feed ingredient origins reflected average Indonesian imports; sensitivity analysis compared Brazilian vs. US soybean meal. Birds of the NC2 treatment were slaughtered at 21d of age due to severe leg issues and depletion, and NC2 was not considered further in the study. For the entire period, broilers fed the NC1 diet had similar BW gain (2369 vs 2389 g/bird; $P = 0.99$), FI (3717 vs 3779 g/bird; $P = 0.93$) and FCR (1.569 vs 1.582); $P = 0.99$) as birds fed the PC diet. Addition of phytase at 500 FTU/kg showed advantages ($P < 0.05$) in restoring FI and BW gain. NC2 + 1000 FTU/kg diet fully restored ($P > 0.28$) FI (3662 and 3662 g) and BW gain (2341 and 2349 g) and had similar performances as birds fed the PC and NC1 diet. LCA results (with Brazilian soybean meal) showed the lowest climate change impact for NC2 + 1000 FTU/kg (3.19 kg CO2eq/kg liveweight (LW)), followed by NC1 (3.37 kg CO2eq/kg LW), NC2 + 500 FTU/kg (3.40 kg CO2eq/kg LW), and PC (3.63 kg CO2eq/kg LW). NC2 + 1000 FTU/kg also had the lowest impacts in 6 out of 7 impacts. Sensitivity analysis with US soybean meal confirmed the best environmental benefits of NC2 + 1000 FTU/kg in 6 impact categories out of 7. The results of this study demonstrated that dietary crude protein reduction strategy together with the use of phytase at 1000 FTU/kg diet improved overall environmental impact without compromising broiler performance.

Keywords: broilers; phytase ; low-protein diet; life cycle assessment; sustainability

2103 Supplementing protected organic acids and essential oils reduces environmental impact in poultry production

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Previous studies conducted at research stations and under field conditions have shown that using protected organic acids and essential oils [P(OA+EO)] as an alternative to antibiotic growth promoters in poultry yields net positive zootechnical effects. These include increased hatchability, improved flock uniformity, reduced infection rates, increased laying rates and egg mass, better feed conversion, and improved meat quality. Building on these zootechnical benefits, this study investigated the potential reductions in environmental impacts linked to improved performance and intestinal health of chickens along the entire Canadian supply chain when basal diets were supplemented with P(OA+EO). The investigation followed an environmental life cycle approach leveraging the Livestock Environmental Assessment Performance guidelines and the ISO 14040/44 protocol. Five environmental impact categories were assessed: greenhouse gas emissions, eutrophication and acidification potential, agricultural land use and fossil resource demand. The results revealed that the environmental impact decreased by an average of 2.2% per kg live weight for 1000-day-old chicks produced from breeder flocks supplemented with P(OA+EO). In broiler chickens, the environmental impact decreased by an average of 3.1% per kg live weight bird supplemented with P(OA+EO). Similarly, in laying hens, the environmental impact was reduced by 4.0% per kg of egg in the shell. The observed impact reduction can be attributed to reductions in farm emission intensities and improvements in productivity due to P(OA+EO). Further statistical analysis based on 500 Monte Carlo simulations demonstrated that the characterized results were significantly different among all the comparative scenarios ($p<0.01$), confirming that the inclusion of P(OA+EO) led to a consistent shift in the emission outcome. In poultry, P(OA+EO) is designed to deliver bioactive ingredients precisely to targeted segments of the intestine. This synergistically helps control pathogenic bacterial populations, promoting better gut health and improving other production variables, consequently leading to sustainable poultry production.

Keywords: Environmental impact; Sustainability; Protected organic acid and essential oils; gut health; poultry production

2104 Effect of decreasing stocking density on sustainability-related parameters in fast growing broilers

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It is widely discussed if a lower stocking density promotes better animal welfare and sustainable farming. Sustainable broiler production needs a holistic approach, where environmental impact and production efficiency are included as well. This trial evaluated the effect of decreasing stocking density on sustainability-related parameters, including animal welfare, ammonia emissions, performance and meat quality, over one rearing period of 43 days.

One-day-old Ross 308 broilers were evenly allocated by sex within pens at four stocking densities: 20 (similar to European industry standards up to 42 kg/m2), 15, 10 (approaching maximum allowed density European Chicken Commitment) and 5 broilers/m2 in a random block design (n=6). Birds were fed a standard three-phase corn-wheat-soy diet ad libitum. Individual body weights and feed intake were recorded to assess growth rate and feed conversion ratio (FCR). Footpad and hock lesions were evaluated according to the Welfare Quality® protocol. Litter quality, dry matter (DM) and ammonia emissions were assessed on d37 and 39. An indicative measurement system was used to measure litter emissions, enabling relative treatment comparisons but not absolute emission rates. On d43, two females and two males per pen were randomly selected to determine carcass yield and meat quality (pH, color, myopathies, water holding capacity and shear force). Data was analyzed using linear mixed models (continuous data) and cumulative link mixed models (scores). Post-hoc ANOVA was used to assess treatment differences, a Tukey's range test was used to obtain adjusted P-values, with level of significance $\alpha=0.05$.

Decreasing stocking density significantly increased slaughter weight, growth rate and feed intake, but increased FCR ($P<0.05$). Mortality, carcass yield and meat quality were not affected. Increasing stocking density significantly reduced litter quality and DM content, while ammonia concentrations increased, resulting in a higher incidence and severity of footpad lesions on d43 ($P<0.05$). Increased litter ammonia concentrations at higher densities resulted in greater ammonia emissions into the air ($P<0.05$). However,

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litter emissions with 15 birds/m2 exceeded those of 20 birds/m2, possibly due to litter crust formation that limits gas exchange with the air.

This study demonstrates that lower stocking densities can enhance animal welfare, based on lower incidence of footpad lesions due to better litter quality, while reducing litter ammonia emissions. However, these benefits come at the cost of increased FCR. The findings highlight the need to balance welfare, environmental and economic aspects in a holistic approach when aiming for sustainable broiler production.

Keywords: Broiler; Sustainability; Stocking density; Trade-offs; Ammonia

2105 Sustainable use of purified rainwater in poultry farming.

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Climate change and recurring droughts put increasing pressure on the availability of groundwater and tap water for poultry farming. Rainwater offers a sustainable option, provided that biosecurity risks - such as microbial contamination - can be managed. Within the LIFE ACLIMA project, the feasibility of using purified rainwater as poultry drinking water was evaluated at the Experimental Poultry Centre (Belgium) using two purification approaches: (1) a multi-step treatment train (MST) for broiler barns (drum filtration, UV disinfection, hydrogen peroxide dosing, sand filtration, activated carbon filtration), and (2) a nanofiltration system (NFS) for laying hens using membrane filters (recovered dialysis membranes) to remove suspended solids and micro-organisms. Both technologies are intended to produce microbiologically safe rainwater for poultry and can be deployed for either broilers or layers.
Both systems were monitored with extensive sampling across seasons. Microbiological parameters -including total germ counts, E. coli, enterococci, yeasts, moulds, and Salmonella- consistently complied with national reference standards for poultry drinking water. Zootechnical performance was assessed in comparative trials against tap water. In broilers (Ross 308, male and female, 8 groups, 1,350 birds/group, five cycles), no significant differences were observed between rainwater (MST) and tap water for technical performance indicators: mortality (2.96±1.82 vs. 3.09±1.60% resp.), feed conversion 2,500g (1.514 ± 0.010 vs. 1.514 ± 0.012 resp.), feed intake (3.80±0.18 vs. 3.78±0.17 kg resp.), water intake (6.41±0.19 vs. 6.41±0.20 L resp.) and final live weight (2.495±0.062 vs. 2.495±0.062 kg resp.). Litter quality and welfare indicators, including footpad and hock health, were also comparable. In laying hens - Dekalb White and ISA Brown - a parallel comparison of nanofiltered rainwater (NFS) versus tap water was assessed (16 groups, 384 hens per group at placement). Available production results (water intake, feed intake and laying %) were comparable between water sources during the monitored period (18-91 weeks of age). Egg-quality assessments conducted at five time points showed no statistically detectable differences between purified rainwater (NFS) and tap water: shell thickness (0.42±0.02 vs 0.42±0.02 mm resp.), shell strength (4.36±0.47 vs 4.32±0.53 kgf resp.) and Haugh units (84.48±0.03 vs 84.70±0.02 resp.). Overall, under the conditions tested in these experimental barns, both rainwater purification methods did not present any disadvantageous results in layers or broiler production systems. Careful monitoring and system maintenance remain essential to guarantee biosecurity and consistent water quality.

Keywords: Rainwater; Purification; Biosecurity; Poultry farming; Sustainability

2106 Circular reuse of cleaning water in broiler chicken farming through biological treatment with membrane bioreactor technology.

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Conventional broiler barns are wet-cleaned after each production cycle, generating a nutrient- and microbe-containing wastewater stream and consuming large water volumes (10–20 L/m2). In the Life ACLIMA project, a containerised (20-ft) membrane bioreactor (MBR) combining biological treatment and membrane filtration was tested at the Experimental Poultry Centre (Belgium) for on-site treatment. Cleaning water was collected in an influent buffer, treated continuously (aerobic nitrification and anoxic denitrification), and the permeate stored for reuse as cleaning water in subsequent cycles. During the test period, indicators showed reductions in Chemical Oxygen Demand (COD) and nitrogen to meet wastewater discharge guideline values. Phosphorus compliance was achieved after FeCl3 dosing. Average total nitrogen decreased from 253 mg/L (influent) to <5 mg/L (effluent), and total phosphorus from 44 mg/L to <2 mg/L. Treated water complied with Belgian requirements for poultry barn cleaning water (e.g., pH 7.74, sodium 67 mg/L, nitrite 0.24 mg/L, iron 0.24 mg/L, total hardness 13.2 DH°). Microbiological quality was within limits. Total germ counts ranged from 6 to 35,000 cfu/mL at 22°C and 9 to 21,000 cfu/mL at 36°C, remaining below the limit of 100,000 cfu/mL. E. coli was consistently very low (<1 to 12 cfu/100 mL, limit 1,000 cfu/100 mL). Yeasts ranged from 12 to 2,200 cfu/mL and moulds from 20 to 120 cfu/mL, both below the limit of 10,000 cfu/mL. Salmonella was not detected (required: absence). Enterococci were frequently below detection (<1 cfu/100 mL) but occasional detections were recorded, indicating that this parameter needs routine operational follow-up. Hygiene assessments during 4 production cycles after cleaning and disinfection (hygiënograms with contact plates on floors, walls and equipment) showed no meaningful differences between barn sections cleaned with treated water and those cleaned with tap water. Comparable scores were maintained when cleaned exclusively with treated water. A farm-level economic assessment balanced CAPital EXpenditures (CAPEX depreciated over 10 years) and OPerational EXpenditures (OPEX) (maintenance, analyses, electricity, chemicals) against avoided costs for tap water and for off-site handling or spreading of untreated cleaning water. The net cost per m3 depends strongly on treated volume (scale), reuse versus discharge, and available investment support.
This study demonstrates that an on-site containerised MBR can reliably produce cleaning water meeting microbiological and chemical requirements and performing comparably to tap water during barn cleaning. Reuse reduces tap water intake but requires continued operational control and routine microbiological follow-up.

Keywords: Circular reuse; Water purification; Membrane bioreactor (MBR); Cleaning water; Sustainability

2107 The potentials of dietary graded levels of Solanum incanum as a phytogenic growth promoter in Cobb500 broiler chickens during hot periods

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nary Teaching Hospital, University of Jos, Jos, Nigeria
Poultry farmers in the tropics suffer huge losses in broiler production, particularly in the post-brooding stage, mainly due to high ambient temperature (HAT). This study evaluated the phytochemical, proximate, and nutritive properties of Solanum incanum fruit meal (SFM) and its effects on the performance, haematology, serum biochemistry, and carcass characteristics of Cobb500 broiler chickens during a hot period in Sokoto State, Northwestern Nigeria. Eighty broiler chicks were randomly divided into four experimental groups (T0 = 0.0% SFM, T1 = 1.5% SFM, T2 = 3.0% SFM, and T3 = 4.5% SFM) in duplicate of 10 birds each, and the feeding trial lasted six weeks. Data were subjected to one-way ANOVA using SPSS version 22. Differences between the mean values were determined using Tukey HSD at a 95% confidence level ($p \leq 0.05$). Data obtained were presented as mean ± standard deviation in tables. SFM was found to be a good source of plant protein, carbohydrates, flavonoids, alkaloids, and antimicrobials such as tannins and showed potential for antioxidant activities. It was observed that although HAT depressed the feed intake of all experimental birds, showing negative residual feed intake of -1207.05±44.50, -1181.05±37.12, -1523.10±47.52, and -1494.05±176.3 for groups T0, T1, T2, and T3, respectively. However, higher inclusions (3.0 and 4.5%) of SFM significantly improved the FCR with an improvement in body weight and body weight gain. Also, SFM had no adverse effects on the health of broiler chickens, as it had no negative effect on the haematological and serum biochemistry of experimental birds. Carcass characteristics of Cobb500 broiler chickens improved with increasing levels of SFM in the diet of experimental birds. Therefore, SFM especially at 3.0 and 4.5% inclusion levels, is a promising phytogenic growth promoter in Cobb500 broiler chickens during hot periods.
Keywords: Broiler chickens; High ambient temperature; Phytonutrient; Solanum incanum fruit; Tropics

General health/Disease/One Health II

2108 Comparative invasion potential of Salmonella serovars using in-vitro cell culture models

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Non-typhoidal Salmonella serotypes are a leading cause of foodborne disease worldwide. Production animals, particularly laying hens, are a significant source of non-typhoidal Salmonella outbreaks in humans. Chickens can be colonised with more than one Salmonella serotype but the role of coinfection on invasion and or shedding remains unclear.
The present study was conducted to investigate the invasion competition between Salmonella Typhimurium (ST) and Salmonella Infantis (SI) using human colorectal adenocarcinoma cells (Caco-2) and Leghorn Male Hepatoma cells (LMH). Ampicillin and rifampicin antimicrobial resistance was induced in Salmonella strains to enable differential plate counting and allowed to invade cells (10 MOI) for over 60 min. Data were analysed using one- and two- way ANOVA in GraphPad Prism version 6.0.
The results of plate counting indicated that ST had significantly ($p < 0.05$) higher invasion capacity compared to SI in Caco-2 cells (with recovery percent of 4.2% for ST compared with 1.6% for SI), whether infected alone or in co-infection. The invasion rates in LMH cells also showed the similar pattern (higher infection rates of ST compared to SI; with recovery percent of 5.9% for ST compared with 1.70% for SI).
Overall, the in-vitro results showed that ST had higher invasion capacity compared with SI both in Caco2 and LMH cells. Future studies are required to elucidate the higher invasion capacity of ST including the mechanisms, which is especially relevant in relation to its persistence and transmission in poultry.

Keywords: Salmonella infantis, Salmonella Typhimurium, Caco-2 cells, LMH cells, Invasion
Keywords: Salmonella infantis; Salmonella Typhimurium; Caco-2 cells; LMH cells; Invasion

2109 Hijacking of the ARIH2-CYBB Axis by Newcastle Disease Virus to Suppress ROS-Mediated Host Defense

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Newcastle Disease (ND) is one of the major infectious diseases threatening the poultry industry. Exploring and identifying host factors that affect the replication of Newcastle Disease Virus (NDV) is crucial for elucidating the pathogenic and host resistance mechanisms of the pathogen. In this study, we performed three rounds of NDV infection on a chicken HD11 whole-genome CRISPR knockout library. Surviving cells were enriched by flow cytometry sorting, followed by high-throughput sequencing and bioinformatics analysis of DNA from these cells. Ultimately, 217 candidate genes were identified, which are primarily involved in biological processes such as the immune system and viral information processing. Among these, we focused on the E3 ubiquitin ligase ARIH2. Initial gene knockdown and overexpression experiments confirmed that ARIH2 significantly promotes NDV replication. To elucidate the molecular mechanism by which ARIH2 enhances viral replication, we conducted IP-MS analysis of ARIH2 under both infected and uninfected conditions, generating a dynamic interactome profile of ARIH2 before and after NDV infection. Within the ARIH2 interactome, we identified the innate immune core effector CYBB. CYBB serves as the terminal executor of the electron transport chain in the respiratory burst, responsible for transferring electrons from cytoplasmic NADPH to oxygen, generating superoxide anions, and initiating the entire ROS cascade, thereby regulating immune processes. We validated the dynamic enhancement of ARIH2-CYBB interaction efficiency upon NDV infection through co-IP experiments. The above findings suggest that NDV may hijack the ARIH2-CYBB interaction, potentially utilizing the E3 ubiquitin ligase activity of ARIH2 to ubiquitinate CYBB, thereby inhibiting the host's ROS-mediated immune killing function and ultimately facilitating viral immune evasion.
Keywords: chicken; disease resistant breeding; Newcastle disease virus; CRISPR knockout library; interacting proteins

2110 Non-typhoidal Salmonella infections in layer flocks in Israel between 2018 and 2023

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Poultry and egg products are major sources of food-borne zoonotic infections caused by non-typhoidal Salmonella (NTS) worldwide, and particularly in Israel, where poultry consumption is higher than in the U.S. and Europe due to religious dietary restrictions. Estimating NTS prevalence and identifying risk factors for infection in poultry are crucial for protecting public health. However, although NTS prevalence in layers is actively monitored, analyses of potential risk factors have not been conducted. We aimed to estimate the prevalence of and risk factors for NTS infections, with a particular focus on proximity to dairy farms, in layer flocks in Israel between

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2018 and 2023.
A cross-sectional analysis was conducted, including 24,500 sampling events from 9,685 laying hen flocks, collected as part of the national active Salmonella surveillance program. The prevalence of and associations with different risk factors were estimated for NTS, and specifically for the serovars S. Enteritidis (SE) and S. Typhimurium (ST), which are considered to pose a high risk to public health. Univariable and multivariable mixed-effects regression models were applied, with nested random effects to account for shared locality, farm ownership, and repeated measures.
The overall NTS prevalence was estimated at 17.74% (4,347/24,500), and the cumulative flock-level prevalence at 33.06% (3,202/9,685). During this period, 1.87% (181/9,685) and 1.31% (127/9,685) of flocks tested positive for SE and ST, respectively. In the multivariable models, older birds (>85 weeks), larger flocks (>10,000 hens), caged housing systems, and sampling during fall were significantly associated with increased NTS detection ($p<0.05$). SE detection was associated with older birds (>85 weeks), farm location in northern Israel, and sampling conducted by Israeli Veterinary Services inspectors compared with Egg and Poultry Board inspectors, while ST detection was primarily associated with older birds (>85 weeks).
Our findings emphasize the importance of flock management and environmental context in the complex epidemiology of NTS in layer flocks. Interestingly, although livestock farms in Israel are often located in close proximity due to the country's small geographic area, distance to dairy farms was not identified as a significant risk factor for infection, even for host generalists such as ST. However, maintaining smaller flocks (fewer than 10,000 hens) in non-caged systems and shortening production cycles may help reduce the prevalence of NTS in laying hens.
Keywords: Salmonella; Risk factors; Public health; table egg; Layer

2111 Transmission of Salmonella Enteritidis in Canada's broiler chicken production chain: An outbreak investigation integrating human and animal surveillance within a One Health framework.
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Objective: To describe an outbreak investigation which identified Salmonella Enteritidis (SE) transmission within Canada's broiler chicken production chain and demonstrate how a One Health approach integrating human, animal, and food surveillance can enhance understanding of disease transmission and inform prevention strategies.
Experimental design: Whole genome sequencing (WGS) analysis conducted by PulseNet Canada revealed a highly related cluster of isolates with ≤3 allele differences using whole genome multi-locus sequence typing (wgMLST). Integration of human epidemiological data with metadata from non-human isolates enabled investigation of potential connections between human illness and broiler production.
Materials and Methods: Human SE cases in this cluster were interviewed to collect epidemiological data (food consumption, travel, and animal contact) in the 7 days prior to illness onset; only cases providing definitive information were included in the analysis. Metadata for non-human isolates (sample source, collection date, and location) was obtained from national surveillance programs (CIPARS and FoodNet Canada) and from passive animal health surveillance.
Results: The identified subcluster included 92 human cases detected between 2024 and 2025. Most cases (83/92) were reported from two neighbouring provinces (A and B). All animal isolates belonging to this subcluster were associated with broiler chicken production in Province A: 4 from broiler breeders, 10 from broiler hatcheries, 12 from broiler farms, and 2 from chicken abattoirs. The same strain of SE was also detected in retail raw chicken meat samples from Province A (4 samples) and Province B (1 sample). The earliest detections were from broiler hatcheries and preceded the first human case by two months. No non-human isolates outside of the broiler production continuum were identified. Among interviewed cases (n = 36), none reported overnight international travel. Chicken meat consumption was reported by 28/31 cases, chicken eggs by 22/28, and contact with live poultry by 3/30 cases.
Conclusions: This study demonstrates how integration of human, food and animal surveillance data can strengthen interpretation of genomic and epidemiological findings and clarify transmission pathways. Genomic and epidemiologic evidence are consistent with SE transmission occurring across multiple stages of broiler production and ultimately reaching consumers. These findings highlight the value of a One Health surveillance approach for identifying points in the production continuum where preventive efforts may be most effective.
Keywords: One health; Salmonella Enteritidis; Broiler production; Outbreak

Withdrawn

2113 Molecular and Pathological studies of chicken infectious anaemia and concurrent infections in layer flocks in Namakkal region of South India
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[This abstract was withdrawn from the program.]

2114 Prevalence and risk factors promoting the onset of Newcastle disease in local poultry farming in north of Togo
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[This abstract was withdrawn from the program.]

2115 Molecular Detection of Newcastle Disease Virus in Backyard Poultry.
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[This abstract was withdrawn from the program.]

2116 Genetic analysis of persistent Salmonella Infantis strains in Austrian broiler farms
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[This abstract was withdrawn from the program.]

2117 Seasonal characterization of Salmonella spp.

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and Enterococcus spp. prevalence in broiler ceca, feed, and farm environments in Québec

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[This abstract was withdrawn from the program.]

2118 Implications of Biofilm Formation and Antimicrobial Resistance Patterns of Atypical Salmonella Infantis Variants

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[This abstract was withdrawn from the program.]

2119 Longitudinal study on antimicrobial susceptibility and biofilm formation ability of Salmonella Gallinarum strains from two laying hen farms

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[This abstract was withdrawn from the program.]

Genetics, Genomics & Epigenomics II

2120 From Selection to Insight: What Seven Generations of Selection Has Taught Us About Water Efficiency in Broiler

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The beginning of any selection program starts with the why and the what: why are we selecting for these traits and what do we expect to change? When it came to selection for water efficiency, the “why” was easy. As average global temperatures increase and freshwater availability becomes limited, finding ways to decrease water usage in livestock and poultry production is imperative for a water sustainable future. A relatively underutilized method to improve water use efficiency in broilers was to look into the genetics as water efficiency related traits were largely under utilized in commercial breeding programs. The “what” became a bit more open ended. At the beginning of the divergent selection program, it became clear that a more water efficient broiler could be selected for. What remained unclear was what other traits may be impacted. After 7 generations of intense selection for water efficiency, several studies have provided additional insight into the feasibility and outcome of selection and its relationship to other economically important traits. The high-water efficiency (HWE) broiler line has exhibited improved water efficiency. Water intake for the HWE line in a 6 week grow out period was 1.79 liters less than the control (MRB) line per bird. Feed conversion ratio was also improved from selection with the HWE line showing a 9-point improvement from the MRB. Investigation into breeder related traits has shown no impact on egg production per hen and no impact on fertility or hatchability. On the male side, HWE males show an improvement in semen volume (0.473 vs 0.322cc) with no impact on mobility or motility. When considering gut integrity, broilers from the HWE line exhibited a decreased amount of FIT-C dextran in their blood, indicating improved gut integrity. Seven generations of selection and selection response has shown promise in creating a more water efficient broiler and a sustainable future but much still remains to be understood in future generations.

Key Words: Broiler, Selection, Water, Efficiency, Sustainability

2121 GWAS-based identification of egg quality SNPs in Darag Philippine native chicken

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Darag native chicken, from Panay Island in Western Visayas Philippines, is popular for its meat and egg qualities and being utilized commercially in various local dishes. This study explored the use of 600K SNP panel in blood samples of 480 laying Darag hens to identify gene markers for egg quality. GWAS was done using mixed linear model (MLM) that accounted for population structure. After quality control, 466,913 SNPs were used in the analysis, while 28,940 LD-pruned SNPs were used to assess the population structure. Heritability was estimated using genomic linear mixed model with genomic relationship matrix (GRM) and REML. To determine the significant association, GCTA was used using the PLINK binary files with MAF > 0.05. Genes located within ±500 kb of the significant SNPs were considered as candidate genes. Results identified five significant SNPs for egg weight, linked to genes such as NOM1, RAP2A, and CNTLN, with potential roles in cell growth and skeletal traits. For yolk weight, seven markers were significantly associated, including regions near MDFIC, which has implications on cell regulation and ovarian tumor development affecting egg quality. Eleven SNPs were linked to eggshell weight and 57 to eggshell thickness, with key genes like ATP2B2, OPTN, and MYH9 involved in calcium transport, mineralization, and mitochondrial function. For egg shape traits, 15 genes—including ROBO1, RIMS2, and AKT3—were associated with egg length and width, possibly affecting follicle development, membrane formation, and overall egg formation. This study represents the first genome-wide documentation of SNPs in Philippine native chickens, providing valuable insights for advancing molecular marker research in native poultry in the country.

Keywords: egg quality; gene markers ; GWAS; smallholder ; village chickens

2122 Expression Dynamics of GH, IGF-1 and PIT Genes in Frizzle-Feather Nigerian Indigenous Chickens Reveal Molecular Basis of Growth and Thermotolerance

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Enhancing tropical poultry productivity and resilience requires a molecular understanding of genes regulating growth and adaptation to heat stress. This study hypothesized that expression dynamics of Growth Hormone (GH), Insulin-Like Growth Factor-1 (IGF-1) and Pituitary-Specific Transcription Factor (PIT) genes underlie growth regulation and thermotolerance in frizzle-feather Nigerian indigenous chickens. The objective was to quantify sex- and tissue-specific variations in GH, IGF-1, and PIT expression and relate them to body weight under tropical conditions. A 2 × 3 factorial experiment was conducted using 60 frizzle-feather chickens (30 males, 30 females) reared under uniform management for eight weeks. Tissues from the hypothalamus, liver, and breast muscle were analysed for gene expression via quantitative real-time PCR (qPCR) using β-actin as the reference gene. Data were analysed using two-way ANOVA and Pearson correlation, with significance at $p<0.05$. Expression of all three genes differed significantly among tissues and sexes. GH was highest in male breast muscle and correlated positively ($r=0.68$) with body weight, while females exhibited higher hypothalamic and hepatic GH expression. IGF-1

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was elevated in female liver and hypothalamus ($p<0.01$), suggesting hepatic mediation of somatic growth through the GH–IGF-1 axis. PIT showed a strong correlation ($r=0.72$, $p<0.01$) with GH, confirming its regulatory role in pituitary-driven growth control. A combined regression model of GH, IGF-1, and PIT explained 82% (Adjusted coefficient of determination=0.82) of body weight variation, highlighting their predictive reliability as biomarkers. The observed expression hierarchy (male breast > female liver > hypothalamus) demonstrates that the GH–IGF-1–PIT axis is central to growth, sexual dimorphism, and thermotolerance. These genes function as molecular indicators for marker-assisted selection, offering a genomic basis for climate-smart breeding of indigenous chickens in tropical environments.

Keywords: Frizzle-feather chickens; Growth Hormone (GH); Insulin-like Growth Factor-1 (IGF-1); PIT gene; Thermotolerance; Gene expression and Precision breeding
Keywords: Frizzle-feather chickens; Growth Hormone ; Insulin-like Growth Factor-1; Thermotolerance; Gene expression and Precision breeding

2123 Single-nucleus Transcriptomic profiling of breast muscle in Yanjin Black-Bone chickens and Jinling Partridge chickens identifies critical melanocyte marker genes

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Objective:
The objective of this study was to determine whether differences in melanocyte composition and melanogenesis-related gene expression contribute to breast muscle pigmentation in Yanjin black-bone chickens and Jinling partridge black-bone chickens by using single-nucleus RNA sequencing (snRNA-seq).
Experimental Design:
Breast muscle tissues were harvested from four individuals each of Yanjin black-bone and Jinling partridge black-bone chickens. Single-nucleus RNA-seq was employed to construct a high-resolution transcriptomic atlas of individual nuclei derived from these tissues. Subsequent cell type annotation and differential gene expression analyses were conducted to pinpoint key genes implicated in melanogenic pathways. This approach offers enhanced resolution over conventional bulk RNA-seq by enabling the dissection of transcriptional heterogeneity at the single-cell level.
Materials and Methods:
Breast muscle samples were obtained from eight healthy black-bone chickens at 60 days of age, including four Yanjin black-bone chickens and four Jinling partridge black-bone chickens. One breast muscle sample was collected from each individual. Nuclei were isolated from muscle tissues, and single-nucleus RNA sequencing libraries were constructed using the 10x Genomics Chromium platform. Sequencing data were processed with Cell Ranger, followed by quality control, clustering, cell type annotation, and marker gene identification using Seurat. Melanocyte populations were further analyzed to characterize the expression of pigmentation-related genes.
Statistical Analysis:
Differential gene expression analysis was conducted using the Wilcoxon rank-sum test implemented in Seurat. Student's t-test was used for comparisons between breeds where appropriate. Statistical significance was defined as $P < 0.05$.
Results:
This study identified 12 distinct cell types within the breast muscle, including myofibroblasts, melanocytes, satellite cells, and vascular endothelial cells. Melanocytes exhibited marked upregulation of key pigmentation-related genes such as PMEL, TYRP1, and EDNRB2, underscoring their central role in melanin biosynthesis. Co-expression network analysis further elucidated melanocyte-specific regulatory modules associated with melanogenesis.
Conclusions:
These results demonstrate that melanocyte-specific transcriptional programs are closely associated with breast muscle pigmentation in black-bone chickens. The identified marker genes, including PMEL, TYRP1, and EDNRB2, provide potential genetic targets for improving muscle pigmentation in poultry breeding programs.

Keywords: black-bone chicken; breast; single-nucleus RNA sequencing; melanocytes; melanogenesis

2124 Regulatory variants in CACNA1C linked to the egg production in chicken

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Introduction Commercial layer breeds, known for their excellent production performance, are raised widely around world. Indigenous chickens are superior in egg quality and flavor but inferior in the productivity. Identifying candidate genes and variants associated with egg production (EP) is therefore essential for improving indigenous chicken breeds. Base on two distinct chicken genotypes, we aim to identify associated genomic variants and explore their potential regulatory mechanism.
Materials and methods We re-sequenced 225 chicken genomes, including five commercial breeds (BB, CC, RR, WW, GF) and four Chinese indigenous breeds (PD, LH, BY, SN). SNPs were called using GATK and imputed using Beagle, yielding 3,722,203 autosomal SNPs. After filtering using hwe 0.00001 (to prevent genotyping errors), geno 0.05, mind 0.1, maf 0.05 and pruning using indep-pairwise 50 10 0.2 in PLINK, population genomic analyses were conducted to assess diversity and structure. Comparative genomic analyses between commercial breeds (WW and RR, high-EP) and indigenous breeds (PD and BY, low-EP) were performed to identify genomic variants under selection. Additionally, multi-tissue transcriptome data and chicken regulatory element annotations were integrated to elucidate the regulatory roles of associated variants in EP.
Results A total of 273,426 pruned SNPs was obtained. PCA, phylogenetic tree and admixture analyses revealed high breed purity and substantial genetic divergence (0.25–0.35) among populations. Nucleotide diversity of genomic variants was ranging from 0.0006~0.001. FST, XP-EHH and XP-CLR identified 22 common genomic regions under selection between high- and low-EP groups. Among them, CACNA1C emerged as a promising candidate gene, significantly enriched in calcium and GnRH signaling pathways related to egg-laying processes. Further $\theta\pi$, Tajima's D, and FST analyses refined CACNA1C containing 14 SNPs under strong selection. These SNPs formed two LD blocks, with haplotype AACTC being dominant in high-EP breeds. An intronic SNP rs315636259, C allele of which was fixed in WW and RR while it exhibits high MAF in PD and BY. RNA-seq data indicated that CACNA1C was differentially expressed in the hypothalamus and liver between WW and BY. Epigenetic annotation further revealed that rs315636259 acts as an enhancer, altering methylation in both tissues, leading to differential expression and EP variation between commercial and indigenous breeds.
Conclusions Our findings provide novel insights into the regulatory mechanism of CACNA1C in egg production and identify potential molecular markers for genetic improvement in chickens.

Keywords egg production, population genomics, comparative genomics, regulatory variants, CACNA1C
Keywords: Egg production; Population genomics; Comparative genomics; Regulatory variants; CACNA1C

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2125 Molecular Stratification Identifies Hepatic Genes and Metabolites Linking Feed Efficiency in Laying Hens

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Residual feed intake (RFI) not only serves as a key indicator of feed efficiency in poultry but also plays a crucial role in enhancing economic sustainability. Its regulation involves complex physiological pathways across multiple tissues. In this study, we analyzed 248 laying hens through an integrative multi-omics strategy, leveraging genomic, transcriptomic(10 tissues), and metabolomic(4tissues) data from tissues of hypothalamus, pituitary, liver, pancreas, duodenum, ileum, cecum, ovary, magnum, and uterus. We found RFI at 100 weeks (100wRFI) had moderate heritability (0.24) using restricted maximum likelihood (REML) analysis. GWAS with the linear mixed model (LMM) identified loci near DGKI (chromosome 1) for 70wRFI and a locus encompassing PCCB and STAG1 on chromosome 9 for 100wRFI, accounted for 4.73% and 13.3% of the posterior inclusion probability respectively. Then we performed eQTL mapping across multiple tissues, following the standardized pipelines established by the ChickenGTEx projects. By integrating eQTL data with GWAS summary statistics via SMR and GIFT analyses, we prioritized key candidate causal genes, including SSBP1 for 70wRFI and PCCB for 100wRFI, which showed consistent associations across multiple tissues. Bidirectional Mendelian Randomization analysis linked hepatic PCCB expression to key metabolites like PE(18:0/20:4) and TMAO. A supervised multi-omics model (DIABLO) based on tissue-specific gene expression and metabolite levels accurately classified hens into distinct RFI subtypes (AUCs 0.97–1.00), verivfied the robustness of our identified genes. Our findings systematically decipher the complex genetic architecture and tissue-specific molecular networks regulating feed efficiency, providing valuable insights and candidate targets for genetic improvement of feed efficiency in poultry breeding.

Keywords: Laying hens; Feed efficiency; Fat deposition; Multi-omics; Integrative analysis

2126 Identification and Functional Characterization of Key Factors Regulating Chicken Growth and Development

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Introduction

Body weight is a key economic trait in chickens, regulated by complex genetic and molecular processes. Understanding the genetic mechanisms controlling growth is critical for accelerating breeding and improving poultry efficiency. In this study, genomic, transcriptomic, and phenotypic data were integrated, and multiple colocalization analyses were applied to construct tissue-specific regulatory variant–functional gene networks. This approach systematically identified key regulators of chicken growth and provides data support for marker-assisted selection of growth traits.

Materials and Methods

An advanced intercross line (AIL) derived from Huiyang bearded and Lingnan yellow chickens, comprising seven generations and 9,978 individuals, was used. Seventy-five growth- and development-related phenotypes were collected. Genotypes were obtained by low-coverage sequencing, and transcriptomes were profiled using bulk RNA-seq. FAANG functional annotations were integrated with TWAS, SMR, and fastENLOC analyses to identify key regulatory genes. Functional validation was performed using myoblast and mouse models.

Results

Based on a 16-generation AIL population, low-coverage whole-genome sequencing, transcriptome sequencing, FAANG annotations, and chicken GTEx data, GWAS were conducted for 75 phenotypic traits, identifying 682 QTLs, of which 154 were located in single-gene regions. By integrating eQTL, TWAS, SMR, and fastENLOC analyses, tissue-specific regulatory networks were constructed, resulting in the identification of 431 functional genes. Among these genes, UCP3 is highly and specifically expressed in skeletal muscle and is progressively upregulated during myogenic differentiation. Functional assays in C2C12 cells and chicken myoblasts indicate that UCP3 inhibits cell proliferation while promoting myotube formation. CRISPR/Cas9-mediated knockout mice for GIP, LCORL, and NCAPG revealed that GIP promotes adipogenesis and fat deposition, whereas LCORL–NCAPG enhances cell proliferation, thereby regulating body weight gain.

Discussion and Conclusion

This study systematically dissected the genetic regulation of chicken growth traits using a 16-generation AIL population and integrated multi-omics analyses. Tissue-specific regulatory variant–functional gene networks were constructed, identifying key genes in muscle and adipose development. UCP3 promotes differentiation and inhibits proliferation in skeletal muscle, suggesting a role in coordinating myoblast proliferation and differentiation. These findings provide functional targets and support molecular breeding in chickens and a framework for comparative studies of growth traits.

Keywords: Chicken; Body weight; Multi-omics analysis; UCP3
Keywords: Chicken; Body weight; Multi-omics analysis; UCP3; GIP

2127 Transcriptomic analysis gains insights into the gene expression profiles of broiler breast muscles affected by growth-related myopathies

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Growth-related myopathies remain a worldwide concern in terms of fast-growing chicken welfare, meat quality, and sustainability. As part of a bigger project aimed at deepening the knowledge about the wooden breast (WB) underpinning biological mechanisms, this study represents a first step focused on a new characterization of the transcriptomic profiles of myopathic breasts collected from male fast-growing broilers (45-d-old; 3.2 kg) at a commercial processing plant in 2024. In detail, 180 breast fillets (90/group) were classified according to their macroscopic appearance as affected by severe WB or not affected (normal; N), and their classification was validated through multivariate analysis of meat quality data (i.e., pHu, color, WHC, and shear force). Among the collected muscles, 10 samples/group were used for total RNA extraction and sequencing. Raw-reads quality control, trimming, alignment to the reference genome, and read counting were performed on the High-Performance Computing platform provided by ELIXIR-IIB HPC@CINECA. After raw-reads processing, a differential analysis was performed (q-value<0.01 and log2 fold change>|1.5|). A total of 193 differentially expressed genes (DEGs) were identified, with 174 being up-regulated and 19 down-regulated in WB samples. Among those, PTX3, a key regulator of inflammation and modulator of extracellular matrix deposition and angiogenesis, has been found as the top up-regulated gene in WB. Also, genes encoding for striated muscle function and contraction (e.g., LMOD2 and MYH15), growth factor activity (e.g., MDK), stress response (e.g., CRH), and extracellular matrix remodeling (e.g., MMP9) were some of the top up- and down-regulated genes. To gain insights into the functional annotation of DEGs, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed. GO analysis identified terms related to muscle contractions

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and neuromuscular transmissions (e.g., “sarcomere”, “contractile muscle fiber”, and “neuron projection”). Also, “Phagosome”, “ECM-receptor interaction”, and “PPAR signaling pathway” were found as significant KEGG pathways. In conclusion, despite years of efforts to mitigate growth-related myopathies, our results largely highlighted persisting alterations in terms of muscular and neuromuscular functions in WB-affected breast muscles, suggesting the persistence of still unknown triggering factors underlying the observed gene expression patterns. Further investigations comparing past and recent transcriptomic profiles may help identify fundamental biological alterations associated with the WB condition. Research supported by NextGenerationEU, National Grant PRIN2022 (Prot. n. 2022EPWEPW).
Keywords: Broiler; Meat quality; Differential gene expression; Woody breast; Transcriptomic profiles

2128 Transcriptomic profiling reveals age as the primary driver of caecal functional maturation in broilers
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The broiler caeca integrate digestive, renal, and immune functions, but their molecular maturation remains largely uncharacterized. While age and diet shape caecal microbial communities, their contributions to caecal tissue development are poorly understood. This study aimed to define how age, sex, and antibiotic or probiotic interventions regulate the caecal transcriptional maturation, defined as age-dependent changes in gene expression reflecting functional development of epithelial and immune pathways. Broilers were assigned to four experimental conditions (n = 5 pens/condition, 45 birds/pen): an antibiotic diet containing bacitracin (0.5 kg/t, T+), an antibiotic-free diet (T-), or antibiotic-free diets combined with in ovo injection of a four-strain (Pb2) or two-strain (Pb1) probiotic mix. A cross-section of the caecal wall encompassing the epithelium and underlying mucosal compartment was collected from one bird per pen on day 10 and day 35 (n = 5 birds/condition/age, total = 40 samples). Total RNA was extracted and subjected to RNA sequencing. Transcript counts were normalized and variance-stabilized prior to multivariate and differential expression analyses (DGEA). Principal component analysis (PCA) and DGEA were conducted using the limma R package, with models including treatment, age, and sex with empirical Bayes moderation and FDR adjustment. PCA revealed no clear separation among treatments but strong clustering by age and sex. In the DGEA model, treatment-associated transcriptional variation was limited. In contrast, the model identified extensive age-dependent transcriptional changes in both sexes, with 3460 and 3059 differentially expressed genes (DEGs, $|\log2FC| > \log2(1.25)$ & FDR < 0.05) between d35 and d10 in males and females, respectively. Gene set enrichment analysis of age contrasts revealed that the transition from d10 to d35 was marked by reduced (FDR < 0.05) transcriptional regulatory activity and chromatin remodeling, alongside the emergence of humoral immune features such as immunoglobulin-mediated responses and B cell receptor signaling (FDR < 0.05), suggesting a shift from a highly plastic, developmentally permissive state toward a more stable and specialized functional state, consistent with the need to maintain long-term immune surveillance and tolerance within the microbially dense caecal environment. Overall, age emerged as the principal driver of caecal functional maturation, outweighing antibiotic and probiotic interventions and highlighting coordinated development of mucosal immunity during post-hatch growth.
Keywords: caeca; functional maturation; mucosal immunity; neuro-immune regulation; RNA-seq

2129 Transcriptomic insights into the molecular basis of sexual dimorphism in body weight of broiler chickens: A multi-tissue RNA-Seq study
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Body weight (BW) is a key economic trait in broilers, and while sexual dimorphism (SD) for this trait has been described genetically and physiologically, its molecular basis remains less clear. We hypothesized that differences in BW between males and females are associated with sex-specific regulation of growth and metabolic pathways across multiple tissues. To test this hypothesis, we compared the transcriptomes of pectoral muscle, liver, and small intestine (ileum) from 35-day-old male and female broilers raised in floor pens (n = 15/sex) under standard conditions. RNA was extracted from tissue samples (n = 15/tissue/sex, 1/pen), quality-checked, and sequenced, and reads were aligned to the chicken genome (GRC7b) using a pseudo-aligner to obtain gene counts. Differential gene expression analysis was then assessed across sexes and tissues using the limma R package with moderated t-tests and Benjamini-Hochberg adjustment for multiple testing, with significance defined as fold change ≥ 1.2 and adjusted $P < 0.05$. Gene set enrichment analysis (GSEA) was also performed using the R package fgsea. Body weight gap between males and females was 17.2% at d 35 (2854 g vs 2435 g, $P < 0.05$). A total of 209, 217, and 313 differentially expressed genes (DEGs) between sexes were identified in muscle, liver, and ileum, respectively, with subsets of DEGs uniquely expressed in a single tissue (9.8%, 10.7%, and 29.8% in the muscle, liver, and ileum, respectively) and others co-expressed between tissues. DEG expression was significantly correlated with BW in both sexes ($P < 0.05$), suggesting a link between these DEGs and BW. GSEA revealed that males exhibited stronger anabolic and proliferative signatures relative to females, with myogenesis, PI3K/Akt/mTOR, MYC targets, and mitotic spindle pathways enriched in muscle; oxidative phosphorylation, protein secretion, and E2F targets in liver; and oxidative phosphorylation, TGF-β signaling, and cell cycle programs in intestine (all $P < 0.05$). Stress-related pathways, including unfolded protein response, p53, and reactive oxygen species, were also upregulated in males ($P < 0.05$). These results suggest that SD in BW arises from males investing in greater growth and metabolic throughput but at the cost of increased cellular stress, whereas females favor stability and stress avoidance over maximal growth.
Keywords: Broiler; body weight; sexual dimorphism; RNA-Seq; growth regulation

2130 Expression of pro-inflammatory and anti-inflammatory cytokines in broiler and local chickens following immune stimulation with lipopolysaccharide
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Understanding the immune response in poultry is essential for developing strategies to enhance disease resistance, particularly in the context of natural alternatives to conventional therapeutics. This study investigated the expression profiles of inflammatory cytokines in two genetically distinct chicken strains: the Ross 308 broiler, bred for rapid growth and high meat yield, and Ghanaian local chicken strain, characterized by slower maturation, greater hardiness, and adaptation to free-range environments. At four

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weeks old, the birds were treated with lipopolysaccharide (LPS), a bacterial endotoxin known to elicit immune responses, and carvacrol, a plant-derived antimicrobial compound with potential immunomodulatory effects. From a population of 100 birds per strain, eighteen were randomly selected and assigned to three treatment groups (six birds/strain/treatment): a control group injected intramuscularly with saline, a group treated with LPS, and a third group administered carvacrol in combination with LPS. Liver tissues were collected for RNA extraction, and the expression levels of key pro-inflammatory and anti-inflammatory cytokines were quantified using real-time PCR. Additionally, histological analysis was performed to assess hepatic inflammation. The results revealed distinct immunological responses between the two strains. Among pro-inflammatory cytokines, IFNβ1 expression remained unchanged across treatments, while IL6 was significantly upregulated in local chicks but not in broilers. IL1β showed increased expression in both strains, with a notably higher response in local chicks. Carvacrol treatment led to an upregulation of IL6 in broilers and a downregulation of IL1β in local chicks. In terms of anti-inflammatory cytokines, broilers exhibited minimal changes except for IL10, whereas carvacrol enhanced the expression of IL1R, IL4, and TGFβ. No significant modulation of anti-inflammatory markers was observed in local chicks following carvacrol treatment. This study demonstrates that genetic background significantly influences cytokine responses to immune stimulation in poultry. The local chicken strain exhibited a more robust pro-inflammatory response to LPS compared to the commercial broiler, while carvacrol modulated cytokine expression in a strain-specific manner. These findings underscore the potential of carvacrol as a natural immunomodulator and highlight the importance of tailoring health interventions to the genetic and physiological characteristics of different poultry breeds.

Keywords: gene expression; cytokines; inflammatory response; lipopolysaccharide; carvacrol

Gut Microbiome and Health II

2131 Asynchronous nutrient utilisation in wheat-based low protein diets alters caecal microbiota and impairs growth performance in broiler chicken

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Gut microbiota are key contributors to growth and health in broiler chickens. Nitrogen (N), a growth-limiting nutrient, is typically supplied by soybean meal crude protein (CP) or partially replaced with non-bound amino acids (NBAA) in low protein diets. While such diets improve N retention and reduce excretion, their benefits are more evident in maize-based than wheat-based diets, where growth performance often declines.

Building on a previous 2 × 2 factorial feeding trial (Chrystal et al., 2021), we examined how diet composition affects nutrient utilisation and caecal microbiota. A total of 144 off-sex male Ross 308 chicks were fed isoenergetic wheat (W) or maize (M) based diets with standard (S: 222 g/kg) or reduced (R: 165 g/kg) CP from day 7 to 35 post-hatch. We hypothesised that imbalanced host utilisation of starch and N-nutrients (protein-bound amino acids and NBAA) in the wheat-based reduced-protein (WR) diet would alter nutrients partitioning between host (reflected by AMEn) and microbiota (indicated by ileal residual), thereby driving shifts in caecal microbiota linked to impaired growth. Starch and N residual in distal ileal digesta was quantified. Caecal contents from randomly selected birds (n = 11-13 per treatment) were collected on day 35 for DNA extraction and bacterial 16S rRNA amplicon sequencing (V3-V4 regions, Illumina MiSeq). Data were processed via a USEARCH-based pipeline (Li et al., 2024) and analysed in R. Birds fed WR had the lowest AMEn and N residual in ileal digesta (Kruskal-Wallis with Dunn's post hoc, Padjusted < 0.05 vs. other treatments), and the highest starch (Padjusted < 0.05 vs. MS and MR). The WR microbiome exhibited a distinct community composition (PERMANOVA, $P < 0.05$), with the N residual identified as key drivers. ASVs explaining the observed community-level shifts were identified (ANCOM-BC2), including Fournierella, Blautia, An181, and Agathobaculum. Notably, these taxa were positively associated with growth performance (Spearman's Padjusted < 0.05, with AMEn, weight gain, and/or FCR), yet reduced in WR-fed bird, with trophic modelling indicating their preference for high N availability.

These findings suggest that asynchronous host utilization of starch and N in the WR diet impaired energy harvesting by both the host and microbiota, ultimately reducing growth. In this model, the host failed to extract sufficient energy due to excessive N uptake but insufficient starch utilisation, leaving limited N in the ileal digesta, which constrained caecal microbial capacity to utilise the residual starch. This highlights the need for a paradigm shift in precision nutrition – emphasising host-microbiota nutrient synchrony to optimise feed efficiency in poultry production.

Keywords: Caecal microbiome; Host-microbiota interaction; Precision poultry nutrition; Low-protein diet; 16S rRNA gene sequencing

2132 Modulation of Microbiome-Gut-Bone Axis in Brolier Via Dietary Suplemantation of Bacillus subtilis DSM 29784 and Phytogenic

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The microbiome–gut–bone axis represents a major micro-ecosystem that plays a critical role in host metabolism, nutrient absorption, immune modulation, and skeletal development. Dysbiosis of this axis leads to impaired bone health in poultry. This study aimed to evaluate the modulation of the microbiome–gut–bone axis in broilers through dietary supplementation with Bacillus subtilis DSM 29784 combined a blend of garlic and essential oil (BsP). 560 birds of 1-day-old male Ross 308 broiler chick were randomly assigned to two dietary treatments, each with 8 replicates with 35 birds per pen: (1) a basal diet supplemented with BsP, and (2) a basal diet (Ctrl). On days 21, 28, and 35, indicators of gut health—

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including cecal microbiome, jejunal tight junction proteins, inflammatory cytokines, and ileal calcium–phosphate receptors—were analyzed alongside femoral bone health markers such as inflammatory cytokine profiles and osteoblast-related gene expression with RT-PCR. Statistic was performed using the multiplet's t-test for growth parameters, gene expression ($p < 0.05$), Adonis and DESeq2 for microbiome and NumPy and Metplotlib for networking. Results found that BsP diet significantly modulated the microbiome–gut–bone axis, enhancing gut health by upregulating tight junction–related genes (CLDN-1, OCLD, TJP-1 and MUC-2), suppressing pro-inflammatory cytokines (TNF-α, NF-κB), and promoting calcium–phosphate absorption via increased expression of CaSR and NaPi-IIb. Cecal microbiome analysis revealed higher richness (Chao1, Shannon indices) in BsP-fed birds, which increased beneficial bacteria including Clostridium butyricum, Enterococcus faecium, Lactobacillus salivarius, Lactonifactor longoviformis and Bifidobacterium longum, while reducing pathogenic species such as Escherichia coli and Enterococcus cecorum. Microbiome function enhanced biosynthesis of serotonin and melatonin, and upregulated L-tryptophan degradation pathways, suggesting activation of the microbiome–gut–brain axis. Regarding bone health, BsP diet downregulated pro-inflammatory cytokines (IL-6, IL-17, TNF-α, NLRP3) and upregulated osteogenic markers (BMP-2, SPARC, SMAD-1, RUNX-2) in the femur. Consequently, BsP exhibited improved feed conversion ratio (FCR), body weight (BW), and body weight gain (BWG). In conclusion, dietary supplementation with BsP effectively modulated the microbiome–gut–bone axis, promoted microbial homeostasis and intestinal integrity, modulated the mineral absorption and osteoblast activity. These findings highlight the potential of BsP as a functional feed additive to enhance intestinal and bone health. Future should address whether BsP can reduce the incidence of BCO in broilers through osteoimmunological mechanisms.

Keywords: Microbiome-gut-bone axis; Bacillus subtilis DSM 29784; Garlic blean botanical; Broiler; Bone-Gut health

2133 The effects of nutritional additives on the performance, microbiota biomarkers, and the severity of Necrotic Enteritis infection in broilers

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Necrotic enteritis (NE) is a major cause of economic loss in the poultry industry. Thus, understanding the role of the gastrointestinal microbiota during a NE challenge is essential for developing targeted interventions. The aim was to assess the impact of different additives on the performance, severity of NE, and identify specific microbiota biomarkers associated with resistance and susceptibility to NE in broilers. A total of 168 d-old-male Ross chicks were randomly allocated to battery cages and divided into four treatments with 6 replicates of 7 birds each. The treatments were: CTRL (control, no additives), PHY (phytogenic, BioStrong BP), PB+S (postbiotic + saponins, Dia-VTMPTplus), and PROB (probiotic Enterococcus faecalis, oral gavage at d 1). Birds had ad libitum access to water and feed from 0-22 d. All birds were challenged with a NE model by using 10x the dose of the Eimeria spp. vaccine on d 14 followed by Clostridium perfringens oral gavage on d 19 and 20. Body weight (BW), feed conversion ratio (FCR), and cloacal swabs were assessed at 7, 14, and 22 d, and intestinal lesions scored at d 22. The microbiota profile was analyzed using a microarray-based platform developed by Cargill called Galleon®. Non-parametric data was transformed prior to statistical analysis. Microbiota data was analyzed by a permutational multivariate ANOVA and LinDa, and performance was analyzed by a linear mixed-effects model (RSudio Software). Statistical significance was set at $P<0.05$. At d 22, PB+S had the highest BW and lowest FCR ($P<0.001$). The different treatments had a different microbiota profile, however the PB+S and PHY presented similarities, observed through the cluster analysis. Additionally, PHY and PB+S exhibited significantly lower intestinal lesion scores in the jejunum and ileum compared to CTRL ($P<0.001$). Analysis of the microbiota revealed distinct microbial profiles associated with NE resistance and susceptibility. Specific bacterial taxa such as Lactobacillus, Bifidobacterium, and Enterococcus were linked to birds that did not develop the disease ($P<0.05$). In contrast, taxa identified in the first wk of life, such as Enterobacter, were shown to have a progressive effect on dysbiosis in birds susceptible to NE. In conclusion, feed additives can effectively modulate the initial microbiota and increase resistance to NE. The identification of important microbial taxa as biomarkers may enable precision interventions to optimize poultry health and productivity.

Keywords: broiler; phytogenic; postbiotic; microbiota; additive

2134 Supplemental phytase and mineral interactions modulate gut microbiota composition in broiler chickens

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The microbial dynamics associated with phosphorus (P) digestion in broiler chickens remain to be understood. The revision of the classification of the Lactobacillus genus in 2020 highlights the need to reassess the host-microbiome interactions and the dietary modulation of bacteria in the gut of broiler chickens. Therefore, our objective was to investigate the dynamics of gastrointestinal (GIT) microbiota of broiler chickens, following phytase supplementation, with a focus on interactions with minerals, such as zinc (Zn) and calcium (Ca). We conducted three independent feeding trials: 1) dietary InsP6 and phytase levels (InsPPhyt), 2) phytase with Zn sources and concentrations (ZnPhyt), and 3) phytase, particle size, and Ca concentrations (PSPhyt). Ross 308 broilers were fed varying phytase levels (0-3000 FTU/kg), supplemental Zn concentrations (0, 30, or 90 mg/kg from ZnO or ZnSO4), Ca (4.9 and 7.2 g/kg), and feed particle size. Digesta samples were collected across the GIT for 16S rRNA sequencing. Statistical analysis included two-way ANOVA and generalized linear regression ($\alpha = 0.05$). Parametric assumptions were confirmed. Phytase increased the abundance of bacteria groups able to produce alkaline phosphatase, such as Ligilactobacillus and Streptococcus, while reducing Lactobacillus abundance (InsPPhyt, ZnPhyt; $p < 0.05$). The divergent response between Ligilactobacillus and Lactobacillus was consistent across trials but not along the GIT, as phytase had no significant effect on Lactobacillus in the ileum of InsPPhyt but did in ZnPhyt. This inconsistency may reflect phytase–Zn interactions, since higher Zn in InsPPhyt coincided with reduced InsP6 degradation. Similarly in the ileum, ZnO interacted with phytase, reducing Lactobacillus by 30% compared to ZnSO4 (ZnPhyt; $p< 0.01$). Ca supplementation (4 - 7 g/kg) increased microbial diversity ($p < 0.05$) (PSPhyt). In the PSPhyt trial, where considerably lower dietary Ca concentrations than the InsPPhyt and ZnPhyt trials were investigated, phytase supplementation reduced Lactobacillus abundance in both the ileum and ceca, suggesting enhanced phytase activity along the GIT. Particle size modulated the abundance of thirteen core taxa present across gizzard, ileum and ceca, predomi-

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nantly belonging to Limosilactobacillus (PSPhyt: p< 0.05).
In conclusion, the ileal microbiota in broiler chickens is modulated by supplemental phytase and its interactions with dietary Zn and Ca. Our results highlight the importance of considering mineral-phytase interactions when formulating diets to enhance P utilization.
Keywords: Phytase; Zinc; Microbiota; Broilers; Phosphorus

2135 Insect larvae meals as alternative protein sources in broiler starters: optimal inclusion levels for performance and gut health

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The objective of this study was to evaluate the use of insect larvae meal as a replacement for soybean meal in the starter phase of broiler chickens and to assess its effects on growth performance and gut health. Two experiments were conducted: one using black soldier fly (BSF) larvae meal and the other using mealworm (MW) larvae meal. A basal diet was formulated to meet all nutritional requirements of broiler chickens. Chicks (Hubbard Redbro) were allocated to seven treatment groups in each experiment, with three replicates per treatment and seven birds per replicate.
The BSF experiment consisted of a control group (basal diet only) and diets containing 2.5%, 5%, 7.5%, 10%, 12.5%, and 15% BSF meal. The MW experiment included the same inclusion levels. The trials lasted ten days (day 1–10), and samples were collected at the end of each experiment. Depending on the variable, data were analysed using linear and mixed models in R ($p < 0.05$) to evaluate the effects of insect type (BSF or MW) and dietary inclusion level (0–15%).
Body weight and average daily gain increased linearly with increasing inclusion level in both experiments, but declined significantly at the higher doses of 12.5% and 15% ($p = 0.004$ and $p = 0.005$, respectively). Daily feed intake and feed conversion ratio were not affected by insect meal inclusion. Irrespective of insect type, villus height and crypt depth in the duodenum and ileum were significantly lower at 2.5% and 5% inclusion levels ($p = 0.001$), increased at 7.5% and 10%, and decreased again at 12.5% and 15% ($p = 0.001$). In the jejunum, villus height increased linearly ($p = 0.001$) with higher inclusion levels of BSF meal.
Overall, BSF and MW larvae meals can replace soybean meal in broiler starter diets, with optimal responses at 7.5–10% inclusion. Higher levels (≥12.5%) negatively affected growth and gut morphology. These results show that insect meals are promising alternative protein sources when used at appropriate inclusion rates.
Keywords: Black soldier fly, mealworm, protein, broiler chicken, gut health
Keywords: black soldier fly; mealworm; protein; broiler chicken; gut health

2136 Genetic line and galacto-oligosaccharide supplementation, but not social stress, alter the cecal microbiota of laying hens

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Severe feather pecking (FP) is a damaging behavior in laying hens that compromises plumage integrity and alters physical appearance. Feather damage from FP negatively impacts hen welfare, health, and productivity and is a global societal concern. The gut microbiota of hens displaying FP differs from non-peckers. In mammals, the microbiota can be shaped by genetics, chronic stress, and dietary fiber, including specific prebiotic oligosaccharides, like galacto-oligosaccharides (GOS). Similarly, FP is influenced by genetics, stress, and diet, but it is unclear how these factors interact to shape the microbiota. This study investigated how genetic selection for FP, GOS supplementation, and social stress impact the cecal microbiota of mature hens. Laying hens (N = 191) of high and low FP (HFP, LFP) lines were housed together in 12 pens. From 29 to 32 weeks of age, hens were offered plain or GOS (Nutrabiotic®,74% GOS; Dairy Crest Ltd., Davidstow, Cornwall, United Kingdom) supplemented water and either underwent social stress by mixing unfamiliar birds or remained undisturbed (three pens/treatment combination). Cecal droppings (n = 16 per group) were collected for 16S rRNA sequencing, and bacterial taxa were identified using DADA2 with the Genome Taxonomy Database, then imported into QIIME2. Alpha diversity was analyzed by mixed models (PROC GLIMMIX, SAS) with fixed effects of supplementation, stress, and genotype (and their interactions) using Gaussian (Chao1 richness), t (Shannon diversity), and beta (Pielou's evenness) distributions. The relative abundance of genera and species were modeled with lognormal distribution (PROC GLIMMIX) and the same fixed effects. Beta diversity (Bray-Curtis) was evaluated by PERMANOVA. The cecal microbiota differed between genotypes in evenness ($P = 0.04$) and richness ($P = 0.05$) and samples from GOS-supplemented hens differed from control hens in richness ($P = < 0.01$) and diversity ($P < 0.01$). Beta diversity differed by genotype ($P < 0.01$) and supplementation ($P < 0.01$), but not by social stress. GOS supplementation increased the relative abundance of Bifidobacterium pullorum ($P = 0.01$) and Methanobrevibacter woesei ($P = 0.01$), an acetate-consuming archaeon, while the genus Lactobacillus was unaffected ($P = 0.72$). Megamonas and Faecalibacterium ($P < 0.01$ and $P = 0.02$, respectively) were more abundant in HFP than LFP hens, suggesting a microbial signature associated with a genetic predisposition for FP. These findings highlight that genotype and prebiotic GOS supplementation, but not short-term social stress, significantly alter the cecal microbiota in mature laying hens. Understanding these microbial shifts may inform strategies to mitigate FP and improve hen welfare.
Keywords: Feather pecking; Laying hen; Microbiota; Prebiotic; Social stress

2137 Synbiotic Water Selection in Laying Hens Under Social Stress: RFID-Tracked Choice Behaviour and Links to Severe Feather Pecking

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Synbiotics are used to support animals under various challenges, including stressful conditions. This study investigated laying hens' preference for synbiotic (SYN)-enriched drinking water and its potential behavioural effects under social stress. A total of 226 White Leghorn hens (37 weeks old) were housed in 12 enriched floor pens with 18–19 birds per pen, and given simultaneous access

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to color-coded jugs containing SYN and plain water. Birds could select between SYN- and control water in each pen, and individual jug visits were measured using radiofrequency identification technology. Further severe feather pecking (SFP) as a behavioural stress reaction was video recorded. After a 2-week associative learning phase and a 1-week washout, the hens underwent a 6-week preference test, spanning a 1-week pre-stress, 3-week stress, and 2-week post-stress phases. Water intake was measured at the group level, and individual jug visits were tracked. Hens consistently preferred SYN water ($p < 0.0001$), with the strongest preference pre-stress and a modest decline during stress. Interestingly, birds exhibiting higher rates of SFP voluntarily visited SYN water more frequently ($p = 0.0288$), suggesting a possible self-medication or coping mechanism. However, SFP rates remained stable, while plumage condition deteriorated over time. Severely damaged plumage increased from 39.4% to 53.5%, and intact plumage declined from 37.6% to 19.9%. These findings demonstrate that SYN supplementation via drinking water is feasible and preferred, even under stress, but may not be sufficient alone to maintain feather integrity.

Keywords: synbiotics; gut brain axis ; laying hens; behaviour; RFID

2138 Lipid profile and gut microbial population of broiler chickens as affected by aqueous leaf extract of Phillathus amarus.

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A 56days feeding trial was conducted to investigate the influence of varying dosages of aqueous leaf extract of Phyllanthus amarus (ALEP) on the lipid profile and gut microbial population of broiler chickens. One hundred and fifty (150) day-old chicks were used for the experiment and thirty (30) chicks each were randomly selected and assigned to each of the five treatments (1, 2, 3, 4 and 5) or dosages of ALEP (0, 25, 50, 75 and 100ml/l of water) with ten (10) chicks per replicate in a Completely Randomized Design (CRD). Treatment 1(T1) was the positive control, while ALEP was administered to the birds at 25, 50, 75 and 100ml/l of drinking water as T2, T3, T4 and T5 respectively. The gut content from the jejunum, ileum and caecum of two birds randomly selected from each replicate were collected and analysed for lactose fermenting bacteria (LFB) and non-lactose fermenting bacteria (NLFB) counts using serial dilution method. Data generated were subjected to a one-way analysis of variance (ANOVA) and differences between means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of probability. Total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (vLDL) values were reduced significantly ($P<0.05$), while the high density lipoprotein (HDL) concentration of broiler chickens was significantly ($P<0.05$) increased by ALEP compared to the control group. The lactose fermenting bacteria (LFB) in jejunum, caecum and ileum were significantly ($P<0.05$) increased by ALEP, but the non-lactose fermenting bacteria (NLFB) in jejunum, caecum and ileum were decreased significantly ($P<0.05$) compared to the control. Therefore, ALEP can be used as a substitute to synthetic antibiotics up to 100ml/L of drinking water as growth promoter for improved health status of broiler chickens.

Keywords: Broiler chickens; caecum; extract; microbes ; triglycerides.

2139 Muramidase Improves performance in poultry: A Review of its mode of action and Physiological Outcomes

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Objective:

Our last research on the mode of action of dietary muramidase has revealed new insights and opened up new ways of thinking animal nutrition. Summarizing findings from multiple trials and the impact on gut functionality, nutrient absorption and looking beyond the gut on systemic immune modulation in poultry.

Introduction:

Muramidase is an enzyme that hydrolyzes peptidoglycans (PGN), a structural component of bacterial cell walls. PGN accumulation in the gut triggers a systemic immune response leading to inflammation, increases a costly mucin secretion, and divert nutrients away from growth. By breaking down PGNs, muramidase reduces Toll-Like Receptor 2 (TLR2) activation and releases muramyl dipeptides (MDP). MDPs on the contrary activate NOD2 and promote immune tolerance. These mechanisms help maintain intestinal homeostasis, supporting better nutrient utilization by the host and thus increasing bird's performance.

Approach:

This abstract compiles data from in vitro and in vivo studies conducted under very different conditions, including PGN challenge. Outcomes reported across studies include immune cell profiles (CD3+, CD45+), goblet cell density, intestinal permeability (FITC-dextran), nutrient absorption (carotenoids), and intestinal morphology.

Key Findings:

Across trials, muramidase consistently demonstrated enzymatic activity on PGN, increasing fragment abundance and MDP release. This was associated with NOD2 activation and reduced inflammatory signaling in the body. Studies reported normalization of intraepithelial lymphocyte counts under PGN challenge, increased goblet cell density, improved gut barrier integrity (lower FITC-dextran translocation), and enhanced carotenoid absorption. Histological observations included reduced mucosa thickness.

Conclusions:

Evidence from multiple studies indicates that muramidase improves intestinal functionality by modulating immune responses and enhancing barrier and absorptive properties. These effects support better nutrient utilization and overall poultry health. However, further research is needed to understand the systemic impact of PGNs as described in the literature. From a practical perspective, incorporating muramidase into feed formulations offers a nutritional strategy to optimize performance beyond the gut leveraging a new era of growth performance for the poultry industry.

Keywords: Muramidase; Peptidoglycan; Intestinal Health; Immune Modulation; Nutrient Absorption

2140 Dietary indole-3-carbaldehyde improves oviductal immune homeostasis and microbial balance in a laying hen salpingitis model

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Indole-3-carbaldehyde (I3A) is a microbiota-derived tryptophan metabolite produced by Lactobacillus spp. with reported immunomodulatory and antioxidant activities. This study investigated the protective effects and underlying mechanisms of dietary I3A supplementation against lipopolysaccharide (LPS)–induced salpingitis in laying hens. A total of 168 healthy 62-week-old Nongda No. 3 laying hens were fed a control diet or a diet supplemented with 15 mg/kg indole-3-carbaldehyde (I3A) for 5 weeks, followed by vaginal lipopolysaccharide (LPS) or saline challenge, resulting in four experimental groups (CON, I3A, LPS, and LPS+I3A). Egg production performance was monitored throughout the trial, and

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egg quality was assessed at week 5. At the experimental endpoint, serum, intestinal, cecal, shell gland, and liver samples were collected for biochemical, histological, microbiome, and gene expression analyses. Microbial community composition in cecal contents and vaginal swabs was characterized by 16S rRNA gene sequencing (V3–V4 region) on the Illumina MiSeq platform and processed using QIIME2 with the DADA2 pipeline. Gene expression was quantified by qRT-PCR, normalized to GAPDH, and analyzed using one-way ANOVA with post hoc tests. I3A supplementation significantly increased egg weight ($P < 0.05$) and tended to improve feed-to-egg ratio ($P = 0.061$). Histopathological analysis revealed that I3A markedly alleviated inflammatory damage in intestinal and shell gland tissues. I3A significantly reduced serum and glandular levels of IL-1β, IL-8, TNF-α, and malondialdehyde while increasing IL-10, total superoxide dismutase, and glutathione peroxidase activities ($P < 0.05$). In addition, I3A enhanced epithelial barrier integrity by upregulating tight junction proteins Claudin-1 and zonula occludens-1 in the shell gland epithelium. Gene expression analysis showed that I3A suppressed Toll-like receptor (TLR) signaling–related genes, including IL-1β, TNF-α, Nrf2, and MyD88, with expression levels comparable to those of the control group. Microbiota profiling demonstrated that I3A modulated microbial composition in both the cecum and shell gland, characterized by increased abundances of beneficial bacteria such as Lactobacillus, Bifidobacterium, and Eubacterium hallii, along with reduced enrichment of inflammation-associated taxa. Collectively, these results indicate that microbiota-derived I3A alleviates oviductal inflammation and microbial dysbiosis in laying hens by modulating the gut–reproductive tract axis and TLR-mediated signaling pathways, highlighting its potential as a functional dietary metabolite for improving reproductive tract health in poultry.
Keywords: Indole-3-carboxaldehyde; salpingitis; Toll-like receptor signaling pathways; laying hens; performance

2141 Potential application of plant extracts on growth, meat quality, gut health, and blood parameters in broiler chickens

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Plant extracts can improve broiler chicken growth performance and meat quality, enhance gut health through beneficial bacteria and antimicrobial properties, and affect blood parameters. This study looked at how various plant extracts affected broiler growth performance, meat quality, gut health, and blood parameters. In a 35-day feeding trial, 500-day-old mixed-sex Arbor Acres broiler chicks were randomly assigned to five groups, each with 100 birds and five replicates per group. We prepared two experimental drinking water samples: the control group (T1) received no plant extract, only standard tap water. Birds received aqueous extract of 0.2% moringa oleifera (T2), 1% aloe vera (T3), 0.2% black cumin (T4), and 0.02% antibiotic (T5) (ciprofloxacin) in a corn-soya-based diet. At 35 days, one bird per replication was slaughtered to analyze meat quality, gut health, and blood parameters. Growth-related parameters, such as feed intake, weight gain, feed efficiency, and survivability, were recorded weekly. Compared with the control, birds given plant extracts showed better feed intake and overall performance, with the best results ($p<0.05$) observed in the 0.2% moringa oleifera-treated group. In terms of meat quality parameters, 0.2% moringa and 1% aloe vera treated groups showed significantly higher ($p<0.01$) meat color (L*, a, b) than other groups. Still, others were not statistically different ($p<0.05$). Birds offered plant extract increased ($p<0.05$) the population of Lactobacillus bacteria while reducing ($p<0.01$) pathogenic bacteria (Salmonella, E. coli) in the cecum compared to the control and antibiotic groups. Additionally, cecal histological studies showed significant ($p<0.01$) alterations, with the moringa- and aloe vera-treated groups exhibiting higher villus height, lower crypt depth, and thinner mucosa compared to the control. Furthermore, birds received 0.2% moringa and 0.2% black cumin, which showed substantial improvements in beneficial serum metabolites and liver health, along with significantly ($p<0.01$) reduced bad cholesterol compared with the control. Taken together, aqueous extract of plant compounds positively influenced broiler growth, improved meat quality, cecal histomorphology, gut microbial profile, and serum metabolites. Among the extracts evaluated in this study, 0.2% moringa oleifera demonstrated superior efficacy. Moreover, all the treated plant extracts showed potential as alternatives to antibiotics. Therefore, we recommend using 0.2% moringa aqueous extract for sustainable broiler production.
Keywords: Broiler; plant extract; growth; meat quality; gut health

2142 Sanguinarine-Based Isoquinoline Alkaloids Improve Gut Health and Growth Performance by Modulating the Bacterial–Fungal Microbiome and Its Carbohydrate-Active Enzyme Profile in Broiler Chickens

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The study evaluated the effects of dietary Isoquinoline alkaloids (IQ) supplementation on growth performance, intestinal barrier integrity, endocrine stress responses, inflammatory signaling, integrated bacteriome–mycobiome dynamics, and microbe–host interaction networks in broiler chickens. A total 400 1-day-old male Ross 308 broilers were randomly assigned to two treatmarnts, each with 8 replicates and 25 birds: 1) basal diet and 2) dasal diet suplemant IQ with 60 mg/kg. Growth parameter was recored. the FITC-d, hormone in serum and gene expresions were evaluated with HPLC and RT-PCR, respectively. Microbiome was analyzed using QIIME2 with DADA2 and Dublur, and functional potential anotated with CAZyme via dbCAN2. Results found that broilers receiving the IQ-supplemented diet exhibited significantly greater body weight and body weight gain, together with a lower feed conversion ratio than control birds ($P < 0.05$). The serum FITC-d level was markedly reduced on days 14 and 35 ($P < 0.001$),enhancing gut barrier. In parallel, corticosterone-to-serotonin ratio was significantly decreased ($P < 0.001$). IQ supplementation significantly downregulated ileal expression of pro-inflammatory mediators; IL-4, TNF-α, and NF-κB, and TLR-1, antioxidant-related signaling ($P < 0.05$). Microbial β-diversity (Bray–Curtis) revealed clear separation between dietary treatments (PERMANOVA, $P < 0.001$, Psedo-$F > 2.51$), accompanied by increased bacterial richness in IQ-fed birds. IQ supplementation enriched beneficial bacterial taxa, Bacillus subtilis, Lactobacillus aviarius, Turicibacter sanguinis, and Akkermansia muciniphila, while reducing potentially pathogenic species such as Enterococcus cecorum and Escherichia fergusoni. The fungal community showed pronounced suppression

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of mycotoxigenic fungi, particularly Aspergillus and Penicillium species. Functional profiling demonstrated enhanced microbial carbohydrate-active enzyme capacity related to lignin-like aromatic oxidation, pectin depolymerization, and cellulose/β-glucan degradation ($P < 0.05$). Integrated network analysis revealed that bacterial and fungal taxa, together with host endocrine and inflammatory biomarkers, formed a structured microbe–host stress–inflammation network, which showed reduced pathogen-centered connectivity and strengthened associations between beneficial microbes and anti-inflammatory markers in the IQ group. In conclusion, dietary IQ improves growth performance, reinforces intestinal barrier integrity, reduces systemic stress and inflammation, reshapes the bacteriome–mycobiome toward a healthier microbial profile, and reorganizes microbe–host interaction networks to favor gut homeostasis and productivity in modern broiler production systems.
Keywords: Isoquinolin alkaloid; broiler microbiome; gut health; gut bacterial-fungal network; carbohydrate-active enzyme

Innovation and Technology II

2143 Cassava peels meal in poultry nutrition: effect of some nutrient enrichment methods

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Cassava peel is an agro-waste produced in large quantity during the processing of cassava roots into various products in Nigeria. It contain substantial amount of energy and minerals, very low in crude protein (CP) and high in hydrogen cyanide (HCN) and crude fibre (CF). We examined the effect of blending ratio and ensiling time of wet cassava peels meal (WCPM) and palm kernel cake (PKC) on its physical quality, proximate composition, HCN, gross energy (GE) and feeding value for broiler chicks. WCPM and PKC blends were ensiled in black polythene bags under room temperature based on the treatments T1 (70:30), T2 (80:20) and T3 (90:10). Each treatment was analyzed for its physical qualities and proximate compositions at 0, 7 and 14days respectively. The 0 and 7days ensiled T3 were later substituted for maize at 25 and 50% levels each in starter broiler diets fed for 0 -14day of age. A 3 x 3 (3 blending ratios and 3 ensiling times) factorial arrangement and a complete randomized design were used in the experiment. Data were analyzed using R2 software. The T3 blend had significantly ($p<0.05$) higher water holding capacity (WHC) (0.91g water-1 sample) and lower bulk density (BD) (0.31 g cm-3) than T1. Ensiling decreased ($p<0.05$) the WHC value at 14 days but BD was unaffected. T3 had significantly ($p<0.05$) higher dry matter (DM) (85.02%) and nitrogen free extract (NFE) (66.85%) compared to T2 and lower crude protein (CP) (8.84%) compared to T1 and T2. Crude fibre (CF) (10.79%) and ether extract (EE), (1.17%) values were decreased ($p<0.05$) in T3 while ash (8.22%) was decreased in T1. T2 and T1 had higher ($p<0.05$) HCN (5.77mg/kg) and GE (4143.04kcal/kg) values, respectively than other treatment groups. Ensiling for 7days, decreased ($p<0.05$) its DM, CP, CF and ash but increased EE and NFE contents, while 14 days decreased ($p<0.05$) HCN and GE contents. The 0day ensiled T3 blend caused progressive increase in growth when compared with the 7 day ensiled and by 14 day, broiler chicks fed diet containing 50% 0day ensiled WCP-BCM weighed 14% more than control. With 50% 7day ensiled, broilers grew slower ($p<0.05$) but feed intake was unchanged. Feed conversion ratio and feed costs /kg gain were better ($p<0.05$) in broiler chicks fed the 50% 0day than the 7day ensiled WCP-BCM diets. At 25 and 50%, 0day ensiled WCP-BCM promoted much better growth and efficiency than 7 days ensiled and values were better than control. We conclude that blending a ratio of WCPM and PKC improved its physical and chemical properties, allowing successful use of 50% 0day ensiled WCP-BCM in starter broilers' diets.

Key words: Wet cassava peels meal, Palm kernel cake, Ensiling, Nutritive value, Broiler performance
Keywords: Wet cassava peels meal; Palm kernel cake; Ensiling; Nutritive value; Broiler performance

2144 Development of an AI Chatbot to assist Poultry producers and production specialists

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The sustainable growth of the Poultry sector requires the development of tools to increase productivity, improve animal health and enhance welfare. Mortality from diseases and poor management practices may be reduced by providing farmers and field specialists instant access to accurate information and guidance.

The development of Large Language Models (LLMs) like ChatGPT is offering tremendous opportunities to develop applications addressing instantly the concerns of producers, veterinarians, nutritionists, and poultry technicians. While off-the-shelf LLMs rely on static training data and may produce generic, outdated, or even erroneous answers, a purpose-designed poultry LLM using Retrieval-Augmented Generation will provide up-to-date information from trusted sources.

A domain-specific LLM model was developed to provide contextualized responses from codes of practice, production guides, scientific publications, and regulatory documents, fused with live domain context such as species, genetic line, age, and production phase. This poultry LLM incorporates more than 40 metadata fields (e.g., species, strain, age, environment, density) and a terminology enrichment engine with over 1,500 poultry-specific terms ensures technically accurate responses.

The system, accessible via web and mobile platforms, is available in 16 languages covering most of the global poultry production. The model was further trained to adapt its tone and response depth to different roles (farmer, veterinarian, nutritionist, supervisor), to refuse or reformulate ambiguous or out-of-scope requests, and to produce structured, data-driven answers with reasoning steps. Users can interact by typing or speaking naturally, receiving answers tailored to their role on complex questions about genetics, nutrition, welfare, health, or production performance.

The system can also analyze images of birds, lesions, or housing conditions and suggest possible health or welfare issues, assisting producers and specialists in identifying potential problems at an early stage and preventing productivity and welfare losses.

The chatbot was validated through alpha testing using expert-reviewed question sets and standardized evaluation criteria (accuracy, relevance, consistency), achieving over 90% expert agreement with reference guidelines and low hallucination rates. A beta testing program in a commercial production environment confirmed the users' engagement and willingness to integrate this tool into daily operations.

Over time, this poultry AI model will develop a deeper understanding of production challenges through daily interactions and user feedback and will assist producers and production specialists with a precision unmatched by general AI models.

Keywords: Artificial Intelligence; Large Language Model; Training; Poultry management; Education

2145 Novel 3D bioprinted eggshell membrane-based biomaterials for wound healing applications

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INTRODUCTION

Wound healing remains a significant clinical challenge, underscoring the need for advanced biomaterials that facilitate active wound repair. Eggshell membrane (ESM), rich in collagen, glycosaminoglycans, and intrinsic antimicrobial proteins, is a promising biomaterial for wound healing applications. We hypothesize that particalized ESM can be incorporated into hydrogel-based bioinks to generate 3D printed constructs with established gradients of ESM, for biocompatibility, antimicrobial activity, and structural stability. The objective of this study is to develop and optimize ESM-integrated hydrogel bioinks for 3D bioprinting of wound healing scaffolds.

MATERIALS AND METHODS

ESM particle dispersions were evaluated in water using OD600 kinetics and extrusion tests. Bioink formulations combining sodium alginate, ESM, and CaCl2 were screened to establish compositional limits of printability. Selected formulations were extruded into multilayered grids (20 x 20 x 0.5mm) using the CELLINK BIO-X6, post-crosslinked in CaCl2 for gelation, and subsequently lyophilized for 48 hours to stabilize scaffolds. The architecture and topography of the ESM-based constructs were visualized using crystal violet staining. Stability of printed constructs was further evaluated in Dulbecco's Phosphate-Buffered Saline (DPBS), DPBS with Ca2+ and Mg2+, and Simulated Wound Fluid (SWF). Wet weight loss was monitored over time to track the breakdown of the material. Group differences were analyzed using one-way ANOVA followed by Tukey post-hoc tests. The significance level for all analyses was $P < 0.05$.

RESULTS

ESM particle aggregation only observed at the highest concentration. Printable bioink formulations were identified within defined alginate/ESM/CaCl2 ranges by calculating the spread ratio to determine shape fidelity. Degradation kinetics revealed progressive breakdown, with complete construct degradation after 3 days in DPBS and 5 days in DPBS with Ca2+ and Mg2+ or SWF, suggesting suitability for short-term wound protection, with periodic reapplication required. Blank and low-concentration ESM-based scaffolds degrade more rapidly, while higher-concentration ESM-based scaffolds exhibit a slower degradation profile, suggesting stabilization at increased ESM concentrations.

CONCLUSION

Preliminary assessments have demonstrated the potential of 3D-printed ESM-based constructs as sustainable biomaterials for next-generation wound healing applications. Future work will evaluate antimicrobial activity and biocompatibility in vitro, followed by acute in vivo assessment of wound closure (Supported by NSERC alliance, Egg Farmers of Canada, and Burnbrae Farms).

Keywords: Eggshell membrane, 3D bioprinting, bioink, wound healing, skin
Keywords: Eggshell membrane; 3D bioprinting; bioink ; wound healing ; skin

2146 Development of the VisioChick Mixed Reality Glasses to simulate chicken's vision

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Chickens experience their environment through a sensory perspective that differs significantly from humans, making it a challenge for humans to easily identify or understand the problem areas in a barn that could negatively impact their behavior. Unlike humans, chickens possess tetrachromatic vision, enabling them to perceive a broader spectrum of colors, a wider field of view, and a higher flicker fusion frequency, which allows them to detect motion faster than humans. These differences mean that chicken perceive their environment different when compared to humans. Aspects of their environment that seem insignificant or invisible to humans might be highly influential to chickens. For instance, lighting conditions, color contrasts, and even minor environmental variations that go unnoticed by human eyes can greatly impact a chicken's stress level, behavior, like feather pecking and cannibalism and overall welfare. Because of these differences in visual perception, it is crucial to develop tools and technologies to bridge this gap, enabling the farmer to better monitor and manage the environments where chickens live, ensuring their needs are met more effectively.
The VisioChick project introduces a system that integrates virtual reality (VR) glasses with multiple cameras and sensors, which are permanently connected. The VR glasses are combined with real-time sensor data, resulting in an augmented reality (AR) technology by creating a hybrid environment where digital information overlays onto the physical world. This integration enables farmers, as well as veterinarians and educators amongst others, to visualize and analyze critical environmental parameters directly in the poultry barns, facilitating proactive management strategies and informed decision-making. The development of mixed reality (MR) glasses has the advantage of making the topic of animal protection and animal welfare more attractive and modern for potential target groups. The glasses serve as an advanced tool for monitoring animal welfare and facilitating innovative knowledge transfer in poultry farming. By adopting the chickens' perspective, these glasses aim to revolutionize husbandry practices, optimizing management strategies and improving overall poultry animal welfare.
Keywords: Environmental monitoring; Agricultural Education; Poultry management; Technological innovation in farming; Animal Welfare

2147 Validating Livestock Counts: Evaluation of a camera system as a useful tool in broiler farming

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In contemporary livestock farming, metrics such as individual feed and water consumption, average daily weight gain, and mortality rates are crucial for risk-oriented flock management. Accurate data on animal counts is essential for these management activities. Camera systems have been proposed to enhance accuracy in tracking bird numbers, but these systems require rigorous validation before practical implementation.

To advance the understanding of automated animal detection, dorsal images of Ross 308 broiler chickens were captured at

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Osnabrück University of Applied Sciences' teaching and research facility in 2024 and 2025. The broilers were kept in eight separate groups, each consisting of 125 birds, resulting in a final stocking density of 35 kg live weight per square meter. The barn utilized for this study featured concrete flooring complemented straw pellets for bedding. Cameras were mounted on the ceiling of the broiler house with a maximum installation height of 3.70 meters to facilitate comprehensive image capture. The collected images were subsequently analyzed using advanced image detection software provided by Wolution GmbH & Co. KG (Altenstadt, Germany).

Step 1: To assess the reliability of animal detection capabilities, a comparative analysis was conducted between software-detected animals and those identified by human observers. This analysis utilized 718 randomly selected images encompassing all life stages of the broilers (day 1 to day 38). The evaluation metrics employed included the discrepancy in the number of animals detected per image, as well as the counts of true positives, false positives and false negatives. Additionally, precision, recall and F1-score were calculated to determine the system's performance. Image annotation was performed using the open-source platform Computer Vision Annotation Tool (CVAT, Fa. CVAT.ai Corporation, Palo Alto, United States of America). To ensure consistency, the annotation process was conducted by five distinct observers. A preliminary inter-observer reliability assessment was performed, resulting in an intraclass correlation coefficient (ICC) of 0.961, with a confidence interval ranging from 0.916 to 0.983 ($p = <0.001$). The F1-score, varied according to the age of the broilers. It ranged from 0.962 to 0.985, with the lowest scores observed during days 1 to 5. In contrast, the highest scores were recorded for birds aged 16 to 20 days. Overall, the analysis revealed a significant impact of the broilers age on animal detection accuracy.

Step 2: As part of an ongoing study, the process of validating animal counting by the software is currently underway. The results are expected to be finalized and ready for presentation in due course.
Keywords: camera; animal detection; animal counting; machine learning; artificial intelligence

2148 Development of optical in-ovo sexing technologies in chickens: A 25-year evolution

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The annual culling of around seven billion day-old male layer chicks worldwide has spurred intensive research into in-ovo sexing technologies over the past 25 years. Initial methods were invasive, relying on biochemical sampling. However, optical techniques have emerged non-invasive alternatives. These advancements were accelerated by legislation, such as the prohibition of day-old chick culling in Germany in 2022, a model that was later adopted and discussed by several countries. This has driven the commercial development of high-throughput systems.

This summary examines the evolution of optical in-ovo sex determination methods, especially hyperspectral analysis, along with performance developments between optical versus biochemical methods. Categories of optical in-ovo sexing encompasses hyperspectral imaging, Raman/fluorescence spectroscopy, infrared spectroscopy, nuclear magnetic resonance imaging, and morphometric analysis.

Hyperspectral analysis identifies sexual dimorphism in feather pigmentation in brown-layer hybrids, achieving 97% accuracy between days 11-14 in literature, leading to the CHEGGY system (AAT Germany) in 2019, which operates at 20,000 – 25,000 eggs/hour/unit. Raman and fluorescence spectroscopy was able to determine sex as early as day 3.5 by analyzing hemoglobin but involves challenges with embryo welfare due to required eggshell windowing. The other methods, like Fourier-transform infrared spectroscopy, faced high hatchability losses and were abandoned. Time-resolved laser-induced fluorescence spectroscopy offers potential for pre day 6 detection, and magnetic resonance imaging with artificial intelligence providing a non-invasive gonadal visualization at day 12 with over 98% claimed accuracy but lacks adequate throughput for commercial needs and sustainability.

Optical methods are advantageous due to their non-invasiveness, rapid analysis and minimal need of consumables, but currently there is only one technique for brown layers that meets all industry requirements. The evolution of in ovo sexing techniques has not yet come to an end, as ongoing research includes advancing Raman and time-resolved fluorescence methods spectroscopy to industrial-scale reliability; improving MRI throughput while maintaining accuracy; and investigating other approaches like volatile organic compound spectroscopy showing sex-specific emissions potentially detectable from day 0.
Keywords: non-invasive in-ovo sexing technologies; spectroscopy; hyperspectral imaging; animal welfare

2149 Evaluation of eggshell-based constructs for bone regeneration in rat calvaria critical size defect model

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Hypothesis and objective: Increasing demand for bone implants has incentivized the development of novel orthopedic biomaterials. The objective of this study is to evaluate the biocompatibility, mineralization, and integration of eggshell (ES) or nanotextured eggshells (NTES)-based hydrogel scaffolds in a calvarial critical-size defect (CSD) model. These experiments will evaluate eggshell-based hydrogels as a potential low-cost, sustainable option for non-load-bearing bone repair.

Experimental design: NTES/ES hydrogel scaffolds and NTES particles are standardized, sterilized through gamma-irradiation, and implanted in a CSD model in 10–12-week-old Wistar rats for 12 weeks, followed by micro-CT and histological evaluation of the extracted bone samples.

Materials and methods: Scaffolds were formed by combining alginate, chitosan, and glutaraldehyde (± ES/NTES particles), followed by freeze-casting and lyophilization. These scaffolds underwent in vitro standardization tests, including pore size assessment through scanning electron microscopy, porosity measurement through a fluid swelling test, and particle distribution analysis by micro-CT. The scaffolds and NTES particles were sterilized (gamma irradiation, 70% ethanol) and implanted in 6-mm bilateral critical-size defects in 10-12-week-old Wistar rat calvaria. Twelve weeks post-implantation, samples will be evaluated by micro-CT to quantify bone volume, mineral density, and tissue distribution, and by histology to assess scaffold degradation, inflammatory response, vascularization, and host integration.

Statistical analysis: Scaffold in vitro properties were evaluated by a one-way ANOVA test to determine statistical significance.

Results: Hydrogels containing ES and NTES particles demonstrated higher porosity, a greater ability to retain liquid, extended stability, and improved structural integrity. Micro-CT analysis revealed no significant differences in the distribution of ES particles across scaffold layers. In vivo studies to determine the impact of ES- and NTES-based scaffold implants on calvarial healing, as compared to blank scaffolds and to empty defects, are ongoing. The presence of osteocalcin, osteopontin, and alkaline phosphatase will be exam-

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ined (immunostaining) to assess osteogenic activity, with relative bone growth quantified from micro-CT data (Dragonfly software). Conclusion: ES and NTES-based scaffolds exhibit promising structural and physicochemical properties for bone regeneration, warranting further in vivo validation as cost-effective, sustainable biomaterials for non-load-bearing bone repair (Supported by Egg Farmers of Canada).

Key words: eggshell, hydrogel-scaffold, bone-regeneration, nano-textured-particles, CSD
Keywords: eggshell; hydrogel-scaffold; bone-regeneration; nanotextured-particles; CSD

2150 Evaluation of non-contact methodologies and in-line implementation of temperature profile probe-based tools to monitor hot pellet temperature in corn-soy based diets

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Previous research has demonstrated that the heat generated during pelleting has an impact on thermo-sensitive nutrients. Even though conditioning temperatures are obtained in real-time, the frictional heat generated by the die can only be estimated using non-contact methodologies. Therefore, the objective of these experiments was to evaluate the current methodologies and novel in-line sensors to measure hot pellet temperature (HPT). In Experiment 1, a corn-soy diet conditioned at three conditioning temperatures (74°, 79°, and 85°C). Experiment 2 constituted a 2×3 factorial arrangement of 2 levels of added moisture (2 and 4%) and 3 varying moisture contents from mixer added water (0, 1, and 2%) and conditioner added steam (0, 2, 4%). In experiment 1 and 2, three non-contact methodologies: Insulated thermos, Styrofoam bucket, and Infrared (IR) gun were used. Experiment 3 constituted a 2×2×3 factorial arrangement of 2 fat levels (1 and 4%), 2 die L:D ratios (6.5 and 10), and 3 conditioning temperatures (74°, 79°, and 85°C). A temperature profile probe-based tool was used to collect HPT. In experiment 4, an Insulated thermos and a Resistance thermometer (RTD) were used to collect HPT at three conditioning temperatures (74°, 79°, and 85°C). In experiment 1 and 2, the lowest HPT was obtained with the IR gun ($P< 0.01$). In experiment 3, interactions were apparent ($P< 0.01$) for all treatments. Furthermore, in experiment 4, a rise in conditioning temperatures increased HPT by 8°C with the RTD sensor ($P< 0.01$). The results of these experiments indicated that HPT readings differed depending on the methodology used.

Keywords: thermo-sensitive; conditioning; pellet die; heat; sensor

2151 Baker's Yeast-mediated Fermentation of Banana Pseudostem Pith: A Sustainable Bioprocess for Enhancement of Feed for Poultry

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Objective

This study investigated banana pseudostem pith (BPSP) fermentation using a proprietary baker's yeast (Saccharomyces cerevisiae) as a novel bioprocess. The objective was to assess yeast-mediated fermentation for nutrient enrichment, fibre modification, antinutritional reduction, and physical quality of BPSP for sustainable poultry feeding.

Experimental Design

A completely randomized design with four treatments and three replicates was employed:

T1: Unfermented oven-dried BPSP (control)

T2: Baker's yeast-supplemented at 0.6%, oven-dried BPSP

T3: Fermented (72 h), oven-dried BPSP

T4: Baker's yeast-supplemented at 0.6%, fermentation for 72 h, oven-dried BPSP

Each replicate contained 500 g of BPSP, fermented under anaerobic, dark conditions. Post-fermentation samples were oven-dried at 60°C for 24 h.

Materials and Methods

Fresh banana pseudostem pith was cut, milled, and partially dehydrated (50% moisture) before fermentation. A proprietary baker's yeast (Instant Success®; 0.6% of BPSP) served as inoculum. Analyses followed AOAC (2010) procedures for proximate composition, fibre fractions, soluble sugars, minerals, vitamins, and phytochemicals. Physical traits: bulk density, specific gravity, and water-holding capacity were determined using standard protocols.

Statistical Analysis

All data were subjected to one-way ANOVA using SPSS v20. Treatment means were separated using Tukey's Range Test at $p < 0.05$. All analyses were performed in triplicate.

Results

Baker's yeast supplemented fermentation significantly improved banana pseudostem pith (BPSP) quality ($p < 0.05$). Crude protein increased from 7.30% to 15.18% ($p < 0.05$), while crude fibre decreased from 31.52% to 29.32% ($p < 0.05$). Antinutrients declined: tannins (201.7→90.4 mg/100 g, $p < 0.05$), oxalates (29.6→24.5 mg/100 g, $p < 0.05$), and cyanide (0.55→0.24 mg/100 g, $p < 0.05$), whereas flavonoids (139→289 mg/100 g, $p < 0.05$) and saponins (1.55→2.19%, $p < 0.05$) increased. ADF and cellulose decreased ($p < 0.05$), hemicellulose increased ($p < 0.05$), and bulk density (0.351→0.465 g/ml, $p = 0.05$) and specific gravity (0.404→0.539, $p = 0.05$) improved, while WHC dropped (655.8→249.8%, $p < 0.05$). Iron, copper, and vitamins B1 and D increased significantly, confirming improved feed quality and microbial efficiency.

Conclusion

Baker's yeast bioprocessing by fermentation effectively converted banana pseudostem pith into a nutrient-rich, safe, and functional feed ingredient for poultry. The optimized 0.6% yeast, 72 h protocol enhanced protein, phytochemicals, and mineral bioavailability while reducing antinutrients; possibly supporting and enhancing poultry feeding, waste valorization, and sustainable circular bioeconomy initiatives.

Keywords: Banana pseudostem pith; Baker's yeast fermentation; Poultry feed; Nutrient enhancement; Circular bioeconomy

2152 Modified Eggshell Membrane as a Novel Gut Health Supplement

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Introduction: The gut microbiome maintains health by regulating digestion, immune responses, and the integrity of the intestinal barrier. A novel hydrolyzed eggshell membrane formulation (HJEM) exhibits significant antioxidant, antimicrobial, and anti-inflammatory effects in vitro with colorectal cancer cells. Our objective is to evaluate HJEM as a dietary supplement for its impact on gut

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health.

Experimental design: After scaled-up production of HJEM and further validation, it will be introduced into a feed study with male C57BL/6 mice for gut response analysis. Mice will receive the processed formulation in a pudding-based diet for 4 weeks, followed by the collection of biological samples for microbiota profiling, gas chromatography, Luminex multiplex assay, quantitative PCR (qPCR), and histomorphology.

Materials and methods: Eggshell membrane was hydrolyzed with KOH, neutralized, desalted and freeze-dried. Protein content was quantified with the BCA assay, characterized by SDS-PAGE, and antioxidant activity assessed by the ABTS-based Trolox Equivalent Antioxidant Capacity (TEAC) assay.

Statistical analysis: One-way ANOVA or two-way ANOVA was used to compare the means between experimental groups, with Tukey's multiple comparison test to determine pairwise significance between means ($p<0.05$).

Results: Preliminary analysis of HJEM demonstrates 82.9% protein, with increased soluble protein from 11.5 to 21.6 mg mL-1 after hydrolysis and simulated gastric digestion. HJEM exhibited ca.15-fold higher antioxidant capacity (0.0077 mg TE mg-1 protein for supernatant of HJEM vs 0.0005 mg TE mg-1 protein for non-hydrolyzed JEM), and an 80% reduction in LPS-stimulated NO production in RAW 264.7 cells. Additionally, we continue to investigate dialysis as an optimal method for scaling up the processing of HJEM for the in vivo feeding study.

Conclusions: The optimized HJEM formulation demonstrates high solubility, anti-inflammatory properties, strong antioxidant effects, and scalability for feeding studies. By integrating biochemical characterization into the animal study, the potential of our ESM-derived formulation will be thoroughly evaluated as a sustainable supplement to maintain gut health. (Supported by Egg Farmers of Canada and Burnbrae Farms).
Keywords: gut microbiome; supplement; intestinal health; HJEM (Hydrolyzed eggshell membrane); eggshell membrane

2153 Alternative Markers to Titanium Dioxide in Broiler Digestibility Studies: Ytterbium Oxide, Acid-Insoluble Ash, Silicon, and Low-Dose Titanium Dioxide

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The index-inert marker method is essential for digestibility studies, but the new EU context for titanium dioxide (TiO_2), which limits its inclusion in broiler diets, calls for validation of alternative markers. A 21-d trial was performed to compare apparent ileal (AID) and apparent total (ATD) digestibility coefficients, using alternative markers: ytterbium oxide (Yb_2O_3; 0.010%), acid-insoluble ash (AIA; 1%), silicon (Si; from AIA), and a low-dose TiO_2 (Ti-Low; 0.0167%), to the standard TiO_2 level (Ti-Std; 0.5%) or the total excreta collection method as a reference (REF). The response parameters were AID of N, P, and Ca and ATD of ash, N, fat, P, and Ca. A total of 504 male Ross 308 broilers were housed in digestibility cages (14 birds/cage) and assigned to six dietary treatments and six replicate cages, in a randomized complete block design. Diets differed only in the marker inclusion. The REF diet had no marker while diets 2 to 6 contained Ti-Std, Ti-Low, Yb_2O_3, AIA, or Ti-Std + Yb_2O_3 + AIA (Mix), respectively. A common starter diet was fed from d0-10. Body weight gain (BWG) and feed intake (FI) were recorded from d10-21. Excreta samples were collected from d18-20 for 24-h or 7-h for the REF and marker treatments, respectively. On d21, ileal digesta samples from birds fed diets 2-6 were collected and pooled per cage. Data will be analysed by one-way ANOVA including treatment and replicate. Means will be separated using Tukey's test ($\alpha=0.05$). Outcomes will conclude whether: 1) alternative markers provide AID and ATD estimates that are comparable to the Ti-Std and REF diets; 2) recovery of Ti-Low is comparable to Ti-Std; 3) marker co-inclusion (Mix) alters digestibility vs. single markers; 4) Si-based estimate is comparable to AIA; and 5) marker inclusion affects performance. Results will be available at abstract revisions or presentation.
Keywords: Nutrient digestibility; Alternative markers; Index method; Total collection; Broilers

2154 Using artificial intelligence to predict and reduce cellulitis rates in broiler chicken farms

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In 2024, 10 million broiler chicken carcasses were rejected at the slaughterhouse due to pathological defects in Canada. 70% of these condemnations are due to cellulitis, a bacterial infection, resulting in more than 22 million Canadian dollars in lost revenue. Cellulitis is caused by Escherichia coli, which enters the skin through wounds or scratches. Several risk factors, such as environment, production, and health can affect its development in chicken barns. However, detecting and correcting these various factors to minimize the rate of cellulitis is complex, and the very short production cycle (about 30 days) leaves little time for adjustments.
Our main objective is to identify AI (Artificial Intelligence) models to predict the cellulitis rate for each production cycle based on chicken rearing conditions.
With producers' consent, we collected 2 years of retrospective environmental data (temperature, humidity, CO_2, etc.) from sensors installed in 35 broiler farms in Quebec, alongside mortality records, final total chicken weights, and final stocking density data from Éleveurs de volailles du Québec. Slaughterhouses provided the cellulitis condemnation rate as the target variable. Data was anonymized, cleaned through imputation and normalization, and integrated into a comprehensive database. Exploratory multivariate analysis (principal components, regression, clustering) identified key associations with cellulitis prevalence to select variables guiding AI model development. AI models were then trained and evaluated to predict cellulitis rates in chicken barns.
Preliminary result (excluding environmental data): Exploratory analysis results using ordinary least squares regression indicate that for each production cycle, higher final total chicken weight (kg) is significantly positively associated ($p<0.05$) with the total mortality count, while greater final stocking density (kg/m^2) is significantly negatively associated ($p<0.05$). The model explains a substantial portion of the variation in the mortality (adjusted R-squared $\approx$ 0.54) but highlights the necessity to incorporate multiple variables into a more complex model to achieve comprehensive, clinically relevant insights. Analysis is ongoing to integrate additional predictors using AI algorithms.
The multifactorial nature of risk factors in poultry barns necessitates advanced modeling beyond univariate or simple multivariate approaches. The final objective is to develop a sophisticated AI model capable of discerning complex, multivariate, nonlinear relationships among risk factors.
Based on the AI-driven model outcomes, recommendations will be formulated for early interventions in poultry production to enhance animal welfare and profitability.
Keywords: Poultry health management; Big data; Artificial Intelligence; Predictive modeling; Broiler

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Nutrition: Amino Acids and Protein II

2155 Moderated net energy and slowly digestible starch decreased fat deposition in broiler chickens regardless of dietary crude protein reductions

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One of the drawbacks of reduced crude protein (RCP) diets for broiler chickens is increased fat deposition. Reducing dietary energy and/or retarding starch digestion rate could mitigate fat deposition without compromising growth performance. This study, therefore, determined how different dietary net energy (NE) levels and starch sources impacts growth performance of broiler chickens offered RCP diets. The study involved a 2 × 2 × 2 factorial arrangement with two NE levels (standard and 75 kcal/kg NE reduction), two starch sources (maize and maize diluted with field peas to elicit rapidly and slowly digestible starch, respectively), and two CP levels (standard and 30 g/kg CP reduction). Each dietary treatment had 7 replicates with 6 birds per replicate, totalling 336 off-sex male Ross 308 broiler chickens over a study period of 11-25 and 25-35 d. Standard NE maintained during d 11-25 and 25-35 periods were 2350 and 2400 kcal/kg, while the standard CP were 205 and 190 g/kg, respectively. Results showed that reducing dietary NE reduced ($P < 0.001$) relative fat pad weights by 9.02% while feeding slowly digestible starch reduced ($P < 0.001$) fat pads by 7.58%. Similarly, total body fat deposition (354 vs 332 g/b, $P = 0.011$) and energy retained in the body as fat (1042 vs 931 kcal/kg0.75, $P < 0.001$) diminished with 75 kcal/kg NE reduction. Although, CP reduction increased ($P < 0.001$) relative fat pad by 18.97%, the RCP-Low NE-slowly digestible starch diets present numerical lower relative fat pad (12.5 vs 12.8 g/kg) compared to the standard diets. Over the whole period (d 11-35), birds offered reduced CP diets had lower WG (2367 vs 2487 g/b, $P < 0.001$), feed intakes (3771 vs 3873 g/b, $P < 0.001$) and higher FCR (1.593 vs 1.558, $P < 0.001$) compared to standard CP diets. However, NE reduction did not have any negative impacts on growth performance.

In conclusion, the study revealed that reducing dietary NE in broiler diets and/or utilizing slowly digestible starch could be viable nutritional strategies to reduce fat deposition. It is also possible to reduce dietary NE by 75 kcal/kg without compromising growth performance; this could increase profitability and sustainability of chicken-meat production.

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Keywords: Energy metabolism; low protein diet; net energy; slowly digestible starch; starch-protein digestive dynamics

2156 Low dietary crude protein in broiler breeders impaired intestinal development associated to suppressed lipid metabolism in hatchlings

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The long-term sustainability of Australian chicken meat production is challenged by the strong reliance on imported soybean meal. Implementing reduced crude-protein (CP) diets provides an opportunity to alleviate this dependency. However, the impact of reducing dietary CP in breeders on the embryonic development of the progeny remains poorly understood. This study, supported by the AgriFutures Chicken Meat Consortium (PRO-016111), investigated the effects of low CP and low soybean meal diets on embryonic development. It was hypothesized that reduced CP diets would enhance chick resilience to low CP diets by inducing adaptive responses in the digestive system.

A total of 587 eggs were collected over last 10 days from Ross 308 broiler breeders fed either 16% (high), 14% (standard) or 12% (low) CP for three weeks (36-38 weeks of age) and incubated for 21 days. At hatch, intestinal weight was recorded (n=12 per treatment). Jejunum samples from six chicks per treatment (12% and 14% CP) were collected for transcriptomics analysis. RNA extraction, library preparation, and sequencing were conducted by the Australian Genome Research Facility (AGRF Ltd., Melbourne, VIC, Australia). Differential expression between treatment groups were quantified using a generalized linear model (GLM). Differentially expressed genes (DEG) were identified at $p< 0.01$; $log2FC>0.5$ or $log2FC<-0.5$ and used for metabolic pathway enrichment analyses using DAVID database.

The results showed that day-old chicks from 12% CP-fed breeders had lighter intestines than those from 14% or 16% CP groups ($p<0.01$). Transcriptomic analysis (12% vs 14% CP) identified 400 differentially expressed genes (DEGs), with 267 downregulated and 133 upregulated in the 12% CP group. KEGG pathway analysis revealed significant effects on peroxisome ($p<0.01$; 12 genes), fatty acid degradation ($p<0.01$; 7 genes), and peroxisome proliferator-activated receptor (PPAR) signalling ($p<0.01$; 11 genes) pathways. ACSL3, ACSL5, ACAA1, ACOX1, and EHHADH were common to all three pathways. ACSL3 and ACSL5 activate long-chain fatty acids, while ACOX1, EHHADH, and ACAA1 catalyse peroxisomal β-oxidation. PPARα, a key regulator of lipid metabolism, was also significantly downregulated in progeny from low CP breeders. It can be speculated from the literature that low maternal CP may restrict amino acids required for lipoprotein synthesis. Thus, disrupted lipid transport and energy utilisation necessary for optimal intestinal development.

In conclusion, feeding broiler breeders a low CP diet (12%) reduced intestinal development and suppressed genes related to lipid metabolism in hatchlings, highlighting the risk of maternal CP reduction.

Keywords: Low protein; broiler breeder; embryonic development; transcriptomics; lipid metabolism

2157 Apparent and Standardized Ileal Digestibility of Amino Acids in Full-Fat Rice Bran Fed to Broiler Chickens

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Rice bran, a readily available byproduct of rice milling, has the potential to be used as an alternative feed ingredient for poultry. However, its effective application necessitates precise estimates of amino acid availability, as conventional measures frequently underestimate true digestibility. This study was carried out to determine the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids in full-fat rice bran (FFRB) fed to broilers. A total of 160 Ross 308 broilers (17 days old) were divided into two dietary treatments in a completely randomized design with eight replicates of ten birds each. Birds were fed either a nitrogen-free diet (NFD) to estimate basal endog-

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enous amino acid losses, or an experimental diet (ED) with rice bran as the sole protein source. Chromium oxide was used as an indigestible marker, and the ileal digesta were collected on day 24 for chemical analysis. Data were analyzed using one-way ANOVA in a completely randomized design to compare treatment effects. The results showed that AID underestimated nutrient availability when compared to SID. Crude protein had an AID of 37.3%, which rose to 42.8% after correction. Lysine digestibility increased from 35.7% (AID) to 71.7% (SID), threonine from 27.9% to 90.6%, and arginine from 46.2% to 90.1%, with cysteine having the lowest values (23.5% to 68.4%). In conclusion, accounting for endogenous amino acid losses significantly improved digestibility estimates, highlighting the importance of SID values in diet formulation. These findings demonstrate rice bran's nutritional value and suitability as a sustainable feed ingredient in broiler production.
Keywords: rice bran; amino acid digestibility; broiler chickens,; ileal digestibility ; feed evaluation

2158 Impact of hybrid rye inclusion in diets for broilers on performance, energy and nutrient availability and digesta viscosity

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Feeding rye to broilers offers potential benefits including improved nutrient utilization, enhanced gut health, and sustainable feed sourcing. Its fibre and prebiotic properties promote beneficial microbiota, while reduced feed costs and environmental impacts enhance production sustainability and profitability. The study aimed at assessing the effect of feeding eight isocaloric and iso nitrogenic diets containing 0, 10, 20, 30, 40, 50, 60, and 70% hybrid rye to male Ross 308 broiler chickens at 10d to 28d of age. All birds were reared following breeders' recommendations (Aviagen Ltd., Edinburgh, UK). During the first 10 days all birds were reared in a communal floor pen and fed the same mash starter diet. At 10 d age, a total of 288 birds were randomly allocated to 48 raised floor pens, six birds in each. All diets were presented in meal form and was fed to six pens following randomisation. Excreta were collected during the last four days of the experiment, oven dried, milled and stored before analyses on gross energy, nitrogen, fat and fibres. At the end of the study, one birds per pen was sacrificed and ileal and caecal digesta were collected and kept in freezer before analyses on viscosity and volatile fatty acids, respectively. The middle part of jejunum was also collected and stored in formaldehyde before measuring villus morphometry. Growth performance variables, including daily feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were measured from the beginning of study, at 10d old, until the end, at 28d old. Data were analysed using Genstat® (23dth Edition) statistical software (IACR Rothamsted). Comparisons among studied variables were performed by one-way ANOVA incorporating orthogonal polynomial contrasts for linear (L) and quadratic (Q) responses to rye levels in diets. In all instances, differences were reported as significant at $p < 0.05$. Diets containing 60 and 70% of rye had lower FI ($P < 0.001$) compared to the rest. Similarly, those birds had lower WG ($P < 0.001$) and utilised the feed less efficiently ($P < 0.001$) compared to diets containing up to 50% of rye. The results suggested that incorporating up to 50% rye in well balanced poultry feed may be tolerated by the birds without significantly affecting bird growth performance. Analyses on dietary metabolizable energy, nutrient retention coefficients, jejunum villus's morphometry, digesta viscosity and caecal volatile fatty acids production will follow. However, further research involving fibre degrading enzymes and/or more mature birds is warranted based on this analysis.
Keywords: Hybrid Rye; Broilers; Growth Performance; Metabolisable Energy; Viscosity

2159 Interaction between low crude protein and raised-without-antibiotics concepts on growth performance, organ weight, and feeding cost in broiler chickens.

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Low-crude-protein feed (LCP) formulations fortified with synthetic amino acids and the raised-without-antibiotics (RWA) concept are gaining traction to mitigate environmental impacts and antibiotic use in broiler chicken production. However, the interactive effects of these concepts remain unexplored. This study evaluated the interactive effects of LCP and RWA on growth performance, organ weights, and feeding costs in broiler chickens. A total of 1,200 Ross 708 male broilers were allocated to four treatments (12 replicates/treatment, 25 birds/replicate) according to a 2 × 2 factorial design. The treatments consisted of two levels of CP (high/standard breeder recommendation and low (reduced by 4%)) diets with or without antimicrobial growth promoters (AGPs). Diets were formulated in three phases: starter (days 1-10), grower (days 11-21), and finisher (days 22-35). The Conventional (CON) group (with AGP) received BMD and an ionophore, whereas the RWA (without AGP) received a blend of short- and medium-chain fatty acids, organic acids, and a polyphenol plant extract, along with a coccidia vaccine. Birds had free access to feed and water. Body weight (BW) and feed intake (FI) were recorded at the end of each phase. On day 35, 12 birds per treatment were euthanized to assess the organ weights. The data were subjected to generalized linear mixed models with fixed factors of CP, gut health-based feeding programs (the use of AGP), and their interactions. Over the 35 days, there was no interaction effect on BW or FI. Independent of the gut health program, high-CP diets had better FCR, higher breast meat yield, and lower feed cost per gain than low-CP diets ($P<0.001$). Broilers fed low CP diets, regardless of the gut health program, had heavier liver and greater abdominal fat deposition (20%) than those fed high CP diets ($P<0.001$). These findings suggest that a blend of short- and medium-chain fatty acids, organic acids, and a polyphenol plant extract can support growth to the same level as AGP, especially at reduced CP levels.
Keywords: Antimicrobial Growth Promoter; Low crude protein; Growth performance; Gut health; Broilers

2160 Dietary raw potato starch inclusion in reduced-protein diets increased body weight during acute phase of Eimeria acervulina infection and modulated gut fermentation responses in broiler chickens

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By resisting pre-cecal digestion, resistant starches can potentially alter cecal metabolite profile, to support gut health. This 35-day study investigates the growth performance, short-chain fatty acid profile, and indices of gut health responses to dietary crude protein (CP) level, resistant starch (raw potato starch, RPS), and Eimeria acervulina challenge. A total of 1,056 male broiler chicks were allocated to eight treatments (2×2×2 factorial) with six replicates per treatment. The factors were CP (standard vs. reduced level), RPS (0 vs. 40g/kg), and E. acervulina challenge (- or +). Challenged birds were orally gavaged with a solution of 50,000/ml E. acervulina on d 12. Body weight (BW) and feed intake (FI) were

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measured during pre-challenge (d 7 – 12), pre-patent (d 12 - 15), acute (d 15 - 18), recovery (d 18 - 28), and finisher (d 28 - 35) phases. Excreta oocysts shedding, plasma FITC-d, duodenal lesion score, cecal content SCFA and soluble protein concentration, and jejunal villi histomorphology were assessed on d 18. Data were analyzed as a three-factor mixed model. At the pre-challenge phase, weight gain (WG) was higher, and FCR was lower in reduced CP diet containing RPS ($P < 0.05$). During the acute phase, E. acervulina challenge significantly reduced WG, and increased FCR ($P < 0.001$) whereas diets with RPS had higher BW ($P \leq 0.05$). In addition, RPS inclusion increased ($P < 0.05$) the BW and WG during the recovery phase. No treatment effect was observed on WG, FI, and FCR during the compensatory growth phase. Overall, WG was higher and FCR was lower in the unchallenged treatment ($P < 0.001$), whereas FCR was lower in the reduced-CP diets ($P < 0.05$). On d 18, there were no treatment effect on oocysts shedding, nor on jejunal villi morphometry. However, the Eimeria challenge increased plasma FITC-d, and duodenal lesion ($P < 0.05$). The cecal content soluble protein concentration was lower ($P < 0.001$) whereas cecal acetate and propionate concentrations were greater in the challenged birds ($P < 0.05$). On d 35, the reduced CP treatment had lower acetate: propionate, and the challenged treatment had higher SCFA concentration ($P < 0.05$). In conclusion, under the conditions of this experiment, the beneficial effect of RPS inclusion on growth performance was more apparent in the early growth phase of the broiler chickens before the Eimeria acervulina challenge, whereas the modulating influences on growth performance and the cecal metabolites following the challenge was largely driven by the challenge itself although this might be dependent on the severity of the challenge.

Keywords: Reduced protein; Raw potato starch; Eimeria acervulina; gut health; SCFA profile

2161 Impact of low-protein diet and arginine levels on growth performance, carcass traits and nitrogen excretion in fast-growing broilers during the finisher phase

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Better protein utilization and precision nutrition are essential pillars for achieving more sustainable poultry production. Reducing dietary protein level in broiler diets is an effective strategy to decrease the environmental footprint, nitrogen emissions and optimizing protein efficiency. This study evaluated the impacts of low-protein diet, supplemented with arginine, during the finisher phase on growth performance, carcass traits, and nitrogen excretion of Ross 308 broilers. A total of 576-day-old male chicks were assigned to four dietary treatments with 12 replicates each, following a 3-phase feeding program: starter (0–9 days), grower (9–21 days), and finisher (21–33 days). A common feeding program was applied until 21 days. During the finisher phase, the control group (CON) received a standard diet with 19% crude protein and a digestible Arg:Lys ratio of 1.15. The three other groups (ALW, AMD, AHI) received low-protein diet containing 16% crude protein, supplemented with arginine to achieve digestible Arg:Lys ratio of 1.05, 1.10, and 1.15, respectively. Average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were measured and calculated for each phase. At 33 days, carcass and breast meat yield were assessed, and nitrogen (N) excretion was determined using a mass balance method. Data obtained were analyzed using ANOVA and means were separated via Tukey's test. No significant difference on performance was observed at 21 days of age ($p > 0.05$). During the finisher phase, no significant difference was found on ADG, while ALW and AHI groups significantly increased FI. All three low-protein diets significantly impacted FCR compared to CON group ($p < 0.0001$) with ALW group showing the greatest degradation (1.66 vs 1.60), while the AMD and AHI groups alleviated the impact (1.62 and 1.63; respectively). At 33 days, only carcass yield was significantly degraded by AHI group compared to CON group (73.7% vs 75.3%; $p = 0.016$). Breast meat yields were similar among the four groups. Low-protein diets supplemented with arginine significantly reduced nitrogen excretion (ALW: 381.61g, AMD: 374.35g, AHI: 363.64g) compared to the CON group (454.07g; $p < 0.0001$). After 21 days of age, it is possible to reduce the dietary crude protein level from 19% to 16% without impairing ADG and breast meat yield. Increasing digestible Arg:Lys ratio in low-protein diet limits the FCR degradation and maintains breast meat yield. In addition, low-protein diets supplemented with synthetic arginine effectively reduced nitrogen excretion. These findings underscore the potential of amino acid–balanced low-protein diets to improve broiler production efficiency while supporting environmental sustainability.

Keywords: Low-protein; Arginine; Nitrogen excretion; Carcass traits; Broiler

2162 In Vitro Evaluation of Pre-Cecal Digestibility and Cecal Fermentation of Protein-Rich Substitute Ingredients for South-American Soybean Meal in Broiler Chickens

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Substitution of Brazilian soybean meal (SBM) by alternative protein sources (APS) in feed formulations is a promising strategy to reduce the carbon footprint of broiler nutrition. A high pre-cecal digestibility of such APS is crucial for an optimal nutrient utilization in birds, paralleled with low protein fermentation in the ceca which is beneficial for gut health. In this experiment a static pre-cecal digestion model was combined with in vitro gas production technology (IVGPT) assays to study digestion and fermentation in the following eight APS: white lupins, faba beans, rapeseed meal (RSM), potato protein, porcine blood meal, insect larvae meal of Hermetia illucens, micro algae of Chlorella sorokiniana and single cell protein of Methylococcus capsulatus, as well as SBM as a reference ingredient. In the static digestion model, air-dried amounts (CP 5.0 g) of each APS were successively mixed with 1.75 L α-amylase solution (pH 5.8), 175 mL pepsin solution (pH 2.7) and 350 mL pancreatin solution (pH 6.5) and incubated (T 41.0°C) for 35, 35 and 170 min, respectively. Digestion was then terminated and the solution was filtered (41 µm nylon gauche) and dialyzed (MWCO 3.5 kDa) in fresh demineralized water (T 4.0°C) for a total of 19 h, after which the dialysate was freeze-dried.

In the IVGPT assays, inocula, which were prepared from cecal content of broilers fed a complete diet including each specific APS, were mixed with amounts (N = 5.0 mg) freeze-dried dialysates of the corresponding APS and gas production was monitored for 48 h. Since ammonia is an important metabolite for cecal protein fermentation, solutions were sampled after 48 h fermentation time and ammonia concentrations were analyzed. Preliminary results showed variation in coefficients of pre-cecal nitrogen digestibility (RSM = 54%, SBM = 60%, white lupins = 60%, insect meal = 60%, faba beans = 61%, blood meal = 66%, potato protein = 67%, micro algae = 74%, single cell protein = 74%) and ammonia concentrations per 5.0 mg nitrogen (single cell protein = 95 mmol/L, blood meal = 114 mmol/L, micro algae = 115 mmol/L, white lupins = 120 mmol/L, faba beans = 120 mmol/L, RSM = 130 mmol/L, insect meal = 136 mmol/L, potato protein = 138 mmol/L, SBM = 140 mmol/L). In several APS, including single cell protein and micro algae, higher pre-cecal digestibility combined with

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lower ammonia synthesis compared to SBM was observed. From these results it can be expected that inclusion of these APS in broiler diets will result in high growth and optimal gut health in birds. Results will be collected in other experiments to compare these in vitro findings with in vivo effects in Ross 308 broiler chickens.
Keywords: Alternative protein sources; Poultry; Soybean meal; In vitro; Fermentation

2163 Determination the metabolizable energy content of a high fat, Lesser mealworm larvae meal with broiler chickens at different inclusion rates

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Insect proteins can be used successfully both in human and animal nutrition. However, their composition is variable, and not all the factors are known that potentially limit their inclusion rates. In this study the metabolizable energy (ME) content of an insect larvae meal product (AdalbaPro), was determined with broiler chickens. The product was a Lesser mealworm larvae meal (Alphitobius diaperinus meal, ADM), containing 58% crude protein, 28% crude fat, 6.6 % chitin and 26 MJ/kg gross energy. Beside a control diet (C), five other diets were used, containing the ADM at 5, 10, 15, 20 and 25%. TiO2, as an inert marker was used in order to calculate the metabolizable energy content of the diets. A total of 48, 42-day-old male broilers (Ross 308) were placed into individual balance cages, and all the experimental diets were fed in 8 replicates. After 3 days adaptation period in the subsequent 2 days representative excreta samples were collected. After the measurement of the gross energy, nitrogen and TiO2 contents of the diets and excreta samples ME content of the diets were calculated. The ME content of ADM was calculated with the difference method and also by the regression method. The ME contents of ADM at the different inclusion rates were compared with one way ANOVA, using Tukey multiple range test. In the case of regression method linear and quadratic regression and was used. The AMEn content of the test diets increased in line with increased incorporation of ADM from 13.55 MJ/kg (diet C) to 14.53 MJ/kg (diet ADM25%). The AMEn content of the larvae meal was around 23 MJ/kg at 5, 10 and 15% inclusions, but declined in tendency at 20% and significantly at 25% incorporation rates. The response of AMEn to the increasing inclusion rates was not linear. The r2 value of the linear regression equation was only 0.597, but if only the first three inclusion rates were evaluated the accuracy of the linear regression equation improved (r2 = 0.994). The quadratic response was more accurate with an r2 value of 0.967, meaning that above 20% inclusion rate ADM impaired the absorption of the energy yielding nutrients. The reason of the response could be the increasing chitin content of the diets but needs further investigation. In conclusion, the ME content of the investigated, high fat ADM cannot be considered as constant.
Keywords: insect larvae meal; broiler chicken; metabolizable energy

2164 Feeding Smarter, Not Richer: Impact of lower metabolizable energy and/or balanced protein on growth and carcass yield in slower-growing chickens

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Improving nutrient efficiency is key to reducing the environmental footprint of slow-growing broiler production. This study investigated the combined effects of lowering dietary metabolizable energy (ME) and/or balanced protein (BP) levels on growth performance, nitrogen excretion, and carcass traits in slower-growing chickens. A total of 1,008 male Rustic Gold day-old broilers were distributed among 48 pens of similar weight at day 0 and randomly assigned to one of eight dietary treatments (21 chickens/pen, 6 pens/treatment). Treatments consisted of two ME levels (100 or 98 % of Ross 308 recommendations) and four BP levels (100 %, 95 %, 90 %, 85 % of Ross 308 recommended CP and digestible AA composition) in a 2×4 factorial arrangement. During the 42-day experimental period, birds were fed a starter (day 0-10), grower 1 (day 10-21), grower 2 (day 21-29) and a finisher diet (day 29-42). Growth performance (average daily gain, feed conversion ratio), and carcass yield were measured. Data were analysed using two-way ANOVA with significance level at $P < 0.05$.
The 2 %-point reduction in ME did not affect growth performance. Reducing BP to 95 % of the recommendation maintained ($P > 0.05$) a comparable growth performance to the 100 % BP group: 60.87 vs. 62.73 g/d for overall average daily gain and 1.426 vs. 1.400 for feed conversion ratio. However, further reductions to 90 % and 85 % BP resulted in reduced ($P < 0.001$) daily weight gain to 58.04 and 53.13 g/d, respectively, and impaired ($P < 0.001$) feed conversion ratio (1.496 and 1.554, respectively). Carcass weight was decreased ($P< 0.001$), but carcass yield was unaffected ($P > 0.05$) by reducing BP levels. However, an effect of the Balanced Protein × Energy interaction ($P = 0.04$) was observed, driven mainly by lower values in chickens fed 90% BP with 100% energy and 85% BP with 98% energy compared with other combinations. In addition, breast yield decreased ($P < 0.01$) as BP was reduced but chickens fed 95% BP did not significantly differ from 100 % BP (30.27 % and 31.01 %, respectively).
A 5 %-point reduction in dietary BP combined with a 2 %-point reduction in ME maintained growth performance and carcass yield, which can potentially lower production costs. These findings show that reducing ME and BP levels is a feasible nutritional strategy for improving sustainability, without compromising feed efficiency and growth of slower-growing broilers.
Keywords: Slower growing broiler; Balanced protein; Metabolizable energy; Performance; Sustainability

2165 Tiny cells, big impact: Replacing soybean meal with bacterial protein in broiler diets

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Bacterial single-cell protein (BSCP) derived from Methylococcus capsulatus is a high-protein feed ingredient, with potential to replace soybean meal (SBM) in broiler diets. We investigated whether the inclusion of BSCP could replace SBM in broiler chicken stages and whether prior adaptation to BSCP could enhance their performance. A total of 480 Ross 308 chicks were randomly assigned to 30 pens and raised for 35 days. In the starter phase, birds received either a BSCP diet (3.125%) or an SBM-based control diet. In the grower and finisher phases, both starter groups were fed either low (6.25%) or high (12.5%) BSCP, along with an SBM-based diet as control. Performance was recorded regularly, while uric acid concentration (UAC) in excreta was analysed by HPLC and ileal amino acid transporter gene expression by qPCR. Data were analysed in R using linear mixed-effects model for growth performance and one-way ANOVA for other parameters, under a 2x2 factorial design and contrasted against the control diet. In the starter phase, Average daily gain (ADG) and Average daily feed intake (ADFI) were similar between SBM and BSCP

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treatment, though FCR (p=0.07) tended to be lower on the SBM diet (1.19 vs 1.23; SEM=0.01). During the grower and finisher phases, there were no significant effects (p>0.05) on ADG, ADFI and FCR. Orthogonal contrasts indicated higher ADG (67.5 vs 64.1g/bird/d; p=0.002) and a tendency for improved FCR (1.31 vs 1.37; p=0.08) in SBM diet during grower phase, and higher ADFI (160.4 vs 152.3g/bird/d; p=0.08) in the finisher phase. UAC across BSCP treatment at starter period was significantly higher (p=0.03) than the SBM group (29.8 vs 21.9 mg/g; SEM=2.45). No significant main or interaction effects were found on UAC in the other phases, suggesting subsequent bird adaptation. During the grower phase, only Aminopeptidase-N (APN) showed an interaction effect (p=0.03), due to its higher expression in birds on high BSCP diet. Other transporter genes were downregulated (p<0.05) compared to the control group, except for BOAT1, EAAT3 and LAT1, suggesting a compensatory regulation of amino acid transporter expression in response to BSCP inclusion. In the finisher phase, APN, CAT2, γ+LAT1, LAT1, and EAAT3 showed interaction effects, with the lowest expression observed in birds that had not previously received BSCP, compared to the SBM diet. Overall, these findings point towards bird adaptation to increasing BSCP levels across the phases, supporting BSCP as a sustainable replacement for SBM at higher inclusion levels in broiler diets at later growth stages.

Keywords: Alternative Protein; Bacterial single-cell protein; Broiler; Performance; Soybean meal

2166 Effects of metabolizable energy and amino acid density interaction on breast muscle myopathies and blood chemistry in Ross-708 broilers during finisher phase (29-42 d)

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Optimizing dietary metabolizable energy (ME) and amino acid (AA) density is important for growth performance in broilers, and could be associated with breast muscle myopathies, including wooden breast (WB), white striping (WS), spaghetti muscle (SM), and blood chemistry (blood glucose and total protein). The aim of the current experiment was to evaluate the effect of various levels of ME and AA on breast muscle myopathies and the blood chemistry of Ross-708 broilers. A total of 1,800 Ross-708 broilers were raised in 72 pens with a common starter (0-14 d) and grower (15-28 d) diet. For the experimental finisher period (29-42 d), birds were redistributed based on body weight with 10 males + 10 females/pen. A central composite design was employed, comprising 13 runs with 8-star points (total = 48 replicates) and 5 central points (total = 24 replicates). Nine different treatments were comprised of five different ME levels (2879, 2943, 3100, 3256, and 3320 kcal/kg) and five different digestible lysine levels (0.95, 0.98, 1.05, 1.12, and 1.15%). At day 42, blood was collected from one male and one female bird/pen for glucose and total protein levels. At day 43, 4 male and 4 female birds /pen were slaughtered for WB, WS, and SM scoring. The data were analyzed using the response surface macro-option of JMP Pro 17. The model for blood glucose (mmol/L) and total protein (g/L) was non-significant, regardless of sex (P > 0.05), suggesting that these variables remained unaffected by dietary treatments. In general, WB responded linearly to both ME (P=0.0151) and AA (P=0.0136). WS showed a significant ME x AA interaction (P=0.0019), while SM exhibited an increasing quadratic response to ME (P=0.0046) only. Interestingly, male and female broilers exhibited different patterns for breast muscle myopathies. For males, only WB showed a significant result, with a linear effect for ME (P=0.0291) and AA (P=0.0064). However, the models for WS (P=0.1563) and SM (P=0.3881) for male broilers were found to be non-significant. Contrarily, female broilers exhibited a significant result for SM with an increasing quadratic effect for ME (P=0.0173) and AA (P=0.0178); however, the model was non-significant for WB (P=0.2286) and WS (P=0.1095). In general, ME and AA levels influenced breast muscle myopathies through linear, quadratic, and interaction effects, but the patterns varied by sex. Males showed a linear ME and AA response for WB, whereas females showed a quadratic ME and AA response for SM.

Keywords: Amino Acids; Blood Chemistry; Broilers; Metabolizable Energy; Muscle Myopathies

Nutrition: Feed Additives II

2167 EFFECT OF AN ESSENTIAL OIL BLEND ON BROILER PERFORMANCE: RESULTS OF A POOLED ANOVA ANALYSIS

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SupplantTM D is an encapsulated combination of three purified phytogenic essential oils formulated to maintain gut health and improve feed efficiency, thereby promoting gut homeostasis and nutrient absorption. These essential oils are known for their antimicrobial and anti-inflammatory properties, which can modulate gut microbiota, reduce oxidative damage, and improve digestive efficiency. To evaluate its efficacy under varied production conditions, a pooled analysis was conducted using six independent trials performed in diverse environments: USA (3), Thailand (1), Spain (1), and Poland (1). Trials included different genetic strains and management systems, reflecting commercial variability. Birds received SupplantTM D at 150 ppm during the starter phase and either 150 or 100 ppm during grower and finisher phases, with 13–18 replicates per treatment. Performance indicators included feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). Data were analyzed using pooled one-way ANOVA. SupplantTM D improved FCR in 100% of trials, with significant reductions during the grower (-5 points; P < 0.001) and finisher phases (-5 points; P < 0.001), and an overall improvement of -4.1 points across the full cycle (P < 0.001). A numerical increase in BWG (+17 g) was observed, while FI remained unaffected. These consistent improvements across multiple climates, genetic lines, and management practices highlight the robustness of SupplantTM D as a practical solution for optimizing broiler performance. By combining targeted phytogenic compounds with controlled-release encapsulation, SupplantTM D ensures stability and gradual delivery throughout the gastrointestinal tract, enhancing feed efficiency and reducing resource use. This approach aligns with industry goals for sustainable poultry production by improving performance without reliance on antibiotic growth promoters, supporting both economic viability and consumer expectations for natural solutions.

Keywords: Broilers; Essential Oils; Functional Ingredients; feed efficiency ; pooled analysis

2168 Palm kernel fatty acid improves growth performance of broiler chickens under necrotic enteritis challenge

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Controlling enteric diseases of broiler chickens is crucial for maintaining productivity and profitability. Necrotic enteritis (NE), an enteric bacterial disease caused by Clostridium perfringens, remains a major challenge, leading to global economic losses exceeding $6 billion annually. Organic acids and their blends, such as short- and medium-chain fatty acids (MCFAs), are gaining attention for combating diseases and improving growth performance in the post-antibiotic era. Palm kernel fatty acids (PKFA, Grolux Lauri, Cargill), a natural source of MCFAs rich in lauric acid (C12:0), have shown potential to enhance performance and gut health in broilers. This study evaluated the efficacy of PKFA under a subclinical NE challenge. A total of 390-day-old male chicks were randomly allocated to 30 floor pens, with five treatments replicated six times and 13 birds per pen. The treatments were: NC (non-challenged control), CC (challenged control), MB (CC plus Maxiban), PKFA (CC plus PKFA), and MB+PKFA (CC plus MB+PKFA). Birds were fed a wheat-corn-soybean meal-sorghum-based diet supplemented with xylanase and phytase. The PKFA were added at 1% during the starter and 2% during the grower, replacing canola oil in the basal diet, while MB was added at 0.05%. The NE challenge was induced by oral gavage of Eimeria spp. on d14 and NetB-producing C. perfringens on d19. Performance data were analysed using one-way ANOVA with Tukey's test, while gut lesion scores were analysed using the non-parametric test. Results showed that, during NE challenge (d10-21) and overall period (d0-32), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were compromised by the NE challenge, with increased intestinal lesions on d21 in the CC group than the NC group ($P < 0.05$), indicating a successful subclinical NE challenge of the birds. Birds fed MB had higher WG and FI, and lower FCR and jejunal lesions than the CC group ($P < 0.05$). During the NE challenge period (d10-21) and the overall period (d0-32), birds fed PKFA had higher WG, FI, and lower FCR compared to the CC group ($P < 0.05$). Over the overall period, WG of birds fed PKFA did not differ significantly from those in the NC or MB groups. However, combined supplementation with MB+PKFA did not provide additional synergistic benefits compared to MB alone. These findings suggest the potential of PKFA supplementation in diets to improve the performance of birds under NE, as indicated by improved WG, FI, and FCR. However, the protective effect of PKFA may not be apparent when MB is present in the feed. Further studies are warranted to elucidate the mechanisms by which PKFA improves performance and modulates gut microbiota, intestinal integrity, and gene expression.

Keywords: Palm kernel fatty acid; performance; intestinal lesion; necrotic enteritis; broiler chicken

2169 Comparison of the Potential of Phytobiotic Substances: Driving for Safe and Sustainable Broiler Production

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In recent years, due to growing global concern over antibiotic resistance and residues, the demand for antibiotic free safe and high quality poultry products has prompted to the traditional broiler production system. Therefore, this study was carried out to examine the potentiality of different phytobiotic substances as alternative to sub-therapeutic antibiotic application for growth performance, carcass, meat quality and serum biochemical indices in broiler chickens fed corn-sybean based diet. In total, 288 one-day old Lohman broiler chicks were randomly allotted into six dietary treatments with four replicates and twelve chicks in each. The six dietary treatments were: control group (basal diet), basal diet supplemented with 0.35 g/kg-1 antibiotic (oxytetracycline), 10 g/kg-1 mushroom, 10 g/kg-1 spirulina, 10 g/kg-1 methi, 10 g g/kg-1 moringa leaf powder in diet, respectively. The growth performance, carcass characteristics, meat and serum quality were measured for 35 days. Data were analyzed using R software. Broiler chickens fed methi diet showed significantly greater ($P<0.01$) body weight (BW) and average daily gain (ADG) compared to dietary treatments on 35 days. The least BW and ADG in control and spirulina fed group had found, however, statistically had no difference in antibiotic, mushroom and moringa treated group. Among the dietary treatments feed conversion ratio (FCR) was significantly differed, with improved FCR observed in methi fed group. Dietary treatments did not affect the dressed weight, neck, breast muscle, liver, kidney, gizzard, abdominal fat and meat quality in terms of breast meat color, drip and cooking loss. However, serum high-density lipoprotein and albumin were to be higher in phytobiotic treated groups while total cholesterol was lowered ($P<0.01$) compare to the control. The finding of the study indicated that among the phytobiotics susbtances, methi can be an effective alternative to antibiotics for promoting growth, improving feed efficiency of broiler chicken for demand-driven safe broiler production.

Keywords: Broiler chicken; Growth performance; Carcass characteristics; Meat quality; Serum biochemical

2170 EFFECT OF L-CARNITINE ON EGG PRODUCTION AND QUALITY OF LOHMANN BROWN LAYING HENS STORED AT DIFFERENT TEMPERATURES

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EFFECT OF L-CARNITINE ON EGG PRODUCTION AND QUALITY OF LOHMANN BROWN LAYING HENS STORED AT DIFFERENT TEMPERATURES

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Abstract

Background

L-Carnitine is an amino-acid derivative known for its essential role in lipid metabolism and energy production through the transport of long-chain fatty acids into mitochondria. Although supplementation of L-Carnitine has been associated with improved growth and egg quality in poultry, results remain inconsistent. This study aimed to evaluate the effect of different dietary levels of L-Carnitine on egg production performance, internal egg quality, and shelf life of Lohmann Brown laying hens.

Methodology

A total of forty-five hens aged 64–76 weeks were assigned to three dietary treatments consisting of 0 mg/kg (control), 100 mg/kg, and 150 mg/kg L-Carnitine supplementation levels. The experiment followed a completely randomized design with five replicates per treatment. Secondary data collected from a previously approved study (Ref: EC027-12, University of Pretoria, 2012–2013) were analysed using the repeated-measures procedure of SAS (Version 9.4). Mean separation was performed using Fisher's LSD

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test at P < 0.05. Eggs were collected daily for four weeks, stored under cold and room-temperature conditions, and assessed for egg weight, albumen height, albumen pH, and related quality parameters.
Results
Supplementation of L-Carnitine at 100 mg/kg and 150 mg/kg had no significant effects (p>0.05) on egg production rate, egg weight, albumen quality, shell characteristics, or egg pH during storage. Although previous studies reported improved albumen quality and yolk modifications with L-Carnitine inclusion, these responses were not observed in the current trial. The lack of significant effects could be attributed to factors such as bird age, diet composition, or environmental conditions.
Conclusion
Dietary supplementation with L-Carnitine at the tested levels did not enhance egg production, internal quality, or shelf life in late-lay Lohmann Brown hens. Future research should investigate higher inclusion levels, different layer strains, and earlier production stages to better elucidate the potential role of L-Carnitine in improving egg quality and storage stability.
Keywords: L-Carnitine, laying hens, egg quality, albumen height, shelf life
Keywords: L-Carnitine; Laying hens; egg quality; Albumen height; Shelf life

2171 The application of a saponin-aluminosilicate blend in vaccinated broilers subjected to a strong enteric challenge
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In broilers, coccidiosis leads to impaired nutrient digestion and absorption, compromised well-being and growth performance, and is often a predisposing factor to secondary diseases such as necrotic enteritis caused by Clostridium perfringens. Globally, the use of natural feed additives has gained much attention as an alternative to support coccidiosis management strategies in broilers.
The aim of this trial was to determine the effectiveness of supplementing a blend of Quillaja saponaria extract (a source of triterpenoid saponins) and activated aluminosilicate on top of a coccidiosis vaccine to reduce the negative effects of an Eimeria challenge in broilers during a rearing period of 42 days.
A total 1500 one-day-old Ross 308 male broilers were divided over 5 treatments, each with 12 replicate floor pens: unchallenged control housed on fresh shavings (UC); Eimeria challenge without coccidia vaccine (CC); Eimeria challenge with coccidia vaccine (CV); Eimeria challenge with saponin-aluminosilicate blend (Excential Sapphire Q, Orffa Additives B.V.) supplemented in the grower and finisher diet (200 ppm) + coccidia vaccine (SQ GF); and saponin-aluminosilicate blend supplemented in all feeding phases (200 ppm) + coccidia vaccine (SQ ALL). All groups except UC were placed on reused litter which contained C. perfringens from a previous trial. Vaccinated broilers received a commercial coccidiosis vaccine prior to placement. Challenged treatments received ~100,000 E. acervulina, ~50,000 E. maxima, and ~75,000 E. tenella oocysts per bird on day 22. At day 28, lesion scores were evaluated in 3 birds per replicate, and blood was collected for FITC-d analysis. Excreta samples were collected on days 14, 21, 28, and 29 to measure the number of oocysts shed per gram of excreta (OPG). Birds and feed were weighed on days 0, 14, 28, and 42 to calculate performance metrics. Results were analyzed using ANOVA and means were separated using LSD procedure at a 95% confidence interval.
Overall, broilers from SQ ALL had a significantly greater body weight gain than broilers from CC and CV (P<0.05) and a numerically higher body gain than SQ GF. FCR of broilers from SQ ALL was improved by 4 points compared to CC (P<0.05) and by 2 points compared to CV and SQ GF. Feed intake was higher for UC than the challenged groups (P<0.05). Vaccinated groups had lower OPG, intestinal lesions, and serum FITC-d compared to CC (P<0.05).
The inclusion of the saponin-aluminosilicate blend in the starter phase did not have a negative impact on the coccidiosis vaccine. The performance outcomes of the current study support the use of the saponin-aluminosilicate blend with a coccidiosis vaccine as part of an enteric health strategy in broiler chickens.
Keywords: Phytogenic; Saponins; Clay; Coccidiosis; Intestinal health

2172 Use of activated diatoms, pronutrients and phenolic antimicrobial molecules for preventing intestinal challenges in broiler chickens
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In recent years, due to genetic selection and productive pressure in poultry, intestinal passage rate is becoming higher, with a negative impact on the gut mucosa integrity and on the microbiota balance. Due to that, several enteric disorders appear in poultry farms, affecting health and performance. Solutions to mitigate this issue are needed so a new technology was developed for this, and it was tested in vivo to assess its efficacy in broiler chickens. A trial was conducted for 42 days to evaluate the efficacy of activated diatoms, pronutrients and phenolic antimicrobial molecules effectiveness to prevent enteric diseases. 400 broilers were distributed into 4 treatments groups with 5 replicate pens each. Treatments were a standard basal diet (SBD) as a Negative Control (NC); a SBD challenged with soybean 3% (SBDC) as a Positive Control (PC); a SBDC with activated diatoms, pronutrients and phenolic antimicrobial molecules at 0.5 Kg/t (SBDC+); a SBDC with Halquinol at 100 g/t (SBDC-). The inclusion of soybean 3% in the diet was due to it contains ANFs and cause intestinal dysbiosis. Statistical analysis was carried out using SAS 9.4 to determine normality and homogeneity of variances. Normality test of the variables was performed using Shapiro Wilk normality test with a significance level of 5%. Analysis of variance, using the Tukey test.
Intestinal retention time was higher in SBDC+ (P<0.05) group of 20 minutes and 24 minutes on average compared to PC and SBDC- groups, respectively. SBDC+ group showed a slightly increase in the digestibility of protein (+ 2%) (P<0.05) compared to PC. Body weight (BW), body weight gain (BWG) and feed conversion (FCR) were significantly better in SBDC+ group than PC and SBDC- (P<0.05). Mortality was significantly lower in SBDC+ group (0%) (P<0.0001) compared to the others. Litter humidity was significantly higher in SBDC+ group (P<0.05) than PC and SBDC- and dirty cloaca was lower in SBDC+ than the others (P<0.0001). Levels E. coli were lower (P<0.0001) in SBDC+ and levels of Lactobacillus were higher (P<0.0001) in SBDC+.
In conclusion, the mentioned active ingredient is effective to prevent gut challenges in broilers improving performance and reducing mortality and pathogen counts.
Keywords: Pronutrients; gut health; gut physiology; feed additives; transit rapid

2173 Enhancing egg yolk quality through the use of moringa oleifera leaves and parkia biglobosa pulp as feed additives in semi-intensive poultry farming in Burkina Faso
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Objective

This study aimed to evaluate the effects of Moringa oleifera leaves and Parkia biglobosa pulp powder used as natural feed additives on egg quality and production performance in Isa Brown layers.

Experimental design

Isa Brown pullets were allocated to 3 groups of 120 birds, corresponding to three dietary treatments, each with four replicates of 30 birds, in a randomized block design. Three iso-protein and iso-energy diets were formulated:

R1: basal diet + 2% Moringa oleifera leaf powder

R2: control diet (no additive)

R3: basal diet + 2% Parkia biglobosa pulp powder

Materials and methods

The first phase consisted of egg collection from farms, restaurants, and retail shops in urban and peri-urban areas of Bobo-Dioulasso, covering seven districts, to assess yolk color. A total of 210 eggs were collected. Yolk color was assessed using the Roche Yolk Color Fan.

The second phase was carried out at Nazi Boni University's animal facility in Nasso village (11°12′ N, 4°26′ W), northwest of Bobo-Dioulasso. A total of 360 Isa Brown pullets (10 weeks old) were randomly assigned to three dietary treatments, each with four replicates of 30 birds.

Data were collected over a 3-month period (February–April), corresponding to the hot dry season.

Statistical analysis

Data were analyzed using MINITAB version 17. General Linear Model Procedure was used in ANOVA to test for treatment effects, and means were separated using Tukey test at 5% significance level.

Results

Yolk color grades 4, 5, and 6 (on the Roche color fan, scale 1–16) were predominant in collected eggs (16.2%, 47.6%, and 27.6%, respectively), indicating poor pigmentation and low vitamin A content. Feed intake was significantly higher ($P < 0.05$) in R1 (70.3 ± 13 g/bird/day) than in R2 (66.1 ± 15 g/bird/day) and R3 (67.5 ± 17 g/bird/day). The R1 group also recorded the highest average daily gain (12.5 ± 3 g/bird/day), and the darker yolk color (grade 7), followed by R3 (grade 5).

Conclusions

This study demonstrates that Moringa oleifera leaves and Parkia biglobosa pulp can serve as natural feed additives to improve yolk pigmentation and overall production performances under semi-intensive production systems in Burkina Faso.

Keywords: Moringa oleifera; Parkia biglobosa; yolk color,; Isa Brown; Burkina Faso

2174 A microencapsulated blend of botanicals and organic acids improves the growth performance and intestinal morphology of broilers in commercial conditions.

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Botanicals and organic acids are widely used in poultry nutrition for their multiple properties (antimicrobial, anti-oxidant, anti-inflammatory) that can help to support growth and health of birds. The aim of the study was to evaluate the dietary supplementation with a proprietary combination of botanicals and organic acids, microencapsulated in a lipid matrix (AviPlus®P) on growth performance and intestinal morphology of broilers in field conditions. The trial was conducted in a commercial farm with a total of 2,218,000 day-old male chicks (Cobb 500/Ross 308) divided in 38 houses (around 58,000 chicks/house at a density of 24 birds/m2) and assigned to 2 dietary groups (19 houses each, around 1,109,000 birds/treatment): control group (CON), fed a basal diet or treated group (TRT), fed the same basal diet supplemented with AviPlus®P at 400-300-200 g/MT of feed in starter (d0-15), grower (d15-30) and finisher (d30-end), respectively.

Necropsies were performed at day 18 and 44 (5 birds from 2 houses/group, n=10) and intestinal samples from duodenum and jejunum were collected for histology analysis (villi length measurement). Data were analyzed with T test (using GraphPad Prism 10) and differences considered significant at $P<0.05$. Growth performance were recorded until birds collection for slaughtering around day 44.

Compared to control, the inclusion of AviPlus®P allowed to improve growth performance in terms of average final body weight (+47 grams, 2,347 vs 2,300 grams total) and average weight produced (+1.26 kg/m2, 56.56 vs 55.30 kg/m2).

Also, TRT group slightly improved FCR (1.71 vs 1.72) and EPEF (346 vs 338) [European Production Efficiency Factor EPEF = (Final body weight*liveability*100)/(days*FCR)].

Regarding histology, no significant differences were observed in villi length between the two groups in duodenum or jejunum at day 18. Conversely, at day 44 group TRT had significantly longer villi than CON group both in duodenum and jejunum ($p<0.02$).

In this trial, the botanicals and organic acids-based feed additive was used in a large commercial setting, allowing an improvement of production yield and intestinal morphology. To conclude, the supplementation with AviPlus®P is an effective dietary tool to sustain growth performance of broilers in field conditions while supporting gut health.

Keywords: Botanicals and organic acids; microencapsulation; field; broilers; gut health

2175 Effect of double buffered sodium butyrate on nutrient digestibility, digestive organ proportions, intestinal histomorphology and ceca SCFAs of layers fed with a low energy and protein diet

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Sodium butyrate associated with buffer salts (DBSB) improved in broilers nutrient digestibility and intestinal histomorphology. However, there are very few studies in laying hens using DBSB. The objective of this trial was to evaluate if DBSB improves energy and nutrient digestibility, gut health and digestive organ proportions in layers using low levels of energy and protein to fed them. A total of 256 Hy-line laying hens of 50 weeks old were distributed following a completely randomized design for 4 treatments (8 replicates of 8 birds) for 84 days. Control group was fed following industrial energy and nutrient levels (CTRL) and DBSB group received the CTRL feed supplemented with 600 g/MT of DBSB (Butylin 54, Dietaxion SAS). LEP group was fed with diets reduced 1,5% in metabolizable energy, crude protein (CP) and main digestible amino acids (Lys, Met+Cys, Thr). LEP-DBSB birds were supplemented with 600 g/MT of DBSB. Traits measured at the end of the experiment were digestibility of dry matter (DM), gross energy (GE), CP and mineral matter (MM), followed by the random slaughtered of one bird per replication to measure digestive organ weights (proventriculus, gizzard, pancreas, small intestine, large intestine), histomorphology of duodenum, jejunum and ileum (villi length-VL and crypt depth-CD), and SCFAs (short chain fatty acids) level in the cecal content (acetate-AA, propionate-PA and butyrate-BA). Data were analyzed using ANOVA followed by Tukey tests. Digestibility of DM, GE and CP was im-

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proved using DBSB (P<0.05), but every digestibility trait was significantly reduced in LEP diet compared to CTRL (P<0.05). DBSB improved gizzard and pancreas proportion (P<0.05), while LEP obtained for all the digestive organs lower proportions compared to CTRL (P<0.05). Although DBSB was not different to CTRL for histomorphological traits (P>0.05), DBSB numerically increased VL, reduced CD and increased the ratio VL/CD in duodenum, jejunum and ileum. The reduction of energy and protein in the diet improved VL/CD in comparison with CTRL at every part of intestines (P<0.05). Regarding the cecal content of SCFAs, results were different for AA and the other two because an interaction was found for PA and BA (P<0.05). For AA results were better using DBSB (P<0.05) and there were no differences between nutritional levels (P>0.05). PA and BA levels were improved using DBSB in the LEP diet (P<0.05). Supplementing 50-62 weeks old laying hens with 600 g/MT of DBSB improved the digestibility of CP, GE and DM, the proportion of gizzard and pancreas, independently of metabolizable energy and CP level, and alleviates the negative impact of a reduced energy protein diet on the propionic and butyric acids ceca levels.

Keywords: sodium butyrate; laying hens; digestibility; SCFAs; digestive organ proportion

2176 Effects of a novel Bacillus-based probiotic blend on growth performance, intestinal morphology, and physiological responses in densely stocked broilers

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Bacillus subtilis probiotic strains are emerging as promising natural feed additives to replace antibiotic growth promoters (AGP) in poultry nutrition. However, there is still limited research on the most effective combinations and dosages of B. subtilis probiotic strains. This study, therefore, evaluated the impact of incremental levels of a B. subtilis probiotic mixture as a replacement for AGP in densely stocked broiler chickens. In a completely randomized design, 900-day-old Ross 308 broiler chicks (43.2 ± 0.37 g liveweights) were distributed evenly into 36 pens (13 birds / 0.75 square-meters), to which six experimental diets were randomly assigned. The experimental diets, provided from the starter through to the finisher phase, comprised: 1. a basal broiler diet without AGP or B. subtilis probiotic mixture (NEGC); 2. the basal diet supplemented with 0.5% (w/w) zinc bacitracin (POSC); and four versions of the basal diet supplemented with B. subtilis probiotic mixture at 0.3 (0.3%PRB), 0.4 (0.4%PRB), 0.5 (0.5%PRB), or 0.6% (0.6%PRB). Data were analysed using PROC GLM in SAS version 9.4 with the pen designated as the experimental unit, and significance declared at P < 0.05 using Tukey HSD test. Polynomial regression analysis was also used to evaluate the data for linear and quadratic effects (P < 0.05). A quadratic effect was noted in the feed intake during the starter phase. The 0.5%PRB treatment improved body weight gain and feed efficiency (P < 0.05) whereas 0.3%PRB increased feed intake and reduced body weight gain. Changes in haemato-biochemical parameters, including increased neutrophils and white blood cells indicate reduced stress and inflammation in broilers fed 0.3%PRB, 0.4%PRB and 0.5%PRB. Carcass traits were statistically unaffected (P > 0.05). The relative duodenal weight and jejunal length were influenced (P < 0.05) with NEGC and POSC promoting the highest weight and 0.6%PRB promoting the longest jejunum. Breast muscles from broilers fed probiotic supplemented diets showed statistically similar results to POSC and NEGC group among meat quality attributes. The inclusion of B. subtilis probiotic mixture in broiler diet had no effects jejunum morphometry, except on villi absorptive surface area (P < 0.05). Probiotics could contribute to better growth performance in poultry, likely by supporting gut health, enhancing digestion, and reducing high stocking density stress. Supplementation with the B. subtilis probiotic mixture at a 0.5% dosage demonstrated better performance compared to control diets. Therefore, it is recommended that B. subtilis probiotic mixture be supplemented to broiler diets at 0.5% (w/w).

Keywords: Antibiotic growth promoters; Broiler chicken; Meat quality; Stocking density; Probiotics

2177 Liver transcriptomic analysis of broiler chickens fed guanidinoacetic acid and L-citrulline and its relationship with growth performance

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Guanidinoacetic acid (GAA) and L-citrulline (L-Cit) are considered alternatives to dietary arginine (Arg) in broiler chickens. GAA is the sole precursor of creatine, for which Arg is required, and L-Cit can be converted to Arg in broilers. This study aimed to investigate the gene expression and physiological pathways through which GAA and L-Cit can influence the growth performance of broilers when used as replacements for Arg. In a 42-day study with male Ross 308 broilers, three dietary treatments were selected: (1) a control semi-deficient Arg diet containing 0.12% less Arg than the Ross 308 recommendations, (2) a GAA-supplemented diet at 0.12%, and (3) an L-Cit-supplemented diet at 0.12%, added to the semi-deficient diet. Both GAA and L-Cit were included as replacements for Arg at a one-to-one ratio. Each treatment was replicated 8 times, with 10 birds per replicate. A total of 32 liver tissue samples (12 per treatment) were collected on day 21 of age. The RNA was extracted and mRNA sequenced using the Illumina platform (150-PE, 20M read depth). The sequenced data were aligned to a reference genome (GRCg7b) using Kallisto and analysed for differentially expressed genes (DEGs) using DeSeq2. A topology-based pathway activation method was used to calculate pathway activation scores, which were used to identify associations with performance. Both GAA and L-Cit decreased (P<0.001) feed conversion ratios (FCR) at the finisher stage (GAA: 1.479 and L-Cit: 1.496) and over the entire study period (GAA: 1.385 and L-Cit: 1.392) compared with the control treatment (1.534 and 1.432). Compared to the control, there were 42 and 37 DEGs (FDR<0.20) in the GAA and L-Cit treatments, respectively. Both GAA and L-Cit exhibited relative inhibition of lipid metabolism and utilization in the liver. Both supplements reduced cellular stress (activation scores: -1.61 for GAA and -1.19 for L-Cit), showed signs of improved redox status, and increased glycolysis. However, GAA had stronger effects on liver health and activation of specific areas of lipid metabolism compared to L-Cit, whereas L-Cit activated cell turnover. The inhibition of liver apoptosis and senescence with GAA (score: -1.25), compared to L-Cit, was associated with beneficial reductions in FCR for GAA (R2=0.69). Both GAA and L-Cit were beneficial in improving broiler performance, with a range of responses captured in the transcriptomic analysis.

Keywords: Transcriptomics; guanidinoacetic acid; citrulline; feed conversion ratio; arginine

2178 Dietary nano-selenium supplementation reduced translucent egg incidence by enhancing eggshell gland function in laying hens

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DRAFT

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Translucent eggs are a common eggshell defect that severely affects egg quality and consumer preference. Selenium, an essential trace element in poultry nutrition, has the potential to improve egg quality. This study aimed to investigate the effects of biogenic nano-selenium with alginate oligosaccharides (Nano-Se) on translucent egg formation. A total of 120 42-week-old Lohmann pink laying hens were selected and assigned to three treatment groups with 10 replicates of 4 hens each: negative control group (NC, score = 0.87), positive control group (PC, score = 2.87), and Nano-Se group (score = 2.71, basal diet + 0.3 mg/kg Nano-Se), and the trial lasted 12 weeks. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test in SAS 9.2. The results showed that the PC group exhibited significantly reduced laying rate (88.53 vs. 96.40%) and egg mass (53.85 vs. 58.62 g/hen/d) compared with the NC group ($P < 0.05$). Supplementation Nano-Se significantly improved translucency sore (1.87), laying rate (93.53%), and egg mass (58.00 g/hen/d) ($P < 0.05$). No significant differences in overall egg quality were observed among the groups, but eggshell strength was higher in both the PC and Nano-Se groups, reaching 4.80 kg/cm2compared with 4.14 kg/cm2in the NC group ($P < 0.05$). Next, eggshell quality analysis revealed that the PC group had lower moisture content (NC, PC, and Nano-Se: 0.65, 1.04, and 0.65%), higher calcium content (NC, PC, and Nano-Se: 7.94, 6.17, and 7.86%) and thicker mammillary layer (NC, PC, and Nano-Se: 32.71, 53.69, and 35.37 μm), whereas Nano-Se supplementation improved the eggshell composition and ultrastructure ($P < 0.05$). The eggshell membrane crude protein and total amino acid contents were reduced by 4% in the PC group, while the Nano-Se supplementation restored them to normal levels ($P < 0.05$). Antioxidant capacity of eggshell gland was significantly enhanced in the Nano-Se group, as indicated by increased GSH, GST, and CAT, and decreased MDA level ($P < 0.05$). Moreover, calcium transport-related genes CA2 and Calbindin-D28k were significantly upregulated in the Nano-Se group ($P < 0.05$). In addition, metabolomics and transcriptomics further revealed that dietary supplementation Nano-Se upregulated the calcium signaling pathway and multiple animo acid metabolism pathways. In summary, dietary Nano-Se supplementation improved eggshell quality by enhancing eggshell gland function, ultimately reducing translucent egg incidence and improving laying performance.

Keywords: Nano-selenium; translucent egg; eggshell quality; eggshell gland; laying hens

Reproduction & Breeders II

2179 Chicken is not only for meat or egg: the application of chicken in basic biology research

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Castration of roosters is a long-standing practice in the poultry industry. However, incomplete removal of the testes may leave residual tissue that continues to develop and and produce functional sperm, indicating strong organ regeneration potential. Regeneration is defined as the process by which an organ or tissue, undergoes re-development to restore its original size, histological structures and biological functions.

To characterize whether the transplanted or residual testicular tissue undergoing proportional growth or regeneration, the excised testes were transplanted subcutaneously to monitor the regenerative process. Our results showed that autotransplanted testes exhibited a significantly greater increase in mass compared to normally developed testes. After 12 weeks, motile sperm were recovered from the grafts, indicating that autotransplanted testes are capable of regeneration and functional restoration of spermatogenesis. Histological analyses further revealed that regenerated testes displayed cellular distributions comparable to those of normally developed testes.

In addition, we also found the age of animal and seasonality also affected the successful rate of testicular regeneration. Therefore, we studied the molecular mechanisms of how aging and different breeding status influenced testis regeneration, and further discovered an approach to re-potentiate the regeneration of aged testis that was not able to regenerate without treatment.

2180 Assessing the genetic improvement of tropically adapted indigenous chicken genotypes of Bangladesh

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Indigenous chickens are valuable genetic resources for sustainable poultry production in tropical environments. This study evaluated genetic progress and performance in the thirteenth generation (G13) of three Bangladeshi indigenous chicken genotypes: Non-descript Deshi (ND), Hilly (HI), and Naked Neck (NN). A total of 1,598-day-old chicks (ND=656, HI=385, NN=557) were produced. Birds were selected at the 8th week based on estimated breeding values for body weight and again at the 40th week using a selection index comprising body weight (BW), egg weight (EW), egg production (EP), and age at sexual maturity (ASM). Selected males and females were mated assortatively at a 1:5 ratio using artificial insemination, avoiding close inbreeding. Performance traits recorded included day-old chick weight, BW at different ages, feed intake, feed conversion ratio (FCR), hen-day egg production (HDEP), fertility, and EP up to 52 weeks. Data were analyzed using a Completely Randomized Design and Generalized Linear Model. Significant ($p<0.001$) genotype effects were observed across traits. NN exhibited the highest day-old weight (31.69±0.12 g), followed by HI (31.20±0.57 g) and ND (30.34±0.12 g). HI showed the highest 8-week BW (males: 860.77±4.51 g; females: 747.80±4.80 g), followed by ND and NN. NN had superior FCR (males: 1.90±0.01; females: 2.52±0.014). EP was highest in NN (132.67±1.32), followed by ND (117.85±1.32) and HI (106.83±1.48). ND matured earliest (143.25±0.97 days), compared to NN (147.99±0.96 days) and HI (152.02±1.8 days). Growth peaked at 10 weeks across genotypes. Expected genetic gains indicated improvements in EP (ND=2.17%, HI=0.68%, NN=1.67%) and EW (ND=0.13 g, HI=0.20 g, NN=0.22 g). Inbreeding remained negligible (ΔF=0.005–0.006). Continuous improvement in growth and egg traits across generations confirms successful genetic selection in tropical environments. These results demonstrate significant progress in growth, reproduction, and egg productivity in indigenous chickens, supporting their potential in sustainable and climate-resilient poultry development in Bangladesh.

Keywords: Indigenous chicken; Genetic improvement; Tropically adapted poultry; Selective breeding; Reproductive performance

2181 Illuminating the invisible: first visualization of sperm storage tubules in ducks using fluorescent-labeled sperm

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Sperm storage represents a remarkable reproductive strategy in birds, enabling females to maintain viable sperm for weeks after

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mating, a phenomenon of particular significance for optimizing fertility in poultry production. While sperm storage tubules (SST) are well-characterized in chickens and readily identifiable at the utero-vaginal junction via dissecting microscope, duck SST have remained elusive to conventional observation, creating a knowledge gap in waterfowl reproductive biology. We hypothesized that fluorescent labeling of sperm with the DNA-binding fluorophore Hoechst 33342 (bisbenzimide, Millipore Sigma) would facilitate identification and mapping of these cryptic SST within female duck reproductive tracts. Our objective was to optimize fluorescent labeling for duck sperm and determine anatomical distribution of sperm storage sites. We employed a two-phase approach using five male and eight female 22-month-old Tsaiya ducks. Phase one optimized labeling examining temperature (4°C versus 37°C) and Hoechst 33342 concentration (20, 40, and 80 µg/mL). Fresh ejaculates diluted in GFT extender were incubated with varying Hoechst concentrations at both temperatures, with motility and fluorescence quantified at 10-minute intervals over 60 minutes. Two-way ANOVA showed temperature enhanced fluorescence at 37°C ($P<0.01$) without affecting motility, while 80 µg/mL compromised motility ($P<0.05$). Optimal conditions were 40 µg/mL Hoechst 33342 at 37°C for 40 minutes, maintaining viability with robust fluorescence. In phase two, we applied this protocol to artificial insemination. Sperm was labeled under optimized conditions, with each female receiving intravaginal insemination of 200×106 fluorescent-labeled sperm on consecutive days. Twenty-four hours post-second insemination, reproductive tracts were examined via fluorescence microscopy. This revealed concentrated fluorescent sperm within mucosal crypts at the utero-vaginal junction, within epithelial invaginations exhibiting characteristic SST morphology. We demonstrated SST localization at the utero-vaginal junction in ducks, corroborating anatomical conservation in galliform species, though structural characteristics distinguishing anatid SST from chicken counterparts warrant further investigation. This provides the first visual documentation of SST distribution in ducks, establishing a reproducible protocol enabling advanced investigations into duck reproductive physiology, including sperm-epithelial interactions and molecular characterization of components maintaining long-term sperm viability.

Keywords: Sperm storage tubules; Hoechst 33342; Tsaiya duck; Fluorescent labeling; Utero-vaginal junction

2182 Ovarian function is sensitive to bile acid signaling, especially under thermal stress

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Hypothesis:Bile acids (BAs) constitute the major organic component of bile. During meals, BAs are released into the digestive tract where they facilitate the solubilization of dietary lipids. Their biosynthesis occurs in the liver, originating from cholesterol through a cascade of enzymatic modifications. BAs function as signaling molecules, mediating their effects primarily via a main receptor: the nuclear farnesoid X receptor alpha (FXRα). For the last decade, in mice, bile acids have been demonstrated to exert effects on functions beyond digestion, including reproduction. Our project focuses on elucidating the dynamics of this liver-ovary axis through BA signaling in avian species. The objective of this study was to investigate the role bile acids may play in ovarian activity, as well as the impact of ovarian steroids on bile acid production by hepatocytes.

Materials and methods: Cell sampling and purification of liver and ovarian cells (theca and granulosa) were performed at different developmental stages: embryonic, post-hatching, and adult. The expression of the various actors of the bile acid pathway (production enzymes, transporters, receptors) was measured. Samples were collected from two populations of chickens (broiler strain) under "control" conditions and a "heat stress" condition, where the chickens were exposed for 10 days to a thermal stress mimicking a heatwave (6 hours at 32°C followed by a temperature decrease during the night period). Histological analyses, immunohistochemistry, expression analysis by RT-qPCR, and ELISA assays were performed to better identify the effects of bile acids. Statistical analysis was performed using one-way ANOVA with GraphPad software.

Results: In adults, the enzymes that produce bile acids (CYP7A1, CYP8B1, HNF4) are expressed in hepatocytes but also in the ovary in the theca cells, but not in the granulosa cells. In hens that have been exposed to heat stress, the expression of these three enzymes decreases in these tissues. The BAs could be transported in theca cells by ASBT and OST transporters. The bile acid receptors (FXR and SHB) are strongly expressed in the theca cells, granulosa cells and also in primordial germ cells. Only the expression of FXR in the theca and granulosa cells is decreased following heat stress, desensitizing these cells to bile acids. In vitro, theca cells exposed to bile acids (Cholic Acid or Chenodeoxycholic Acid) alter the steroid synthesis, which impact folliculogenesis. In conclusion, our results suggest a dual effect of bile acids, which act on digestion, liver activity but also directly on ovarian function to produce better quality eggs. This project is supported by Region Centre Val de Loire, APR IR FERTICHAUD project.

Keywords: bile acids; ovary; germ cells; heat stress

2183 How Oviposition Time Shape Shell Traits, Translucency, Speckling Patterns and hatchability in Broiler Breeder Eggs at 35 weeks old

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Oviposition timing is associated with a hen's laying sequence and has been reported to influence egg quality. Similarly, eggshell translucency has been linked to reduced hatchability. However, the interaction between these two factors has not been well characterized. The objective of this experiment was to evaluate how translucency and overall egg quality vary according to oviposition time, and to determine whether translucency at specific oviposition times affects egg quality, hatchability and chick quality. In the first part of this study, a total of 5,400 eggs from Cobb500 hens of 35 weeks old were assessed for translucency level (T1 = low, T2 = medium, T3 = high) and the presence of speckling at two oviposition times: Morning (9:00 AM) and Afternoon (2:00 PM) and then incubated for hatchability and chick quality assessment. For the second part of the study, 180 eggs were analyzed for internal and external egg quality traits. The egg tester ultimate was used to determine egg weight, eggshell strength, Haugh unit, yolk color, and shell thickness. A cuticle blue staining method and an electronic colorimeter was used to determine cuticle coverage. Data were analyzed using the GLM and GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means. A difference between the means was considered when $P \leq 0.05$. Results showed that eggs from the morning had a lower incidence of T3 (10% vs. 38%, $P = 0.0153$) and speckling (9% vs. 28%, $P = 0.0024$), but a higher incidence of T1 (50% vs. 25%, $P = 0.0215$) when compared to afternoon-laid eggs. T2 incidence was not affected by oviposition time. No statistical differences in hatchability or chick quality were observed by translucency effect ($P = 0.0594$), oviposition time ($P = 0.8501$) or its interaction ($P = 0.8501$). In terms of egg quality, T3 eggs had 0.022 mm thicker shells ($P < 0.0001$), 0.81 Kgf stron-

ger shells, darker yolks (P<0.001), 0.98 g heavier eggs, and 0.42 percentage points higher shell weight (P < 0.0034) than T1 eggs. An interaction between oviposition time and translucency was observed for several egg quality parameters, with T3 eggs laid in the afternoon showing the thickest shells (0.3662 mm; P = 0.0004), the strongest shells (4.76 Kgf; P = 0.0433), and the greatest shell weight (5.58 g; P = 0.0125). In conclusion, oviposition time affected translucency and speckling incidence at 35 weeks of age. However, at this flock age, no differences in hatchability were detected due to translucency, oviposition time, or their interaction.
Keywords: Oviposition time; Hatchability; Shell quality; Speckling; Translucency

2184 Use of Phytase, probiotic and essential oils to restore performance in nutrient-deficient breeder hens during early and peak production

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Breeder operations are a critical part of any integrated, semi-integrated, or stand-alone poultry enterprise. Parent flocks represent a strategic part of the production system with direct impact on hatchery outcomes and broiler performance. Proven feed additives that help reduce feed costs and/or improve health status and productivity are desirable. This study evaluated whether supplementing a nutrient-deficient breeder hen diet (NC) with phytase (APG), probiotic (EP), and essential oil (EO) could restore performance to the level of a nutritionally adequate control (PC) during early (25–30 weeks) and peak (31–40 weeks) production. 1224 Ross 308 breeders (1080 females and 144 males) were allocated to 6 dietary treatments with 12 replicate groups (15 female and 2 males) per treatment. A mixed grain positive control (PC) diet meeting the nutritional requirements of the breed was formulated. A negative control (NC) diet reduced by 0.19% AvP, 0.19% Ca, 0.023% lysine, 0.04% Na and 52Kcal/kg ME was formulated. The NC diet was fed either as is or supplemented with 600 FTU/kg of a consensus 6-phytase (PHYG), 600 FTU PHYG + 150,000 cfu of multistrain bacillus (EP), 600 FTU/kg PHYG + 100mg/kg cinnamaldehyde and thymol blend (EO) or a combination of the 3 products (PHYG+EP+EO). Breeder performance and egg production, including hatchability, were monitored weekly, from 25 to 40 weeks of age. Data was analyzed using general linear model of SPSS (v 26.0) with means separation by Tukeys. Probability of P<0.05 was taken as significant and P<0.1 as a tendency. FCR was unaffected statistically but numerically improved in supplemented groups. Significant improvements in egg weight were seen from 25-30 weeks. Hens fed the EP, EO and PHYG+EP+EO diets produced heavier eggs (53.16, 52.84 and 52.95g respectively) than those on the NC diet (51.81 g), with egg weights numerically exceeding the PC group (52.46g). Eggshell strength (ESS) at 40 weeks was significantly reduced for the NC versus the PC (3.66 kgF vs 4.06 kgF). PHYG (4.28 kgF), EP (4.13 kgF) and PHYG+EP+EO (4.02 kgF) all improved ESS versus NC. From 31 to 40 weeks the PC group maintained a high hatchability (95.28%), and while the NC group achieved a comparable value (94.61%), significant improvements were seen with the inclusion of PHYG (96.45%), EP (95.91%), EO (95.04%) and PHYG+EP+EO (95.62%). Benefits of PHYG, alone or in combination with EP and EO, were more pronounced during peak lay. Improvements in hatchability and maintenance of ESS support the role of these additives in partially compensating for dietary nutrient restrictions, with APG contributing the most consistent benefits across parameters, particularly during peak production.
Keywords: Breeders; Phytase; Performance; Bacillus; Hatchery

2185 Impact of Artificial Insemination and Body Weight Variations on Reproductive and Progeny Performance in Broiler Breeders

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The objective of this study was to test the hypothesis that Artificial Insemination (AI) can mitigate the negative effects of body weight variation on reproductive performance, welfare, and progeny output in broiler breeders, and that its efficacy is dependent on the stage of the laying cycle. The experiment was conducted using a 2 x 3 factorial arrangement under a Completely Randomized Design (CRD). The factors were two mating methods and three body weight categories, assessed across early (30w), mid (45w), and late (60w) laying cycles.

A total of 3,000 Ross-308 breeder hens and 360 males were used. Hens were divided into two main groups: Natural Mating (NM, male:female 1:10) and Artificial Insemination (AI, male:female 1:7). Each group was subdivided into three body weight categories: Underweight (UW), Standard Weight (SW), and Overweight (OW). Data were collected on productive performance, welfare (pododermatitis, hock burn, feather score), egg quality parameters (egg weight, egg shape index, long diameter, short diameter, shell thickness, shell weight, specific gravity, eggshell membrane weight,albumen height, albumen weight, haugh unit, yolk weight, yolk diameter, yolk index) fertility, hatchability, and post-hatch broiler performance. Data were analyzed using a two-way ANOVA in R-software for quantitative traits, with means separated by Tukey's HSD test. Qualitative welfare data were analyzed using the Chi-Square test. A significant interaction (P<0.05) between mating method and body weight was observed. At 45 weeks, fertility was significantly higher in AI groups for Underweight (94.5% vs. 89.1%) and Overweight (93.2% vs. 87.5%) hens compared to natural mating. Overweight hens had significantly (P<0.01) higher egg production but also higher incidences of pododermatitis (Score 3-4) and floor eggs (8.44%) compared to Standard Weight and Underweight hens. At 60 weeks, the total number of chicks per hen was significantly highest in the Underweight-Artificial Insemination group. Progeny from Overweight parents had a significantly higher (P<0.01) average daily gain and European Broiler Index. AI is not recommended in the early laying cycle but is a highly effective strategy during the mid and late cycles to optimize fertility in flocks with body weight variations. While Overweight hens produce heavier chicks, they present greater welfare challenges. A targeted AI strategy can significantly enhance overall chick output and economic efficiency.
Keywords: Broiler Breeder; Artificial Insemination; Body Weight; Fertility; Welfare

2186 Effect of Bio-Available Silicium on Number of Day Old Chicks per Broiler Breeder Hen

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Optimal egg quality is vital for broiler breeder performance, by improving egg production, hatchability and chick quality. Silicium contributes to eggshell and membrane formation through its role in collagen cross-linking and biomineralization.

The objective of this study was to assess the effects of bio-available silicium (Poulsil®) on key egg quality parameters, hatchability, and total chick output in broiler breeder hens. A total of 40,804 broiler breeder hens (16 weeks old) were housed in two identical houses. Both groups followed identical management and diets. From weeks 22 to 61, one house received Poulsil (150 g/ton),

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while the other served as a control. At week 30, 40 and 50 at Zootests 500 eggs per house were analysed individually. At week 33, 39, 41 and 51 at the hatchery the data were collected for 450 eggs per house, summarized for 150 eggs. Data were assessed using R project, version 4.5.1.

Bio-active silicium supplementation significantly improved the different parameters for eggshell quality. Static stiffness (N/mm), shell weight, shell thickness, shell percentage, and shell index improved significantly (P<0.001) overall. Also, higher fracture force improved significantly overall (P<0.009). Bio-active silicium also improved egg production during the entire production. Laying rate increased from 88.9% to 89.9% at peak and from 65.9% to 67.6% at 60 weeks (+1.7%), while hatchability rose from 78.4% to 79.9%. Cracked eggs decreased to 1.6–2.2% compared with 1.8–3.6% in the control group.

Poulsil supplementation (150 g/ton) improved eggshell quality, laying rate, and hatchability, resulting in +4.33 day-old chicks per hen housed. The observed improvement increased with age, which is consistent with the well-documented decline in silicium and collagen content observed in aging humans*. The supplementation of bio-available silicium seems an interesting nutrient to improve breeder and layer performance. Further investigation is needed to substantiate its efficacy in animal nutrition particularly in physiological processes where collagen metabolism is of critical importance, such as bone formation, cartilage, membranes (e.g. vitelline membranes) and connective tissues.

Keywords: Broiler breeder; bio-available silicium; collagen; eggshell quality; hair cracks

2187 Impact of body weight variation on sexual maturity of layer pullets raised in a commercial aviary

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Sexual maturation in laying hens is traditionally triggered with an increase in light intensity and duration (photostimulation). However, faster growth can lead to an earlier onset of lay prior to moving to adult housing. We hypothesize that in a commercial rearing aviary, moderate body weight (BW) uniformity may lead to unsynchronized sexual maturation, which could impact production and bird health long term. This study shows the relationship between body weight and reproductive traits of Hy-line Brown pullets during the initiation of sexual maturation. The trial took place at a commercial farm in southern Ontario (Canada), in an aviary-style pullet barn divided into three sections. Three categories of birds were selected for sample collection per section as per the flock's average BW (below mean –1 SD; mean ±1 SD; and above mean +1 SD) at 16, 18, and 20 weeks of age (woa): N = 12, 9, 9 birds/section, respectively. At 14 woa, three birds per section were selected at random as a baseline measure. Body weight, fat pad, and comb height were recorded from the selected pullets, and after euthanasia, reproductive organs (oviduct and ovaries) were collected and weighed, and the large yellow follicles (LYF) were counted. Body weight CV for the whole flock was between 10.4-10.8% for all ages, and sample birds' BW ranged from 1190-1310 g, 1190-1690 g, 1280-1780 g, and 1540-2000 g at 14, 16, 18, and 20 woa respectively. Data were analyzed in R using Pearson's correlations, which showed a relationship between BW and primary and secondary reproductive traits during critical ages. At baseline, no associations were detected. At 16 woa, fat pad (r = 0.67, P < 0.0001), comb (r = 0.36, P = 0.03), and ovary (r = 0.38, P = 0.02) all increased with BW, though the oviduct weight did not (r = 0.25, P = 0.15). At 18 woa, three out of 27 birds had LYF, and these more mature birds were heavier (1767 g) than those without LYF (1502 g). The correlations were more pronounced for all traits at 18 woa, including ovary (r = 0.53, P = 0.004), oviduct (r = 0.53, P = 0.004), fat pad (r = 0.83, P < 0.0001), and comb (r = 0.51, P = 0.007). By 20 woa, 26 out of 27 birds had LYF and averaged 1739 g compared to 1580 g for the one without LYF. At this age, only the fat pad (r = 0.62, P = 0.001) had a strong association with BW. These results showed that birds with higher BW sexually matured earlier and BW influenced primary and secondary sexual traits within the same age. These findings demonstrate that BW differences in the timing of sexual maturity can lead to an uneven onset of lay, thus impacting early egg production, and long-term performance. The presented data represent the first step of a comprehensive study with multiple breeds.

Keywords: Pullets; body weight; reproductive traits; uniformity; rearing system

2188 Effect of dietary manipulation and vaccination of turkey breeder hens on growth and immunity of turkey poults

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Two hundred turkey breeder hens and twenty-four viable toms of 30-35 weeks of age of small white variety were distributed into two groups (NRC-1994 diet, High immune diet-HI) having four replicates of 25 hens and three toms in each treatment. The diets were fed to turkey birds for about eight weeks. After six weeks of experimental feeding two replicates from each treatment group were vaccinated with ND (R2B). At the end of experimental feeding, 370 fertile eggs were collected from 4 groups (NRC-V, NRC-NV, HI-V and HI-NV). After hatch the four groups were further subdivided into two sub groups; conventionally vaccinated (CV) and non-vaccinated (NCV). Poults hatched from the turkey breeders fed HI diet had significantly higher (P<0.05) body weight from 4th week onwards compared to those hatched from breeders fed NRC diet. Total immunoglobulin in response to sheep red blood cells was significantly higher (P<0.01) in poults hatched from breeders maintained on HI diet than those hatched from breeders fed NRC diet (7.52 vs 5.22). Poults hatched from breeders fed HI diet (0.51) had significantly (P<0.01) better cell-mediated immune response (in vivo PHA-P) than those fed NRC diet (0.36). Haemagglutination inhibition titer (log2) values against ND, R2B vaccination were significantly higher (P<0.05) in the poults from breeders fed HI diet than those fed NRC diet at day old, 28th, 42nd and 49th day of age and apparently higher at 14th and 21st day of age. Further, the titer values were apparently higher in maternally vaccinated poults than non-vaccinated poults throughout the experiment. Conventionally vaccinated poults had apparently higher HI titer on 14th, 21st, 42nd day and significantly higher (P<0.01) HI titer on 49th day than non-vaccinated poults. Interaction of breeder diet and breeder vaccination on ND HI titre at 0 day of age shows poults from HI diet and vaccinated breeders had significantly higher (P<0.05) HI titer compared to those from non vaccinated NRC diet breeders. Hence it may be concluded that breeders may be fed HI to enhance post hatch-growth and immune response in turkey poults. Further breeder vaccination may elicit better post hatch immunocompetence traits in their offspring.

Keywords: Turkeys; Diet; Vaccination; Growth; Immunity

2189 Prolonged Survival of Symbiotic Gut Bacteria in Egg White Suggests a Potential Pathway for Vertical Transmission

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While the gut microbiota of chickens is well researched, the reproductive tract microbiota has only recently come into focus. We previously demonstrated that live bacteria can be cultured from different parts of the reproductive tract of Cobb broiler breeders, and that fewer bacterial strains were culturable from the magnum compared with the infundibulum and shell gland. Of note, some bacterial phylogenetic groups were culturable from magnum samples whereas others were not. Because the main function of the magnum is to secrete egg white, which contains antimicrobial proteins, we hypothesized that these egg white secretions are responsible for the magnum's bacterial selectivity.

To determine whether egg white is the selective factor, complex bacterial communities from adult cecal content were incubated in egg white. Cecum content from broiler breeders and layer hens was separately mixed with freshly collected egg white from the same chicken line and incubated under anaerobic and aerobic conditions at 37°C for 1 day and 1, 2, and 4 days, respectively. Input samples were also collected. All samples were cultured on YCFA agar and surviving bacteria were identified by 16S rDNA sequencing.

We found that only a limited fraction of the cecal microbiota survived incubation in egg white. In broiler breeders, 65 ASVs were detected prior to egg white incubation, and 14 survived after 1 day, mainly Lactobacillales and Bacteroidales. In layer hens, 61 ASVs were detected before egg white treatment, and 20, 11, and 22 ASVs were cultured after 1, 2, and 4 days, respectively, including Lactobacillales and Pseudomonadales. Notably, 5 ASVs that survived 1 day in the broiler experiment also persisted for 4 days in layers experiment, indicating shared egg white resistant bacteria. Moreover, at least two of these ASVs were previously shown to be the main colonizers of two-day old chicks.

These results imply that bacteria which survive egg white might integrate into the forming egg and become the first inoculum for the developing chick. This would represent a new mechanism for vertical transmission of gut symbionts, complementing transmission by interaction with adults and survival on the eggshell. Furthermore, these results show that the chicken's physiology, egg white secretions, selects a subset of gut symbionts to become the first inoculum of the chick. Ongoing statistical and diversity analyses will further clarify the relationship between bacterial phylogeny and survival time and are expected to reveal specific traits that enable persistence in this unique antimicrobial environment.
Keywords: reproductive tract microbiota; magnum; egg white; culturomics; vertical transmission

Welfare and Behavior II

2190 Relative preferences of growing pullets for different perch diameters and locations for nighttime roosting

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Roosting in elevated locations at night is an innate behaviour that evolved as an anti-predator response in wild jungle fowl. In natural environments, elevated vantage points, such as those in trees, allow the birds to remain vigilant of their surroundings. Although current Codes of Practice require that perches be provided from one day of age for birds in non-cage systems, there are no recommendations and little research on perch characteristics for young birds. This study aimed to assess the relative nighttime roosting preferences of growing pullets for round perches of varying diameters and locations. Dekalb White (n=75) and Bovans Brown (n=75) laying hen chicks were housed in groups of 12 or 13 in 6 floor pens/strain (N=12). Each pen had 3 round wooden perches of differing diameters: 1.91 cm (S), 2.54 cm (M), and 3.18 cm (L), affixed to the walls at 81 cm above the ground using metal brackets. The perch locations were rotated weekly among three pen walls (A: wall closest to the room entrance, B: wall opposite pen door, C: wall furthest from the room entrance) to account for location bias. Video recordings of 48 h per week over three 3-week long periods (W5-7, W8-10, and W11-13) were scanned once per hour from lights off to lights on to determine the average percentage of birds within each pen on each perch (S, M, L) and at each location (A, B, C). Data were analyzed using a Generalized Linear Mixed Model with fixed effects of diameter, strain, location, period, and their interactions in SAS Studio Statistical Software version 9.4. As expected, nighttime perch use increased over time ($P<0.001$, $F=19.01$, $DFden=32$), with more pullets in each pen on perches and fewer on the ground as they aged (W5-7: 30.7±0.94%, W8-10: 66.8±0.91%, W11-13: 80.9±0.56%). During W5-7, there was no difference in the average percentage of pullets on S, M, or L ($P=0.319$, $F=1.16$, $DFden=64$). In W8-10, more pullets tended to use M (23.9±2.32%; $P=0.068$, $F=3.31$, $DFden=64$) and L (23.7±2.32%; $P=0.082$, $F=3.31$, $DFden=64$) rather than S (19.2±2.32%), while in W11-13, M (29.1±2.32%) tended to be used more than S (24.2±2.32%; $P=0.061$, $F=2.86$, $DFden=64$). There was a significant interaction between strain and location ($P=0.007$, $F=5.00$, $DFden=276$). Both brown and white pullets roosted at B the most (B: 28.1±2.02%, W: 28.3±2.02%), but whites also preferred A over C ($P=0.001$, $F=25.79$, $DFden=276$) whereas browns did not ($P=0.970$, $F=37.21$, $DFden=276$). Pullets used all diameters equally while young; as they grew, they used the smallest diameter less. They also showed a clear preference for the perch located along the back wall, which provided a vantage point out of the pen.
Keywords: Pullet; Perching; Perch diameter; Roosting; Behaviour

2191 How does UV light supplementation influence hen physiology, behavior, and welfare?

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Conventional artificial lighting in poultry facilities is primarily designed for human vision and often lacks ultraviolet (UV) wavelengths, which are crucial for avian physiology and welfare. Birds have broader spectral sensitivity, including UVA perception, and can synthesize vit-D through UVB exposure, which supports bone health. While free-range hens have the opportunity to obtain vit-D naturally through sunlight exposure, indoor hens rely entirely on dietary supplementation. Introducing full-spectrum artificial UV light (UVA/B) could replicate natural lighting, but its effectiveness requires further validation before widespread application. A study was conducted to investigate the effects of UVA/B light supplementation on laying hens during early lay. The study hypothesized that UVA exposure would promote positive behaviors and reduce stress and fear, while UVB exposure would improve egg produc-

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tion, egg and bone quality, and overall health compared to conventional UV-deficient lighting. A total of 252 ISA Brown pullets of 16 weeks of age (WOA) were housed under three lighting conditions across 18 pens (14 birds per pen, 6 pens per treatment) until 27 WOA: UV0 (control, UV-deficient LED-white light), UVA (LED-white light with supplemental UVA), and UVA/B (LED-white light with full-spectrum UVA/B). For physiological assessments, egg production, egg quality, plasma calcium and phosphorus levels, serum 25(OH)D3 (vit-D) external health, and tibia bone parameters were measured at different age points. For behavior and welfare, behavioral time budgets in the home pens at 18 and 24 WOA, tonic immobility (TI), open field (OF), novel object (NO) tests, and heterophil/ lymphocyte (H/L) ratios, were assessed at 20, 23, and 26 WOA. Data were analyzed using JMP 16.0 using GLMMs with □ level set at 0.05. Results showed that UVA/B supplementation had limited benefits, with a slight increase in egg production and vit-D relative to control hens (both P<0.05). However, considerable variation was observed between pens within treatment groups. An interaction between light exposure and age revealed more comb wounds at certain time points under both UV treatments (P<0.001), suggesting potential unintended behavioral consequences. Both UV treatments reduced TI duration (P=0.01) and vocalizations in the OF test (P<0.001), indicating decreased fearfulness. UVA/B exposed hens also had lower H/L ratios than UV0 hens (P=0.01), suggesting reduced physiological stress. In conclusion, UVB exposure appears beneficial for vit-D synthesis, but behavioral and welfare impacts of UV supplementation require further investigation. Future research should refine the optimal intensity and duration of UV exposure to maximize UV benefits.

Keywords: Behavior; Egg production and quality; Laying hen; UV light; Welfare

2192 Influence of light-exposure during late incubation and early feeding on performance of on-farm hatched layer chicks in a multitasking test

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On-farm hatching (OFH) is expected to expand into the layer sector due to advances in in ovo-sexing, but knowledge on how factors around hatching, such as lighted incubation and early feeding, influence behavioral and cognitive development remains limited. In this context, we investigated the effects of continuous light exposure and early feeding during OFH on multitasking abilities using a 2×2 factorial design across two trials, with the following factors: 1) immediate post-hatch feed and water access (FW+), 2) no feed and water (FW-), 3) lighted incubation (L+), and 4) no light (L-). Hatching eggs (n=1,280; parents: Super Nick, 32–33 weeks old) were exposed to one of the following treatment combinations from 18–21 days of incubation (4 pens/group): FW+L+, FW-L+, FW+L-, or FW-L-. We hypothesized that FW+L+ and FW-L+ chicks would show better multitasking abilities than FW+L- and FW-L- chicks, as lighted incubation induces visual laterality, potentially increasing behavioral control. A total of 128 pairs of chicks (n=4 pairs/pen) were subjected to a multitasking test and habituated to the arena from 3–9 days of age (DOA). Within each pair, a focal chick was selected for observations. During testing, pairs could forage while predator silhouettes were presented at 10, 11, 12, or 13 DOA. Latency to spot the predator (PRED) and return to pecking (PECK) were recorded in seconds, assessing vigilance and habituation. Eye preferences to spot and sustain view of the predator were observed on camera and recorded when chicks looked upward, serving as a laterality proxy. Data were analyzed using generalized mixed-effects models with the two treatments as fixed factors and pen nested in trial as a random term. PRED and PECK were affected by L (p=0.0001; p=0.006, resp.) but not by FW (p=0.99; p=0.11, resp.). L+ chicks were more likely to have faster PREDs than L- chicks across both FW levels (41.2%, p<0.0001), indicating higher vigilance, as they detected sudden environmental changes sooner. L+ chicks also had faster PECKs than L- chicks across both FW levels (52.8%, p=0.003), suggesting better foraging while monitoring for predators. Eye preferences were not affected by FW, L, or their interaction (spot: p=0.32, p=0.18, p=0.18; sustain view: p=0.32, p=0.49, p=0.20, resp.), likely due to binocular testing allowing switching between eyes. In conclusion, shorter latencies/faster responses suggest that L+ chicks may have higher behavioral flexibility and are less easily distracted or stressed than L- chicks. Effects of FW+ or FW- on multitasking abilities were not detected during this test. In commercial settings, higher behavioral flexibility could help chicks to better adapt to daily stressors and improve welfare.

Keywords: on-farm hatching; laterality; perinatal environment; dual task; layer chicks

2193 Gradient lighting in broiler houses allows for functional zones to promote natural behaviours

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Light programs are a key husbandry factor in broiler production, influencing welfare and performance. Research into broiler preferences for differing light intensities is growing, with experimental studies indicating that intensity may be associated with specific behaviours being performed, such as darker areas being preferred for inactive behaviours, and brighter areas for active behaviours. Thus, we investigated the impact of providing zones with varying light intensities on the behaviour and welfare of slower-growing broilers. The experiment took place during 2 cycles on a commercial broiler farm in the Netherlands, with 4 broiler houses, each housing ~8,500 Hubbard JA757 broilers. All houses had the same farm management and layout. There were two treatment houses (GRAD), which had 3 similarly sized zones with differing light intensities: bright > 100 lux; gradient 25 – 50 lux and dark < 10 lux. Control (CON) houses had uniform lighting throughout the house, ~20 lux. Measures included behaviour, fearfulness tests, a play behaviour test, and clinical welfare indicators (currently being analysed). Behaviour observations and tests were performed at week 1, 3, 5 and 7 (only wk 7 in cycle 1). Behavioural data and data from the behaviour tests were analysed with a generalised linear mixed model (beta-binomial and negative-binomial, respectively). A significant age x treatment interaction for explorative behaviours (P=0.01) was found, at 1 wk there was a higher probability of GRAD birds (5.7%) performing explorative behaviours than CON birds (1.7%) but not at later ages. Overall, GRAD birds were more active, showing more locomotion than CON birds (P<0.01) but GRAD did not affect other behaviours. Additionally, an Analysis of Deviance was used to determine marginal means for behaviours in equivalent zones between treatments, e.g. dark zone in GRAD versus the equivalent zone in CON. This showed GRAD birds have a higher probability of performing more explorative (P<0.01) and locomotion (GRAD: 9.8%, CON: 5.9% P<0.01) behaviours and less resting (GRAD: 62.1%, CON: 71.5% P=0.03) in the bright zone compared to CON birds. Limited treatment effects were found on fear and play responses, only more GRAD birds approached the novel object (P=0.04) than CON birds. In conclusion, GRAD only affected behaviour to a limited extent, however, a difference in behaviours performed in specific lighting zones indicates that there may be a preference for certain light zones according to behaviour

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performed. Additionally, at all visits, birds were observed to be present in all light zones. Thus, providing a choice of differing light zones within broiler houses may enhance broiler welfare by encouraging natural behaviours.
Keywords: Broiler; Light; Welfare; Behaviour; Preference

2194 The effect of light wavelength on the production and health of Nicholas Select beak-treated and non- beak treated turkey hens reared to 11 weeks

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Limited research has been conducted studying the effect of LED light wavelength on turkeys. This research objective was to determine the effect of 3 LED wavelengths (L): blue (B; peak=455nm), green (G; peak=510nm), and white (W) on production and health of infrared beak treated (IR) and non-beak treated (Non) Nicholas Select turkey hens (0-11wk of age). Hens (n=2,916/trial; 2 trials) were housed in 9 (3 rooms/L) individual environmentally controlled rooms (n=324/room), each divided into 2 pens according to beak treatment (trt). Birds were fed commercial diets with ad libitum access to feed and water, and pen weights were taken at 0, 3, 5, 8, and 11wk. At 8 and 11wk, a random subsample of 180 hens was individually weighed to assess flock uniformity. Feed consumption and efficiency were calculated for 0-3, 3-5, 5-8, and 8-11wk. Health assessment included litter quality, gait score, foot pad score, feather condition, and feather cleanliness (8 and 11wk). Dead and cull birds were necropsied for cause. The experiment was a 3x2 factorial arrangement in a RCBD (trial as block). Data were analyzed using ANOVA (SAS 9.4) (replicate unit room=L and pen=beak trt). Differences were significant when $P \leq 0.05$. Birds reared under B had higher average weight gain and feed consumption compared to G or W from 8-11wk ($P<0.01$). Non birds consumed more feed from 3-5wk ($P=0.05$). Feed efficiency differences were noted between IR and Non from 0-3wk ($P=0.02$) and 3-5wk ($P<0.01$), with IR having improved feed efficiency. Hens reared under B demonstrated improved feed efficiency compared to hens under G ($P=0.02$) at 3-5wk. Cannibalism was the highest cause of mortality under W and G compared to B ($P<0.01$), while abnormal etiologies was the highest in B compared to G or W ($P<0.01$). Interactions between L and beak trt were noted for metabolic ($P=0.03$) and infectious ($P=0.03$) mortality causes and total mortality ($P=0.02$). Average litter quality was better in W compared to G ($P=0.02$). Foot pad scores were worse under B compared to G or W at 8wk ($P=0.04$). Gait score was not affected. Feather cleanliness was poorer under B compared to W or G at 8wk ($P<0.01$) and 11 wk ($P<0.01$). In conclusion, B enhanced weight gain and feed consumption, particularly between 8 and 11wk, while also reducing total mortality in comparison to W. Cannibalism was more prevalent under W. However, turkeys under B demonstrated poorer litter quality, footpad scores, reduced feather cleanliness, and higher mortality due to abnormal etiologies. Non consumed more feed, while IR exhibited better feed efficiency.

Key words: Mortality, cannibalism, performance, abnormal, health indicators

Keywords: Mortality; Cannibalism; Performance; abnormal; health indicators

2195 Effect of a specific Light Spectrum and Sex on the Performance of Broilers under standardized light conditions

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The so-called 'jungle light' is frequently evaluated positively regarding broiler performance; however, the term lacks a uniform definition. In many studies parameters and measurement methods are either unspecified or do not align with current European regulatory standards.

The aim of the present study was to investigate the effect, of the spectrum of a so-called 'jungle light' on broiler production parameters under standardized lighting conditions, comparing both as hatched and sexed broiler flocks.

In total 2,160 broilers (Ross 308) were housed as one day old chicks, either as hatched (trial 1; n=720) or sexed (trial 2; 720 male, 720 female), with a final stocking density of 35 kg/sqm on day 40. The birds were reared under conventional standards and subjected to two lighting variants, both emitting a spectrum of 390–800 nm. The first variant was standard white (SW, Zeus LED GEN2 cool/warm IP69K by Big Dutchman) and the second was 'jungle light' (JL, LED 'Gallux 50W' by M.E. International) with an increased proportion of green light in the total spectrum. The average light intensity was maintained at 20 lux at bird height. Ultimately, this resulted for each sex group (n=3; as hatched/male/female) under each light treatment (n=2) in four replicates (n=90 birds/replicate; in total 360 birds/light treatment=720 birds per sex group=2,160 birds).

Animal-related parameters were recorded daily, e.g. mortality, whereas body weight (BW) was measured weekly and slaughter and carcass parameters were assessed once. Statistical analysis was conducted considering the factor light in trial 1, and both sex and light in trial 2. Differences in BW were assessed using the Kruskal–Wallis test for the non-normally distributed as-hatched dataset and ANOVA for the normally distributed sexed dataset considering the factors light and sex and its interaction, using SPSS v28.

Results of selected parameters: Mortality rates ranged between 1.1–3.3% in SW groups and 3.3–5.5% in JL groups, respectively. Differences in mean BW at the end of rearing were not significant in the as-hatched groups (SW 2,982.9 g ± 380; JL 3,037.6 g ± 352; $p=0.07$), indicating that light had no effect on final BW. In the sexed groups, the effect of light on day 40 was not significant (mean for females, SW: 2,808.2g ± 214, JL: 2,787.5 g ± 216; $p = 0.210$; mean males, SW:3,340.3 g ± 261, JL: 3,348.3 g ± 237, $p = 0.679$). While the main effect of sex was significant ($p < 0.001$), the interaction of light and sex was not ($p = 0.258$).

In conclusion, the defined spectrum of jungle light used in this study did not affect BW at the end of fattening. The factor of sex should be considered in future studies when evaluating management recommendations in broiler husbandry.

Keywords: light spectrum; broiler; standardization; lighting

2196 Laying hen preference for different pecking blocks in commercial aviaries does not change as they age

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Pecking blocks (PB) are edible enrichment provided to poultry to create foraging opportunities and choice for nutrients. PB vary in chemical composition, texture, colour, and density. We measured

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foraging behaviors and PB replacement rates on a commercial egg farm to determine relative preferences between 3 commercially available PB [A. fiber and mineral, B. fiber, mineral, and molasses, and C. mineral-based] and whether laying hens' interest in the different PB changed over time. Lohmann Brown hens were housed in pens of ~9,000 with 4 pens/flock (3 flocks). There were 3 treatment groups: YY (PB introduced at 12 weeks of age (WOA) with continued access in the layer barn), NY (no PB in pullet barn, PB in layer barn introduced at 20WOA), and NN (no PB in pullet or layer barns). PB were evenly distributed on the littered rows of the pens at a ratio of 1PB/500 hens (18PB/pen). Foraging directed at each PB and surrounding litter (area=0.53m2) in YY and NY, and in 0.53m2 litter area in identical pen locations in NN was quantified for 1 day at 30, 40, and 50WOA through scan samples. Every 15 minutes the number of hens foraging (pecking or scratching) the PB or litter areas was counted. Data were analyzed with a GLMM at each age to assess the number of hens foraging with fixed effects of treatment and row. Treatment affected foraging at 30WOA with more foraging/scan observed in YY (5.49 ±0.077 hens) and NY (5.01 ±0.070 hens) compared to NN (1.07 ±0.023 hens) ($p<0.0001$). At 50WOA, YY treatment groups were lost due to disease. NY (3.85±0.067 hens) still had higher number of hens foraging/scan compared to NN (0.76±0.023 hens) ($p<0.0001$). As the hens aged, NY's foraging decreased from 5.01±0.070 hens at 30WOA to 3.85±0.067 hens at 50WOA ($p=0.053$). To analyze relative preference for PB, a GLMM was run to compare block pecking directed at each PB in NY and YY treatments, where fixed effects were PB type, treatment, age, and their interactions. PB preference was not affected by age ($p=0.288$) or treatment when comparing NY with YY ($p=0.197$). Pecking was highest at PB type B (5.02±0.058hens), then A (3.12±0.063hens), then C (2.44±0.055hens) ($p=0.0001$). Data for replacement rates of PB were analyzed with a GLMM with fixed effects of season, age, pen, row, and their interactions. Replacement rate results aligned with preference, with PB type B being replaced the most ($p<0.0001$). Season interacted with PB type ($p<0.0001$) with PB type B replacement rate higher in the summer compared to other seasons combined. Overall, the results show that PB are attractive edible enrichments that stimulate foraging. While preference for PB did not change with treatment or age, amount of foraging at PB tended to decrease over time.

Keywords: pecking blocks; laying hens; non-cage system; foraging; enrichment

2197 Highlights on nest use in laying hens measured by a Radio Frequency Identification (RFID) system

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Nest use of laying hens may be influenced by both individual preferences and social dynamics. Understanding whether associations arise from social attraction or from shared temporal patterns would improve hen management and welfare. To this purpose, the present study evaluated frequency of nest-use in laying hens of two genotypes (Brown and White) housed in 2 pens (225 hens per pen; 1 pen per genotype) before the oviposition (19-21 weeks of age) and after oviposition (22-52 weeks of age). Radio-frequency identification (RFID) technology was used to quantify individual and collective nest-use behavior focusing on nest preference, temporal activity, and potential social associations, automatically recording the nest use by individual passive tags attached to the hen legs. Individual entries and exits were continuously recorded by RFID in four nests of the two pens (two nests per pen). Raw data were cleaned to remove artefacts. For hens with ≥10 nest visits, individual chi-square tests compared nest-use patterns to the overall population distribution ($\alpha = 0.0003$). Potential social structuring was assessed with a Poisson model estimating expected copresence frequencies under random conditions. Data were analyzed using Python 3.12.2, with pen and time of nest access treated as fixed effects, since the analysis aimed to directly compare the two genotypes and their temporal laying patterns. In contrast, individual nests within each module were considered a random effect, as the number of accesses per nest was normalized to account for instrumental variability. Before oviposition, 26.0% of white hens and 16.8% of brown hens exhibited nest preferences ($P < 0.001$). During oviposition, 28.6% of white hens and 38.3% of brown hens showed nest preferences ($P < 0.001$). No evidence of structured social behavior was found Poisson-based copresence tests yielded median $P = 1.0$ in all cases, indicating random association patterns. Temporal analyses revealed distinct circadian rhythms, with two main activity peaks: one between 06:00 and 10:00h in the weeks before oviposition, and another between 05:00 and 08:00 in the weeks during oviposition, consistently with the circadian laying cycle. Overall, the RFID-based analytical framework effectively detected individual nest-use ($P < 0.05$), providing a robust basis for behavioral monitoring in laying hens. In conclusion, this preliminary study highlights the potential of the use of RFID system to monitor nest use in laying hens in an aviary system. Acknowledgements: Agritech National Research Center, European Union – NextGenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022).

Keywords: laying behaviour; precision livestock farming; social interactions

2198 Influence of different locations in the broiler house on occurrence of broiler comfort behavior

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To assess the welfare of broiler chickens, evaluation criteria such as feed and water consumption, mortality during fattening and animal-based welfare indicators including footpad quality and hock burns are commonly used. More recently, animal-based measures have been increasingly applied. For broiler chickens, several behavioral characteristics associated with comfort behavior have been described in the literature (EFSA, 2023). However, these behaviors have rarely been studied under practical commercial conditions. Therefore, the objective of this study is to assess the occurrence of comfort behavior in a commercial broiler house and to evaluate the influence of broiler age and weight, spatial distribution and house management.

A total of 12,000 Ross 308 broilers were observed in a commercial broiler house from day 7 to day 42 of rearing. Chicken behavior was continuously recorded using video cameras installed throughout the barn. Every two days, three 10-minute video sequences from two camera locations were analyzed: (A) the end of the house and (B) a side area with feeders and drinkers. The comfort behaviors dust bathing, preening, wing and leg stretching, stationary wing flapping, wing flapping with locomotion and interactive running with conspecifics were evaluated and related to the number of animals present in the observed area. Data were analyzed using JMP statistical discovery software (SAS). In total, 720 minutes of

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video material were evaluated.
Comfort behavior was observed throughout the entire fattening period. Older broilers showed more sedentary behaviors such as leg and wing stretching, preening and dust bathing. Observation location influenced dust bathing behavior, which decreased during periods of high activity. A one-way ANOVA showed a significant effect of location, with higher dust bathing at the barn end (A: 3.8%; B: 0.5%). This effect was less pronounced for preening (A: 3.3%; B: 3.3%) and leg stretching (A: 8.4%; B: 5.5%). For wing flapping (all types combined), no clear location effect was found (A: 23.8%; B: 24.9%), suggesting that time of day and house management influenced this behavior. Wing flapping declined with increasing broiler age. In conclusion, all described comfort behaviors can be observed under practical conditions. However, their prevalence depends on location and timing. Further studies will be needed to clarify the influence of management, season, and animal distribution within the broiler house.
Keywords: Behavior of broiler chickens; Animal welfare; Housing conditions; Age; comfort behavior

2199 Enhancing laying hen welfare and longevity through optimized enrichment: Insights from the OMELETTE project

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Environmental enrichment supports behavioural needs and may contribute to extended hen longevity. We hypothesize that continuous automated monitoring of enrichment use can provide real-time insight into engagement patterns, detect behavioural deviations, and support precision management in extended-lay systems.
As part of the OMELETTE project, enrichment strategies were evaluated within a broader package of interventions aimed at promoting hen longevity. The study assessed temporal patterns of enrichment use and compared manual assessment with development of automated detection methods.
Eight semi-commercial aviary test units (960–1325 hens; Dekalb White, ISA Brown, Warren Brown) without outdoor access were equipped with alfalfa hay bales, pecking stones ad libitum, and rotating enrichment items to explore how provision diversity influences sustained engagement. Dahua overhead cameras (DH-SD1A203T-GN) captured images every 30 min, offering a top-down view for clear and detailed observation of the free-range area where the enrichment materials were placed. Manual scoring quantified hens interacting with enrichment from October 20, 2024, to January 31, 2025. Parallel development of computer-vision tools targeted hen and enrichment recognition and interaction classification. In this abstract, we report only the results related to pecking-stone use, with analysis of other enrichment types ongoing.
A consistent diurnal rhythm in pecking-stone use was observed. Interaction remained low during early light hours and increased progressively throughout the day, peaking during the final three hours of the 15-hour light period (lights on 03:00–18:00). Late-afternoon activity was significantly higher than morning and midday periods ($P < 0.001$), demonstrating a clear daily behavioural pattern. While test units showed the same overall rhythm, unit-specific differences were evident, including a delayed but more pronounced peak in one unit. Peak engagement (~16:00; mean 8.3 ± 2.1 hens) aligned with expected physiological calcium demand during eggshell calcification and increased behavioural time availability after feeding, drinking and oviposition.
These findings support the hypothesis that pecking-stone use is biologically motivated and highlight the role of enrichment in facilitating self-regulation of mineral intake and coping with physiological demands in extended-lay systems. Manual monitoring confirmed biological drivers of enrichment motivation. Ongoing automation will enable continuous, objective tracking and early detection of behavioural deviations, supporting welfare and resilience in long-life flocks.
Keywords: Laying hens; Enrichment; Longevity; PLF

2200 Evaluation of broiler disturbance induced by darkling beetles during the night period: a new approach to raise farmers awareness

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Alphitobius diaperinus (darkling beetle) is commonly found in broiler farms and recognized as reservoir for pathogens (Salmonella spp., E. coli, IBD virus). Currently, control relies on the application of insecticides. However, habituation to high infestations is often observed, despite associated health risk. This nocturnal insect becomes active when birds are resting. It is therefore hypothesized that these activities may disturb broilers welfare and performance. This study aims to assess the darkling beetles impact on broiler welfare during the night period.

Two successive flocks of broilers were monitored in high infested commercial house. A larvicidal/adulticidal insecticide treatment based on Spinosad (ELECTOR® Elanco) was applied between the 2 flocks. Infestation level was measured in the same area through samples collected at D14, D21, D28, and D35 under the feeders (equivalent to a handful) and in TENEDROP traps. Each sample was weighed, and the proportion of adults/larvae was estimated by counting on a homogenized subsample. In parallel, night-time videos (20 sec/20 min) were recorded with an infrared camera at day 14, 16,18, 21, 23, 26,30 and 33. Manual video analysis was conducted following an disturbance behaviours ethogram developed in preliminary study. The objective was to quantify atypical nocturnal activities, including birds remaining active during the night, individuals exhibiting slight movements (e.g., waddling, wing flapping, awakening) and preening behaviour. In total, 120 video recordings, each divided into four distinct observation areas, are analysed. Behaviours frequency per square was compared between flocks and age using Wilcoxon test.

Infestation level in flock 2 (after Spinosad application) was lower than in flock 1 at all sampling ages (mean = -92±9%). Also, different colonisation pattern was observed. In flock 1, until D21, majority of adults were present (≈ 95%). Massive larvae emergence was observed from D28 (92%), while it occurred at D35 in low proportion (10%) in flock 2. In parallel, behavioural analysis revealed an increase ($P < 0.05$) in abnormal nocturnal activity at D21, D23, and D26 in broilers from flock 1, compared with flock 2.

ELECTOR® demonstrated its efficacy against both larvae and adults. While adult darkling beetles do not appear to affect the broilers' nocturnal rest, an increase in atypical nocturnal activity was observed in coincidence with the massive appearance of larvae. This new approach, based on an innovative behavioural monitoring technique, makes it possible to visualize the impact on broiler welfare and thus raise farmers' awareness of the importance of effective beetle control, both for welfare improvement and for limiting health risks.
Keywords: Darkling beetle; Night disturbance; Broilers; Pest; Spinosad

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2201 Assessing the factors stocking density and thinning on the behaviour of both conventional and slower growing broilers under semi-commercial controlled conditions

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Stocking density is a key factor influencing broiler welfare and the expression of natural behaviours such as foraging, resting, standing, walking and comfort behaviours (dustbathing, preening, stretching, wing flapping). The Better Chicken Commitment requires slower-growing breeds at lower stocking densities (≤30 kg/m^2) compared to conventional systems (e.g. 42 kg/m^2 max in Belgium). Partial depopulation ('thinning') is widely used in the EU to balance stocking density and productivity, yet its full behavioural and welfare implications remain unclear. Two trials were conducted at the Experimental Poultry Centre (Geel, Belgium) with conventional (Ross 308) and slower-growing (Hubbard Redbro) broilers under semi-commercial, climate-controlled conditions (8 rooms; 16 experimental groups in total). In trial A, both breeds were placed at 42 kg/m^2 (n = 1,180 birds/group; 21 birds/m^2) and 30 kg/m^2 (n = 850 birds/group; 15 birds/m^2) with thinning in all groups 1 week before depopulation. In trial B, Ross 308 were reared at 42 kg/m^2 (with thinning, n = 1,200 birds/group; without, n = 920 birds/group) and Redbro at 30 kg/m^2 (with thinning, n = 850 birds/group; without, n = 650 birds/group). During both trials, thinning for Ross 308 was done at 34d (where applicable) and depopulation at 41d, and for Redbro at 41d and 50d of age, respectively. The designs of each trial yielded 4 pseudoreplicates (i.e. 2 rooms) per treatment. Behavioural observations followed the ethogram by de Jong & Gunnink (2018): direct observations (trial A), or a 2-min video per group per day (made in the afternoon to control for diurnal variation in bird behaviour) analysed using BORIS (trial B) at 6d (Ross 308/Redbro), 28d (Ross 308), 32/35d (Redbro), 39d (Ross) and 47d (Redbro) of age. Data were analysed using mixed-effects models; fixed effects tested with Type III Wald χ^2 tests and significant effects explored with Tukey-adjusted post-hoc comparisons ($\alpha = 0.05$). In trial A, lower density increased foraging ($p < 0.03$) and reduced resting ($p < 0.01$); resting and comfort behaviours increased with age ($p < 0.01$). Redbro showed more preening ($p < 0.01$). In trial B, foraging was higher ($p < 0.01$) and resting lower ($p < 0.01$) in Redbro than Ross 308. Resting increased ($p < 0.01$) and walking decreased ($p < 0.01$) with age; preening was more frequent in Redbro ($p < 0.01$) and rose with age ($p < 0.05$). Only eating behaviour differed with the factor thinning, with more birds observed to be eating in groups with thinning ($p < 0.01$). No perching was observed. Lower stocking density (30 kg/m^2) consistently promoted natural and comfort behaviours across trials and breeds, indicating a clear net welfare gain under semi-commercial conditions.

Keywords: Thinning; Stocking density; Broiler behaviour; Fast and slower growing; Semi-commercial

Trouw Nutrition Symposium: From Insight to Impact: Transforming Poultry Nutritio

Feeding for Performance: Principles of Poultry Nutrition and Modeling

Nayara Ferreira*1 1Trouw Nutrition, , Netherlands

Optimizing Trace Mineral Feeding for Poultry

Gavin Boerboom*1 1Selko, , Netherlands

PhytoComplexes: Harnessing the Power of Plants to Support Animal Health and Resilience

MARLIEN DE KOCK*1 1Selko, , Netherlands

Q&A

Anitox Symposium: Connecting Gut Health to Growth: Performance, Meat Quality, and Molecular Responses in Broilers

Dr. A. Thomas

General Health/Disease/One Health III

3000 The Role of Vertical and Hatchery Transmission of Bacteria in Health and Disease Transmission

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Microbiota in the hen to chick continuum influence pioneer colonizing bacteria that influence both short term and long term health of production flocks. Incubation and hatching systems somewhat limit microbial transfer to bacteria present on and within eggs, and present opportunity for vast horizontal transmission during the hatching process. Previous work has shown that early exposure to bacteria in hatching cabinets shapes immune function and resistance to opportunistic pathogens. Next, it has been shown that breeder flocks greatly influence the microbiome of hatching birds and its pliability in response to probiotics application, suggesting a strong influence on both disease susceptibility and plausibility that prevention methods will be effective. More recent studies characterized the microbiome of broiler breeder hens and day-of-hatch chicks to assesses the potential for bacterial transmission between them. Specifically, Enterococcus cecorum was traced from hen organs, including crop, small intestine, ceca, reproductive tract, and cloaca. Though present in all organs, Bray–Curtis PERMANOVA showed that the cloaca and small intestine were the hen sites most similar to the day-of-hatch GIT, with only marginal or non-significant differences ($q = 0.05–0.17$), suggesting limited maternal contribution and some continuity along the intestinal axis. Eggs and hatchery fluff showed similar microbial profiles ($q = 0.21$) and were not significantly different from the small intestine or day-of-hatch GIT ($q = 0.05–0.21$). Other studies of chick microbiome from day of hatch to 10d demonstrated that the Enterococcus genus was present at 3.73% on day of hatch, which increased significantly in the upper intestine (UI) to 53.39% by D3, and remained relatively high at 37.68% on D10, indicating that low-level presence of Enterococcus at DOH can lead to significant presence as broilers age, potentially contributing to disease. Patterns of direct egg-based transmission from hens to offspring highlight the need to control initial colonization of young birds through breeder flocks and microbiological management in hatcheries.

3001 Cracking Down on Contamination: Québec's Model for Salmonella Enteritidis Prevention

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Over the past 25 years, Québec's egg industry has developed a multi-tiered strategy to monitor and control Salmonella Enteritidis (SE), combining a strong collaborative, producer-driven initiative and science-based interventions. This integrated approach includes biosecurity protocols, environmental testing, traceability system, and structured response plans, each contributing to the province's long-standing success in preventing SE-related outbreaks.

At the heart of this strategy is a certification program that incorporates national standards and HACCP principles. Producers must comply with strict hygiene and pest control measures, ensure the purchase of SE-negative pullets, and maintain cold chain protocols

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for egg storage. Hatcheries are specialized to prevent cross-contamination between meat and egg sectors, and transport systems are dedicated to pullet movement.
Environmental sampling and PCR testing are conducted regularly across all production stages. When Group D Salmonella is detected, eggs are traced and seized at grading stations, and further testing determines whether they can be returned to market or must be pasteurized. Confirmed SE cases trigger egg pasteurization, flock depopulation, barn disinfection, and empty barn testing before repopulation. These measures are supported by compensation and logistical assistance to ensure not only producer compliance but also a comprehensive support, and to minimize economical disruption.
A unique traceability system enables rapid identification and segregation of potentially contaminated eggs, ensuring rapid containment and protecting public health. Despite ongoing environmental detections, which appear to be increasing with the new housing systems, Québec has not recorded any human outbreaks linked to egg consumption since the implementation of this program in1997. The success of Québec's SE control program lies in its adaptability, stakeholder collaboration, and commitment to continuous improvement, especially with the transition to alternative housing systems. It demonstrates how an integrated approach, combining surveillance, biosecurity, and rapid response, can effectively safeguard food safety while supporting industry sustainability.
Keywords: Salmonella Enteritidis; biosecurity; traceability; monitoring program; HACCP

3002 Characterizing virulence in emerging APEC Isolates using three different challenge models with histopathological analysis

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Avian Pathogenic E. coli (APEC) causes colibacillosis in poultry, leading to systemic or localized infections such as airsacculitis, septicemia, pericarditis, and salpingitis. Serogrouping, based on O-antigens, is used to classify APEC and correlate with disease pathogenicity.
While O1, O2, and O78 are the most common serogroups, our APEC collection from Georgia poultry has identified emerging serogroups. Therefore, we evaluated their pathogenicity using embryo lethality (ELA), chick, and chicken challenge assays.
We selected 21 novel APEC strains, including O11, O17, O25, O15, O91, O152, O161, O86, O88, O115, Onovel12:H4, O149, and O45. In the ELA, 12-day-old embryonic eggs were injected with 300-500 CFU/0.1 mL via allantoic fluid. Embryos were monitored daily for five days, and deaths recorded. At 17 days, yolk sacs were collected for histopathological analysis.
ELA results showed O17 (n=1) caused 93.3% mortality and O45 (n=2) caused a mean mortality of 86.65%. O88 and O91 had mean mortality rates of 65% and 60%, respectively.
Histopathological analysis of yolk sacs revealed marked swelling and disruption of epithelial endodermal cells, multifocal loss of hematopoietic islands, and plumped, reactive endothelium. Periodic Acid-Schiff (PAS) staining showed decreased PAS-positive material (glycogen), suggesting reduced absorption from endodermal cells to blood vessels. These changes were not significantly different among strains but were significantly different from PBS and APEC control groups.
A chick challenge assay was performed on twelve one-day-old chicks per group, inoculated subcutaneously with 100 µL (0.1 mL) of 108 CFU bacterial strain. Pathogenicity scores (PS) were generated by combining death times and clinical scores. Isolates killing >50%, 10–50%, and 0–10% of chicks were classified as virulent, moderately virulent, and avirulent. A one-way ANOVA found that O15, O91, and O88 had significantly lower PS ($p < 0.05$) than the APEC O18 positive control, while O25, O152, O115, and O45 had numerically higher PS.
The challenge assay in 3-week-old chickens involved intratracheal injection of 108 CFU/mL. PS were calculated from death times and clinical scores. Isolates killing >60%, 10–60%, and 0–10% of chickens were considered virulent, low virulent, and avirulent. Interstingly, only O91 caused 66% mortality and was identifed as a virulent strain.
Overall, novel APEC strains exhibited different degrees of pathogenicity across the various models and impacted yolk sac development. Some strains were highly virulent in all assays, while others were not. Further investigation into the pathogenicity of novel APEC in birds is needed to establish new protection plans against emerging serogroups.
Keywords: Avian pathogenic E. coli; Poultry; histopathological ; Colibacillosis; Georgia

3003 Reduction of APEC cecal colonization and quantification of silver residues in broiler breast muscle following therapeutic administration of biogenic silver nanoparticles

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Avian pathogenic Escherichia coli (APEC), including the globally disseminated and high-risk clone ST131, poses an important threat to broiler production and food safety, especially in major exporting countries such as Brazil. In this context, biogenic silver nanoparticles (bio-AgNP) have emerged as a promising antimicrobial alternative. This study aimed to evaluate the therapeutic efficacy of orally administered bio-AgNP in reducing APEC cecal colonization in experimentally infected broilers and to quantify silver residues in pectoral muscle at slaughter age. Forty-eight one-day-old broiler chickens were housed for 42 days and distributed into four groups (n = 12): G1 – negative control; G2 – challenged with APEC; G3 – treated with bio-AgNP; and G4 – challenged with APEC and treated with bio-AgNP. Broilers were experimentally infected with an APEC ST131 strain with induced resistance to nalidixic acid and rifampicin (10^9 CFU/mL; gavage) on days four and five. Bio-AgNP were administered therapeutically on days seven, eight and nine (51 mg/kg). At 42 days of age, broilers were euthanized for sampling of cecum and pectoralis major muscle. Cecal samples were serially diluted in a 1:10 scale and plated on selective media supplemented with the antibiotics used for induced resistance, with bacterial counts expressed as log10 CFU/g. For silver quantification, approximately 500 mg of pectoral muscle were dehydrated, macerated, and subjected to closed-vessel microwave acid digestion, followed by analysis using graphite furnace atomic absorption spectrometry (GFAAS). Data was evaluated by one-way ANOVA followed by Tukey's test, considering $p < 0.05$. Broilers challenged and treated with bio-AgNP (G4) showed a statistically significant reduction in cecal APEC counts compared with the challenged untreated group (G2) ($p = 0.0057$). As expected, no APEC recovery was observed in the non-challenged groups (G1 and G3), confirming proper infection control. Silver concentrations in breast muscle remained low, with mean values ranging from 0.030 µg/g in G3 to 0.021 µg/g in G4, while no detectable silver residues were found in the negative control groups, demonstrating low tissue deposition resulting from the therapeutic regimen. Therapeutic administration of bio-AgNP effectively reduced APEC intestinal colonization in broilers, with low silver accumulation in

edible tissue at slaughter age.
Keywords: nanotechnology; bio-AgNP; ST131; broiler chickens; antimicrobial therapy

3004 Intrapulmonary delivery of live Escherichia coli at hatch in broiler chickens to protect birds against Escherichia coli septicemia during the grow-out period, exploiting the gut-lung axis

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Escherichia coli infections in the broiler chicken industry are a major disease problem, causing a significant economic loss worldwide. Current control measures against E. coli infections are not adequate. The gut-lung axis (GLA) is a bidirectional communication system that links the respiratory system and gastrointestinal system, playing a crucial role in modulating the immune system. We have recently demonstrated the potential of exploiting the GLA concept to control necrotic enteritis in broiler chickens. The objective of this study was to evaluate the immunostimulatory effects of intrapulmonary (IPL) delivery of E. coli at hatch in neonatal broiler chickens against E. coli septicemia, exploiting the GLA context. Live lyophilized and freshly prepared E. coli (1x105 colony forming units (CFU)/bird) formulations were delivered by the IPL route at hatch (n=40), while the control group received saline by the IPL route (n=40). All groups were challenged with E. coli (1x106 or 1x107 CFU/bird) by the subcutaneous route on day 27 of age, and survival and mortality rate was monitored. Survival data were analyzed using the log-rank (Mantel-Cox) test. Birds that received IPL delivery of E. coli had significant protection compared to the saline control. The results of this study demonstrated that IPL delivery of both formulations, fresh ($P < 0.0032$) and lyophilized ($P < 0.0001$) E. coli induced immunoprotection against E. coli septicemia. The mechanism of protection is mediated through GLA. Overall, the findings of this study lay the foundation for a novel control strategy against E. coli septicemia and yolk sac infections in the broiler chicken industry.
Keywords: Escherichia coli; Intrapulmonary delivery; Broiler chickens; Gut–lung axis; Septicemia

3005 Molecular detection and characterization of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli from poultry and farm environments in Bangladesh

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Objectives: This study hypothesized that poultry farms and their surrounding environments in Mymensingh harbor ESBL-producing Escherichia coli with significant antimicrobial resistance and important virulence genes. The objective was to determine their prevalence, antimicrobial resistance pattern, and associated genetic characteristics.
Problem Description: A cross-sectional study was conducted to isolate, identify, and characterize ESBL-producing E. coli from multiple poultry farm sources. The problem addressed was the growing public health threat posed by antimicrobial-resistant bacteria circulating between poultry, the environment, and humans.
Materials and Methods: A total of 197 samples-cloacal swabs, litter, feed, water, and farmers' hand wash-were collected from 40 poultry farms. Standard bacteriological and biochemical tests were performed to identify E. coli. ESBL production was confirmed phenotypically, followed by molecular detection of resistance genes (bla-TEM, bla-SHV, bla-CTX-M-1, bla-OXA-1). Pathotyping (stx-1) and serotyping (O26) were also performed.
Statistical Analysis: Descriptive statistics were used to determine prevalence. Comparative statistical analyses (e.g., one-way ANOVA or chi-square where appropriate) were applied to evaluate differences between ESBL-positive and ESBL-negative isolates.
Results: Overall, 30.91% of isolates were ESBL-positive. Among these, bla-TEM was present in 100%, while bla-SHV (5%), bla-CTX-M-1 (25%), and bla-OXA-1 (20%) were detected. Multidrug resistance was observed in 50.98% of ESBL-positive and 34.21% of ESBL-negative isolates. Additionally, 45% of ESBL-positive isolates carried the stx-1 gene, and 5% belonged to the O26 serogroup.
Conclusions: The study reveals widespread ESBL-producing and multidrug-resistant E. coli in poultry farms, posing substantial risks to farm workers and consumers through environmental and food-chain exposure. Strengthened biosecurity and prudent antibiotic use are imperative to reduce the spread of these resistant pathogens.
Keywords: ESBL-producing Escherichia coli; Multidrug resistance (MDR); Poultry farm environment; Antibiotic resistance genes; Public health hazards.

3006 Isolation and characterization of Enterococcus faecalis isolated from cases of vertebral osteomyelitis in broiler chickens.

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Enterococcus spp. are Gram-positive, non-spore-forming bacteria commonly present in the intestinal microbiota of birds; however, several species have emerged as opportunistic pathogens associated with septicemia and locomotor disorders, leading to significant sanitary and productive losses in poultry systems. Among these, Enterococcus cecorum and Enterococcus faecalis are frequently implicated in vertebral osteomyelitis in broilers and breeder chickens and are recognized as important reservoirs of clinically relevant antimicrobial resistance genes. This descriptive study aimed to isolate and phenotypically characterize Enterococcus spp. recovered from vertebrae of broiler chickens presenting paresis and/or paralysis and macroscopic lesions compatible with osteomyelitis of the free thoracic vertebra, originating from six distinct farms. Vertebral samples exhibiting compatible lesions were cultured on BBL Enterococcosel™ agar and incubated at 35 °C under microaerophilic conditions for 48 h. Presumptive colonies (n = 6) were subjected to biochemical characterization, including carbohydrate fermentation, amino acid decarboxylation, and Gram staining, and were subsequently identified to the species level by MALDI-TOF mass spectrometry. Antimicrobial susceptibility was evaluated by the disk diffusion method using tetracycline, linezolid, ciprofloxacin, erythromycin, lincomycin, sulfamethoxazole/trimethoprim, florfenicol, and fosfomycin. Biofilm formation was assessed using the crystal violet microplate assay in TSB broth, with spectrophotometric reading at 546 nm. All isolates were identified as Enterococcus faecalis. Resistance was observed to lincomycin (6/6), consistent with intrinsic resistance, erythromycin (6/6), tetracycline (5/6), sulfamethoxazole/trimethoprim (4/6), ciprofloxacin (3/6), and linezolid (2/6). Resistance to fosfomycin was detected in a single isolate (1/6), whereas all isolates remained susceptible to florfenicol. All isolates exhibited resistance to three or more antimicrobial classes, characterizing a multidrug-resistant profile. In addition, all isolates demonstrated strong adhesion and

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biofilm-forming capacity. In conclusion, E. faecalis was isolated from vertebral osteomyelitis lesions in broilers from different farms and exhibited multidrug resistance combined with marked biofilm-forming ability. These phenotypic traits may contribute to bacterial persistence within the host and underscore the importance of routine identification of E. faecalis in cases of locomotor disorders in poultry.
Keywords: bacterial resistance; diagnostics; locomotor disorders; Enterococcus faecalis; biofilm formation

3007 Detection of multidrug-resistant Enterococcus spp. in litter and Alphitobius diaperinus in broiler poultry houses
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Poultry litter plays a critical role in broiler production systems, directly influencing bird welfare, performance, and health status. When improperly managed or reused, litter may function as an environmental reservoir for pathogenic and antimicrobial-resistant bacteria. Within this context, Enterococcus spp. are of particular concern due to their environmental persistence, frequent multidrug-resistant phenotypes, and documented association with osteomyelitis and locomotor disorders in poultry. In addition, the lesser mealworm (Alphitobius diaperinus) has been implicated in the maintenance and dissemination of pathogens within poultry house environments. This descriptive study aimed to report the isolation and phenotypic characterization of Enterococcus spp. recovered from poultry litter and lesser mealworms collected from a broiler farm with a history of locomotor disorders. Composite samples (40 g) of litter and lesser mealworms were collected from four distinct locations within the poultry house (n = 4 per sample type), homogenized, diluted 1:10, and plated onto BBL Enterococcosel™ agar. Plates were incubated at 35 °C under microaerophilic conditions for 48 h. Presumptive colonies were subjected to biochemical confirmation, including carbohydrate fermentation, amino acid decarboxylation, Gram staining, and esculin hydrolysis. Following genus confirmation, antimicrobial susceptibility was assessed by the disk diffusion method using tetracycline, linezolid, ciprofloxacin, erythromycin, lincomycin, sulfamethoxazole/trimethoprim, florfenicol, and fosfomycin. Isolates resistant to three or more antimicrobial classes were classified as multidrug resistant. Eight isolates displaying phenotypic characteristics consistent with Enterococcus spp. and Gram-positive cocci morphology were recovered. Reduced susceptibility was observed for lincomycin (8/8), consistent with intrinsic resistance, as well as for ciprofloxacin (7/8), tetracycline (6/8), erythromycin (5/8), sulfamethoxazole/trimethoprim (3/8), and fosfomycin (3/8). Linezolid resistance was less frequent (2/8), whereas all isolates remained susceptible to florfenicol. All isolates (8/8) exhibited resistance to three or more antimicrobial classes, characterizing a multidrug-resistant profile. The detection of multidrug-resistant Enterococcus spp. in poultry litter and lesser mealworms highlights their presence across distinct environmental components of the poultry house and supports their potential role as environmental reservoirs. These findings reinforce the importance of effective litter management and biosecurity strategies to mitigate the persistence and dissemination of antimicrobial-resistant bacteria in broiler production systems.
Keywords: biosecurity; antimicrobial resistance; broilers; performance; pathogen

3008 Establishment of an reproductible Enterococcus cecorum infection model under farm conditions to test alternatives solutions to antibiotics
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E. cecorum (EC) has been recognized as a major emerging pathogen in fast-growing broiler chickens. The clinical signs have a significant impact on animal welfare, zootechnical performance, and economic outcomes and antibiotic consumption. Development of alternative solutions requires the establishment of a robust experimental infection model. The aim of this study was to select one EC strain, among five isolated in 2023 from Ross 308 broilers lesions, based on its ability to reproduce clinical signs under commercial farm conditions.

An experimental farm authorized to perform this inoculation received 1,536 male Ross 308 chicks on day (D)0, which were randomly allocated into six groups, each with 4 replicates of 64 birds. Five groups (infected area) were individually inoculated by intra-crop route with a different EC strain (same dose and volume). The sixth received sterile water (non-infected area). Both areas were physically separated and equipped with specific feed pans and nipples. In infected area, each group was separated by a one-meter-wide walkway. High biosecurity level was maintained to prevent human contamination between groups. At D7, intestinal colonisation was monitored by EC PCR on cloacal swabs. Clinical signs (lameness assessed by Gait Score, mortality, underweight) and zootechnical performance were recorded on D21 and D34. Necropsies were performed on underweight or lame birds. Isolated EC strains were compared with the inoculated ones using MALDI-TOF. Chi2, Kruskal Wallis and ANOVA were used to compare all the variables.

Two groups showed clinical signs in a repeatable way with their inoculated strains. Infection dynamics differed between these two strains. The chosen one caused clinical signs earlier (≈20% at D21) and induced both mild and severe lameness. Model reproducibility has been validated in a second trial using this strain with the same protocol. For the 3 other groups, re- isolated EC strains on lesions did not all match to their inoculated ones, including two groups that had inconsistent intestinal colonization at D7. These results suggested aero diffusion inside the farm and the ability of certain strains to outcompete others within the same environment.

This EC infection model with the chosen strain reproduced what could be observed on the field in commercial farms. This tool could allow testing alternative solutions to prevent EC clinical signs and reduce the use of antibiotic treatment. Beyond that, for the first time, 5 clinical strains were tested in the same environment and exhibited very different behavior, raising questions about the conditions of EC pathogenicity.
Keywords: E. cecorum; Experimental infection; Broilers; Alternative solutions; Pathogenesis

3009 Field Evidence Challenges the Role of Immunosuppression in Recurrent Enterococcus cecorum Infections
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Enterococcus cecorum (EC) osteomyelitis/spondylitis is an emerging condition in broilers and the pathogenesis is poorly understood. This study hypothesized that immunosuppression is an important risk factor in the development of EC infection and that chickens

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with damaged or atrophied bursa of Fabricius (BF) are more likely to develop EC-associated lameness. The objective was to collect the BF from healthy and lame chickens on EC positive farms and determine if there is an association between BF scores and health status. A field study was conducted on commercial broiler farms with recurrent EC episodes. EC positive barns were selected by poultry veterinarians based on a history of confirmed EC infections in previous flocks. During the third week of age, veterinarians visited a total of 16 farms and as part of the sampling protocol, collected BF from lame and healthy birds. The BF were collected, fixed in formalin, sectioned, stained with hematoxylin and eosin, and examined histologically by a poultry pathologist. BF lesions were scored on an ordinal scale ranging from 0 (normal) to 5 (marked atrophy). Statistical comparisons between healthy and lame birds were performed using the nonparametric Mann-Whitney U test, and a Kruskal-Wallis test, followed by a post hoc Holm-Bonferroni test, was used to compare the farms.
Bursal lesion scores varied from 0 to 5 across all birds and flocks. Lesion scores of 5 are unusual for three-week-old chickens and were observed in at least one BF on six farms. There was no significant difference between healthy and lame chickens ($p = 0.67$), with bursae from healthy broilers ($n = 59$) presenting a mean score of 1.95 (SD = 1.67) and bursae from lame chickens ($n = 49$) presenting a mean score of 1.90 (SD = 1.37). Scores varied significantly among farms (Kruskal–Wallis test, $H = 36.24$, $p = 0.0016$). However, post hoc pairwise comparisons with corrections did not identify significant differences between individual farms. This suggests that the observed variability was distributed across farms rather than being driven by specific outliers. Our results indicate that immunosuppression, as assessed by bursal lesion scores at three weeks of age, was not associated with EC-related lameness. Birds without clinical signs of lameness had bursal scores comparable to those of lame birds within the same flock, suggesting that bursa atrophy does not predict susceptibility to EC. These findings indicate that immunosuppression is unlikely to be a primary risk factor for EC infection, pointing instead to the involvement of other risk factors in the development of EC-associated lesions and lameness.
Keywords: Enterococcus cecorum; immunosuppression; Bursa of Fabricius; broiler chickens; field study

3010 Translating Field Conditions to Laboratory: Establishment of an in vivo Model of Colibacillosis in Laying Hens

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Avian colibacillosis, caused by avian pathogenic Escherichia coli (APEC), represents a major challenge for the egg industry, leading to economic losses through elevated flock mortality, compromised hen health, and reduced egg production, particularly during peak production at 27–28 weeks of age. Predominant serogroups include O1, O2 and O78, with virulence of strains mediated through expression of adhesins, siderophore-mediated iron uptake, and protective surface structures. The absence of consensus regarding inoculation conditions to reliably reproduce extra-intestinal APEC infections in laying hens at peak production remains a limitation for establishing a reproducible experimental model. Yet, such a model is essential for evaluating efficacy of alternative antimicrobial strategies and optimizing dosage regimens of selected antimicrobials used in this sector.

The present study aimed to establish inoculation parameters capable of reproducing colibacillosis in laying hens, including clinical signs and lesions typically observed under field conditions in Canada. Based on results of pilot studies comparing different inoculation routes, 60 28-week-old white Lohmann hens were purchased from a commercial poultry farm and separated in six groups of 8-12 hens. Following an acclimatization period, groups were exposed to a light stress (100 lux) for 6 to 12 hours prior to inoculation. Hens were then inoculated intraperitoneally (IP) with 1x109 colony-forming units of an exponential-phase, rifampicin-resistant APEC O78:H9 strain suspended in 0.5 mL of 0.9% NaCl. Clinical signs, mortality, lesion scores and bacterial loads in the ileum and cecum (for both generic E. coli and the challenge strain) were measured to evaluate the model's ability to reproduce infection. Mortality ranged from 16.6% (2/12) to 41.6% (5/12) across infected groups, with higher rates observed in hens subjected to light stress. T-test analysis of lesion scores at 24, 48, and 72 hours post-infection (PI) revealed significant differences between infected and control groups. Two-way ANOVA showed differences in total lesion scores among infected groups only at 48 hours PI. No significant differences were detected in generic E. coli loads, suggesting IP inoculation did not trigger intestinal dysbiosis.

Together, our results suggest that IP infection of APEC O78:H9 in exponential phase is sufficient to induce characteristic lesions of extra-intestinal infections in hens at peak of production. While colibacillosis often arises as a secondary infection under predisposing stress conditions, incorporating pre-infection light stress into the model appears to better simulate field conditions, as reflected by increased mortality among stressed groups.
Keywords: Avian pathogenic Escherichia coli (APEC); Colibacillosis; Laying Hens; In vivo; Model

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3011 Why Bug about Salmonella? – a microbiota database exploration study

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Modern poultry production conflicts with the fact that bird microbiota frequently includes Salmonella. Salmonella may or may not impact birds' health and performance but pose a high risk for food safety. Salmonella is a genus of Enterobacteriaceae family of bacteria with 2 species, S. bongori and S. enterica. Salmonella enterica is divided into six subspecies and more the 2650 serovars. From those, about 50 serovars are relevant for food safety because they cause infections in humans. Salmonella enterica subsp. Enterica serotype enteritidis causes most human infection cases in the USA. Each serovar can have many strains, which makes the story even more complex. Their differences are mostly based on antigen recognition or sequence typing. Since the DNA sequences used by these methods are not in the 16S gene, Salmonella identification in many microbiome studies is limited. A commercially available microbiota analysis platform called Galleon™ covers hundreds of pre-selected bacteria biomarker DNA probes designed based on 16S gene sequences. This list of biomarkers was complemented by adding 2 probes to identify Salmonella designed based on sequences of heat shock protein genes. The method used is a high-throughput fluorescence microarray assay. Extensive use of this platform generated a database with more than 10,000

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observations for broiler alone, with 13 countries represented, 4 breeds and 6 time points or ages (7, 14, 21, 28, 35 and 42 days). A database exploration exercise focusing on Salmonella calculated how frequently Salmonella was found in commercial and research conditions and what is the ratio Salmonella positive birds in flocks. This was possible after a Salmonella risk threshold was created and validated in controlled conditions for the platform, where Salmonella was confirmed either by culturing or by qPCR analysis. After classifying observations in the database based on Salmonella level, machine learning models were trained to classify new samples for Salmonella level using microbiota data. Results showed that in the database we have 41 datasets split into 24 R&D trials and 17 commercial trials. Salmonella prevalence was evaluated per flock. For R&D trials each trial was considered a flock, so we have 24 flocks. Commercial trials sampled different houses, so each house was considered a flock so within the 17 trials we have 88 flocks. It was noticed that in commercial datasets there was no data for 14 days so there were only 5 time points. In commercial conditions, 32% of them had at least 1 positive bird (28 out of 88 flocks). As far as prevalence per age, commercial flocks had more than 10% positive birds at 7, 21 and 28 days and 1% in older birds (35 and 42d). Within the 28 positive flocks, there was, on average, 18% of positive birds per flock. In the case of R&D trials, 100% of them had at least 1 positive bird (24 out of 24 flocks). The prevalence per age was similar to commercial flocks with 13% positive birds from 7 to 28d and 7% positive birds in older birds (25 and 42d). There were clearly 3 groups of R&D flocks based on Salmonella prevalence, 40% highly positive (>15%) and 30% with less than 5% positives, and 30% between 5 and 15% Salmonella positive birds. Investigating what the highly positive trials had in common revealed that those were trials where major shifts in diets were applied, being both in ingredients and/or feed additives. It was possible to train AI models for Salmonella classification (positive vs. negative) for birds at 7 and 21d. These models were highly accurate (>95%) even when Salmonella information was removed from the datasets. This means that there is a Salmonella associated microbiota signature that is consistently different between positive and negative birds. The most important biomarker for the models at both ages was Enterococcus hirae. It was a surprise to find more flocks with positive birds in R&D trials compared to commercial flocks but that indicated that extreme dietary interventions give opportunity for Salmonella to colonize. The prevalence based on age matched expectations based on previous research data, indicating that efforts to control Salmonella should target flocks before 28 days of age. This example shows the value of exploring large databases to identify and confirm patterns of prevalence and design future interventions.

3012 Small players, big signals: a low abundance biomarker balance (LABB) captures intestinal responses to necrotic enteritis in broilers

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Hypothesis or objective: Low-abundance intestinal taxa were hypothesised to act as biomarkers of gut balance in broilers, and a network-weighted Low-Abundance Biomarker Balance (LABB) to distinguish healthy birds from those challenged with necrotic enteritis. Experimental design: Cloacal swabs were collected from broiler chickens classified as Healthy (non-challenged) or Challenged (co-infected with Eimeria spp. and Clostridium perfringens) across five experiments (31 Healthy and 30 Challenged birds). No feed additives were used. Materials and methods: Bacterial communities were profiled by 16S rRNA gene sequencing; reads were classified to genus level, and relative abundances were calculated per sample. Candidate genera were first screened in the dataset as low-abundance but prevalent taxa (<0.5% mean relative abundance, ≥20% prevalence) differing between Healthy and Challenged birds. From these, 12 genera with consistent beneficial or detrimental associations with intestinal health or enteric disease, reported in multiple peer-reviewed studies, were retained and labelled as positive (n = 7) or negative (n = 5) biomarkers. Focusing on low-abundance taxa aimed to capture otherwise hidden information about the intestinal ecosystem and to provide specific signals for gut health improvement. A genus-level Spearman co-occurrence network ($|r| \geq 0.5$) was built to estimate a weighting coefficient for each biomarker, which was rescaled to a 0.2–1.0 weight reflecting putative ecological impact. For each sample, a raw LABB score was computed as the network-weighted sum of positive minus negative biomarkers and then linearly rescaled to a 0–10 scale. Statistical analysis: Between-group differences in genus-level abundances and LABB were tested using Mann–Whitney U tests with false discovery rate correction for multiple comparisons. Results: Literature-based low-abundance biomarkers represented <0.5% of total reads individually but were detected in >20% of samples. LABB differed between Healthy and Challenged birds ($P < 0.001$), with Challenged birds concentrating lower scores and Healthy birds exhibiting a subset of high-balance profiles (LABB values approaching 10). Conclusions: Integrating literature-derived functional polarity with network-based weights for low-abundance taxa yielded a Low-Abundance Biomarker Balance that captured microbiota responses to Eimeria and C. perfringens challenge in broilers, highlighting the contribution of low-abundance community members to microbiota interpretation and to the design of targeted intestinal health interventions.

Keywords: broiler chicken; intestinal microbiota; low-abundance taxa; biomarker balance; Clostridium perfringens

3013 EFFECT OF TURMERIC SUPPLEMENTATION ON GROWTH PERFORMANCE, WATER INTAKE AND THERMO-PHYSIOLOGICAL INDICES OF BROILER CHICKENS CHALLENGED WITH SALMONELLLA GALLINARIUM

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Most commercial farms utilize antibiotics as a preventative measure and therapy for Salmonella gallinarium, a prevalent poultry disease that primarily affects young birds between days 14 and 21. Therefore, the purpose of this study was to assess how supplementing feed and drinking water with turmeric affected the growth performance, water consumption, and thermo-physiological indicators of broilers infected with Salmonella gallinarum.
A total of 144 broiler chicks of abor acre strains were randomly assigned to four treatments consisting of 36 birds each, two groups (T3 and T4), were infected via gavage with 0.1ml of 2 x 105 Salmonella gallinarium endospores suspended in water. Treatment one (T1) served as control and was given normal medication (NM) for broilers. Treatment two (T2) were given turmeric supplement (TS), treatment three (T3) received NM with Salmonella gallinarium challenge (SGC), treatment four (T4) received TS and SGC. Broiler feeds were treated with Turmeric powder at 2g/kg, while ethanol extract of turmeric were included in drinking water at 0.5g/L at any point antibiotics were administered to the control treatments (NM). The pathogen was administered to the birds by oral gavage at 1ml/bird at 21 days old. Daily water intake, daily rectal temperature, respiratory rate (RR) and pulse rate (PR) were measured for 21 days post infection (DPI). Daily feed intake (DFI) and weight

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gain were collected and used to calculate feed conversion ratio (FCR) and feed efficiency (FE). Data generated were subjected to one-way analysis of variance in a completely randomized design (CRD) using the graphpad prism software. Average water intake ranged from 288.93 (T4) - 353.33 (T3) ml/day, though significant differences (P=0.0181, 0.0068, and 0.0084) were obtained on the 8, 18 and 21 dpi respectively. Mean DFI ranged from 692.57 (T1) -793.79g (T2), with significant differences (P=0.03, 0.05, 0.03, 0.02, and 0.02) observed on 2, 4, 5, 12, and 19 dpi. T4 had the best ADG and FCR with T3 recording the least for ADG and FCR respectively. RT were similar but were significantly different (P=0.0001, 0.0283, 0.0001, 0.0013, and 0.0155), on 1, 2, 4, 6, and 7dpi. RR sharply increased from 42.26 and 42.16 on 2dpi to 114.2 -121.3 on 3dpi for T3 and T2 respectively, and dropped on 4dpi to 81 and 58 bpm with significant differences (P=0.0011, 0.0021, and 0.0056) on 2, 4, and 6 dpi respectively. The PR of birds were normal, but only differed significantly (P=0.0040) on 1 dpi. The study suggests that supplementation of turmeric in both feed and water can improve FCR and normalize thermo physiological parameters in broiler production.

Keywords: Phytogenics; curcumin; Bacteria infection; antibiotic resistance; Salmonella

3014 Broiler litter conditions influence the efficacy of probiotics used for Salmonella reduction

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Probiotics are prophylatic alternative to antibiotics to reduce the proliferation of food-borne pathogens. However, we do not know how environmental factors influence the efficacy of probiotics or if probiotics have a broad or narrow specificity towards targeted pathogens. We raised 768 one-day old broiler chicks on litter composed of either new pine wood shavings (PWS) or reused litter (used to raise 2-4 flocks) that was amended with a Bacillus velezensis proLitterbiotic at a rate of 2.7 x 109 CFU/ft2. The study was conducted in two separate trials with 96 birds/treatment (48 birds/trial; 24 birds/pen). At 2-days old, six chicks from each group were orally inoculated with a cocktail of 104 Colony Forming Units (CFU) of nalidixic acid (NAL) resistant strains of Salmonella serovars Enteritidis, Heidelberg, Infantis, and Kentucky. At days 7, 14, and 28 cecal samples were collected from six chickens per treatment. At day 35, feed was withdrawn for 6 h and fresh fecal droppings were collected from 6 chickens per treatment. Salmonella prevalence and abundance was determined by plating cecal and fecal slurry onto Xylose Lysine deoxycholate agar supplemented with 64 ppm NAL. We used multiplex qPCR to estimate the prevalence of the 4 Salmonella serotypes at harvest. Salmonella prevalence was higher in trial 2 (65.4%) than trial 1 (34.5%) ($P < 0.001$). Data was analyzed using PROC GLM. Irrespective of trial, broilers raised on reused litter had lower Salmonella prevalence (21%) and abundance (log10 1.58± 0.08 CFU/g) in their ceca and feces than broilers raised on PWS (Prevalence = 79%; abundance = log10 2.63± 0.18 CFU/g) ($P< 0.0001$). There was a significant interaction between trials and treatments. In trial 1, broilers raised on litter amended with proLitterbiotic had higher Salmonella prevalence (71.4%) than chickens on unamended litter (28.5%) ($P < 0.01$). In trial 2, Salmonella prevalence in the proLitterbiotic group was lower (43.4%) compared to broilers raised without proLitterbiotic (56.6%). qPCR-based serotyping revealed that most of the Salmonella isolates recovered from broiler feces post-feed withdrawal in trial 2 were S. Heidelberg (39/53), S. Enteritidis (11/53), S. Kentucky (2/53) and unknown (1/53) strains. S. Heidelberg was prevalent in the feces than others raised on unamended litter while S. Enteritidis was more prevalent in litter amended with proLitterbiotic ($P < 0.0001$). In this study, broilers raised on reused litter were less likely to harbor Salmonella than chickens raised on new pine wood shavings suggesting the efficacy of probiotics was influenced by the litter conditions. Probiotics putatively affected diversity of Salmonella serotypes that are able to colonize a broiler flock.

Keywords: Salmonella; Probiotics; Litter; ProLitterBiotic

3015 Synergistic Effects of Quercetin and Zinc Sulfate on Alleviating Intestinal Damage Induced by Salmonella Infection in Broilers

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Newly hatched broilers are highly susceptible to Salmonella infection due to the immaturity of their immune system and intestinal microbiota. To identify effective antibiotic alternatives, this study investigated the synergistic protective effects and mechanisms of quercetin, zinc sulfate, and vitamin C against Salmonella challenge in broilers based on the process of salmonella invasion into intestinal mucosa. A total of 540 one-day-old male Ross 308 chicks were randomly assigned to nine treatment groups: a control (CON) group, a Salmonella-infected (ST) group, and groups supplemented with single (Q, Zn, VC) or combined additives (QZ, QC, ZC, QZC). Growth performance, antioxidant capacity, inflammatory responses, intestinal morphology, and microbiota composition were comprehensively assessed. Data were statistically analyzed by one-way ANOVA, along with the Tukey HSD multiple comparisons test. Salmonella infection significantly markedly reduced average daily gain (ADG) and increased feed conversion ratio (FCR) during both the 22-42 d and 1-42 d periods compared with other treatments ($P < 0.001$). It also impaired thymic development and elevated hepatic and intestinal reactive oxygen species (ROS) levels, accompanied by increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities compared with the CON group ($P < 0.05$). Relative to the ST group, QZ group markedly increased ADG and decreased FCR during 1-42 d ($P < 0.05$). QZ also reduced hepatic ROS levels and serum AST and ALT activities ($P < 0.05$). Villus height was markedly increased in Q and QZ groups compared with the ST group ($P < 0.011$). Compared with the ST group, Q and QZ groups markedly reduced ileal ROS levels ($P < 0.001$); Q group significantly upregulated nuclear factor erythroid 2-related factor 2 (NRF2), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase 2 (GPX2), and GPX3 expression ($P < 0.05$), enhancing total antioxidant capacity (T-AOC; $P = 0.032$). QZ group decreased ileal MDA concentration ($P = 0.008$) and increased GPX2 expression relative to ST ($P < 0.001$). Q and QZ groups significantly downregulated NF-κB and IL-6 expression ($P < 0.05$) while upregulating IL-10 ($P = 0.008$). Combined supplementation with Quercetin and Zinc Sulfate further enhanced α-diversity in early-stage broilers and altered the relative abundance of Fournierella (day 5) and Turicibacter (day 42) ($P < 0.05$), suggesting improved microbial stability. In conclusion, combined supplementation of quercetin and zinc sulfate synergistically alleviates Salmonella-induced oxidative and inflammatory damage, enhances intestinal antioxidant and immune defenses, and maintains gut microbial homeostasis in broilers.

Keywords: Quercetin; Zinc sulfate; Salmonella infection; Intestinal damage; Broiler

3016 Recombinant Lactococcus lactis secreting FliC protein nanobodies for resistance against Salmonella Enteritidis invasion in the intestinal tract

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Salmonella Enteritidis is a major foodborne pathogen worldwide, and the increasing prevalence of antibiotic-resistant strains poses a serious threat to public health and poultry production. In this study, nine nanobodies targeting the flagellar protein FliC of S. Enteritidis were identified, among which FliC-Nb76 exhibited the strongest activity, with EC50 values of 1.37 × 10-4μM for FliC and 4.65 × 10-4 μM for S. Enteritidis. In vitro assays showed that FliC-Nb76 significantly inhibited bacterial motility at a minimum effective concentration of 0.2 mg/mL and markedly reduced adhesion to and invasion of human intestinal epithelial cells (HIEC-6), murine macrophages (RAW264.7), and chicken intestinal epithelial cells, including multidrug-resistant clinical isolates ($P < 0.05$). A recombinant nanobody-secreting strain, L. lactis-Nb76, was subsequently constructed. In a chicken infection model, oral administration of L. lactis-Nb76 reduced mortality from 26.7% in infected controls to 0%, significantly decreased intestinal colonization and fecal shedding of S. Enteritidis, alleviated intestinal pathological damage, and downregulated pro-inflammatory cytokines (IL-1β, IL-8, TNF-α, and IL-17A) while upregulating IL-10 ($P < 0.05$). These results demonstrate that nanobody-secreting Lactococcus lactis represents an effective, antibiotic-independent strategy for controlling S. Enteritidis intestinal infection in poultry.
Keywords: S. Enteritidis; Recombinant Lactococcus lactis; FliC protein nanobodies; Targeted bacteriostasis

3017 Genomic comparison of clinical and non-clinical Enterococcus cecorum strains isolated in West of France between 2023 and 2024

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E. cecorum (EC) is a commensal but also a major pathogen for fast-growing broilers worldwide. The existence of biomarkers differentiating clinical strains (C, isolated from typical lesions) and non-clinical strains (NC, isolated from the intestines of healthy chickens before slaughter) could allow the development of early detection and control strategies for infection. The aim of this study was to perform a molecular comparison of C and NC strains collected within a restricted temporal and geographical space.

During 2023 and 2024, 54 strains were collected across Brittany production area. Protein fingerprints obtained by MALDI-TOF allowed the grouping of strains into MALDI-groups according to their degree of similarity. Five pairs of strains (= 100% homologous with this method) were subsequently selected within five MALDI-groups. Whole-genome sequencing (WGS) was performed on each of the pairs, including C/C (1 pair), C/NC (3 pairs), and NC/NC (1 pair) combinations. Genome analysis was used to search for the presence of virulence factors (VF) and to perform a single nucleotide polymorphism (SNP) analysis.

MALDI-TOF analysis did not allow differentiation between C and NC strains on several EC specific protein biomarkers. SNP analysis revealed significant genetic diversity between strains belonging to different MALDI-groups (3,000 to 12,000 SNPs). Comparison of the SNP profiles of paired C/C strains showed no polymorphism: they were epidemiologically linked to their hatchery of origin. Surprisingly, only minor differences were observed between paired C/NC strains (58, 76, and 690 SNPs). In contrast, 7,014 SNPs were found between the genomes of the paired NC/NC strains.

No notable differences were observed in the VF profiles of the paired strains, unlike what was observed between the different MALDI-groups. Recent publications showing discriminating VFs between strains C and NC are not confirmed in our collection. This implies poor reproducibility of these VF.

Unlike other recent studies, no differentiation pattern between C and NC EC strains based on the molecular criteria was observed. The restricted temporal and geographical scope of the strain collection may partly explain this. C strains did not show a stronger genetic proximity than with certain NC strains, and some even appeared closer to NC strains than to other C strains.

Genetic differences seen between the strains of our study could lead to a certain type of classification that isn't stackable with C and NC criteria. Molecular determinants of clinical strains were not confirmed. Some environmental parameters could, however, have a greater influence on the EC clinical expression.
Keywords: E. cecorum; Broilers; Clinical and non clinical strains; Whole genome sequencing; Virulence Factors

3018 Evidence of Vertical Transmission of Enterococcus cecorum in Broiler Breeders Based on Whole Genome Sequencing

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Enterococcus cecorum has emerged as a significant pathogen in modern broiler production, contributing to skeletal disorders, mortality, and substantial economic losses. Despite its growing clinical importance, its transmission dynamics remain poorly defined, with vertical transmission recognized as a critical yet unexplored route. Building on our previous 16S rRNA findings indicating possible vertical transmission of Enterococcus spp., this study specifically investigated E. cecorum at the strain level using whole-genome shotgun sequencing. We characterized the distribution of E. cecorum across gastrointestinal (GIT), reproductive, and hatchery sites in broiler breeders and day-of-hatch (DOH) chicks to elucidate its potential for vertical transmission. Samples from five 69-week-old hens, eggs, day-of-hatch chicks, and hatchery fluff were processed for whole-genome sequencing. DNA was extracted via bead beating and enzymatic lysis, libraries prepared with Nextera XT and sequenced on the Illumina NextSeq 2000. Quality control included FastQC, Trim Galore, host-read removal, and taxonomic assignment via Kraken2/Bracken for species and strain-level resolution. Our results showed that E. cecorum was detected across all sampling sites, supporting potential vertical transmission. Within sampled hens, relative abundance of E. cecorum ranged from 0.06–0.85% (cecum), 0–2.37% (cloaca), and 0.09–1.85% (crop). Further downstream, E. cecorum was present at relative abundance levels of 0.78–7.69% in eggs, 0.23% in fluff, 0–0.11% in the intestinal tract of day-of-hatch chicks, and 0–5.34% in the small intestine. On average, eggs exhibited the highest relative abundance (4.87%), followed by small intestine (4.23%), crop (0.39%), cecum (0.34%), cloaca (0.25%), fluff (0.23%), and day-of-hatch GIT (0.02%). E. cecorum abundance differed significantly among sample groups (Kruskal–Wallis $p = 0.01636$), with eggs showing the highest median levels despite non-significant FDR-adjusted pairwise comparisons. ANCOM also identified eggs as a significantly enriched site. With E. cecorum at ~1% abundance, strain tracing was not possible; however, SGB 7961 was found in all sample types, indicating a conserved lineage with

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vertical-persistence potential. E. cecorum was present in all cecal, crop, and egg samples, 80% of cloacal and small intestinal samples, and 40% of DOH chick gastrointestinal samples, highlighting breeder and hatchery stages as key control points. While individual hen-to-chick linkage and virulence confirmation weren't possible, consistent egg enrichment supports putative vertical transmission. Future work will map breeder-to-chick routes and differentiate commensal versus pathogenic strains.

Keywords: Enterococcus; vertical transmission; microbiome; whole genome sequencing; broiler

3019 Genomic diversity of Enterococcus cecorum isolated from broiler chicken clinical cases in Quebec, Canada

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A constant increase in osteoarthritis, spondylitis, and polyserositis cases in broiler chickens and broiler breeders is observed in Quebec, Canada. Enterococcus cecorum (EC), a Gram positive, facultative anaerobe, and part of the normal intestinal microbiota from chickens was determined to be the causative agent. Recently, Borst et al. 2015 identified cpsO, a gene unique to pathogenic EC strains and used to distinguish pathogenic from commensal strains. However, in some clinical cases, only cpsO- EC strains can be isolated. We hypothesized that factors other than cpsO could be involved in the pathogenesis of EC infections. We sought to assess the phylogenetic relationships from cpsO+ and cpsO- EC isolates from different clinical cases. In addition, we compared the presence of antimicrobial resistance genes (ARGs), virulence factors (VFs), and mobile genetic elements (MGEs) to identify differences between both groups. A total of 92 EC isolates (n=47 for cpsO+ and n=45 for cpsO- isolates), were cultured and whole-genome sequenced. All genomes were assembled and annotated using an in-house pipeline. Phylogenetic analysis was realized based on a core genome alignment performed by roary using raxml-ng. Prediction of antimicrobial resistance genes (ARGs), virulence factors (VFs), and mobile genetic elements (MGEs) was performed using bioinformatics tools (CARD, Resfinder, VFDB, MOBsuite, ISescan). A Shapiro-Wilk test was performed for ARGs, VFs, and MGEs to determine if data had a Gaussian distribution. Next, a Mann Whitney test was conducted for ARGs and VFs (non-Gaussian) and an unpaired t test with Welch's correction was conducted for MGEs (Gaussian) to assess if differences between groups were statistically significant. All differences with $P<0.05$ were considered significant. A high genomic diversity was observed at the core genome level between isolates, but no clear distinction based on the cpsO status, or the sample nature, was observed. Although isolates from different organs of the same clinical case generally clustered together, we also observed the opposite. No significant difference was observed ($P>0.05$) between the total number of ARGs, VFs, and MGEs per isolate for the cpsO+ and cpsO- groups. Since all EC isolates came from clinical cases and because a high genomic diversity was observed between them, these results pointed to an opportunistic lifestyle rather than a pathogenic one in EC. Furthermore, EC infection appeared to be systematic and caused by multiple isolates rather than by a single dominant isolate.

Keywords: Enterococcus cecorum; osteomyelitis; broiler chicken; genomic diversity

3020 Gut microbiota–associated development of lymphoid structures in the avian cecal tonsils

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Cecal tonsils (CTs), which are enriched in both B and T lymphocytes, represent a major inductive site for mucosal immune responses in the avian intestine. CTs are composed of two anatomically distinct compartments, the follicular region (FR) and the interfollicular region (IFR). The FR is predominantly populated by B cells, whereas the IFR contains large numbers of T cells together with B cells. Previous observations suggest that FR development is closely associated with microbial colonization, as this structure remains poorly developed during the first two weeks after hatching. In this study, we therefore investigated the relationship between gut microbiota and FR formation in CTs.

To characterize the three-dimensional organization of CTs during development, tissues were collected from male chickens at 7, 21, 35, and 50 days of age and analyzed using histological reconstruction with Amira software. To assess the influence of gut microbiota on CT morphogenesis, newly hatched chicks were allocated to three groups: non-treated (NT), antibiotic-treated (ABX), and fecal microbiota transplantation (FMT) after antibiotic treatment. Chickens in both ABX and FMT groups received a broad-spectrum antibiotic cocktail (vancomycin, ampicillin, neomycin, and metronidazole) via drinking water until day 21. From day 22, chickens in the FMT group were orally administered fresh fecal suspensions prepared from NT donors once daily, whereas ABX treatment was continued in the ABX group. Cecal tonsils were collected at day 28 for immunohistochemical analysis, and cecal contents were subjected to microbiome profiling. Statistical analyses were performed using two-tailed Mann–Whitney U tests or Kruskal–Wallis tests, with $P \leq 0.05$ considered statistically significant.

Three-dimensional analysis revealed that FR structures first appeared around day 7 on the muscular side of the CTs and progressively expanded toward the luminal side during maturation. The total FR volume increased markedly between days 7 and 21 and remained relatively stable thereafter. Notably, expansion of the T-cell–rich regions toward the lumen preceded the enlargement of FR. Antibiotic treatment was associated with a marked reduction in both the number and volume of FR as well as in T-cell–rich regions within CTs. In contrast, FMT was associated with the restoration of both B- and T-cell compartments, together with recovery of the cecal microbial community.

Collectively, these findings demonstrate a strong association between the presence of intestinal microbiota and the development of the microarchitecture of cecal tonsils during post-hatch maturation, suggesting that gut microbes contribute to the organization of lymphoid structures in the avian intestine.

Keywords: cecal tonsil; B cell; T cell; cecal microbiome; Amira

3021 Bursa of Fabricius-independent B cell genesis in avian GALTs shape gut microbiota in chickens

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Bursa of Fabricius, the primary lymphoid organ for initial B cell development is found only in birds, supplies IgM+ mature B cells to the peripheral lymphoid tissues. Historically, B cell development in chickens has been regarded as entirely bursa-dependent. However, bursa undergoes regression relatively early in life, with the process initiating much earlier. This study aimed to demonstrate the bursa-independent B cell genesis in chickens, and identify its contribution to host gut health. Firstly, B-cell phenotypes across multiple organs in chickens (Ross strain, male) at 35 days of age were investigated by flow cytometry, and CXCR4 expressing immature pre-B cells (bursa-independent B cells) found in the cecal

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tonsils (CTs) were further characterized by scRNA-seq and immunohistochemical analysis. Secondly, the number and distribution of bursa-dependent and -independent B cells in the CTs of chickens treated with surgical removal of bursa and/or CXCR4 antagonist AMD3100 which inhibits CXCL12/CXCR4-mediated chemotaxis were investigated at both 21 and 50 days of age. The number of IgA-secreting cells in gut was also quantified in all groups (n = 6–15/group). Finally, to determine the bacterial specificity of intestinal IgA produced by bursa-dependent or -independent B cells, we performed Bug-FACS/IgA-Seq. Data were analyzed with the non-parametric two-tailed Mann–Whitney U test or the Kruskal–Wallis test. A P-value of ≤ 0.05 was considered significant. Contrary to general avian immunology, our recent studies revealed that CTs consist of immature IgM- pre-B cells, which highly expressed CXCR4. The distribution depended on the B cell type in the CTs, as pre-B cells were predominantly detected in the B cell follicular (FR) region whereas most B cells in the intra-follicular (IFR) region were IgM+ mature B cells. Using bursectomy chickens, we found that FR were fully developed in the CTs even without bursa. In contrast, treatment with a AMD3100, which interferes pre-B cell migration from bone marrow completely inhibited the formation of FR in the CTs, indicating that FR formation was dependent on bone marrow (not bursa). Furthermore, the combination of bursectomy and AMD3100 treatment completely abolished B-cell accumulation in the CTs. Importantly, the contribution of bursa-independent B cells to mucosal immunity progressively increased during chicken maturation. Specifically, at 50 days of age, bursa-independent IgA highly coated SCFA-producing microorganisms (e.g., Lachnospiraceae), and shape the gut microbiome composition associated with metabolic functions. Together, our data indicate that bursa-independent B cell genesis in the CTs regulates the gut microbiome and contributes on the chickens' health.
Keywords: B cell; Bursa of Fabricius; cecal tonsil; IgA; gut microbiome

Incubation Technology and Physiology I

3022 Frontiers in poultry production: Incubation biology and hatchery innovations

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Poultry hatching eggs contain all the nutrients necessary for the developing embryos. However, the quality of day-old chicks that emerge from the eggs depend on several factors, which may include the genotype, breeder age, hatching egg management before incubation, and incubation conditions. Incubation temperature, humidity, turning and ventilation are widely investigated. Indeed, the effects of incubation factors such as exposure of the embryo to high or low levels of these environmental variables, duration of exposure, the amplitude of variations and embryonic developmental stage and post-hatch performance need more investigations. In addition, chick quality is discussed in the context of the parameters for determining it and the factors that may affect it. Recent incubation practice factor such as In ovo feeding may also affect incubation outcomes. It is well known that poor day-old chick quality as well as the delay in feed and water access might affect post-hatch performance, adversely. Thus, several factors affect chick embryo physiology and developmental trajectory and consequently the quality of the hatched chicks and post-hatch performance. Furthermore, few reports have indicated that embryo and post-hatch growth trajectory is gender depended. This suggests that incubation conditions may need to be adapted to the gender in order to improve their productivity. But the main challenge is being able to determine the embryo gender early and to investigate the effects of incubation factors with regard to level of exposure vis-a-vis the embryonic developmental stage. The potential application of adapted incubation conditions for the improvement of post-hatch performance up to slaughter age is also discussed. It is concluded that incubation conditions affect embryo parameters and consequently post-hatch growth differentially according to exposure duration and stage of embryonic development.

Key words: Incubation conditions, embryo development, chick quality, post-hatch performance

3023 Gradual warming from storage to incubation temperature restores broiler embryo development after prolonged egg storage

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Hatching eggs are typically stored at the hatchery before incubation begins. The duration of egg storage depends on the availability of hatching eggs, hatchery capacity, and the demand for day-old chicks. In some cases, it is inevitable to extend the egg storage duration beyond 7 days, a practice referred to as 'prolonged storage'. Prolonged storage is associated with decreased incubation results, such as a lower hatchability, delayed hatching, and worse chick quality at hatch. The extent of these negative effects may be influenced by the rate at which eggs are warmed from storage temperature (18°C) to incubation temperature (37.8°C), a process referred to as the 'warming rate' (WR). The objective of this study was to investigate the combined effects of broiler egg storage duration and WR on morphological embryo development and survival. An experiment was carried out using a 2×3 factorial design, comparing two storage durations (4 and 14 days) and three WR treatments (10, 24, and 144 hours). Morphological embryo development was not statistically assessed. All other data was statistically analyzed using a general or generalized linear mixed model, depending on their distribution.

Embryos from 14-day-stored eggs showed morphological progression during the storage period, but during subsequent warming and incubation, their development lagged behind compared to embryos from 4-day-stored eggs. A WR of 24 and 144 hours enabled embryos to progress morphologically before reaching incubation temperature, without impacting their growth rate during incubation. In 4-day-stored eggs, hatchability increased from 93.6% to 96.0% for the 10-hour WR compared to the 144-hour WR, respectively (P=0.04). In 14-day-stored eggs, hatchability was similar for all WR treatments. No interaction between storage duration and WR was observed for chick length at hatch, however, chicks from 4-day-stored eggs were longer than chicks from 14-day-stored eggs (19.6 vs 19.5 cm; P=0.04) and chicks from a 144-hour WR were longer than chicks from a 10-hour WR (19.6 vs 19.5 cm; P=0.04). A 144-hour WR shortened the incubation period, defined as the duration at incubation temperature, with 77 hours (P<0.01) compared to a 10-hour WR due to the progression of morphological embryo development before reaching incubation temperature. Incubation duration increased for the 10-hour and 24-hour WR in 14-day- compared to 4-day-stored eggs (2 and 9 hours respectively; P<0.01), but this was not observed for the 144-hour WR. The lack of a hatch delay indicates that a 144-hour WR may have counteracted the developmental delay usually observed after prolonged storage. The underlying physiological mechanisms still need to be explored.

Keywords: Incubation; Egg storage; Warming rate; Embryo development ; Hatchability

3024 Optimal paraformaldehyde levels for disinfection of

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eggs used in vaccine production

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The objective of this study was to evaluate the effects of paraformaldehyde levels on the disinfection of fertile eggs from breeders intended for vaccine production. We hypothesize that minimal concentrations of paraformaldehyde can achieve effective bacterial decontamination while minimizing adverse effects on embryonic viability and egg mass. By refining disinfection protocols, this study seeks to enhance the safety and efficiency of fertile egg preparation in vaccine production, meeting both sanitary and economic standards. A total of 4,368 fertile eggs were divided into seven treatment groups: nondisinfected eggs and eggs disinfected with 0, 2, 3, 4, 5, or 6 g/m3 of paraformaldehyde, with 624 eggs per treatment. Disinfection was carried out by fumigation, followed by incubation. On the 10th day of incubation, various parameters were measured, including egg mass loss, absolute and relative weights of the embryo and yolk sac, embryo length, embryonic mortality, and fertility. Bacterial counts were also assessed at three points: before disinfection, one hour after disinfection, and on the 10th day of incubation. Data were analyzed via ANOVA using the general linear model (GLM) in SAS® (SAS Institute Inc., Cary, NC, USA). Significant means (F test at 5% probability) were further evaluated via orthogonal contrasts. For microbiological analyses, frequency analysis was performed via Fisher's exact test with 5% significance, dividing the eggs into mesophilic bacterial counts less than or greater than 300 CFU. Results showed that eggs disinfected with paraformaldehyde had significantly lower bacterial counts compared to nondisinfected eggs. One hour after disinfection, treatments without fumigation or those with fumigation without paraformaldehyde exhibited 100% of bacterial counts exceeding 300 CFU, while the treatment with 2 g/m3 of paraformaldehyde had 83.33% of bacterial counts exceeding 300 CFU. Higher paraformaldehyde levels significantly reduced bacterial counts, with the treatment of 3 g/m3 being the most effective, where only 8.33% of samples had bacterial counts exceeding 300 CFU. The optimal concentration for achieving the lowest bacterial counts was estimated to be 6.07 g/m3. In terms of egg mass, nondisinfected eggs experienced greater mass loss compared to fumigated eggs. Additionally, the best embryo length was observed at a concentration of 2.67 g/m3 of paraformaldehyde. Based on these findings, it is recommended to use 6.07 g/m3 of paraformaldehyde to effectively reduce bacterial contamination in fertile eggs used for vaccine production.

Keywords: fertile egg; formaldehyde; fumigation; incubation; vaccine production

3025 Effects of Different Disinfectant Types on the Eggshell Microbiome and Bacterial Load of Hatching Eggs

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Microbial contamination of hatching eggs poses a significant threat to embryo survival, hatchability, and chick health. This study evaluated the efficacy of two disinfectants—3% hydrogen peroxide spray and formaldehyde fumigation—on bacterial load, microbiome composition, egg quality, and hatchability of Rhode Island Red eggs. Ninety (90) eggs were randomly assigned to three treatment groups: hydrogen peroxide, formaldehyde, and an untreated control. Microbial samples were collected at three stages: pre-disinfection, post-disinfection, and after 14 days of incubation. The target microorganisms were Escherichia coli, Salmonella spp., and Staphylococcus spp., while fungal contamination, primarily Aspergillus spp., was negligible across all treatments. Hydrogen peroxide significantly ($p < 0.05$) reduced bacterial load and maintained the lowest microbial load after incubation, compared to formaldehyde and the control. Both disinfectants preserved eggshell and internal quality traits. However, eggs treated with hydrogen peroxide showed the highest ($p < 0.05$) hatchability (50%) and lowest embryo mortality, while formaldehyde, despite a higher fertility rate (75%), had the poorest hatchability (10%) due to late embryo loss (65%). These findings indicate that 3% hydrogen peroxide is a safe and effective alternative to formaldehyde fumigation, offering improved hatchability and microbial control without compromising egg quality.

Keywords: eggshell microbiome; hydrogen peroxide; formaldehyde fumigation; bacterial load; egg quality

3026 Effect of Heat Stress and biofortified Orange Corn during pullet rearing on growth performance

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Each year the U.S. is breaking temperature records resulting in more high heat days causing heat stress in poultry, especially, laying hens. The laying hen life cycle predisposes them to more instances of experiencing heat stress, including during pullet rearing. Heat stress disrupts the metabolism of chickens, impairing digestive functionality. Dietary ingredients, such as biofortified orange corn, may mitigate heat stress disruptions. The objective of this study was to assess whether heat stress during pullet rearing disrupts pullet growth and if orange corn can mitigate that disruption. 1,600 LSL white pullets were raised in cages (10/cage) in three identical rooms. Pullets were fed one of two isocaloric diets varying in corn source: traditional yellow corn (YC) or biofortified orange corn (OC). Pullet cages were weighed weekly throughout the pullet rearing period. At 9 and 14 weeks of age (woa), room temperatures were increased for 4 days to achieve a temperature heat index (THI) of 82, extreme heat stress zone, for at least 10 hours with nighttime THI of at least 76, alert heat stress zone in one naive room. At 12 hours and then every 24 hours during the heat stress (HS) period and 4 days recovery, liver and gastrointestinal tracts (GIT) were weighed from euthanized pullets. Data were analyzed with two-factor ANOVA. YC birds had heavier body weights than OC pullets ($P = 0.0477$) but not at any specific week. During the 9 and 14 woa HS periods, body weights decreased in heated rooms ($P < 0.04$). During the 14 woa HS, OC pullets were lighter than YC pullets at 12 hours during HS ($P = 0.0046$). At 17 woa, pullets that experienced HS were lighter than pullets in the thermoneutral room ($P < 0.0001$). Liver and GIT weights decreased with HS ($P \leq 0.01$) and during recovery ($P \leq 0.033$). OC pullets had lighter organ weights than YC pullets ($P < 0.02$). In this study, HS during pullet rearing did not disrupt overall growth of pullets but did cause short term growth and metabolic reductions. Orange corn had no effect on overall growth but did seem to mitigate heat stress disruption during the heat stress periods.

Keywords: pullets; heat stress; growth; Orange Corn; organ weights

3027 Effect of eggshell temperature variation during the mid-phase of incubation on hatching profile and post-hatch parameters of male and female sasso broiler chickens

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Incubation is defined as the process of applying heat to eggs and controlling this process is essential for successful hatching. Some factors influence the metabolism of the embryo during incubation, including the sex of the animals.The time at which 50% of females had hatched was about 3 hours earlier than the 50% hatching time for males. This clearly indicates that each sex requires a crucial incubation condition for its embryonic development. Few studies on thermal manipulation during incubation have considered sex as a factor.The present study aimed to evaluate the trajectory of embryonic sexual differentiation in Sasso broiler chickens subjected to varying eggshell temperature profiles during embryonic day 13 (ED13) today 18 (ED18) of incubation. A total of 1,800 eggs obtained from 43-week-old Sasso breeders were numbered, weighed and placed into three of 600 egg-capacity Royal Pas Reform incubators of six replicates of 100 eggs each. The control group (Tc) was incubated at a standard temperature of 37.5°C, the low-temperature group (Tl) at 36.5°C, and the high-temperature group (Th) at 38.5°C, with a relative humidity of 60% across all groups. Between 444 and 501 h of incubation, hatching events were recorded, and chick quality parameters were determined. Increasing the eggshell temperature (38.5°C) resulted in higher egg weight loss at day 18 ($P < 0.05$), leading to increased embryonic mortality and a lower hatchability rate. Male chicks were more abundant under low temperature (53.13%) compared to female chicks (39.29%). Eggs subjected to the Tl group increased chick weight at hatch, particularly in males (39.52 g), narrowed the hatch window, and shortened incubation duration ($P < 0.05$). Female chicks had higher T4 hormone levels in the Th group than the males ($P < 0.05$). Male birds in group Tl had higher body weight, better feed conversion ratio and lower mortality rate during post-hatch ($P < 0.05$). A continuous low eggshell temperature (36.5°C from E13 to E18) presents substantial growth benefits for male Sasso broiler chickens. This study provides remarkable evidence of the effect of eggshell temperature on the chick sex ratio at hatch.

Keywords: Eggshell temperature; Incubation; Sasso chicken; Sex ratio; Embryonic sexual trajectory

3028 Early thyroid hormone variation before HPT-axis formation modulates embryonic development and metabolic response in broiler embryos

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In avian embryos, thyroid hormones, triiodothyronine (T3) and thyroxine (T4), regulate cell proliferation, tissue growth and embryonic endothermic heat production. Since the embryo's thyroid system becomes functional only around embryonic day 10, the embryo depends only on maternal thyroid hormone deposits in the yolk during early development. We compared maternal serum T3 and T4 and corresponding yolk T3 and T4 deposits among layers, slow-growing L1986 broilers and commercial broiler breeders. Our findings indicate that commercial broilers had lower serum T3 and T4 and deposited significantly less T3 and T4 in their yolks compared to slower-growing lines. This suggests that commercial broiler embryos begin development in a relatively hypothyroid state, influencing their physiology and metabolic patterns.

This study, therefore, examines how early in-ovo thyroid hormone supplementation affects development and metabolism in modern commercial broilers.

600 Cobb500 broiler eggs were incubated under standard conditions and injected on embryonic day 3 with four treatments: T4 (100 ng/ml), T3 (50 ng/ml), 6-propyl-2-thiouracil (PTU, 0.25 mg/ml; a T3 and T4 antagonist), and saline control. The embryos were sampled for breast muscle, liver, and heart on embryonic days (E)12, 14, 16, 18, and at hatch, while temperature and heart rate were monitored to assess metabolic activity. Data were analyzed by one-way ANOVA (Tukey HSD) using JMP 18.0, and the results are presented as mean ± SEM ($\alpha < 0.05$).

T3 and T4 injections significantly increase the relative weight of the yolk, embryo, and breast muscle ($P < 0.05$) particularly at hatch. From day 12 to 16, T3- and T4-treated embryos had higher relative heart and liver weight ($P < 0.05$), then returned to saline levels by hatch. PTU treatment reduced relative embryo weight but increased relative breast muscle weight ($P < 0.05$) while maintaining liver and heart weights.

T3 and T4 had no effect on the embryo's heart rate and egg temperature, but PTU treatment reduced temperature from E12 to E16 and heart rate from E16 to E18, suggesting lower metabolic activity. It seems the broiler embryo is still capable of regulating thyroid hormone levels while maintaining stable metabolic activity but it shows limited protection against low thyroid hormone levels following PTU injection, making it more susceptible to hypothyroidism.

These findings highlight the critical role of thyroid hormones in regulating embryonic development and metabolism. Increasing hormone availability before HPT-axis formation promotes embryo growth and balanced metabolism. However, further research is needed to understand the molecular mechanisms and post-hatch performance under different environmental conditions.

Keywords: Thyroid hormone; embryonic development; metabolism; broiler embryo; HPT-axis

3029 Field-optimized cryopreservation protocol for duck semen: toward practical application in Pekin, Muscovy and Mule duck breeding

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Cryopreservation of duck semen remains a major challenge for avian genetic resource preservation and commercial breeding programs. This study aimed to establish an efficient, field-adapted freezing protocol for Pekin (Anas platyrhynchos domesticus), Muscovy (Cairina moschata), and interspecific crosses (Muscovy × Pekin) used for Mule duck production.

Semen was collected from 30–50-week-old drakes and diluted (1:2 or 1:3) in four extenders Buffered Hepes Saline Vehicle(BHSV), Fowl Extender Buffer (FEB), Buffered Phosphate Saline with EDTA (BPSE). Dimethylacetamide (DMA, 6%) or dimethylformamide (DMF, 8%) were used as cryoprotectants. Cooling was performed at 4 °C (10–15 min), followed by freezing at −60 °C/min for 3 min until a final temperature of −140 °C was reached. Thawing was carried out in water at room temperature (20°C) for 30 s. Post-thaw sperm motility and viability were evaluated using an IVOS system. Fertility tests were performed by artificial insemination (100 µL or 200 µL per female, repeated at 48 h intervals) using straws stored in liquid nitrogen for 10–15 days prior to insemination, on Pekin, Muscovy, and Pekin females for hybrid (Mule) production. Data were analyzed using a one-way ANOVA, followed by a Tukey's multiple comparison test when a significant effect was detected. Differences were considered significant at $P < 0.05$.

In Pekin drakes, the best post-thaw fertility was obtained with FEB extender + DMA 6%, achieving 84–92% fertility depending on

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insemination volume (200 µL > 100 µL, $p < 0.05$). DMF yielded significantly lower rates (73%, $p < 0.05$). Optimal dilution (1:2) gave 85% fertility, confirming robust sperm survival and in vivo efficiency. In Muscovy drakes, BHSV extender + DMA 6% provided the best results with 76% fertility on Muscovy hens, while the Lake C extender reached only 19%. Crossbreeding trials (Barbarie × Pekin) produced 35% fertile Mule embryos with BHSV + DMA 6%, compared with 3% using Lake C. These findings demonstrate that extender composition, cryoprotectant type, and equilibration time are decisive factors for cryosurvival and fertilization success. This study provides the first comprehensive, field-compatible protocol for duck semen cryopreservation, enabling reproducible fertility above 80% in Pekin and over 50% in Muscovy under routine farm conditions. The optimized BHSV + DMA combination is also suitable for producing fertile Mule duck embryos. These results pave the way for operational cryobanks and secure preservation of pure lines and hybrid breeding potential in response to sanitary crises such as avian influenza.

Keywords: Duck; semen cryopreservation; fertility; cryoprotectant; mule duck

3030 Effect of fenugreek seed on the production and reproduction parameters of breeding chickens

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The productivity of breeding chickens is a major challenge for the sustainability of the poultry sector in Togo. However, the prolonged use of antibiotics as growth promoters poses serious public health and environmental problems due to the emergence of bacterial resistance. This situation creates an urgent need for natural, safe, and effective alternatives to boost poultry performance. In this context, fenugreek seed (Trigonella foenum-graecum), known for its digestive, metabolic, and reproductive properties, appears as a promising solution. This study aims to promote its use in the feed of breeding chickens in Togo.

The experiment was conducted at the Regional Center of Excellence for Poultry Science (CERSA) in Lomé, on a total of 132 subjects: 120 Ross 308 females aged 58 weeks and 12 Sasso males aged 62 weeks. The chickens were divided into four groups according to the level of fenugreek seed powder supplementation in their feed: T0 (0 g/kg, control), T10 (10 g/kg), T20 (20 g/kg), and T30 (30 g/kg). Measurements were taken regarding weight gain, laying parameters, egg quality, and hatching performance. The data were analyzed using Prisme 8 software, and a one-way ANOVA was used to compare means with a significance threshold of $p < 0.05$.

The results show that weight gain was significantly higher ($p < 0.05$) in groups T20 and T30 than in groups T0 and T10, with no significant difference between T20 and T30 ($p > 0.05$). The laying rate increased significantly ($p < 0.05$) in batches T30 (80%) and T20 (78%) compared to batches T0 (60%) and T10 (63%). The feed conversion ratio (FCR) was significantly lower ($p < 0.05$) in batches T20 (2.5) and T30 (2.3), indicating better feed efficiency. The average egg weight of batch T30 (65 g) was also significantly higher ($p < 0.05$) than that of batches T0 (58 g), T10 (59 g), and T20 (60 g). Similarly, the yolk weight, albumen weight, thickness, height, and diameter of eggs in batch T30 were significantly greater ($p < 0.05$) than those in the other batches. The fertility rate (90%), chick hatching weight (42 g), and hatching rate (85%) were also significantly higher ($p < 0.05$) in group T30.

In conclusion, supplementing the feed ration with 20 to 30 g/kg of fenugreek seed powder significantly ($p < 0.05$) improved the production and reproductive performance of breeding chickens, with no notable difference between these two high supplementation levels.

Keywords: Fenugreek (Trigonella foenum-graecum); Breeding chickens; Livestock performance; Fertility and hatching; Feed supplementation

3031 Science supporting SetCare: A new start of incubation

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Large-scale artificial incubation has grown rapidly since the last century. Ever since then, batches of hatching eggs are collected from breeder farms and transported to hatcheries, where eggs are generally stored at ≤65°F to preserve their quality until incubation is initiated. To initiate incubation, eggs have to be warmed to 100°F eggshell temperature (EST). As hatching eggs already contain a blastoderm at oviposition, the embryo will be subjected to a temperature transition of approximately 35°F at the start of incubation. Embryos are poikilotherm, meaning that their development is highly dependent on ambient temperature. However, remarkably enough, effects of the warming rate from storage to incubation temperature have barely been studied until now. In 20 consecutive experiments with a total of 153,920 eggs, the optimal warming rate was investigated as follows. Eggs were first linearly warmed in 5 hours from storage temperature to 85°F EST to prevent condensation and because it has previously been shown that the warming rate during this first temperature transition is irrelevant for embryo development. Subsequently, eggs were warmed linearly from 85°F to 100°F by decreasing the rate from 0.88°F EST / hour (= control) to 0.08°F EST / hour stepwise in consecutive experiments. For each experiment, eggs originated either from broiler (Ross 308) or layer (Dekalb White or Lohmann Brown) parent flocks of various ages (26-58 wk), and eggs were stored between 0 to 23 days prior to incubation. Results indicated that the optimal warming rate was 0.11°F EST / hour, expressed by a significantly ($P \leq 0.04$) lower early embryo mortality (1.5 – 31.3 %) in 16 out of 20 experiments, higher hatchability (between 1.8 – 33.9 %) in 13 out of 16 experiments, and longer chicks with improved uniformity in 6 out of 9 experiments. In conclusion, the optimal warming rate with which hatching eggs are warmed from storage to incubation temperature takes 6 days (=0.11°F EST / hour), leading to higher hatchability and improved chick quality in both broilers and layers. SetCare, a patented setter system that can conduct this very gradual warming process, can be applied to advance hatchery results regardless of hatching egg storage duration. However, benefits were shown to be largest when hatching eggs were stored for longer durations, meaning that this new start of incubation can contribute to resolving the current US hatchability crisis.

Keywords: Prewarming; Hatchability; Embryo development ; Poultry incubation; Eggshell temperature

3032 The Quality Scan, a novel approach to early detection of embryo viability

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For decades, hatcheries around the world have followed a standard incubation timeline: eggs are set in setters and remain there for the first 18 days of incubation, after which the eggs are candled and all eggs containing a viable embryo are transferred to hatchers for the hatching process. This incubation timeline requires revision due to the application of in-ovo sexing in the layer industry, which takes place during the second week of incubation. Ideally, eggs are candled prior to this in-ovo sexing process to maximize efficiency and accuracy.

Since all existing candling systems were designed and optimized

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for incubation day 18, the Quality Scan was developed to accurately assess embryo viability during earlier stages of incubation with the use of artificial intelligence driven vision software. The objective of the current experiment was to determine the performance of the Quality Scan in layer hatching eggs between incubation day 9 and 12.

A total of 47,941 Dekalb White eggs were set and between incubation day 9-12 4,800-19,000 eggs were scanned per day with the Quality Scan. On each day, Quality Scan results were verified by manual candling and breakout analysis. Only descriptive statistics were performed to analyze and visualize the data. The number of correctly classified viable and unviable embryos and the number of incorrectly classified viable and unviable embryos were collected to form a confusion matrix.

On average, 99.7% of the eggs were correctly identified as either viable (90.4%) or unviable (9.3%) between day 9 and 12 of incubation. The 0.3% wrongly classified eggs consisted of 0.2% non-viable embryos that were classified as viable and 0.1% viable embryos classified as non-viable. Additional performance data of the Quality Scan in layers, as well as in broilers, will be collected in the near future and are presented at the conference.

These results show that the Quality Scan can determine embryo viability with a high reliability between day 9 and 12 of incubation for layer eggs, making it suitable for layer hatcheries that apply in-ovo sexing. The system's accuracy and ability to distinguish various egg quality parameters are expected to further increase, as the size and variation of the training set of the underlying neural network will be extended. Furthermore, early fertility and embryo viability detection can play an important role in the optimization of the hatchery workflow of all poultry breeds and the realization of other early in-ovo techniques (e.g. feeding or vaccination).
Keywords: Candling; embryo viability; fertility; vision software; incubation

Innovation and Technology III

3033 Data, Images, Algorithms – Areas of Innovation in the Poultry Sector

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Production systems are increasingly transitioning from reactive management, addressing problems after they arise, to proactive and predictive decision-making based on continuous streams of data. The integration of imaging technologies, spectroscopy, and machine learning is enabling new levels of precision, transparency, and efficiency across the poultry sector.
Hyperspectral imaging technologies are being developed for in-ovo sex determination, allowing non-invasive identification of embryo sex during early incubation. Likewise, near-infrared spectroscopy combined with artificial intelligence offers promising approaches for automated process control, such as precision vaccination.
In parallel, sensor-based monitoring systems, from acoustic and optical devices to environmental and behavioral sensors, enable early detection of deviations in animal welfare, health, or housing conditions.
New breeding techniques based on the complete knowledge of genomes (such as Genomic Selection and Intentional Genomic Alteration), which were first developed and used with plant DNA, are also being explored and discussed as a way of providing answers to the challenges of today.
Beyond individual applications, the convergence of data acquisition, image analysis, and predictive algorithms enables integrated decision-support systems. These systems promote resource-efficient production, strengthen quality assurance and traceability, and facilitate robust documentation. Systematic analysis of large datasets also opens new avenues for modeling biological processes and optimizing management practices at both farm and hatchery levels.
However, implementing these technologies raises important challenges. Data availability and standardization, robust model validation under commercial conditions, economic feasibility, and practical acceptance within the industry remain critical factors for successful adoption. Crucially, technological systems should be understood as tools that augment human expertise. At the same time, there is an ongoing societal and regulatory debate about which biotechnological methods are approved and accepted for practical application.
Against this backdrop, data, images, and algorithms represent key areas of innovation that will shape the next generation of poultry production systems, combining scientific insight with practical applications to enhance sustainability, animal health, and production efficiency worldwide.

3034 Electron Beam (eBeam) Intervention Reduces Foodborne Pathogens in Poultry Pre- and Post-Harvest Systems

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Foodborne pathogens, particularly Salmonella and Campylobacter, remain persistent challenges in poultry production and processing. Electron beam (eBeam) technology, an FDA-approved non-thermal intervention, has shown potential for pathogen control across the poultry supply chain. This study evaluated (1) the use of eBeam-inactivated pathogens as killed vaccines for pre-harvest reduction of foodborne bacteria, and (2) the efficacy of eBeam treatment for post-harvest decontamination of poultry meat.
Pathogens were exposed to lethal eBeam doses to generate inactivated cultures for vaccine development. In pre-harvest trials, birds vaccinated with eBeam-inactivated pathogens exhibited a 99% reduction of target organisms. For post-harvest assessment, ground poultry meat was inoculated with five Salmonella serovars and five Campylobacter strains (7–8 log CFU) and treated with up to 4 kGy. A 3 kGy dose achieved a 99.9999% reduction in both pathogens, demonstrating robust lethality at FDA-approved irradiation levels.
These results indicate that eBeam technology is a promising intervention for controlling Salmonella, Campylobacter, and decomposer microbes in both pre- and post-harvest environments. Implementation of this technology could reduce foodborne illness risks, extend shelf life, and mitigate economic losses within the poultry industry.
Keywords: Salmonella; Campylobacter; eBeam; eBeam-killed vaccine; irradiation

3035 A novel method for efficient isolation of avian primordial germ cells

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A novel method for efficient isolation of avian primordial germ cells

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Much effort has been taken to reserve the biodiversity of animals. The cost effective strategy for mammals is to cryopreserve the gametes and the fertilized embryos. Due to the big size of the avian oocytes, cryopreservation has not been successful. Therefore, it was proposed that preservation of the avian embryo primordial germ cells (PGCs) as an alternative approach. The current methods of isolating PGCs from avian embryos has only limited to several poultry species as the collected PGCs from embryos were mixed with many somatic cells, either blood or gonad somatic cells, and specialized medium was needed to cultivate and isolate PGCs. As the avian PGCs can respond the SDF-1 concentration gradient and travel from germinal crescent to gonads during their early stage migration, we utilized this property and established an artificial SDF-1 gradient in vitro to attract only the PGCs. For convenient harvesting of PGCs, we employed a hydrogel that is able to gelate above 25°C degree and liquefy at 4°C to minimize the high temperature stress to the PGCs. By placing the HH18 chick embryo torso to the hydrogel with SDF-1 gradient, we were able to obtain above 1000 PGCs from one embryo within 24 hours. These isolated PGCs were subsequently cultured and analyzed for their expression of DDX4 and the ability to proliferate and migrate back to gonad when reintroduced to a host embryo. We found the PGCs isolated with hydrogel showed similar properties as those isolated with conventional methods.
Keywords: primordial gem cells; isolation; SDF-1; hydrogel

3036 Challenging a broiler growth model designed for technical and economic decision support

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Mechanistic growth models are increasingly used in broiler nutrition, but their adoption depends on demonstrated biological and economic relevance. A mechanistic broiler growth model was evaluated for its ability to predict performance and economic outcomes. To express the between-trial variation from Trouw Nutrition Research Centre (PRC), we calculated the mean absolute percentage error for body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). The model evaluation involved 11 trials (including PRC and literature sources, totaling 199 phase observations), where simulated data were compared against observed data using MAPE and the coefficient of determination (R2). Delta Analysis were conducted to compare observed and predicted changes between adjacent diets when SID lysine or ME varied, expressed as a percentage of Trouw Nutrition Feed Advice. Additionally, data from the dedicated trial using 1,760 male Ross 308 from 0-52 d was analyzed. Four feeding programs were tested: a five-feed program meeting genetic recommendations for ME in kcal/kg (3000, 3100, 3200, 3225, 3225) and SID Lys in g/kg (12.8, 11.5, 10.2, 9.6, 9.3), plus three feed programs generated by the model by altering only ME and SID Lys to maximize final BW (2868/13.8, 2961/11.5, 3061/10.2, 3082/9.1, 3082/10.3), minimize FCR (3138/10.7, 3208/12.4, 3309/11.1, 3323/10.5, 3327/10.2), or maximize margin over feeding cost (MOFC-2915/11.9, 2960/11.1, 3061/10.8, 3082/9.2, 3082/8.4). Baseline between-trial variation averaged ≈10–11% MAPE for BW, BWG and FI and 15% for FCR. Across the mixed-dataset evaluation, the model showed MAPE <10% and R2 >0.90 for all traits, with errors within typical between-flock variation. For SID Lys and ME changes, delta analyses gave MAPE ≤~10% and R2 ≥0.95 (≥0.90 for FCR), indicating that the model effectively captured both the direction and magnitude of performance responses within practical nutrient ranges. However, the dedicated trial average variation was ~2% MAPE for BW and ~2% for FCR. In the dedicated trial, the BW-optimized feed program yielded similar BW compared to the control (4670g vs 4630g, P>0.05), the FCR-optimized feed program reduced FCR (1.44 vs 1.50, P<0.05), and the MOFC -optimized feed program returned an extra 10 cents per bird (P<0.05). Within validated ME and SID Lys ranges, the model can be used to compare feeding strategies and design programs aligned with technical or economic objectives.
Keywords: simulation; optimization; prediction; accuracy; poultry

3037 Ytterbium oxide as a low-dose indigestible marker for ileal and total tract digestibility in broilers

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Indigestible markers are routinely used to estimate nutrient digestibility instead of total collection, but concerns regarding titanium dioxide (TiO2) have stimulated interest in alternative markers such as ytterbium oxide (Yb2O3). This study compared TiO2 and Yb2O3 for estimating nutrient flows at ileal and total tract level in broilers fed diets based on different cereals and processed animal proteins (PAP). A total of 576 Ross 308 broilers were allocated to 96 cages (6 birds/pen) at d1 of age and fed an adaptation diet (d1-15). From d16 to 25 of age, birds received the experimental diets in two periods (d16-20 and d21-25). Twenty-four diets were cereal-based differing in cereal species (barley, rye and wheat) and variety (4 per cereal), included at 40% with or without in-feed enzymes. Eight other diets contained 8 PAP (6 meat meals and 2 feather meals) included at 8%, resulting in 32 experimental diets that included 50 mg/kg Yb2O3 and 2 g/kg TiO2. At the end of each period, excreta and ileal digesta were collected. Yb2O3 and TiO2 were assayed in diet, digesta and excreta acid hydrolysates using ICP-OES 5900. Dietary energy and nutrient flows in ileum and excreta (kcal or g per kg diet dry matter) were calculated using the index method for each marker. Data were evaluated using mixed models to test diet and marker effects and linear regression to assess agreement between markers. No significant interactions were observed between marker type and cereal species, cereal variety or enzyme supplementation for DM, OM, energy, N, starch, EE, P or Ca flows in either ileal digesta or excreta (P>0.05). Likewise, no significant differences (P>0.05) were detected between TiO2 and Yb2O3 in the estimates of these flows. In the PAP dataset, no interaction between marker and diet was observed for any component at ileal or excreta level, and marker type did not affect the estimated flows (P>0.05). Across all diets (cereals and PAP; N=192), ileal DM digestibility ranged from 26.7 to 77.4% and total tract from 59.1 to 80.6%; regression of Yb2O3 on TiO2 values showed slopes close to 1 and high coefficients of determination (P<0.001). For example, excreta flows showed strong correlation for energy (R2=0.89; Y=0.89X+153.01), N (R2=0.91; Y=0.95X+0.07) and P (R2=0.95; Y=0.93X+0.02), and ileal flows showed similar agreement for N (R2=0.95; Y=0.90X+0.06) and P (R2=0.99; Y=0.91X+0.01). These results indicate a strong agreement between TiO2 and Yb2O3 over a wide range of diet compositions and digestibility values. Marker choice did not change diet ranking or interpretation of cereal, PAP or enzyme effects at either ileal or total tract level, supporting Yb2O3 as a reliable low-dose marker in broiler digestibility studies.
Keywords: Inert marker; Ileal digestibility; Ytterbium oxide; Titanium dioxide; Broiler chickens

3038 Testicular organoids, a tool for preserving germ stem cells

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Hypothesis or objective : Birds possess unique anatomical characteristics, including in their testicles, as they produce spermatozoa in 15 days (unlike the mouse, 35 days, and 74 days in humans) and their testicles are located in the abdominal position. The quality of the feed or its environmental contamination affects the formation and survival of germ cells. To test and evaluate dietary components or molecules present in the environment on the gonad, we have developed a method alternative to animal experimentation which uses "mini-testicles" from chicken embryos placed in culture, thereby avoiding the use of live animals.

Experimental design : This system is based on the culture of cells derived from gonads and sampled from E14 chicken embryos. At this age, where the neuronal system is poorly developed, the procedure related to animal experimentation is not necessary.

Materials and methods : Cells were dissociated from gonad by enzymatic digestion. The cells were seeded on different matrices: 1/ an artificial Extracellular Matrix (ECM) of the Matrigel type, 2/ on a PET-type insert, and 3/ in low-binding wells. The culture is performed in a DMEM medium enriched with vitamins, hormones, and antibiotics allowing for the conservation of germ cells and the activity of somatic cells. The organoids were analyzed by expression analyses such as RT-qPCR, and by analysis of immunolabeling using confocal microscopy. Cell interactions were visualized by electron microscopy. Metabolomic analyses are currently being performed. Statistical analysis was realized by one-way ANOVA (GraphPad).

Results: The organoid 'mini-testicles' grow and survive for nearly 4 weeks. The immunostaining of DDX4 and electron microscopy analysis described live primordial germ cells in the organoid. Expression of DDX4, SSEA1, and OCT4 markers are expressed along the culture, and Sertoli cells and germ cells interact together and present points of communication. After 4 weeks of culture, the cells inside the organoid show stress, probably related to a lack of vascularization. Cryopreservation tests have shown that the organoids can be frozen in liquid nitrogen and then thawed later to be cultured.

In conclusion, this innovative methodology allows 1/ the preservation and freezing of germ cells, and also 2/ the rapid and low-cost testing of dietary components, environmental substances, or temperature variations on the quality and multiplication of germ cells. This in vitro model thus facilitates decision-making in poultry research and helps identify effects on fertility.

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Keywords: Organoid; Gonad; Embryo; germ cells

3039 Can NIR be used for coccidiosis prediction in broilers? A proof of concept

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Coccidiosis is a major problem in poultry production, caused by the protozoan Eimeria spp., which damages the intestinal tract and leads to poor growth and feed conversion ratio. Current diagnosis relies on oocyst per gram (OPG) counts in excreta and/or lesion scoring in culled animals. However, both techniques are laborious and require specific expertise. We hypothesized that Near-Infrared Spectroscopy (NIRS) and chemometrics applied to broiler excreta could predict the presence of eimeria oocysts. In a controlled trial conducted in metabolic cages, 192 birds were allocated to 24 cages across 4 treatments with six replicates of 8 birds each. In three treatments, birds were challenged with 1 mL of a solution containing sporulated oocysts (62,500 E. acervulina, 12,500 E. maxima, and 12,500 E. tenella), while unchallenged birds received 1 mL of saline solution. Excreta samples were collected at 14 days of age (day zero, pre-challenge) and daily up to 10 days post-challenge, totaling 264 samples. A total of 261 samples (3 were lost) were pre-dried, ground, and their spectra collected using NIRS equipment (FOSS, model 2500), comprising 178 samples from challenged and 83 from unchallenged birds. The spectra were pre-processed using Savitzky-Golay (SG) smoothing, Standard Normal Variate (SNV), and SG 1st derivative before modeling. Principal Component Analysis (PCA) was used to reduce dimensionality, showing that PC1 and PC2 explained 74.3% of the variation. A Partial Least Squares Discriminant Analysis (PLS-DA) model was built to classify the samples. Model validation was performed using Stratified 10-Fold Cross-Validation to assess robustness and prevent overfitting. The PLS-DA model achieved a global accuracy of 90%. The precision values to discriminate unchallenged and challenged broilers were 88% and 91%, with a recall of 81% and 95%, and an F1-score of 84% and 93%, respectively. In conclusion, this proof of concept demonstrates that NIRS analysis of excreta is accurate, non-invasive, and could be a rapid method for distinguishing between coccidiosis-challenged and healthy broilers. These findings suggest that NIR technology has the potential to become a viable alternative, though further evaluation under commercial field conditions is required.

Keywords: Eimeria; Spectroscopy; Chemometrics; Machine learning; Poultry health

3040 Decoding the Genetic Regulation landscape of chicken single cells using foundational models

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Introduction

In recent years, an increasing number of foundation models have emerged in the biology. However, how to leverage these models to elucidate biological mechanisms remains underexplored. Current foundation models are typically built and validated in mammalian model organisms—humans and mice. As an important avian model species in developmental biology and modern agriculture, the chicken has not yet seen systematic validation of such models. Based on this, we pose the question: can we build a chicken-specific foundation model and use it to dissect the molecular and regulatory mechanisms underlying complex traits, thereby providing a feasible path for applying foundation models to real biological problems?

Materials and methods

Using all single-cell RNA-seq (scRNA-seq) data collected in the Chicken Cell Atlas (ChCA) that comprising over six million single cells and nuclei from 56 tissues from 100 individuals, we trained a chicken single-cell foundation model based on the xtrimo-cell architecture. We also constructed a pseudobulk expression matrix from all scRNA-seq data and associated metadata, used for fine-tuned the Borzoi DNA model with the chicken genome sequence. A subset of the scRNA-seq data has been used to deconvolve bulk RNA-seq from the Advanced Intercross Line (AIL) and to perform single-cell-eQTL (sc-eQTL) mapping.

Results

The single-cell foundation model converged stably and outperformed baseline approaches across benchmarks such as cell type annotation and data enhancement. Cell embeddings increased cluster separability and aligned with known cell markers. Using the gene embeddings, we built robust gene modules that were

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consistent across datasets and recapitulated canonical pathways (e.g., proliferation, lipid metabolism), with cell-type–specific modules enriched for relevant TF motifs. The Borzoi DNA model fine-tuned on the chicken genome with pseudobulk matrix showed accurate sequence-to-expression predictive performance (Pearson r≈0.78; AUROC≈0.80). Deconvolution-guided mapping yielded a curated catalog of sc-eQTLs. Effect directions and impacted regulatory elements were corroborated by the fine-tuned DNA model, supporting cell-type–specific regulatory mechanisms underlying complex traits.

Conclusion

These results demonstrate that a chicken-specific foundation model is feasible and effective. Integrating these models yields a catalog of cell-type–specific pathway from genetic variation to gene modules, delineating regulatory mechanisms underlying complex traits in a non-mammalian model.

Keywords: chicken; scRNA-seq; foundation model; pseudobulk; fine-tuning

3041 Research on High-Precision Phenotypic Measurement of Live Poultry Driven by Artificial Intelligence Methods

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In the process of biological research and agricultural development, phenotypic information, as a direct manifestation of biological traits, is experiencing a growing demand. However, traditional measurement methods are generally plagued by problems such as time-consuming and labor-intensive processes, destructiveness, and the inability to obtain internal phenotypes of live poultry in real time, which severely restrict the refined development of the poultry breeding and farming fields. To address this issue, this study innovatively proposes to use artificial intelligence technology to build a full-process solution for automated and accurate phenotypic quantification, enabling efficient, non-destructive, and precise phenotypic measurement of live poultry.

Specifically, first, multimodal professional imaging equipment, including high-resolution image scanners, is deployed to comprehensively collect two-dimensional DR images and three-dimensional CT data of poultry in their natural activity states. Subsequently, cross-domain learning is integrated to construct an image enhancement model, which performs operations such as normalization and denoising on the data, significantly improving image clarity. Next, a multi-object segmentation network is built based on the CNN-Transformer architecture to achieve accurate identification and segmentation of key parts such as chicken breasts, drumsticks, and wings. Finally, a feature extraction algorithm is designed to extract features from the segmented target regions. Multiple algorithms including support vector machines, random forests, and deep learning regression are integrated to construct a multi-task prediction model, achieving high-precision fitting of real phenotypic values.

Based on the above methods, this study developed a complete technical process for non-invasive measurement of phenotypes such as the weight of key parts of poultry. Taking white-feathered broilers as the research object, after training and verification with 500 sets of annotated data, this method achieves an average segmentation accuracy of over 97.5% for target regions such as breasts, drumsticks, and wings in two-dimensional DR images, and the segmentation accuracy for internal muscle regions in three-dimensional CT also reaches over 92%. In the fitting analysis of two-dimensional DR images, the prediction correlations for the weight of breasts, drumsticks and wings are as high as 91.7%, 81.2%, and 83.5% respectively.

Experimental results shows that compared with traditional measurement methods, the method proposed in this study not only improves measurement efficiency and reduces the errors of traditional subjective measurement but also achieves completely non-destructive measurement.

Keywords: Phenotypic Measurement; Artificial Intelligence

3042 The Effect of feed form on growth performance and efficiency in modern broiler genetics

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The objective of this study was to evaluate how feed form influences growth performance and feed efficiency of modern broiler genetics. A total of three dietary treatments were compared using Cobb 500 broilers: (1) crumb in the starter phase followed by pellets, (2) crumbs for all phases, and (3) mash for all phases. Body weight (BW) was recorded at days 10, 21, 28, 35, 42, and 49. Cumulative feed conversion ratio (FCR) was calculated for 0–35, 0–42, and 0–49 days. Breast meat yield (BMY) was measured at day 35 and 49. The experiment followed a completely randomised design, and data were analysed using one-way ANOVA with means separated at $P < 0.05$.

Feed form had a significant effect on broiler performance. Birds receiving crumb-pellet diets achieved the highest BW at all time points (305g at day 10; 1918g at day 28; 4198g at day 49), while the mash treatment produced the lowest BW (210g; 1594g; 3967g at the same ages). The crumb-only treatment yielded intermediate BW although not significantly different to the crumb-pellet treatment. FCR was poorest for the mash treatment across all periods (1.563, 1.599, and 1.740 for 0–35, 0–42, and 0–49 days, respectively), whereas the crumb/pellet treatment achieved the best FCR (1.456 from 0–35 days, 1.503 from 0-42 days and 1.688 from 0-49 days) but was not significantly different to the crumb-only treatment for the same periods (1.484, 1.557 and 1.717 for 0–35, 0–42, and 0–49 days, respectively). There was no significant difference in BMY at 35 days between the crumb-pellet and crumb-only treatment (25.56% and 25.7% respectively), the same was seen at 49 days (27.8% and 27.9% respectively). While at 35 and 49 days the mash treatment had a significantly lower BMY(23.6% and 26.7%)

In conclusion, feeding pellets remains critical for expressing the growth potential and yield of modern broilers. Although the BW, FCR and BMY differences between the crumb-pellet and the crumb treatment were not significant, performance was improved by using a pellet. While mash markedly impaired both BW, FCR and BMY throughout the trial. These findings reinforce the importance of feed form optimisation, particularly pellet quality and early-life feed structure for maximising biological and economic performance in broiler production.

Keywords: broiler nutrition; feed form; pellet quality; growth performance; feed efficiency

Keywords: Broiler Nutrition; Feed Form; Pellet Quality; Growth Performance; Feed Efficiency

3043 Statistical evaluation of equations to predict the net energy value of complete feeds for broiler chickens

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This study evaluated predictive net energy (NE) equations for complete broiler chicken feeds through a formal statistical approach of error decomposition analyses. A total of 384 hatched male Cobb 500 chickens were used, randomly assigned to 12 experimental treatments, with four repetitions and eight birds per repetition. At 14-d-old, the birds were fed with the experimental diets formulated under two criteria: (i) six diets formulated to optimize variation on the AMEn (from 2900 to 3200 kcal/kg); and (ii) six diets

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formulated to optimize variation on predicted NE=0.815*AMEn-12.8*CP+14.76*EE (E1). The feeds included non-traditional ingredients (e.g., corn DDG, sorghum grain, wheat grain, and citrus pulp) to flexibilization the formulation process, ensuring variation of crude protein (CP, 21.7 to 29.8%), ether extract (EE, 3.9 to 23%), and crude fiber (CF, 3.3 to 6.6%). At 19d, the birds were allocated to open-circuit respirometry chambers, to heat production (HP) determination using the Brower equation (1965). During the same period, excreta were collected to determine the AME and AMEn (corrected by nitrogen balance). On the last day (25d), fasting heat production (FHP) was measured, and the heat increment (HI) was calculated as HP minus FHP. The difference between the AME and HI determines the NE. AMEn, CP, EE, and CF were used as inputs in the selected equations: E1, E2 - Wu et al. (2019), E3 - Noblet et al. (2024), and E4 - Tay-Zar et al. (2024). The regression (predicted-observed) was performed, and individual errors were calculated for each equation and the mean squared error of prediction (MSEP) was determined. Error decomposition was performed into: ECT (error of central tendency), ER (regression error), and ED (distribution error). The results of the predicted-observed regression indicated that the R2 was lower for E2 (0.63) than for E1 (0.58); however, the slope was closer to 1, and the intercept (-715) was closer to zero. Meanwhile, Eq. 1 showed a relatively high error (14.6%), while the other equations showed an error of 13.4% (no large difference). However, E1 showed a higher ECT (15%) than the other equations (3.24 and 6.6% for E2 and E3). These reflect a compensatory effect, where E1 showed a lower ER (7.6%) than the other equations, which ranged from 13.3% (E2) to 16.8% (E3 and E4). All the models presented the ED as the principal source of error. In conclusion, an error-decomposition-based evaluation of the predictive NE equation provided a comprehensive assessment of the models, showing that the NE prediction equation demonstrated strong predictive power, with the principal difference occurring in the CTE and RE, where Eq. 1 performed better than the other equations.

Keywords: energy value; feed formulation; prediction; indirect calorimetry

Nutrition: Amino Acids and Protein III

3044 Source and relevance of endogenous amino acid losses in commercial poultry production

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Endogenous amino acid (AA) losses represent a substantial and biologically unavoidable component of ileal amino acid flow in poultry and are increasingly recognised as a key determinant of precision feed formulation. These losses arise primarily from secretions and cellular turnover within the gastrointestinal tract, including mucin glycoproteins, digestive enzymes, bile, immunoglobulins, and sloughed epithelial cells. In some definitions, losses related to bacterial biomass may also be considered. Collectively, these sources are particularly enriched in threonine, serine, proline, glycine and cysteine, reflecting the amino acid composition of mucin and structural proteins, while lysine and methionine are less implicated. From a nutritional perspective, failure to account for endogenous losses leads to systematic underestimation of true dietary AA availability. This limitation underpins the transition from apparent ileal digestibility (AID) to standardized ileal digestibility (SID) systems, in which basal endogenous losses are quantified and corrected for, thereby improving additivity of digestible amino acids across ingredients in least–cost formulation. Basal losses are diet independent and driven by intake and physiology, whereas diet–specific endogenous losses vary with ingredient characteristics such as fibre, antinutritional factors, protein solubility and heat damage. Importantly, several feed additives, including exogenous proteases, phytase, emulsifiers, organic acids and gut health modulators, may reduce diet–specific endogenous losses by improving nutrient digestion, reducing mucosal irritation, or lowering inflammatory turnover of intestinal tissue. Understanding both the sources and nutritional implications of endogenous AA losses is therefore central to optimizing efficiency, robustness and sustainability in modern poultry production systems and in improving formulation approaches to maximise precision.

3045 Establishing the Rank of Dietary Non-Essential Amino Acids Limitation in Broilers

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A Plackett–Burman (PB) experimental design can be used to screen for influential factors affecting a response variable when many factors are under consideration. Reducing broiler diet CP lowers non-essential amino acids (NEAA) levels, and optimal NEAA needs are poorly characterized. Consequently, the inferior performance often observed when broilers are fed reduced CP diets may be attributed to deficiencies in NEAA. Therefore, the objective of this experiment was to rank the relative importance of NEAA in reduced CP broiler diets. Cobb 500 broiler chicks were placed in floor pens containing new pine shavings, nipple drinkers, and were given ad libitum pan feeder access. After a 14 d common feed, 72 pens with 11 birds each (0.10 m2/bird) were fed 12 treatment diets from 15 to 35 d of age (6 replicate pens/treatment). To create dietary treatments (T), a positive control diet (T1; 21.2% CP, 3,000 kcal/kg ME and 1.16% digestible Lys) and a negative control diet (T3; 17.2% CP, 3,000 kcal/kg ME and 1.16% digestible Lys) were formulated to contain a 4.0 percentage unit difference in CP. Minimum constraints were placed on all essential amino acid ratios. An intermediate control (T2; 19.2% CP, 3,000 kcal/kg ME, and 1.16% digestible Lys) was created with a 1:1 blend of T1 and T3. To create T4-10, L-Ala, L-Asp, L-Gln, L-Glu, Gly, L-Pro, and L-Ser were added in various combinations to equal T1 levels at the expense of an inert filler following an 8-run PB design matrix with 7 factors. A NEAA control (T11) was created by supplementing all 7 NEAA in question. An energy control (T12) was formulated using the same ingredient profile and nutrient specifications as T11 but with a reduction of 75 kcal/kg ME. Treatments were assigned in a randomized complete block design with pen as the experimental unit. Live performance was assessed from 15 to 35 d and processing yields (4 birds per pen) at 35 d of age. Treatments 3-10 were subjected to the PB analysis, and pre-planned orthogonal contrasts compared T1, T2, T3, T11, and T12. All data were analyzed using SAS 9.4 with significance considered at $P \leq 0.05$. Reducing the CP content by 4.0 percentage units compromised ($P < 0.05$) weight gain, FCR, peritoneal fat yield, and breast filet yield by 9, 12, 46, and 8%, respectively. Supplementing NEAA ameliorated ($P < 0.05$) live performance and breast filet yield losses, but failed ($P > 0.05$) to restore fat yield, despite an ME reduction. Alanine, Gly, and Pro improved ($P < 0.05$) live performance parameters, and Gly had improved ($P < 0.05$) processing yield implications. In conclusion, this study provides direction for future research elucidating optimal NEAA levels in reduced CP broiler diets.

Keywords: broiler; non-essential amino acid; alanine; glycine; proline

3046 L-Cysteine, L-Histidine and L-Tyrosine suppressed pathogens and promoted beneficial bacteria in the chicken gut

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Opportunistic pathogenic and beneficial bacteria are integral components of the chicken gut microbiota. Amino acids are one nutritional strategy to modulate microbial growth, yet the effects of individual amino acids on specifc bacteria remain poorly defined. We hypothesised that specific amino acids could suppress pathogenic bacteria without adversely affecting beneficial ones.

Separate experiments were conducted for pathogenic and beneficial bacteria. For pathogens, the effects of 20 proteinogenic amino acids were assessed on seven species—Avian pathogenic E. coli (APEC), Clostridium perfringens, Enterococcus cecorum, Enterococcus faecalis, Enterococcus faecium, Salmonella enterica and Staphylococcus aureus. For each species, at least four independent isolates were tested, and each isolate was cultured in triplicate in 96-well plates. Each culture well was considered the experimental unit. Growth at 1 g/L versus 0 g/L was compared using Student's T-test or Mann-Whitney tests, depending on data normality.

For beneficial bacteria, four amino acids—L-alanine (Ala), L-cysteine (Cys), L-histidine (His) and L-tyrosine (Tyr)—were evaluated. Three Lactobacillus strains (L. salivarius DSM20555, L. reuteri DSM108983 and L. paracasei DSM46331) were cultured in triplicate in chemically defined media with the amino acid concentrations adjusted to 0, 0.2, 0.4, 0.6, 0.8, or 1 g/L in 96-well plates. Each culture well was considered the experimental unit. One-way ANOVA was used to assess the effects. Amino acid impacts were classified as essential (E: no growth at 0 g/L), conditionally essential (cE: growth observed at 0 g/L but increased with concentration), suppressive (S: decreased growth with increasing concentration) or no effect (N). Growth in all experiments was measured by optical density at 600 nm.

Several amino acids showed suppressive or promoting effects ($p \leq 0.05$) on bacteria. For pathogens, Cys suppressed the growth of APEC, C. perfringens, S. enterica and S. aureus, Ala suppressed S. aureus, His suppressed APEC, and Tyr suppressed E. cecorum and S. aureus. For beneficial bacteria, the four selected amino acids modulated Lactobacillus growth. In the order of L. salivarius, L. paracasei and L. reuteri: Ala was cE, N, and E; Cys was N, E, cE; His was E, N, E; and Tyr was E for all three species. Additionally, increasing the concentration of Ala suppressed L. reuteri growth.

In conclusion, Cys, His and Tyr suppressed specific pathogens while supporting the growth of Lactobacillus species. These findings suggest a targeted nutritional approach for microbiota modulation and enhanced gut health in chickens.

The study was supported by the AgriFutures Chicken Meat Consortium (PRO-016111).
Keywords: proteinogenic amino acids; bacteria; pathogens; Lactobacillus; aminobiotics

3047 Effects of Digestible Lysine, Metabolizable Energy, and Amino Acid Ratios on the Zootechnical Performance, Environmental Impacts, and Yields of Finishing Turkeys: A Central Composite Design Approach

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The balance between digestible lysine (dLys), metabolizable energy (ME), and amino acid ratios (AA) plays a critical role in determining feed intake, feed efficiency, and muscle deposition in turkeys. This study aimed to determine the responses of these nutrients as well as their interactions on zootechnical performance, environmental impacts, and carcass yields in finishing turkeys based on a central composite design (CCD). CCD provides a 3D analysis allowing for an efficient exploration of nutritional responses and their interactions using central, factorial, and axial points to define the experimental domain, setting extreme values for the treatment variables. This study addresses a 3-factor and 5-level CCD with 6 repetitions for the central point and 3 repetitions the factorial and axial points. A total of 528 male Aviagen Premium turkeys, 63 days of age, were distributed into 48 pens with 11 turkeys each. At 81 days, birds were reallocated to obtain 10 turkeys of equivalent live weight per pen. The experimental phase lasted from 81 to 123 days of age. The turkeys were fed 15 experimental diets varying in dLys content relative to balanced protein (from 0.94 to 1.17%), ME (from 2950 to 3400 kcal/kg), and dMet+dCys/dLys and dThr/dLys ratios (from 88 to 122% of the requirement). Results were analyzed according to a response surface methodology using polynomial regression model. Over the experimental period, from 81 to 123 days, feed intake was positively impacted by lysine (+10 g/day for +0.10% dLys; P=0.002) and negatively by energy (-15 g/day for +100 kcal/kg ME; P<0.001). Average daily gain was mainly improved by lysine (+8 g/day for +0.10% dLys; P<0.001), and a trend was observed between ME and AA (P=0.064). Feed conversion ratio was positively impacted by lysine (P<0.001), energy (P<0.001), and AA ratios (P=0.049) independently, and the lowest FCR was observed at the highest levels of lysine, energy, and AA ratios. Nitrogen efficiency was maximized at a low lysine level or a high energy level (P<0.001). Carbon impact per ton of live weight was minimized with a low lysine level and a low energy level (dLys×ME; P=0.002). Breast meat yield was negatively affected by energy (-0.3 points for +100 kcal/kg ME; P<0.001) and positively by lysine to balanced protein (+0.3 points for +0.10% dLys; P<0.001), thus mainly driven by amino acid intake. This study confirms that finishing turkeys precisely regulate their energy intake according to the dietary energy concentration, which consequently influences amino acid intake. Therefore, digestible lysine and energy requirements vary depending on the performance criteria targeted—such as live weight, FCR, carcass yields, or environmental impacts.
Keywords: Turkey; Digestible Lysine; Metabolizable Energy; Performance

3048 Impact of methionine supplementation and deficiency on growth, immune reactions, and metabolic processes of broiler chickens challenged with lipopolysaccharide

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Methionine, an essential amino acid for broiler growth, requires dose adjustments based on health conditions. This study examined how methionine deficiency and supplementation influence broiler growth performance, methionine metabolism, and immunity following lipopolysaccharide (LPS) challenge. Five hundred and four Arbor Acres one-day-old male broilers were placed into four different treatment groups, each comprising six replicates. The groups were as follows: 1) The positive control (POS) group received a basal diet (100% methionine); 2) The negative control (NEG) group received a basal diet (100% methionine) but was also exposed to LPS; 3) The methionine deficiency (MD) group consumed a diet lacking methionine (60% methionine) and was also subjected to LPS; 4) The methionine supplementation (MS) group was provided a diet rich in methionine (140% methionine) and was challenged with LPS. The experiment lasted for 21 days.

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Data that conformed to normality and homogeneity of variances were statistically analyzed by one-way ANOVA, along with the Tukey HSD multiple comparisons test. For non-normally distributed data, such as liver histological scores, significant differences were determined using the non-parametric Kruskal-Wallis H test, followed by Dunn's T3 post-hoc test. The MD group's feed conversion ratio (F/G) was notably elevated (P = 0.007) compared to POS before LPS induction (days 1–17). Additionally, the ADG was significantly lower in NEG and MS groups during the LPS challenge (days 17–21) (P = 0.018). Interestingly, the levels of serum alanine aminotransferase (ALT), aspartate transaminase (AST), LPS, corticosterone (CORT), interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α), along with liver malondialdehyde (MDA), were markedly reduced in the MD group compared to NEG (P < 0.05). Furthermore, the levels of serum IL-10, along with liver catalase (CAT) and superoxide dismutase (SOD) activities, as well as total antioxidant capacity, were markedly elevated in the MD group compared to NEG (P < 0.05). Similarly, the hepatic expression of SOD, glutathione peroxidase (GPX), and kelch-like eh-associated protein 1 (KEAP1) was also significantly increased in the MD group (P < 0.05). Conversely, hepatic levels of 5,10-methylenetetrahydrofolate reductase (MTR) and cystathionine β-synthase (CBS) were elevated in the MD group compared to POS and NEG (P < 0.05). Conclusively, these results indicate that, during the acute response period following LPS injection, reducing dietary methionine to 60% enhances antioxidant capacity, alleviates hepatic burden, and helps maintain metabolic stability.
Keywords: Antioxidant capacity; Broiler chicken; Growth performance; Methionine metabolism; Methionine supplementation and deficiency

3049 Methionine source-dependent modulation of TNF-α-induced intestinal damage in avian organoid model

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This study evaluates the cytoprotective potential of varying methionine (Met) sources against tumor necrosis factor-α (TNF-α) mediated intestinal injury in broiler organoids. Jejunal segments were collected from 2-week-old broilers. High-purity crypts were isolated and cultured to establish a stable three-dimensional organoid system. Organoid numbers and area were quantified using ImageJ software following image conversion and threshold adjustment. Organoids were treated with a range of TNF-α concentrations (0, 100,150, 200, 250 μg/L) followed by repetition of the experiment with even higher TNF-α concentrations (300, 500, and 1500 μg/L) to determine the optimal concentration for subsequent testing. Extremely high concentrations of TNF-α did not exacerbate cytotoxicity. Based on these observations, 300 μg/L TNF-α was selected as a suitable concentration. After establishing an intestinal injury model, the reparative effects of three different sources of Met i.e., DL-Met, L-Met, and 2-hydroxy-4-(methylthio) butanoic acid (HMTBa), at concentrations of 25, 50, and 100 μmol/L were evaluated, respectively. One-way ANOVA was performed to assess statistical differences among groups, followed by Tukey's test. Exposure to 300 μg/L TNF-α for 24 h markedly reduced organoid numbers without inducing complete cell death (P<0.05). TNF-α suppressed expression of the Paneth-cell marker LYZ (P<0.05) and down-regulated intestinal stem-cell markers LGR5 and SOX9 (P < 0.05), suggesting the impairing effect on stem-cell proliferation and differentiation. All Met treatments at 100 μmol/L significantly decreased organoid number and area compared to the control and TNF-α only groups (P<0.05). Treatments with varying concentrations of L-Met and DL-Met did not alleviate the damage caused by TNF-α induced injury. However, at 25 μmol/L, HMTBa significantly increased organoid number without reducing organoid area compared to other Met sources (P<0.05), indicating a potential protective effect at lower concentrations. Likewise, HMTBa reversed TNF-α-induced down-regulation of LYZ and LGR5 gene expressions compared to DL-Met and L-Met (P<0.05). Tight junction protein expression showed that TNF-α did not down-regulate Claudin1 or ZO-1 expression compared to the control while Occludin expression was significantly increased through TNF-α exposure (P<0.05). HMTBa treatment significantly upregulated Claudin1 and ZO-1 expressions relative to both the control and TNF-α groups (P<0.05). Taken together, our findings suggest that HMTBa at 25μmol/L promotes intestinal stem-cell proliferation and differentiation by activating the Paneth-cell–stem-cell regulatory axis, thereby ameliorating TNF-α-induced intestinal damage in these conditions.
Keywords: Intestinal organoids; TNF-alpha; multi-source methonine; stem cell proliferation and differentiation

3050 Effect of dietary supplementation of an active blend of guanidinoacetic acid on performance of commercial broiler chicken

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This study was aimed to investigate the effect of dietary supplementation of an active blend of guanidinoacetic acid (GAA) at 0.06% on growth performance, feed conversion ratio (FCR), carcass characteristics, breast muscle creatine, and serum creatinine level of commercial broiler chicken fed corn-soybean meal (SBM) based diets with or without animal protein source. A total of 192 commercial broiler chicks were randomly allocated to six dietary treatments, each comprising four replicates with eight birds per replicate. The treatments included: T1 (corn–SBM control diet); T2 (corn–SBM diet with an active blend of GAA); T3 (corn–SBM diet with 50 kcal/kg reduced metabolizable energy (ME) and an active blend of GAA); T4 (corn–SBM–meat and bone meal (MBM) diet); T5 (corn–SBM–MBM diet with an active blend of GAA); and T6 (corn–SBM–MBM diet with 50 kcal/kg reduced ME and an active blend of GAA). The experimental data were analysed using one-way ANOVA to determine significant differences among treatment means. Body weight was significantly increased (p<0.05) in T5 (2190.08 ± 46.41 g) and T2 (2183.04 ± 38.50 g) compared to control T1 (2011.59 ± 58.11 g). Reduced metabolizable energy (ME) diets supplemented with GAA also maintained comparable body weights (T3: 2053.43 ± 45.65 g; T6: 2027.74 ± 40.52 g). Feed conversion ratio (FCR) exhibited numerical improvements with active blend of GAA supplementation. The best FCR was recorded in T2 (1.64 ± 0.04), showing 9.39% improvement compared to the control T1 (1.81 ± 0.05) followed by T6 (1.66 ± 0.04). Carcass characteristics i.e. dressing percentage was significantly (p<0.05) improved, significantly (p<0.05) highest dressing percentage was recorded in T2 (68.14%) and T5 (67.92%) compared to the control T1 (63.60%). Breast muscle weight was significantly (p<0.05) higher in T2 (345.10 g) compared to control T1 (260.98 g). Active blend of GAA supplementation increases breast muscle creatine concentration compared to control. The highest creatine deposition occurred in T5 (2810.09 ± 83.94 mg/kg) and T6 (2716.78 ± 53.28 mg/kg), both significantly greater than control T1 (1932.23 ± 164.51 mg/kg), T2 (2090.22 ± 118.83 mg/kg), T3 (1948.59 ± 29.43 mg/kg), and T4 (2226.03 ± 138.49 mg/kg). Serum creatinine concentration was non-significant across different treatments. The recorded range of serum creatinine (0.24–0.31 mg/dL) remained

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within normal physiological limits for broilers. Overall, dietary supplementation of an active blend of guanidinoacetic acid at 0.06% (600 g/tonne of feed) effectively enhanced growth performance, feed conversion ratio, dressing percentage, breast muscle weight, and breast muscle creatine concentration in commercial broiler chicken.

Keywords: Guanidinoacetic acid; Broiler; Plant based diet; Body Weight; Feed Conversion Ratio

3051 Sulfur amino acids requirement of slow growing birds: prediction from meta-analysis

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Methionine (Met) is the primary limiting amino acid in broiler chickens in practical feeds. However, despite its importance, little data is available to assess the total sulfur amino acid (TSAA) requirements of slow-growing chickens. These strains are generally promoted for animal welfare, meat quality and genetic diversity. The objective of this study is therefore to estimate the TSAA requirement of slow growing chickens through a meta-analysis. To be included, trials had to be focused on slow growing chickens or native strain, provided specific information such as diet composition or growth performance and tested a basal diet supplemented with at least 4 levels of Met, with dose of L-Met, DL-Met or 2-hydroxy-4-(methylthio) butanoic acid (HMBTA). A database of 24 trials and 151 lines was then constructed with 5 published articles and 3 internal experiments. The SID digestible coefficient of amino acids were recalculated based on INRAE-AFZ (2018) tables and used to recalculate the SID TSAA level from published analyzed values. The quadratic (QD), and curvilinear-plateau (CLP) models were tested to estimate the daily TSAA intake (g/d) and the SID TSAA:Lys (%) requirement using the average daily gain (ADG) and feed conversion ratio (FCR) as response variable, with the trials as a random effect. The linear relationship between the estimated SID TSAA intake required to maximize ADG and the corresponding ADG values recalculated for each trial was examined to assess how TSAA intake requirements vary with increasing ADG. The strains used in the database in order of importance are: Lingnan (13 trials), JA757 (4), Guangxi (2), Qingyuan (2) and Korat chickens (1). The average dietary SID Lys was 9.5 ± 0.86 g/kg. According to the QD model, optimal ADG and FCR were achieved at SID TSAA:Lys 77.1% and 73.5%, respectively. In contrast, the CLP model estimated SID TSAA:Lys at 63.3 ± 1.5% for ADG and 61.0 ± 1.4% for FCR. The estimated SID TSAA intake required to optimize ADG increased linearly with ADG, following the equations $Y = 0.238 + 0.010 \times ADG$ ($P < 0.001$) for the QD model and $Y = 0.235 + 0.007 \times ADG$ ($P < 0.001$) for the CLP model. Chickens with an average ADG of 16 g/d during the starter phase (1–22 days), 37 g/d during the grower phase (23–43 days), and 46 g/d during the finisher phase (44–64 days) required, on average per feeding phase, 0.40, 0.61, and 0.70 g/d of SID TSAA intake according to the QD model, and 0.35, 0.49, and 0.55 g/d according to the CLP model. In conclusion, this study delivers a comprehensive assessment of TSAA requirements in slow-growing chickens, providing key insights to support more precise and efficient nutritional strategies.

Keywords: Slow growing broilers; Methionine; Requirement

3052 A low crude protein diet allows to maintain growth performance or mineralization, with different importance regarding the dietary amino acid tested in laying pullets

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Efficient nutritional management is essential for optimizing feed intake and growth in future laying hens. A high warning during the starter period is critical for muscle and skeletal development, with dietary crude protein and amino acid levels playing a decisive role. This trial had two different objectives. The first one was to evaluate the effect of a two points reduction of crude protein with or without secured levels of amino acids. The second one was therefore to identify the next limiting amino acid to maximize growth, feed intake and bone strength. A total of 2,496 Lohmann Brown laying pullets were randomly allocated to eight iso-energetic treatments, with six replicates of 52 pullets per pen, from hatch to seven weeks of age. The first three groups were: a high crude protein diet (CP=20.6) with secured levels of amino acids (CP+AA+), a low crude protein diet (-2 pts of crude protein reduction) without any formulation constraint on amino acids, besides Lys and Met (CP-AA-) and the same low crude protein diet with secured levels of amino acids (CP-AA+). The reduction of dietary CP content was achieved by replacing soybean meal with sunflower meal, wheat bran and free AA. The five others treatment were formulated based on the CP-AA+ diet, without a formulation constraint on each individual amino acid tested: Threonine (CP-AA+Thr-), Arginine (CP-AA+Arg-), Tryptophan (CP-AA+Trp-), Isoleucine (CP-AA+Ile-) et Valine (CP-AA+Val-). The statistical analysis was executed with the software R studio with a linear mixed effect model, considering the random effect of pen or the pullet. Results indicated that there was no effect of treatment on mortality, litter score, or keelbone deviation score. At seven weeks of age, pullets from the CP-AA- group had lower body weight than those from the CP+AA+ (-40.3 g, $P<0.0001$) and CP-AA+ (-14.7 g, $P<0.0001$) groups. There was no significant difference regarding feed intake or skeletal development between these three treatments. That means that securing the dietary amino acid supply in a low crude protein diet allows to maintain growth performance in starter pullets. The CP-AA+Arg-, Val- and Thr- groups had the significantly lowest growth rates (ADG 0-7 weeks = 12.16, 12.28 and 12.13 g/day, respectively, $P<0.0001$) and the lowest feed intake (ADF 0-7 weeks = 28.81, 29.03 and 28.72 g/day, respectively, $P=0.32$). At seven weeks of age, the CP-AA+Val- pullets had the shortest tarsus length (4.43 vs. 4.60 cm for CP+AA+, $P=0.02$). In conclusion, these findings suggest that Arginine, Valine and Threonine seem to be the next limiting AA in starter period, underscoring the need for precise amino acid formulation to support optimal growth and bone development in laying pullets.

Keywords: Pullet; Bone mineralization; Protein; Amino Acid; Growth

3053 Effects of Supplementing Urea or Alanine in Low-Protein Diets to Replace Partial Soybean Meal on Production Performance and Nitrogen Metabolism in Laying Hens during the Peak Laying Period

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Moderately reducing dietary crude protein (CP) while supplementing limiting amino acids can conserve protein feed and reduce nitrogen emissions in laying hens without compromising performance. However, excessive CP reduction causes an amino acid imbalance. This study investigated supplementing low-protein (LP) diets with alanine (Ala) or urea to balance nutrition and elucidate their roles in nitrogen metabolism, aiming to optimize LP diets and improve nitrogen use efficiency. In this experiment, 140 40-week-old Jinghong No. 1 laying hens were randomly allocated to 7 dietary treatments with 20 replicates (1 bird per cage). The treatments were: 16% normal protein, 15% LP, 15% LP + Ala, 15% LP + urea, 14% LP, 14% LP + Ala, and 14% LP + urea. Ala or urea was added to restore CP to 16%. A one-week adaptation period was followed by a 7-week experimental period. All data were subjected

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to one-way ANOVA based on means using SPSS 26.0. Significant differences among treatment means were separated by Duncan's multiple range test, with the significance level set at $P < 0.05$. The results indicate that reducing CP to 14% significantly decreased the laying rate ($P < 0.05$), which was partially alleviated by Ala or urea supplementation. Ala supplementation also raised the yolk ratio. The LP groups exhibited significant decreases in serum TP, ALB, GLB, and BUN ($P < 0.05$), accompanied by notable increases in IgA, IgM, and IgG ($P < 0.05$). Ala restored serum TP, ALB and BUN levels, while urea increased AST level. Hepatic enzyme ALT, GS and GDH activities were higher in Ala supplemented group ($P < 0.05$). Urea supplementation significantly upregulated the mRNA expression level of TOR, S6K1, and 4E-BP1 genes ($P < 0.05$). The proteolysis-related gene MAFbx was significantly upregulated only in the 14% LP group ($P < 0.05$), an effect that was reversed by Ala supplementation. Although the LP diet negatively affected jejunal amino acid transporter expression, urea supplementation significantly enhanced the expression of multiple amino acid transporters ($P < 0.05$), whereas Ala had minimal impact. After urea supplementation, urea nitrogen levels normalized in the proximal ileum of 15% LP + urea group and in the jejunum of 14% LP + urea group, indicating that urea concentration influences its absorption site. In conclusion, supplementing Ala or urea to LP diets can partially mitigate negative effects on performance by modulating intestinal urea nitrogen metabolism, hepatic enzyme activities, and jejunal amino acid transporter expression. Ala supplementation was most effective in maintaining production performance and optimizing nitrogen metabolism, while urea supplementation was more effective in restoring the laying rate.

Keywords: Low Protein Diet; Urea; Alanine; Production Performance; Nitrogen Metabolism

3054 Effect of low soybean meal levels with varying levels of non-bound crystalline amino acids on broiler growth and feed efficiency

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Reducing soybean meal in broiler diets has become a key challenge for the European feed industry, driving efforts to cut its soybean meal import dependence, shrink environmental footprints, and push poultry production toward sustainability. The aim of this study was evaluated the extent to which soybean meal could be replaced by a wide range of essential and partially non-essential crystalline amino acids (Lys, Met, Thr, Trp, Arg, Ile, Val, Leu, Gly). A total of 400 one-day-old Ross 308 chicks of both sexes were housed in floor pens and randomly assigned to three dietary treatments, each consisting of six replicates. From 0 days to 10 days chicks were fed with commercial starter diet (22.9 % CP; 12.4 MJ/kg ME, SID Lys 1.41 %). Experimental design included grower (11-24 d) and finisher (25-35 d) diets with different soybean meal levels 5%, 10%, and 15 % and with different total summary of essential and partially non-essential amino acids (85.2; 57.5; 44 g/kg in grower diets and 75.7; 49.8; 36.5 g/kg in finisher diets). The experimental feed mixtures were optimized at the level of standardized ileal digestibility (SID) of amino acids. Growth performance, including body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined on days 10, 17, 24, 31 and 35. Data were analyzed using GLM, and means were compared using Scheffé's test. In the starter period, growth parameters did not different between groups. During the growing phase (17-24d) and finisher phase (25-35d), statistically significant differences in chicken body weight (BW) were observed between the 5%, 10%, and 15% groups (541 vs. 681 vs. 749 g; $p<0.001$) and ADG (187.27 vs. 330.67 vs. 384.43 g; $p<0.001$), with the diet with the highest SBM level and lowest crystalline amino acid content promoting higher ADG and BW. The highest final live BW was observed in the 15 % group (2 360 g) exceeding the recommended target values for Ross 308, which was supported by higher cumulative FI over the entire period (2 938 g; $p<0.001$). FCR was significantly higher in the 5% group compared to the others during the 10–17 d period (1.76 vs. 1.4 and 1.38; $p=0.0071$), and the trend continued during the 17–24 d period (1.7 vs. 1.38 and 1.3; $p=0.0032$), reflecting differences in FCR between groups. Over the entire trial period, the 15% group demonstrated superior growth performance and cumulative feed efficiency ($p=0.001$). In conclusion, it can be said that by optimizing feed mixtures according to SID AA and meeting amino acid requirements, the SBM level can be reduced to 15% without loss of growth performance and feed efficiency.

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Keywords: soybean meal; amino acids; broiler chickens; growth performance; feed efficiency

Nutrition: Feed Additives III

3055 Utilization of exogenous enzymes to unlock the prebiotic potential of alternative feedstuffs in poultry

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The withdrawal of antibiotic growth promoters from poultry production has increased the need for sustainable nutritional strategies to support gut health and performance. Additionally, alternative feed ingredients, such as coproducts and fibrous grains, are increasingly used in poultry feeding programs to maintain industry competitiveness. However, their use is limited by high levels of non-starch polysaccharides, which often act as anti-nutritional factors by increasing intestinal viscosity and encapsulating nutrients. As a result, poultry's gastrointestinal tract cannot naturally digest these feedstuffs efficiently.

Nevertheless, these fibers have latent prebiotic potential that can be unlocked through the strategic use of exogenous enzymes. This process involves enzymatic depolymerization of complex fiber matrices into smaller, biologically active, soluble oligosaccharides. These fragments serve as fermentable substrates for beneficial gut bacteria, effectively functioning as in situ-produced prebiotics. Such fermentation increases the production of short-chain fatty acids (SCFAs), which serve as energy sources for intestinal epithelial cells and play crucial roles in maintaining gut integrity and modulating immune responses. Additionally, SCFAs have been shown to modify gut microbiota composition, promoting beneficial bacteria and suppressing pathogenic microorganisms. Studies have shown that enzyme supplementation in fibrous feedstuffs can lead to significant improvements in gut morphology, including increased villus height and a higher villus-to-crypt ratio, indicators of enhanced nutrient absorption capacity. Moreover, the synergy between exogenous enzymes and alternative feedstuffs has been demonstrated to improve growth performance and immune responses, effectively mimicking the benefits of traditional antibiotic growth promoters. Therefore, by combining exogenous enzymes with alternative feedstuffs, the poultry industry can improve nutrient utilization and support a resilient gut-immune system without relying on antibiotics. It supports the concept that fiber can be transformed from an anti-nutritional factor into a functional dietary component when properly managed.

In conclusion, using exogenous enzymes to convert complex fibers into functional prebiotics offers a sustainable strategy to optimize poultry health, performance, and resource efficiency in modern

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poultry production systems. However, despite these positive outcomes, enzyme efficacy can vary with factors such as dosage, dietary composition, and poultry age. Therefore, more research is needed to optimize enzyme supplementation strategies and fully elucidate the mechanisms underlying their beneficial effects.
Keywords: Dietary fiber, enzymes, prebiotics, intestinal health, immune response

3056 Impact of Dried Apple Pomace on Growth Performance and Nutrient Digestibility in Broiler Chickens

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The rising demand for natural and affordable feed ingredients has stimulated an interest in fruit by-products, which can serve as functional additives to enhance poultry health and performance. This study examined how different drying methods and inclusion levels of apple pomace (AP) interactively influenced broiler weight gain, feed efficiency, and nutrient digestibility during a 21-day trial. 300 one-day-old Ross 308 chicks were randomly allocated to ten dietary treatments arranged in a 3 x 3 + 1 factorial design. These included a control diet (0% AP) and diets with 3, 6, or 9% of hot air dried (HAD), freeze dried (FD), or electrohydrodynamic dried (EHD) AP. Each treatment had six replicates, with five birds per cage, using the cage as the experimental unit. Growth performance parameters, including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were measured weekly. Relative organ weight and apparent nutrient digestibility of crude protein (CP) and gross energy (GE) were also assessed. All data were analyzed using a completely randomized design (CRD) in a MIXED procedure of SAS. The BWG, FI, and FCR were strongly influenced ($P<0.05$) by the drying process, inclusion levels, and their interaction across the experimental periods. BWG and FI decreased as the inclusion level increased, while FCR values increased with higher inclusion levels. BWG decreased linearly from 795 g (control) to 419 g at 9% inclusion of FDAP, while the FCR increased from 1.27 (control) to 1.73 (9% inclusion of FDAP). The relative weights of the spleen and liver were not significantly affected ($P>0.05$) by the drying method, inclusion levels, or their interaction. However, gizzard weight increased by 20% (from 22.4 to 27.4 g/kg) and was significantly affected ($P < 0.0001$) by both inclusion levels and the interaction between drying method and inclusion levels, with values increasing as AP inclusion level increased. The apparent digestibility of CP and GE was significantly influenced by drying method, inclusion level, and their interaction, with digestibility decreasing with increasing inclusion level, showing significant ($P<0.05$) linear and quadratic effects. In conclusion, inclusion levels higher than 3% impaired growth and feed efficiency, increased gizzard weight, and had no effect on liver or spleen development. HAD AP at 3% yielded the best performance and nutrient digestibility, suggesting it as a possible feed additive in broiler chickens' diets.

Keywords: apple pomace drying method; broiler chicken; growth performance; nutrient digestibility; sustainable feed ingredient

3057 Effect of a commercial triple-strain Bacillus-based probiotic on egg quality, performance, welfare and livability of laying hens

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Persistency of lay cannot be achieved without due consideration of how to sustain egg quality, health, and welfare of the hen during the whole laying cycle. Optimum feeding strategy including the use of effective probiotics since the first day of life does not only help establishing a robust and balanced microbiota but does also influence bird productivity and health. The aim of the study was to assess the impact of feed supplementation with a commercial triple-strain Bacillus-based probiotic on performance, egg quality, livability, and well-being of laying hens. 360 Leghorn day-old chicks were allocated to two different treatment groups: one control group (CON) receiving a standard diet and a second group fed with the same diet including the probiotic (PRO, 1.6×106 CFU/g of finished feed) from day 1 (D1) up to 42 weeks of age (W42). 30 replicates including 2 cages per replicate and 3 birds per cage were used for each treatment group. Data were analyzed using a general linear model (JMP 18 Software) and Dunnett test was used for means comparison. The group of hens fed with PRO did show better zootechnical performance compared to CON ($p< 0.05$) with higher hen-day egg production (87,3 vs. 85,9 %), higher hen-housed production (85,9 vs. 84,1%) as well as better feed efficiency with a lower feed consumption per dozen eggs (1,2023 vs. 1,245 Kg) at W42. Regarding egg quality parameters, egg weight was also in favor ($p< 0.05$) of PRO (56,442 vs. 55,894g), but there was no significant difference between PRO and CON for Haught unit score (87,751 vs. 86,421 respectively) and eggshell thickness (0,420 vs. 0,413 mm respectively). Furthermore, PRO group did show higher circulating levels of serotonin (+20%) at W16 and lower mortality rate (-4% points) compared to CON ($p< 0.05$) during the whole trial period. In conclusion, supplementing the feed with the commercial triple-strain Bacillus-based probiotic from D1 did improve productivity and egg quality while supporting livability and welfare of commercial layers.

Keywords: Laying hens; Probiotic; Productivity; egg quality; well-being

3058 Beyond growth performance; early intestinal development using xylanase

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For broilers time is of the essence. There is little time for natural development of the intestinal microbiome and maturation of the largest immune system, the intestinal immune system. Targeted dietary interventions have emerged as a promising strategy to strengthen barrier function and enhance immune development through feeding the developing microbiome. This 11-day study sought to evaluate the impact of varying doses of non-starch polysaccharide degrading enzyme, xylanase, on performance, gut barrier and immune development in early life. A commercially relevant wheat-soy based control diet (Control) was formulated to be nutrient adequate and tested against the same diet with three xylanase doses; 2000U, 4000U, and 8000U (Danisco Xylanase) with a 100kcal reduction. In total 1088 Ross 308 male broilers were placed in 32 pens divided over 8 houses (34 birds/pen, 4 pens/house). In each house, pens were randomly assigned to one of 4 treatments. Body weight (BW) and feed intake (FI) were determined per pen and mortalities recorded. On d11, 2 birds/pen were sacrificed and ileal tissue collected for the evaluation of tight junction protein (TJP) gene expression Claudin-1 (CLDN-1) and Zonula Occludens-1 (ZO-1), Toll-like receptors (TLR-2,4 and 21) and cytokines (interleukin (IL)-10, IL-1β, and IL-22). Performance data was analyzed using JMP (v.16) with treatment as a fixed effect and house as random effect. Wilcoxon-Test was used for host markers. Pearson correlation was used to determine correlation coefficients among different markers. BW increased and FCR improved with increasing xylanase dose, 8000U showing significant

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differences vs Control (300 and 323g for BW (P = 0.016) and, 1.19 vs 1.11 (P = 0.042) for FCR, respectively). Increases in ZO-1 with both 4000U and 8000U (P=0.012) and CLDN-1 (P=0.051) were observed vs Control. Increases in TLR-2, 4 and 21 were observed in all treatments with the greatest increases reported in 4000U and 8000U in TLR-4 and TLR-21 (4.46 and 3.35 fold increase in TLR-4 (P<0.1) and 3.98 and 2.8 fold increase in TLR-21 (P<0.1)). These increases in TLRs were associated with moderate to strong positive correlations to cytokine and TJP gene expression. Noticeably, TLR-2 was associated with increases in IL-10, IL-22 (0.617 and 0.529) which further demonstrated a 0.738 correlation to CLDN-1. TLR-4 was associated with increased IL-1β (0.585) and IL-10 (0.639) with subsequent correlations of 0.541 and 0.504 to ZO-1 and CLDN-1 respectively. These results indicate that higher enzyme supplementation enhances TJ integrity and innate immune activation in the ileum, with upregulated TLR signaling strongly correlating to increased anti-inflammatory cytokines and improved barrier function.

Keywords: Xylanase; Intestinal development; feed efficiency ; Microbiome; Broilers

3059 Improving thermoregulation in broiler chickens during summer through dietary guanidinoacetic acid supplementation

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Guanidinoacetic acid (GAA) improves weight gain, muscle energy conservation, and vasodilation in poultry under heat stress, owing to its osmolytic function, regulating cellular fluid balance, contributing to better thermoregulation and lower core body temperatures. With increasing heat stress challenges in chicken meat production during hotter summer periods, this study hypothesised that dietary GAA supplementation would reduce thermoregulatory demands in broiler chickens by lowering core body temperature. The aim was to evaluate the efficacy of GAA supplementation in improving heat tolerance and growth performance by continuously monitoring body temperature in real-time and measuring performance indicators during a summer grow-out period.

496, day old Ross 308 broiler chicks were supplemented with either no GAA (Control) or 0.06% GAA (600 g/t Creamino; GAA) from 0 to 50 d of age (8 pens, 4 replicates/treatment). Internal body temperature was recorded continuously using a subcutaneous temperature-sensitive pit-tag, implanted on the right breast at d14 (n=96, 12/pen), via antennas positioned underneath the litter in each pen.

At d50, blood samples were collected for biochemical profiling, specifically Creatine Kinase (IU/L). Weekly body weight, average daily gain and weekly pen FCR were measured for all birds. Statistical analyses were conducted using linear and generalized linear mixed models to evaluate treatment effects on thermoregulation, growth performance and blood biochemistry, with significance set at $P \leq 0.05$ and trends noted at $P < 0.1$.

Overall, chickens supplemented with GAA tended to have lower body temperatures compared to control birds. A significant treatment × sex interaction was observed ($P < 0.001$) with GAA males having the lowest body temperatures (39.83 ± 0.03°C vs. 40.22 ± 0.04°C). No differences were observed in females.

GAA birds were heavier ($P < 0.05$) on d 7, 21 and 29 and tended ($P = 0.052$) to be heavier on d 43. Growth rates were higher in GAA birds compared with control birds ($P < 0.05$). Pen feed conversion ratio tended to be higher in control birds than in GAA birds but was not statistically significant. Plasma creatine kinase tended ($P = 0.055$) to be lower in GAA birds compared to controls. GAA supplementation shows promise as a nutritional strategy to mitigate potential heat-stress during Australian summer grow-outs. The observed reduction in body temperature, improved growth performance and muscle health suggests physiological benefits, although further research is needed to clarify the underlying mechanisms and to optimize dosage strategies for commercial application.

Keywords: broiler; guanidinoacetic acid; thermoregulation; growth performance; heat stress

3060 Microbiota-mediated metabolic activation of plant polyphenols in poultry and their potential application

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The overuse of antibiotics in poultry production has led to serious issues such as antimicrobial resistance, drug residues, and environmental risks, highlighting the urgent need for effective antibiotic alternatives. Plant extracts, rich in polyphenolic compounds with antioxidant, anti-inflammatory, and gut microbiota-regulating activities, have shown great potential as natural feed additives. However, their generally low bioavailability in poultry limits their practical efficacy, and the underlying metabolic mechanisms remain unclear. In this study, broilers and laying hens were used as models to investigate the metabolic transformation pathways of representative polyphenolic compounds and their interactions with gut microbiota. For the laying hen trial, a total of 640 61-week-old Hyline Brown laying hens were randomly divided into 5 groups and fed a basal diet supplemented with 0, 100, 200, or 400 mg/kg chlorogenic acid (CGA) for 12 weeks. Blood and ileal content samples were collected for analysis. For the broiler trial, a total of 18 21-day-old male broilers were individually housed. Half received an antibiotic cocktail in their drinking water for 7 days, while the other half received plain water. These groups were used for pharmacokinetic studies of icariin. Mass spectrometry analysis revealed that after CGA supplementation, its initial metabolic step in the laying hen intestine was ester bond hydrolysis, producing caffeic acid, which subsequently underwent reactions such as reductive hydrogenation and dehydroxylation to form 3-hydroxybenzoic acid (3-HBA). After icariin supplementation, compared with the antibiotic-treated group, icariin was metabolized via multiple gut microbiota-mediated pathways (e.g., deglycosylation, glycosylation, demethylation, and hydroxylation) into various metabolites, with baohuoside I, formed by the removal of one glucose molecule, being the predominant metabolite. In contrast, the antibiotic group showed mainly the parent form of icariin. Metagenomic analysis further demonstrated that Lactobacillus, Enterococcus, and Escherichia coli (Firmicutes) were primarily responsible for glycosidic bond cleavage, while Bacteroides and Phocaeicola (Bacteroidetes) were involved in ester bond hydrolysis. These findings elucidate a “microbiota-mediated metabolic activation” mechanism of plant polyphenols in poultry, providing new insights into improving the efficacy of plant extract-based feed additives and developing efficient antibiotic alternatives for sustainable poultry production.

Keywords: polyphenols; gut microbiota; bioavailability; feed additives

3061 Optimum dosage, formulation, and administration route of essential oils to improve growth performance, meat quality, and gut microbiota of broiler chickens

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This study investigated the optimum dosage, formulation, and administration route of an essential oil (EO) blend containing lavender, eucalyptus, and pine oils on growth performance, meat quality, and gut microbiota of broiler chickens. It was hypothesised that bioavailability and efficacy of EO differ across feed, water-soluble, and nano-emulsified delivery systems, influencing performance, carcass yield, and intestinal microbial balance. A 42-day trial was conducted using 256 Ross 308 male broilers randomly assigned to 32 pens (4 treatments × 8 replicate pens × 8 birds/pen) under controlled environmental conditions. Treatments included a control, EO blend in feed (200 g/ton = 200 ppm), EO blend in drinking water (200 ppm), and nano-emulsified EO in water (200 ppm). Birds were fed standard wheat–soybean meal diets meeting all nutritional requirements, with ad libitum access to feed and water. Growth performance was measured bi-weekly, and at day 42 one bird per pen was sampled for carcass and breast meat evaluation, including pH, colour, water-holding capacity (WHC), cooking loss, shear force, and proximate composition. Cecal contents were analysed using a 16S rRNA sequencing to assess microbial shifts. Data were analysed using two-way ANOVA with Tukey's post-hoc test ($P < 0.05$).

Both water-soluble and nano-emulsified EO treatments significantly improved final body weight and average daily gain ($P = 0.01$) compared with the control, with Nano-EO yielding the greatest effect. Feed intake increased ($P = 0.02$), while feed conversion ratio tended to improve. Cold carcass weight ($P = 0.004$) and breast WHC ($P = 0.01$) were enhanced in EO-Water and Nano-EO groups, indicating superior meat quality. Microbiota analysis revealed increased abundance of Lactobacillus johnsonii and reduced Romboutsia ilealis and Streptococcus alactolyticus in EO-Water and Nano-EO treatments, suggesting improved microbial balance and gut health. Feed-supplemented EO produced moderate effects, highlighting the importance of solubility and dispersion for optimal EO bioactivity. The better efficacy of the nano- emulsified EO are likely linked to the smaller particle size and greater intestinal absorption, and shifts in gut microbiota resulting in improved gut functionality.

In conclusion, based on our findings, adding 200 ppm EO and nano-emulsified EO in drinking water enhanced broiler productivity, carcass yield, and gut microbial ecosystem in an antibiotic-free production system. Both nano-emulsified and water-soluble forms demonstrated superior efficacy to feed-supplemented EO.

Keywords: broiler chicken; essential oils; nano-emulsion; gut microbiota; meat quality

3062 N-ACETYLATED CHITO-OLIGOSACCHARIDES: NOVEL OLIGOMERS WITH ENDLESS APPLICATION OPPORTUNITIES

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Chitin derived compounds such as NACOS (N-acetylated chito-oligosaccharides) are hypothesized to trigger biological effects which make them great as potential dietary supplements for animal nutrition and health. The mechanisms underlying the bio-function of NACOS are not well documented, owing to their limited availability and poor characterization in literature. Hereto, two different NACOS mixtures were produced (NACOSA & NACOSB) by mechanochemical grinding and were well characterized using ultra high-performance size exclusion chromatography (UHPSEC) coupled to refractive index (RI) and mass spectrometry (MS) at Univeristy of Münster (Germany). Both NACOS mixtures have a degree of polymerization (DP) ≤ 20 or less, a degree of acetylation of ≥ 70%, and a polydispersity (PDI) index of ≤ 10. For NACOSA, the milling time was 2 times higher as compared to NACOSB. In experiment 1, the effect of NACOS on in vitro IPEC-J2 cells was evaluated using a H2O2 induced stress model. Cell viability was screened via MTS assay. In experiment 2, NACOS has been added to freshly excysted Eimeria tenella sporozoites. In experiment 3, NACOS immunostimulatory potency was tested by using lipopolysaccharide (LPS) and a macrophage cell line. In experiment 4, the effect of NACOS on intestinal CaCo-2 cell health and gene expression was evaluated. The results showed that efficacy of NACOS clearly depended on oligomer profile. In experiment 1, NACOSB had a bigger antioxidant impact than NACOSA. Experiment 2 revealed that especially NACOSB served as highly potent compound for control of avian coccidiosis. Main takeaway from experiment 3 is that NACOSB can function as anti-inflammatory agent, which is reflected in lower nitric oxide (NO) production after LPS trigger ($P<0.0001$). In experiment 4, NACOSB improved intestinal cell viability ($P<0.02$) and triggered gene expression in CaCo-2 cells more effectively than NACOSA ($P<0.05$). Overall, these data show that the NACOSB clearly outperforms NACOSA in terms of intestinal health related read-outs. The more oligomers are included, the more effective the NACOS product will be. NACOS have demonstrated many biological effects including antioxidant pathways, macrophage inflammatory effects, coccidiosis prevention and intestinal cell health, which make NACOS (especially NACOSB) great candidates of dietary supplements for human and animal health. Especially, the impact on Eimeria/coccidiosis is remarkable. Coccidiosis is one of the most economically devastating diseases of poultry industry, with losses exceeding 3 billion US dollars annually due to a combination of low productivity, mortality and high costs required for effective prophylactic and therapeutic interventions.

Keywords: : N-acetylated chito-oligosaccharides; intestinal tract; immunity; oxidative stress; coccidiosis

3063 Dietary Guanidinoacetic Acid Enhances Performance and Intestinal Health of Broilers Under Reduced-Energy Diets and Heat Stress

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Heat stress remains one of the most critical biological and economic challenges in broiler production. Guanidinoacetic acid (GAA; as Creamino®) has emerged as a promising nutritional strategy that enhances cellular energy status via creatine synthesis, particularly when dietary energy is limited or during thermal stress. This study evaluated the effects of GAA supplementation in broilers fed standard or reduced metabolizable energy (ME) diets under thermoneutral or heat-stress conditions, focusing on growth performance, carcass traits, intestinal morphology, and microbial populations. A total of 1,536 day-old Ross 308 broilers were assigned to a 2 × 2 × 3 factorial design with eight replicates per treatment (16 birds/pen) and fed experimental diets from day 1 to 28. The factors included temperature (thermoneutral vs. heat stress from day 21–28; +10 °C midday), dietary ME (standard: 3015/3075 kcal/kg vs. reduced: 2915/2975 kcal/kg for starter/grower phases, respectively), and Creamino® supplementation (0.00%, 0.06% or 0.09%). Data were analyzed using ANOVA with main effects and interactions evaluated. Heat stress significantly reduced body-weight gain (657 g vs. 713 g; $P < 0.001$) and increased feed conversion ratio (1.42 vs. 1.34; $P < 0.001$) between days 21–28. Dietary ME and GAA showcased a statistically significant interaction affecting the performance parameters. Under reduced-energy diets, 0.06% GAA increased feed intake (990 g), and body-weight gain (737 g), while decreasing feed conversion ratio (1.34). These results outperformed both unsupplemented and 0.09% GAA treatments.

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GAA supplementation at 0.09% on reduced-energy diets showcased a lower FCR compared to standard-energy diets (1.32 vs 1.44; P<0.001). Intestinal morphology was significantly influenced by the three-way interaction among environmental temperature, dietary energy level, and GAA supplementation. Birds receiving both GAA levels under standard energy diets during heat stress conditions exhibited the greatest ileal villus height, surface area, and villus: crypt ratio, significantly surpassing unsupplemented birds under the same conditions. Additionally, dietary GAA supplementation modulated microbial populations. Lactobacillus spp. counts increased with 0.06% and 0.09% GAA under heat stress, while Clostridium perfringens, E. coli, and Salmonella spp. were reduced in GAA-fed groups indicating enhanced gut microbial balance. These findings highlight the potential of GAA, particularly at 0.06%, as a nutritional intervention to enhance performance, gut health, and thermotolerance in broilers facing energy restrictions and heat stress.

Keywords: Guanidinoacetic acid; Heat stress; Energy; Broiler; Intestinal morphology

3064 Influence of a chestnut-quebracho tannins blend on performance, litter moisture, and fecal moisture in commercial broiler chickens in Salmonella challenged conditions

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Plant-based extracts can be natural alternatives to antibiotic growth promoters, due to their bioactive compounds that support gut health and nutrient utilization, as well as control Salmonella in poultry. This study evaluated the effects of a blend of chestnut (Castanea sativa) and quebracho (S. balansae or S. lorentzii) tannin extracts (TE) (Silvafeed Nutri P, Silvateam S.P.A., Italy) on growth performance, litter moisture, and fecal moisture in Salmonella-challenged broilers. A total of 1,200 day-old male chicks (Ross 708) were assigned to 4 dietary treatments using RCBD with 12 replicate pens of 25 birds each: (1) negative control (NC; unchallenged), (2) positive control (PC; orally challenged with nalidixic acid–resistant Salmonella Typhimurium and S. Infantis at 105 CFU/birds on day 0), (3) bacitracin methylene disalicylate at 100 mg/kg feed with Salmonella (PC+BMD), and (4) PC+TE (TE; 1000 g/ton feed). Corn-soybean meal diets were formulated according to the nutrient requirements of Ross 708 broilers for starter (d0-14), grower (d15-28), and finisher (d29-35) phases. For fecal moisture analysis, one bird from each of 8 replicate pens per treatment was transferred to individual cages (d13, d34). Litter and fecal samples were weighed and oven-dried at 75°C until a constant weight was achieved, and the moisture percentage was calculated based on weight loss after drying. Data were analyzed by ANOVA, and means were separated using Tukey's HSD at P≤0.05, with a tendency defined at P≤0.1 in R software (version 4.4.3; R Core Team, 2025). At d7, feed conversion ratio (FCR) differed among treatments (P=0.0135), with the BMD group showing the best FCR, followed by TE, while PC had the worst. The TE group did not differ from the BMD group, and no significant differences were observed between PC and either TE or BMD. FCR and feed intake at later time points were not significantly different. At d14, body weight gain (BWG) was affected by treatment (P=0.0051); TE-fed birds had significantly higher BWG than PC and were comparable to the BMD group. Over the 35-day period, BWG tended to differ (P = 0.0634), with numerically higher gain in TE-fed birds compared to PC and BMD. No significant differences in overall performance (P>0.05) were observed between the NC and the PC group. Litter moisture at d35 differed (P=0.0415), in pens with birds fed TE having lower litter moisture values than other treatments. Fecal moisture at d34 showed a similar reduction tendency (P=0.0962), indicating improved water balance in birds fed the TE blend. Chestnut and quebracho tannin extracts may offer a viable alternative to antibiotics in their ability to enhance performance and litter quality of Salmonella-challenged broilers.

Keywords: chestnut and quebracho tannin extracts; non-antibiotic growth promoters; production performance; fecal and litter moisture; Salmonella challenge

3065 Targeted delivery of butyric acid: Intestinal health markers response in broilers fed different protected forms of butyric acid

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Butyrate is a gut health stimulator due to its properties of stimulating intestinal epithelium, gene expression, and microbiota modulation. This study aimed to evaluate the effects of three different forms of protected butyric acid on gut health marker responses in broilers. A total of 600 one-day-old Hubbard broilers were randomly assigned to 4 dietary treatments with 10 replicates each: a negative control fed standard diet (NC); NC + sodium butyrate protected by medium-chain fatty acid (DC, Dicosan+, Norel Animal Nutrition); NC + tributyrin (TB, ProPhorce SR130, Perstorp); and NC + sodium butyrate protected by buffer salts (BB, Butirex C4, Novation). Products were dosed to meet a 54% butyric acid level. The trial lasted for 35 days. Blood samples were collected for antioxidant levels analysis [Total Antioxidant Capacity (TAC), malondialdehyde (MDA) and glutathione (GSH)]. Duodenal and ileal tissues were analyzed for villi height and crypt depth, and mRNA tight junction protein expression: MUC-2 and ZO-1. Cecal content was collected and analyzed for short-chain fatty acids (SCFA), and bacterial count of lactobacillus, coliform and Clostridium. Data were analyzed by one-way ANOVA with Tukey's post hoc test of JMP software. Blood antioxidant analysis showed the highest levels of TAC for DC and TB among the other treatments (P<0.05), and the lowest level of MDA for DC among the other treatments (P<0.05). However, GSH value was higher in all treatments except for NC (P<0.05). Villi height at the duodenal level was the highest in DC (P<0.05) among the other treatments. At the ileal level, villi height was higher for DC and TB (P<0.05) than for NC and BB. Crypt depth was the lowest for TB at the duodenal level among the other treatments (P<0.05), and no difference was observed at the ileal level. Tight junction protein expression MUC-2 was the highest for DC and lowest for NC, and was intermediate for TB and BB. For the ZO-1 expression, it was the highest for TB and lowest for NC, and intermediate for DC and BB. Microbial count analysis showed a higher lactobacillus count for DC compared to NC (29%), followed by TB (25%) and BB (14%). Clostridial and coliform counts were lower for all butyrate treatments (average 63.8 and 46.3%, respectively) compared to NC. For SCFA, acetate and propionate were higher for DC, and lower in NC. However, butyrate value was the highest for TB and the lowest for NC, while it was intermediate for DC and BB. In summary, protecting sodium butyrate with medium-chain fatty acids promoted the most gut health markers, indicating better intestinal homeostasis, which is crucial to improve the production efficiency of broilers compared to other forms of protected butyrate.

Keywords: Broilers; Butyric Acid; gut health; tight junction; villi

Small Scale Family Poultry Farming

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3066 Strengthening Small-Scale Poultry Systems: Health, Biosecurity, and Food Safety

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Small-scale and backyard poultry systems contribute significantly to household nutrition, local food security, and rural livelihoods worldwide. In the United States, interest in backyard poultry has grown rapidly over the past few decades, with thousands of small flocks maintained for egg production, food sovereignty, and community-based agriculture. In states such as Connecticut and across the Northeast, these systems range from small backyard flocks to diversified farms integrating poultry into sustainable and organic production systems. While these operations provide important social, economic, and nutritional benefits, they also present unique challenges related to poultry health management, biosecurity, and food safety.

Backyard and small-scale flocks often operate with limited infrastructure and varying levels of management training, which can increase risks associated with infectious diseases, including highly pathogenic avian influenza (HPAI), Salmonella transmission, and environmental contamination. Additionally, inconsistent biosecurity practices, informal egg handling and storage, and close human–animal interaction can create pathways for both animal health and public health risks. Addressing these challenges requires practical, science-based strategies that are accessible and adaptable for small-scale producers. The approach should include the role of extension education, stakeholder engagement, and collaborative research in translating poultry science into practical management tools for small-scale producers.

Drawing from ongoing extension and applied research programs focused on poultry health, egg safety, and farmer training, this project discusses approaches such as targeted biosecurity education, producer workshops, risk communication during avian influenza outbreaks, and development of accessible training resources for backyard flock owners. By integrating scientific knowledge with extension-driven outreach, these efforts aim to strengthen small-scale poultry systems while protecting animal health, food safety, and consumer confidence. The goal is to provide a framework for understanding the interconnected roles of poultry health, biosecurity, and safe food production in small-scale systems, promoting subsequent case studies and regional perspectives on family poultry production from around the world.

3067 Family Poultry as a Catalyst for Food Security, Nutrition, and Resilience in Vulnerable Communities

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Family poultry—small, low-input, and widely accessible backyard poultry production—plays a critical role in enhancing food security, achieving Zero Hunger targets, and supporting emergency nutrition programs, particularly in low- and middle-income countries. As an integral part of rural and peri-urban livelihoods, family poultry provides a continuous supply of nutrient-dense foods, including eggs and meat, essential for combating malnutrition, micronutrient deficiencies, and hidden hunger. Women and children, who are often the primary caregivers of backyard flocks, directly benefit from improved access to animal-source protein and income, thereby strengthening household dietary diversity.

Our multiple field experiences with small-scale family poultry in Pakistan demonstrate that supporting highly vulnerable communities with as few as ten birds can significantly improve maternal and neonatal health. In several intervention sites, access to eggs and poultry meat was associated with reduced risk of pregnancy-related complications, including lower chances of abortion in women, and improved growth and health outcomes in neonates. These findings highlight the strong nutrition-sensitive impact of family poultry on women and child health in resource-poor settings.

Within emergency contexts—such as natural disasters, conflict situations, and humanitarian crises—family poultry becomes a strategic intervention for rebuilding food systems. The short production cycle, mobility of birds, and minimal infrastructure needs allow for the rapid restoration of household food production. Providing improved breeds, feed, vaccines, and basic training facilitates quick flock establishment, enabling families to resume food production within weeks. Eggs, being an immediate source of high-quality protein, are particularly valuable for infants, pregnant women, and vulnerable populations, making family poultry a cornerstone of emergency nutrition strategies.

Family poultry interventions aligned with One Health principles ensure disease control, stronger biosecurity, and reduced zoonotic risks. Improved housing, vaccination programs, and balanced feeding enhance both productivity and resilience. Integrating work toward achieving the UN Sustainable Development Goals, particularly SDG 2 (Zero Hunger), integrating family poultry into national food and nutrition security policies offers an affordable, scalable, and climate-resilient pathway to improved livelihoods and community well-being. Overall, family poultry remains a vital instrument for sustainable development, hunger reduction, and emergency nutrition support across diverse regions.

Keywords: Family poultry; Backyard poultry; Small-scale poultry production; Food security; Zero Hunger

3068 Challenges and opportunities for more mature and sustainable family poultry value chains in resource-limited settings

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Family poultry (FP), which make up more than 80% of the about 29.2 billion heads of world's poultry stocks in 2023 and kept in small numbers ranging from as few as one up to about 20, are critically important in low- and lower middle-income countries (LLMICs) of Africa, Asia, Latin America and the Pacific. With native poultry (NP) breeds kept in FP systems contributing about US$ 289 billion of gross meat production value globally in 2022, the socio-economic and environmental values of the FP value chains (FPVCs) are remarkable, as evidenced by their contributions to Sustainable Development Goals (SDGs). NP breeds hold particular significance as they are hardy, adaptable to diverse agro-climatic zones and resource-limited settings, possessing strong immunity, brooding characteristics, foraging capacity and disease resistance potentials. Besides, the versatility of FPVCs is exemplified by their key roles in food and nutrition security, wealth creation, sustainable livelihoods and conservation of NP breeds, underpinning FPVCs' needs for more maturity and sustainability. Although many opportunities abound, FPVCs continue to grapple with notable challenges associated with resource management, biosecurity, climatic conditions, infrastructure and logistics, breeding, regulatory policy, health practices and disease surveillance systems. Furthermore, the diverse NP breeds with complex roles in human societies and ecosystem services need to be strategically addressed by exploiting innovation-driven, digitally focused and sustainability-oriented approaches. Moreover, the pressing need for climate-smart resilient FP flocks amid challenging climatic conditions also acts as a catalyst for adopting sustainable management practices. Using advanced biotechnological tools in FP farming redefine the future of FPVCs by fueling swift improvements in productivity, feeding management, disease management, genetic manipulation, research and diagnostics. Thus, leveraging strategic strengths and address-

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ing challenges are crucial for securing more maturity and long-term sustainability of FPVCs. This paper explores the challenges and opportunities to make FPVCs more mature, environmentally sustainable and resilient under resource-limited conditions and harsh environments.

Keywords: Family poultry; Mature poultry; Small-scale farming; Sustainable poultry; Value chain

3069 Production performance of improved native chickens at selected coastal areas of Bangladesh

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Bangladesh Livestock Research Institute has improved native chicken which refers to a chicken lines developed by at least 10 generation studies of best to best selection, resulting in improved genetic performance. The purpose of the study was to determine the production performance of improved native chicken under four selected coastal villages of Bangladesh. The experimental design was in 4 villages (Mulghor, Koyekha, Andharmanik, Biskhali) x 2 types chicken (CD, NN) x 8 farmers having 40 chickens in each farmer with female: male (35:5). Hence, a total of 1280 pullets at 14 weeks of age were distributed among 32 women farmers under four villages. All the collected data were analyzed under Completely Randomized Design (CRD) by using General Linear Model (GLM) Univariate Procedure in SPSS Computer Program. The results indicated that the average body weights of chicks were significantly ($P<0.001$) difference at 1st wk of age of Common Deshi (CD) 56.80g and Necked Neck (NN) 60.57g. The average body weights of growing chicks at 7th weeks of age among male and female were significantly different ($P<0.001$) of CD (M-655g/F-551g) and NN (M-630/F-558g). Although the daily feed (Least-cost ready feed) intake was affected by genotypes at 1-14 weeks of age but there were no significant ($p>0.05$) differences between the genotypes. The analyzed results (15-50 Wks.) indicated that Hen-housed egg production percentage also differed significantly ($P < 0.05$), with Koyekha (33.91%) and Andhermanik (31.85%) performing better, while Bishkhali and Mulghor recorded the lowest (29.12%) & (24.26%) respectively. Egg weight was varied significantly ($P < 0.001$) among locations. The study revealed that the higher egg weight was observed under Andharmanik village (45.04 g), followed by Bishkhali (44.09 g). Mulghor (41.72 g) and Koyekha (41.95 g), respectively. The body weight of birds varied from 1487.74 g and 1642.39 g, and difference was non-significant among villages ($P = 0.38$). Egg consumption per household was similar ($P = 0.35$), ranging from 168.28 eggs in Andhermanik to 200.20 eggs in Mulghor. Further, the results exhibited that income from egg sales was significantly different among villages ($P< 0.05$). The highest income from egg sales was found in Koyekha (BDT 28,237.88; $230.72), followed by Mulghor (BDT 26,694.90; $ 218.11) and Andhermanik (BDT 26,650.00; $ 217.75), respectively. However, the lowest income from egg sales was observed in Bishkhali (BDT 22,989.06; $ 187.83), which was due to lower egg production. From the above results, it may be concluded that Non-Descript Deshi chicken was better in terms of egg production compared to Naked Neck in the coastal areas of Bangladesh.

Keywords: Native; chicken; performance; adopted; coastal areas

3070 Preserving the Himalayan Gene Pool: A Conservation Outlook for Uttara Chicken

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India is globally recognized for its substantial poultry sector, ranking second in total egg production and fourth in total meat production. The current per capita availability stands at 106 egg numbers per annum and 3.68 kg per annum of chicken meat. The National Bureau of Animal Genetic Resources (NBAGR), Karnal, India, has registered 20 indigenous chicken breeds, including several lesser-known native populations. Among these, the recently registered Uttara breed from Uttarakhand represents a unique genetic resource predominantly maintained by nomadic and economically marginalized communities. Uttara chickens are highly adapted to harsh free-range environments, exhibiting pronounced agility and vocal behaviour. The breed is characterized by dark black plumage, shank feathering, and the presence of a crest or crown in approximately 20% of individuals. Phenotypically, the birds possess white skin, yellow-black eyes, and predominantly single combs (88%), with a minority displaying pea combs (12%). Adult males and females attain average body weights of 2.0 kg and 1.6 kg, respectively. Annual egg production ranges from 165 to 170 eggs, with an average egg weight of 52 g and a light brown shell colour. Blood biochemical parameters, including globulin (2.7 g/dl), cholesterol (115.2 mg/dl), and glucose (237.9 mg/dl), were comparable between sexes. Uttara meat is reported to be highly palatable with lower cholesterol content, enhancing its suitability for health-conscious consumers and cardiac patients. Owing to its hardiness, disease tolerance, low-input requirements, and superior product quality, the Uttara chicken represents a valuable birds for backyard poultry systems in Uttarakhand state. Conservation and promotion of this indigenous germplasm can significantly contribute to livelihood security among small and marginal farmers, particularly in remote and hilly regions where commercial poultry farming is less feasible.

Keywords: backyard poultry system; germplasm conservation; indigenous poultry; native chicken genetic resources; Uttara, India

3071 Household chicken keeping in Ghana: a case study of the Ashanti region

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This research's objectives were to identify the production practices, trait preferences, benefits and challenges of household chicken keeping in Ghana. Four (4) districts were chosen to represent the north, south, east and west of the Ashanti region. One hundred and twenty (120) respondents; 30 from each district, were provided with questionnaire; designed to collect both qualitative and quantitative data. Descriptive statistics were obtained using

SPSS. With 88 (73.3%) males and 32(26.6%) females as household heads; the data indicated that 65% of homes raised indigenous breeds and 35% raised exotic breeds. Average years of keeping home poultry was 14 years and management systems are extensive (75%) and semi-intensive (25%). Flock sizes averaged 25-30, with full (100%) reliance on children and spouses to care for the flocks. Trait preferences are disease resistance (40%), higher egg laying ability (35%) and good mothering ability (25%). Household chickens provide meat and eggs and ready cash for emergency expenditure. Challenges include diseases, predator attack and theft. Keepers call for Veterinary support to help them deal with seasonal disease outbreaks.

KEY WORDS
Household poultry, Trait preferences, Meat and eggs, Diseases, Veterinary support
Keywords: Household poultry; Trait preferences; Meat and eggs; Diseases; Veterinary support

3072 Small-Scale Family Poultry Production in Rural KwaZulu-Natal: Ethnoveterinary and Improved Management Practices

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Hypothesis or Objective: To evaluate ethnoveterinary remedies and improved management practices for enhancing productivity, flock health, and resilience in small-scale family poultry systems in rural KwaZulu-Natal, South Africa.
Experimental Design / Description of the Problem: Family poultry systems are vital to rural livelihoods but limited by preventable disease, poor housing, and weak veterinary support. From 2020–2024, a participatory field initiative engaged 90 farmers in five rural communities to assess local ethnoveterinary treatments and introduce complementary best-practice management approaches.
Materials and Methods: On-farm trials combined observation and extension-led training. Farmers documented the use of Aloe ferox, chili-garlic mixtures, and ash alongside improved housing, feed supplementation, and vaccination. Data on morbidity, mortality, egg output, and management adoption were collected quarterly. Quantitative data were analyzed using descriptive statistics and one-way ANOVA ($p < 0.05$); qualitative adoption trends were evaluated thematically.
Statement of Statistical Analysis: Differences in flock survival, egg production, and adoption indices among treatment and control households were tested using one-way ANOVA ($p < 0.05$).
Results: Integrated ethnoveterinary and management interventions improved flock survival and egg output by 10–15 % relative to baseline ($p < 0.05$). Aloe ferox and chili-garlic mixtures reduced respiratory and digestive disorders. Farmer participation raised knowledge scores and confidence in disease prevention by 13 %, enhanced youth, and women's involvement, thereby reduced dependence on external veterinary inputs.
Conclusions: Validated ethnoveterinary practices integrated with improved management offer a sustainable pathway to strengthen small-scale poultry in resource-limited settings. Participatory training empowers farmers, enhances welfare and productivity, and supports income stability. Institutionalizing blended approaches within extension programs can improve food security across rural South Africa.
Keywords: Ethnoveterinary practices; Family poultry; Management practices; Rural development; KwaZulu-Natal

3073 Consumer Awareness of Egg Quality and Animal Welfare: Educational and Market Challenges for Small-Scale Poultry Producers in Serbia

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Eggs are a food of high biological value, easily accessible and widely consumed, representing an excellent source of protein, with global production estimated at around 91 million tons per year. This study aimed to identify key factors influencing consumer preferences and perceptions of egg quality in relation to animal welfare and more sustainable, small-scale poultry production systems. A voluntary, descriptive cross-sectional online survey was conducted in Serbia in 2025 to collect sociodemographic data, information on the frequency and place of egg purchase, preferences regarding egg characteristics (size, color, cleanliness, freshness), and attitudes toward animal welfare and ethical aspects of production. Data were analyzed using descriptive statistics, correlation analysis, one-way ANOVA and multiple regression. Results indicated that consumers considered egg freshness, shell integrity, and domestic origin as the most important quality indicators, while price and packaging design were less influential. Female respondents assigned slightly greater importance to free-range systems compared to males ($t = 2.14$, $p = 0.034$). ANOVA revealed no statistically significant differences among age groups regarding the perceived importance of eggs produced in free-range systems ($F = 1.82$, $p = 0.146$). Regression analysis showed that the perceived importance of animal welfare legislation ($\beta = 0.29$, $p = 0.002$) and the belief that animal welfare is directly linked to food safety ($\beta = 0.25$, $p = 0.006$) were the strongest predictors of consumers' preference for free-range eggs, while the importance of price exhibited a weak negative association ($\beta = -0.11$, $p = 0.094$). These findings indicate that Serbian consumers increasingly recognize animal welfare as an integral component of egg quality, substantially influencing their purchasing behavior. However, limited consumer knowledge and awareness still pose challenges, particularly for small-scale and family poultry producers seeking to compete in a market dominated by larger systems. Strengthening education and transparent communication about welfare-friendly and sustainable production practices could enhance consumer trust, improve market differentiation, and support the long-term viability of small-scale poultry farming.
Keywords: Egg quality; Animal welfare; free-range systems; small-scale poultry production; sustainable farming

3074 Rodent chew card monitoring around pasture-housed laying hens

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Pasture-based poultry systems offer substantial welfare and ecological benefits but can also increase hens' exposure to wildlife and rodent pests, posing potential biosecurity and disease transmission challenges. Traditional rodent monitoring methods such as motion-triggered cameras or tracking tunnels can be expensive, time-intensive, and impractical for many egg producers to deploy regularly. This project aimed to evaluate chew cards as a low-cost, farmer-friendly method to monitor rodent activity in pasture-based layer systems from before and after hens are placed on pasture. The study was conducted at Purdue University's Animal Sciences Research and Education Center (ASREC) using three mobile

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pasture-based poultry units. Rodent activity was assessed using canola-oil–dipped 10 cm2 gridded chew cards deployed along the perimeter every 10 meters for 24 hours, and gnawing was quantified as the percentage of each card chewed. Across all units and sampling periods, rodent activity was extremely low. Binary detection rates (any gnawing vs. none) remained sparse: Unit 1 decreased from 16.98% before placement of hens into the units to 11.11% post-placement of hens, Unit 2 increased from 24.39% to 37.50%, and Unit 3 declined from 6.25% to 0%. Since detections were rare, logistic regression and zero-inflated/binomial models could not converge due to quasi-complete separation. Therefore, Fisher's Exact Tests were used to compare pre- and post-placement periods within each unit, and none showed statistically significant differences (Unit 1: P = 0.72; Unit 2: P = 0.42; Unit 3: P = 1.00). These consistently low detection levels likely reflect genuinely low rodent pressure at the site, due to low local rodent populations, availability of alternative food sources, and the open-field placement of the pasture units. These results highlight chew cards as a simple, low-cost monitoring tool capable of verifying low rodent presence and supporting more efficient, data-driven rodent control programs that can reduce management costs and enhance the sustainability of pasture-based egg production.
Keywords: Pasture-based Poultry; Rodent Monitoring; Chew Cards; Management; Egg Production Systems

Waterfowl I

3075 Duck duck goose! Insights into the poultry industry from studies on duck brain, hormones and welfare

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Duck is one of the most consumed animal proteins globally. Despite this, there are relatively few investigators who participate with duck research. Our lab has done so for decades and focused on brain, behavior, and welfare of Pekin ducks. The purpose of this lead-off lecture for the Waterfowl Section is to provide an overview of past research on Pekin duck housing, physiology and welfare. Data will be provided to show that depending upon age and sex of the duck, corticosterone may not be the best, or only measure of stress. Further, the talk will provide evidence that colored LED lights may not be ideal to house ducks and suggest that a scotophase is essential for duck growth and welfare. Current evidence for these past outcomes will be provided based upon new insights on retinal physiology of the Pekin duck compared to its wild progenitor, the Mallard. This lead-off lecture will spotlight ongoing studies on alternative measures of stress and affective state in poultry, and possible epigenetic mechanisms of stressors to impact future generations.

3076 South African Ostrich research highlights

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Objective: To summarize recent ostrich research findings.
Materials and methods: The pedigreed ostrich flock maintained at the Oudtshoorn Research Farm in South Africa were used to study various aspects of ostrich production. This includes the application of assisted repoduction technology, field experiments, behavioural studies, gut microbiome analysis and reproduction output. Chick body temperature was monitored via implanted intra-coelomic data loggers, while behaviour and physiological responses were recorded. Hatching-assistance strategies and differences in incubator and eggshell temperature were also assessed.
Statistical analysis: Mixed-model quantitative genetic analysis were applied to reproduction and thermoregulatory traits, while behavioural and physiological shade-response data were analysed using mixed-effects models accounting for treatment and repeated measures. Microbiome data were evaluated using diversity indices, ordination and differential-abundance analysis. Incubation trials were analysed with linear mixed models.
Results: Progress in assisted reproduction technology demonstrated that chick production through artificial insemination is possible; however, outcomes remain lower than those obtained from naturally pair-mated females. It was shown that extreme temperatures reduced egg and sperm numbers, although gamete quality remained stable. Heat and cold tolerance showed significant heritability, but an unfavourable genetic correlation limits concurrent improvement. Female thermoregulation was heritable and positively correlated with egg production. Significant spatial variation in incubator temperature were demonstrated, while eggshell temperature consistently exceeded incubator temperature, Assisting chicks to hatch have lasting effects, with unassisted hatchlings having the lowest heart rates and superior growth. High-density shade substantially reduced heat load in chicks, lowering body temperature, suppressing heat-dissipation behaviours and increasing shade use with rising ambient temperature. Microbial diversity increased with age, while dysbiosis characterised chicks that died.
Conclusions: Temperature-related reproductive constraints, heat-load pressures in chicks, microbiome instability in early life and suboptimal incubation conditions remain central challenges in ostrich production. Genetic selection offers potential for long-term adaptation to rising temperatures, while providing high-density shade is a practical and effective heat-mitigation strategy for chicks. Unassisted hatching and early-life establishment of a healthy microbiome is critical for chick survival, while stricter control of incubator temperatures is needed to avoid compromised hatchability.
Keywords: Ostrich production; thermoregulation; incubation; reproduction; gut microbiome

3077 Positive combinational- impact of amino acids and anti-Oxidant on Immune-competence of In Ovo primed ducklings beyond juvenile growth across genotypes

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This study evaluates the combinational impact of amino acids (L-Lysine and DL-Methionine) and the antioxidant N-acetyl cysteine (NAC) on immune competence in in-ovo primed ducklings across two established genotypes of ducks, i.e. White Pekins and a synthetic breed (Kuzhi) popular across Indian Poultry production system. Four treatments were tested: LM (amino acids combination of L-Lysine plus DL-Methionine @10mg/egg each), C (NAC: N Acetyl Cysteine @7.5mg/egg each LMC (combo of LM with NAC at respective dosages), and sham control (SC), using 60 fertile eggs (per each Genotype-treatment combinational group), all administered in Ovo, at day 23–24 of incubation. Post hatch, all experimental ducklings were reared uniformly, using a standard duck starter diet till 6th week of age. Traits- wise, besides weekly liveweight records and livability, humoral Immune competence of ducklings was assessed via antibody titres (anti-sRBC Hemag-

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glutination [HA] during 5th week of age, NDV-Hemagglutination Inhibition [HI at 6 weeks age], and DPV vaccinal-antibody titre during 8th week of age) vis a vis growth parameters up to 6 weeks of age. Resultant data were statistically analyzed using one-way ANOVA and means comparison conducted by Tukey's test. Results showed that, LM treatment led to the highest juvenile growth, with body weights at 4 and 6 weeks surpassing other groups, visible across both genotypes. LMC and C groups did not outperform LM in growth but showed a trend toward improved NDV-HI titres, especially in the synthetic breed (5.2 vs 3.7 log e2 titre for Pekins). DPV antibody titres were numerically higher in synthetic ducks regardless of treatment. Anti-sRBC HA titres were highest for LMC, then LM and C, but differences were not statistically significant. Inter-genotype differences in immune responses were consistently more pronounced in synthetic ducks, in contrast to the usual significant growth-rate advantages of Pekins over Kuzhi breed. In conclusion, it is inferred that, in ovo amino acid supplementation (LM) boosted growth and immune competence, with the addition of NAC (LMC) providing subtle but notable immune advantages, particularly in terms of specific antibody responses. The synthetic duck breed exhibited superior immune responses over the Pekins, suggesting genotype-specific effects being more desirably impacted by the In-Ovo treatments. In conclusion, this combinational approach offers a smart, sustainable strategy to enhance duckling productivity (weight-gain) and survival at juvenile stages, highlighting the importance of both nutrient selection and genetic background in optimizing in ovo interventions.

Keywords: Amino acids; Anti-oxidants; in Ovo; Immunecompetence; Ducks

3078 Innovatively designed Nano-Selenium molecules aid in improving immune-competence and growth in Pekin Ducks

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Nano-elements are gaining global attention in poultry research for their enhanced bioavailability, targeted delivery and reduced toxicity compared to conventional supplements. Among these, Nano-Selenium (NSe) and its intelligently designed composite forms offer improved cellular uptake and stability. In Ovo administration of such nano-formulations presents a novel strategy for early immune programming, for White Pekin ducks, a species of importance in India. This study aimed at evaluation of NSe and a Nano-Fe-Se composite (NCm) on embryonic development, post-hatch growth, survival and immune competence under controlled experimental conditions. Total of 450 fertile White Pekin duck eggs were randomly allocated into four groups (@~112 eggs/group), including NSe and NCm (@25 µg/egg) via air-cell injection on day-23 of incubation. Two additional groups served as sham and uninjected controls. All hatched ducklings from NSe, NCm, and control groups were further divided to receive either standard feed or feed supplemented with 0.2 ppm of the respective nano-compounds for 3 weeks (@30 ducklings/Nano-dietary treatment group, covering 3 replicates each). Growth performance and survival were monitored weekly for 6 weeks post-hatch, while immune competence was assessed via sRBC antibody titers (humoral) and foot-web thickness indices (CMI from PHAP injections). Statistical analysis (One-way ANOVA) indicated significant differences ($p < 0.01$) across nano-elements for weight-gain and fitness traits. Among 4 nano-molecules screened, only NSe and NCm supported improved embryonic development, with hatchability @~60%. The NSe group achieved the highest 6-week body weight (1840 g), followed by NCm (1698.8 g) and control (1516.4 g), reflecting a growth differential of 21.3%. Survival was highest in NCm group (99%), followed by NSe (93%) and controls (88–90%). Feed consumption and FCR showed no statistically significant trends, though both nano-treatments exhibited higher appetite. Immune competence was notably higher in NCm ducklings, with elevated sRBC titers and CMI correlating with improved survival. Ducklings receiving only In-Ovo nano-doses performed comparably to ducks receiving additional supplementation. While NSe demonstrated superior growth-promotion, NCm excelled in enhancing immune responses. In conclusion, In Ovo administration of Nano-Selenium; either alone or as a composite, significantly improved survival and neonatal growth in Pekin ducks. Notably, the composite form showed greater immunomodulatory potential, while NSe favored growth. These findings underscore the importance of nano-structural design in optimizing biological outcomes with promises of tailored In-Ovo nano-nutrition for duck production.

Keywords: Nano-Selenium; growth; immune-Competence; Innovative design; Pekin duck

3079 Enhancing the livelihoods of rural populations through duck rearing under the Indian tropical environment

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In India, duck, turkey, quail, and Guinea fowl production has gained popularity as an alternative to chicken production. Duck production is characterized by low input and low maintenance costs, compared to other poultry species. A study was performed to comprehend the duck management practices employed and to determine the limitations felt by duck farmers in Northern Tamil Nadu, South India. Data was collected by conducting a personal interview with a pretested interview schedule that was specially designed for this purpose. The majority of farmers who rear ducks were young people (78 %). An all-in-all-out system and thatched or Mangalore roofing tiles for night shelters were being used by a majority of farmers (95 %). Under deep litter rearing, coir pith was used by 80 % of duck farmers, while groundnut hulls were used by 20 %. Sanyasi and Keeri variety ducklings were purchased from the local hatchery at a price of US $ 0.35. For up to 10 days, ducklings received commercial broiler starter feed at a price of US $ 14.11. The study revealed that the majority (95.56 %) of the duck farmers fed locally available immature paddy, broken rice and cooked rice from 10 days to 2 months. From the 3rd month onwards, ducks were not provided with hand feeding and allowed to forage. A large majority of respondents (89.33%) had flocks of around 500-700 with a ratio of 1:12 between drake and duck. The annual production of Sanyasi and Keeri duck eggs produced by natural foraging ranged from 130 to 140 eggs per duck. Vaccinating their ducks with the Duck Plague vaccine was a common practice among duck farmers, costing US $ 1.23 for a first dose at 35 days of age, followed by a booster dose at 140 to 150 days. The ducks reached a body weight of 1.2 kg during the 90-day period and were sold. The cost of producing duck for table purposes was around US $ 1.75. The price of one duck was US $ 2.90, while the price of an egg was US $ 0.07. The constraints as perceived by the duck farmers were recorded. Garret's ranking technique was adopted to analyses the constraints in duck production. Mortality due to the incidence of disease was the major constraint reported by all respondents, followed by attacks by predators during brooding, non-availability of superior duckling and duck feed, loss of eggs due to laying in improper places, poor transportation facilities, inadequate knowledge about the scientific rearing of ducks and credit facility. Duck rearing is a low-input, low technology that

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will certainly improve the livelihood of the duck farmers in South India.

Keywords: Duck rearing ; Sanyasi and Keeri ducks; Management practices; Production performance; Constraints

3080 Exploitation characteristics and phenotypic diversity of the common guinea fowl (Numida meleagris) in Cameroon

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The diversity of natural biological resources is at the heart of concerns relating to food security and climate change, hence the interest in characterizing local species and their exploitation systems. It is in this context that the present study, which took place from April 2020 to December 2022, aimed to evaluate the exploitation characteristics of guinea fowl as well as the analysis of their phenotypic diversity in Cameroon. For this purpose, a random sample of 509 voluntary farms for a total of 1021 guinea fowl was characterized in three agro-ecological zones. The data collected through surveys, direct observations and measurements were subjected to descriptive statistics, contingency test, Student test, multi-way ANOVA and Duncan test. The main results showed that: the breeding of guinea fowl in Cameroon is not subject to any prohibition and is practiced mainly for self-consumption (37%) and marketing (27%) ; it is an extensive family farm type. Plumage constitutes the main criterion for choosing guinea fowl. Natural incubation of eggs and/or capture-taming of non-domestic flocks constitute the primary source of supply. The mortality of guinea fowl (20.24%), the low brooding ability of guinea fowl (17.49%), the low level of in-depth technical knowledge (15.13%) and diseases (12.77%) are the main constraints of mixed farming. The morphometric characteristics of guinea fowl vary ($p < 0.01$) depending on region, sex, plumage coloring and genetic type. The pearl gray coloring of the plumage (23.02%), the bluish-red barbels (29.09%), the black eyes (36.04%) and the black tarsi (39.18%) are more abundant. The development of the barbels and the shape of the crest are determinants of sex ($p < 0.01$), as well as the live weight which presents a weight dimorphism in favor of females. The main average body measurements (in cm) were: horn length (2.79±0.03), barbel length (3.35±0.04), wing span (41.82±0.32) , thigh diameter (9.17±0.10), tarsus length (6.62±0.04), body length (40.13±0.15), live weight (1.68±0.02). The correlations between measurements are varied. Principal Component Analysis revealed that the first 03 components better explain the total phenotypic variability (50.21%). The F1 factor constitutes the most discriminating variables (89.40%). Discriminant Factor Analysis and Hierarchical Ascending Classification made it possible to detect 3 subpopulations which can be grouped into 2 subgroups on the basis of intra-population variations (77.15%) and genetic distances (1 and 2=17.78 ; 1 and 3=17.62 ; 2 and 3=6.80). Thus guinea fowl constitutes a natural genetic resource adapted to environmental conditions and has the variability necessary for its genetic improvement, even better, its preservation.

Keywords: Exploitation characteristics; Phenotypic variability; Genetic structure; Guinea fowl; Cameroon

3081 Dietary water spinach inclusion on turkey growth performance and meat quality traits of different heritage turkey varieties

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Turkeys' natural foraging in semi–intensive systems can replace part of commercial feeds, which account for 65% of production costs. This approach lowers expenses while promoting sustainable poultry farming. The present study was conducted to evaluate the effect of different inclusion levels of dietary water spinach (Ipomoea aquatica) as a partial dry matter (DM) replacement in turkey diets. A total of 144 turkeys (8 weeks old), individually tagged, were randomly allocated to four dietary treatments, where water spinach replaced 0% (Control), 4%, 8%, or 12% of the DM content of the basal diet. The trial continued for 12 weeks, with each treatment comprising four replicates of nine birds each. At 20 weeks, 16 birds (4 from each treatment) were slaughtered, and carcass characteristics, meat quality, texture profile analysis (TPA), proximate composition and amino acid profiles of turkey meat were evaluated. Amino acids were analyzed by ultra-performance liquid chromatography (UPLC, Waters, USA) with a PDA detector. Data were statistically analyzed using one-way ANOVA in RStudio (R software version 4.5.0). No statistically significant differences were observed among treatment groups in live weight (LW), dry matter intake (DMI), feed conversion ratio (FCR), dressing percentage, texture profile (TPA) and physicochemical parameters except drip loss, meat lightness, maximum fracture force, gumminess, and chewiness. However, the groups receiving 4%, 8% and 12% water spinach inclusion showed numerically improved growth performance, with final BW ranging from 3091 to 3540 g, LWG from 2383 to 2473 g, DMI of 640-653 g, FCR between 3.40 and 4.12, and dressing percentage of approximately 65%. Furthermore, these groups showed significantly lower drip loss (1.5-1.7%), improved meat color (L*=48.5-50) and enhanced meat tenderness characterized by lower force maximum 98.87-11.230, fracture force (13.87-15.30), gumminess (6.64-7.06) and chewiness (5.56-6.05) values compared to the control. A significant elevation in ether extract content of breast meat with higher dietary inclusion levels ($P < 0.05$) was observed, whereas moisture, crude protein, crude fiber and ash contents were not notably altered. Amino acid profiling of meat revealed significant increase in several amino acids, including alanine, aspartic acid, glycine, histidine, isoleucine, lysine, tyrosine and valine, particularly in the T2 diet ($P < 0.05$). In conclusion, water spinach can replace up to 8-12% of dietary dry matter in turkey diets, reducing feed costs by ~9% without compromising growth, carcass traits, meat quality, texture profile, nutritional profiling, providing a cost-effective and sustainable alternative to conventional feeds.

Keywords: Turkey variety; dietary water spinash; growth performance; meat color; dressing percentage

3082 Evaluation for efficacy of different vaccines against challenge with Newcastle Disease Virus in pigeon of Bangladesh

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Newcastle disease virus (NDV), the sole member of Avian Paramyxovirus type 1 (APMV-1), is a globally distributed pathogen affecting domestic and wild birds. This study evaluated the protective efficacy of commercially available live and inactivated NDV vaccines in pigeons.The experimental design combines a

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randomized controlled trial for causal inference on vaccines with an observational study for field factor analysis. The statistical tests validate differences in immune response, correlate titers with protection, and rank the efficacy of the interventions. The experimental design involved vaccinating birds with either live vaccines (Cevac New L or Nobilis Clone 30) or inactivated vaccines (killed Ranikhet Disease Virus (RDV/BCRDV) or Imopest/Cevac New K). Serum antibody titers were assessed using the Haemagglutination Inhibition (HI) test at defined intervals post-vaccination. Vaccine efficacy was ultimately determined via a challenge test with a virulent NDV strain. Statistical analysis of HI titers (detailed in Tables 6-13) evaluated the effects of several factors: vaccine nature (live vs. killed), manufacturer source (private vs. government), specific vaccine type, pigeon breed, age, flock size, treatment protocols, and farm biosecurity levels. Results indicated that inactivated vaccines elicited a rapid serological response ("instant immunity"), defined as a significant rise in HI titer within one week post-vaccination, and provided immunity lasting more than two months. Overall, HI titers were significantly higher for inactivated vaccines than for live vaccines. Vaccines from private manufacturers performed better than those from government sources. The hierarchy of vaccine performance based on mean HI titers was: Imopest < RDV/BCRDV < ND Clone 30 < Cevac New L. Superior antibody responses were associated with local breeds, older pigeons, smaller flock sizes (<100 birds), veterinary-supervised health plans, and farms with good biosecurity. In conclusion, the inactivated NDV vaccines provided more effective and durable protection compared to the live vaccines in this challenge model. While live vaccines induced a rapid initial immune response, the immunity conferred by the inactivated vaccines was longer-lasting.
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Keywords: Vaccines; Newcastle disease; Efficacy; titre ; Pigoen

3083 Quail farming in Ghana: a case study of the Ashanti region

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This research's objectives were to identify the production practices, trait preferences and challenges facing quail farmers in Ghana. A random sample of sixty (60) quail farmers from the Ashanti region (commercial and cultural center of Ghana) were provided with questionnaire; designed to collect both qualitative and quantitative data. Descriptive statistics were obtained using SPSS.

There were 55 (91.6%) males and 5 (8.3%) females; with an average flock size of 90 quails. Utilizing intensive battery cage production systems, farmers also had an average of 5 years' experience in quail keeping and preferred traits of higher egg laying ability (83% of respondents) and low mortality (17% of respondents). Challenges included lack of veterinary services (100% of respondents) and financial support (20% of respondents)

The relatively short generation interval of quails, high fecundity and the low capital requirement for starting a modest aviary; makes quail farming a potent tool for poverty alleviation. This seeks to address the following Sustainable Development Goals: SDG1-NO POVERTY. While the high protein content of their meat and eggs also address SDGs 2 and 3; ZERO HUNGER and GOOD HEALTH AND WELLBEING respectively.

Ghanaian quail farmers call for veterinary support and better governmental involvement to reduce hunger and poverty and promote good health and wellbeing.

KEY WORDS

Quails, SDGs 1,2 and 3; Egg production, Low mortality, Battery cage system

Keywords: Quails; SDGs 1,2 &3; Battery cage system; Egg production; Low mortality

3084 Lysine Requirement of Meat Type Japanese Quail Breeders in Cages

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Lysine is a limiting amino acid for poultry species that is required for rapid body weight gain and for high reproductive performance assessed by rate of egg production, egg size, and feed efficiency that determine profitability in a breeder farm. A study to assess the digestible lysine requirement of meat type Japanese quail breeders was conducted in a commercial farm. A total of 13500 Japanese quail (1500 M + 12000 F) reared in cages were subjected to the experiment from 9 to 24 weeks of age. In each cage compartment, two males and eight females were allowed with a male: female sex ratio of 1:4 and a floor space allowance of 90 cm2 per bird. Birds in 150 cages were subjected to each of the 3 different experimental diets containing 0.85, 0.95 and 1.05 percent digestible lysine while digestible methionine + cystine levels were maintained at 92 percent of digestible lysine. The amino acid levels in the experimental diets were calculated using standard figures available and adjusted by adding synthetic amino acids. The diets were iso-caloric with 2650 Kcal M.E./kg. Adlibitum feeding was practised. The data were analysed and compared by analysis of variance. Mean (+ S.E.) egg production were 72.18+01.06, 74.56+01.15 and 76.84+01.18 respectively which were significantly different from each other. Respective mean feed consumption of 35.52+00.62, 35.64+00.48 and 35.69+00.58 g did not differ between experiments. Mean egg weights were 12.21+00.16, 12.45+ 00.18 and 12.58+ 00.21 g at 16 weeks of age and did not show any significant difference. Mean percent fertility of 88.67+01.28, 90.61+01.39 and 93.08+01.44 were significantly different between treatments. Similarly, mean percent hatchability of respective treatments on fertile eggs of 78.45+01.01, 80.28+00.98 and 82.43+01.11 were significantly different. Mean chick weights at hatch were 7.48+0.17, 7.63+0.16 and 7.85+0.19 respectively and were not different significantly. Livability percent also did not differ significantly between the different dietary treatments employed. It is concluded that, in diets meant for meat type Japanese quail breeders, a digestible lysine level of 1.05 percent has proved to be optimal with 0.97 percent of digestible methionine + cystine.

Keywords: Japanese Quail Breeders ; Meat type; Lysine requirement; Egg production; Hatchability

3085 Pesticide Residues in Wild Pheasant Egg Yolks: Implications for Embryonic Viability and Ovarian Physiology

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In France, populations of pheasants and partridges have shown a marked decline, as evidenced by monitoring data collected by the Loir-et-Cher Hunting Federation. These species are chronically exposed to a wide range of pesticides, which may impair their reproductive performance. Analyses of partridge blood and eggs have revealed multiple contaminations including residues of banned compounds over several years, suggesting potential mixture or synergistic effects on avian health and population dynamics.

The objectives of our study were to collect unhatched wild

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pheasant eggs in order to screen for over 600 chemical compounds in the yolk using mass spectrometry, and to assess their effects both in ovo on embryonic development and in vitro on ovarian cell activity.
To assess their effects, a pesticide mixture at concentrations corresponding to those detected in egg yolks was injected into embryonated pheasant eggs after 5 days of incubation. After 16 days of incubation, embryo and organ weights were recorded, and a morphological analysis of the gonads was performed. After 16 days of incubation, cholesterol and triglyceride levels were also quantified in the embryonic livers. In parallel, pheasant granulosa and theca cells were exposed in vitro to the same pesticide mixture.
A total of 15 pesticide-related molecules were detected, some of which had already been identified in partridge eggs in 2011, and approximately half of which have been banned in Europe for several years. The mixture did not affect embryo or organ weights, but it induced an increase in hepatic cholesterol and triglyceride concentrations, as well as alterations in gonadal morphology. In vitro, cell viability was only slightly affected; however, cell proliferation decreased significantly following exposure, while progesterone and testosterone production increased significantly in granulosa and theca cells, respectively.
Thus, exposure of pheasant eggs to a mixture of pesticides detected in wild eggs disrupts embryonic development and ovarian function, particularly by impairing cell proliferation and altering steroidogenesis. These findings suggest that such contaminants may contribute to the decline of bird populations in agricultural landscapes.
Keywords: Pesticides; Pheasant; Embryonic Development ; Ovarian Activity

Welfare and Behavior III

3086 The power of light for meat bird production and welfare – wavelengths in turkeys vs broilers

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Since the easily available light emitting diode lights (LED) have come on the market, and since they can be purchased in many colors, the use of various light wavelengths in poultry houses has become a significant area of interest in both research and commercial production. Because turkey research is expensive, labor-intensive and difficult in many cases, decisions for turkey management is often based on research conducted in broilers.
Should it be? This presentation will discuss two large-scale research studies that have been conducted (albeit at different times) in the same research facility with the same light colors and similar data collected in each. Overall, the longer period of time turkeys are exposed to blue lighting resulted in some positive areas of production and welfare that were not noted in broiler birds. However, other areas changed for the negative, including an unusual behavior that resulted in loss of body condition in a small number of turkeys housed under blue light. Overall – the presentation will suggest that we should not be using broiler data to manage turkeys – rather management decisions for light color must be based on specific species.

3087 Investigating the combined effect of stocking density and thinning on gait score in fast- and slower-growing broilers under semi-commercial, controlled conditions

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Stocking density is a key management factor influencing broiler welfare, as higher densities have been linked to poorer gait scores due to restricted movement and deteriorated litter quality. Thinning is commonly applied in EU broiler production to manage late-cycle density and maintain productivity, yet it may introduce handling stress and biosecurity risks. Few studies have examined the combined effects of stocking density and thinning on gait, although gait score is an important animal-based welfare indicator that typically worsens with faster growth and higher density. This study assessed gait in fast-growing (Ross 308) and slower-growing (Hubbard Redbro) broilers under semi-commercial, controlled conditions at the Experimental Poultry Centre (Geel, Belgium). The facility comprised eight climate-controlled rooms, each divided into two experimental groups (16 total). Ross 308 and Redbro were placed on the same day. Thinning (25%) was conducted in 4 Ross 308 groups at 34d. Depopulation of Ross 308 as well as thinning (25%) of 4 Redbro groups was done at 41d. Depopulation of Redbro took place at 50d. Ross 308 were reared at 42 kg/m^2 (with thinning (T), n = 1,200 birds/group; without (no), n = 920 birds/group) and Redbro at 30 kg/m^2 (with thinning (T), n = 850 birds/group; without (no), n = 650 birds/group), resulting in 1 control (Ross 308(T)) and 3 treatments (Ross 308(no), Redbro(T) and Redbro(no). Gait scoring (n = 20 birds/group) was performed based on the six-point Bristol method where direct observations are used to rate a bird's ability to walk on a scale from '0' ('normal') to '5' ('complete inability to walk'). The gait scores reported here were carried out 5-7d pre-thinning (at 28-29d for Ross 308 and 32-35d for Redbro) and 1.5d before depopulation (post-thinning; at 39d for Ross 308 and 48d for Redbro). Data were analysed via mixed-effects models with Type III Wald χ^2 tests and Tukey-adjusted post-hoc comparisons ($\alpha = 0.05$). Pre-thinning, model-adjusted means (± SE) showed higher gait scores for Ross 308(T) (2.33 ± 0.08) than Redbro(no) (1.96 ± 0.08) and Redbro(T) (1.99 ± 0.08) ($p < 0.05$), while Ross 308(no) (2.52 ± 0.08) did not differ significantly after adjustment. Post-thinning, model-adjusted mean (± SE) gait scores averaged 3.09 ± 0.08 (Ross 308(T)), 3.11 ± 0.08 (Ross 308(no)), 2.46 ± 0.08 (Redbro(T)) and 2.42 ± 0.08 (Redbro(no)). Gait scores were significantly higher in Ross 308 than Redbro ($p < 0.05$), but thinning had no significant within-strain effect. Under semi-commercial conditions, thinning did not materially affect gait within strains, whereas strain (and associated growth rate) remained the primary driver of higher - thus worse - gait scores.
Keywords: Gait score; Broilers; Stocking density; Thinning; Fast- and slower-growing

3088 Using the Quality of Life framework to assess the behavior of free-range chickens

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The concept of Quality of Life (QOL) represents a recent perspective in animal welfare, focusing on holistic evaluation of the emotional state and behavioral expression over time. This approach recognizes that negative experiences cannot be entirely avoided, but should be minimized to allow animals greater opportunities for positive experiences. Thus, animal welfare can be assessed by balancing positive and negative experiences.
The aim of this study was to characterize the behavior of four slow-growing (SG) chicken genotypes reared under free-range conditions by applying the QOL framework. A total of 400 one-

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day-old chicks belonging to four SG genotypes (A, B, C and D) were housed in eight pens with access to a pasture area (2 pens per genotype, 50 birds each—25 females and 25 males). Animals were reared indoors until 35 days of age, after which they had free access to the outdoor area. Behavioral observations were carried out following an adaptation phase, from 42 days until the end of the rearing cycle (81 days). For each pen, 2-hour videos were recorded per week, for a total of 10 per genotype. Behaviors recorded over extended observation periods were classified as positive or negative affects based on their underlying motivation and whether they were associated with rewarding experiences or with physical or emotional discomfort; their frequency was also evaluated to identify possible over- or under-expressions. Descriptive statistical analyses were used to present the data, while the occurrence of each behavior was categorized using data binning. An indicator reflecting the balance between positive and negative affects was also calculated.

The results confirmed a substantial behavioral variability among SG genotypes. In particular, A birds exhibited the highest occurrence of static behaviors ($p<0.01$), along with lower levels of active behavior compared with B and C birds ($p<0.01$). Notably, sheltering behavior was categorized as a negative affect, as its occurrence and frequency were substantially higher in D birds compared with the other genotypes, without any apparent functional explanation. Overall, positive affects occurred more frequently than negative ones across all genotypes.

This study highlights that behavioral classification should not be predetermined but should consider multiple factors, including frequency of expression, genotype, and rearing conditions. However, applying the QOL approach remains challenging, and further research is required to refine the classification of behaviors into positive and negative affects and to develop robust indicators of their balance.

Keywords: Quality of Life ; behavior; welfare; slow growing chicken; affective state

3089 Analysis of Broiler Behavioral Patterns Through a Multi-Camera Tracking System

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Understanding how broiler behavior changes across the grow-out period is essential for evaluating health, welfare and environmental effects. In this study, a multi-view tracking system quantified three behaviors (activity, feeding, and drinking) over time, assessing activity dynamics, distance traveled, diurnal patterns, and responses to heat stress (HS). Four production cycles were conducted with 1,120 male Ross 308 broilers (280 per cycle), housed in two climate-controlled pens per cycle (140 birds/pen; 14 kg/m2). Daily observations were grouped into morning (09:00–12:00), midday (13:00–16:00), and evening (18:00–21:00). From days 29–33 and 36–40, one pen was exposed to HS, while the paired pen remained thermoneutral (TN). A multi-camera pipeline (YOLOv11 detection; SORT data association) generated tracklets that were projected to ground-plane coordinates and merged across views to yield continuous trajectories. Tracking performance was high (MOTA = 0.81; IDF1 = 0.89). A total of 1,641 three-minute video segments recorded between days 8–40 were analyzed. Outcomes (drinking, feeding, activity) were analyzed with separate linear mixed-effects models in Python, with age and high-temperature condition as fixed effects and production round as a random intercept. Behaviors were classified from movement features and proximity to feeders/drinkers, and metrics were averaged per segment to enable scalable analysis. From days 8–40, activity declined with age ($\beta = -0.168 \pm 0.009$, $p < 0.001$), with high-intensity activity decreasing fastest ($\beta = -0.134 \pm 0.006$, $p < 0.001$). Heat stress increased drinking ($\beta = 3.68 \pm 0.30$, $p < 0.001$) and reduced feeding ($\beta = -3.03 \pm 0.27$, $p < 0.001$), while overall activity was unaffected ($p = 0.257$). Drinking and feeding increased with age ($\beta = 0.277 \pm 0.013$ and 0.077 ± 0.012; both $p < 0.001$), and feeding rebounded in the evening after HS ($\beta = 1.995 \pm 0.460$, $p < 0.001$).

Keywords: precision livestock farming; poultry; well-being; technology; climate change

3090 Behavioral differences between fast- and slow-growing broiler chickens reared in cages at six weeks of age

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Behavior is an important parameter for assessing animal welfare. The aim of this study was to compare the behavior of fast- and slow-growing broiler chickens at the same age. A total of 136 broilers from the Ross 308 (fast-growing) and Hubbard JA57 (slow-growing) genotypes (68 birds per genotype) were included in the study. Animals were reared in eight plastic slatted floor cages (155 × 240 cm). Behavioral observations were conducted when the birds reached six weeks of age. Each pen contained 17 birds, from which two focal animals were randomly selected for detailed observation. All behavioral assessments were performed by a single, previously trained observer to ensure consistency. During observations, the observer quietly approached the pens to a distance of 1 m and waited for 2 minutes to allow the birds to acclimate. A total of 16 birds across eight pens were observed for 80 minutes in total, with each focal bird monitored for 5 minutes. The behaviors recorded included sitting/lying, standing, walking, feeding, drinking, feather pecking, pecking objects, foot stretching, fighting, and feather ruffling. The duration of each behavior of six-week-old broilers was compared between Ross 308 and Hubbard JA57 genotypes ($n = 8$ per genotype). No significant differences were observed in the average duration of sitting/lying, feeding, drinking, feather pecking, pecking objects, stretching, fighting, or feather ruffling behaviors ($p > 0.05$). However, significant differences were detected in standing (Hubbard: 60.75; Ross: 33.75; $p = 0.002$) and walking behaviors (Hubbard: 14.63; Ross: 9.69; $p = 0.038$), indicating higher activity levels in the slow-growing genotype. It should be noted that comparisons were conducted at the same age rather than at similar market weights. Consequently, differences in live body weight and stocking density expressed as kg per square meter between genotypes may have contributed to the observed behavioral patterns. These findings suggest that growth rate influences broiler activity at six weeks of age, while further studies comparing birds at similar market weights are needed to clarify the independent effects of growth rate and body weight on behavior and welfare-related outcomes.

Keywords: activity; behavior; broiler; genotype; welfare

3091 Sequential Probability Ratio Test for real-time stress detection and individual resilience quantification

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Efficient identification of resilient animals is essential for genetic improvement and precision management in poultry systems. This study aimed to develop and evaluate a statistical framework for detecting stress and quantifying resilience using high-frequency feeding behaviour data. We hypothesized that variability in daily feeding duration (DFD), when modelled with a two-component

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Gaussian Mixture Model (2-GMM) and integrated into a Sequential Probability Ratio Test (SPRT), could provide objective indicators of stress onset and resilience capacity.
Feeding behaviour of 60 Lohmann Breeder hens was continuously video-monitored for 17 days (1.86 M feed events). After standard cleaning and aggregation, DFD was computed per hen and day, and log-coefficients of variation (log-CV) were obtained using overlapping 5-day windows. For each window, a 2-GMM was fitted to classify stable and stressed behavioural states. Posterior probabilities informed two indicators: (i) a one-sided SPRT (S1) accumulating evidence only when the stressed-state probability exceeded 0.9, and (ii) a two-sided SPRT (S2), from which the log-area under the curve (log-AUC) was derived as a resilience index.
The 2-GMM yielded two distinct distributions (means: −2.25 stable; −1.30 stressed). S1 identified the first stress signal in the second window, when 5% of hens exceeded both the 0.9 stressed-state probability and the 97.5% S1 quantile. Seven hens displayed stress episodes; two recovered, one deteriorated before death, and one showed progressive worsening—indicating that S1 may function as an early-warning tool for compromised individuals.
The resilience index (log-AUC) also revealed biologically coherent patterns. Low-resilient hens (log-AUC ≈ 4.35) exhibited markedly higher variability (CV = 0.64) and lower mean DFD (6821 s), while highly resilient hens (log-AUC ≈ 0) showed lower variability (CV ≈ 0.12) and higher mean DFD (13 057 s). These contrasting profiles support the capacity of the proposed indicator to capture behavioural stability and recovery performance.
Overall, this framework demonstrates the potential of integrating mixture-model state estimation with SPRT-based evidence accumulation for continuous, objective stress detection and resilience assessment. However, the limited sample size and short monitoring period restrict generalization; larger datasets are needed to validate robustness, refine thresholds and assess applicability in breeding and management programmes.
Keywords: Resilience; Sequential Probability Ratio Test; Feeding behaviour; Gaussian mixture; Precision Livestock

3092 Biosecurity compliance and broiler farmers' knowledge, attitudes, and practices on animal welfare in the Northwest region of Cameroon

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Infectious disease represents the main hindrance in poultry production in Cameroon. As a solution, recent research suggested improving biosecurity implementation in chicken farms. This cross-sectional study, conducted on 65 commercial broiler farms in Mezam Division (Northwest region of Cameroon), assessed biosecurity compliance, the reasons for not implementing biosecurity and farmers' knowledge, attitudes, and practices (KAP) regarding animal welfare. Data were obtained through structured questionnaires during a face to face interview on farm followed by technical observations. Biosecurity was assessed using a binary scoring system, with farms scoring ≥ 50% classified as having good biosecurity. Farms scoring below 50% were considered to have poor knowledge, negative attitudes and inadequate practices whereas those scoring 50% or above were deemed to exhibit satisfactory animal welfare understanding. Descriptive statistics, including frequencies, percentages, means, were used to summarize socio-demographic biosecurity scores, mortality rates, and animal welfare KAP scores. Wilcoxon and Kruskal–Wallis test were used to compare the averages of two and more than two independent groups, respectively. To examine associations between continuous variables, the Spearman correlation test was used. Overall, biosecurity implementation was good on 86.2% of farms, with an average score of 76.1 out of 100. Higher scores were associated with older (>50) and more experienced farmers, poultry farming as the primary activity, and regular veterinary follow-up. Biosecurity measures related to protection of litter and bacterial auto control were applied in all the farms. While compliance with biosecurity was generally good, weaknesses persisted in all-in all-out management, carcass disposal, and disinfection barriers. The main reasons provided by farmers for not implementing biosecurity were related to the high cost for implementing the measures and the lack of knowledge of the advantages of these measures. Farmers demonstrated positive welfare attitudes (60%) and adequate practices (70%) but poor knowledge (38%). These findings highlight the need for targeted farmer training to address welfare knowledge gaps and enhance sustainable poultry production in the region.
Keywords: Biosecurity; Cameroon; disinfection; Poultry; Welfare

3093 Do first impressions count? Social responses to phenotypic appearance in laying hen pullets

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Phenotypic appearance influences social dynamics in laying hens, but it is unknown when and how archetype recognition serves as a basis for social referencing. This study aimed to determine if social archetypes are fixed by imprinting during the first week post-hatch or if a longer socialization period is needed. Whether the archetype is flexible depending on flock phenotypic diversity during early development was also investigated. ISA Brown day-old chicks (n=720) were randomly assigned to 30 groups of 24 birds. Two early social exposure treatments: 1 wk imprinting (1WK) or 6 wk imprinting plus early socialization (6WK), were combined with 5 phenotypic appearance treatments: 100% non-altered (NA), 100% altered (A), 84% A, 84% NA or 50% A (3 replicates/treatment combination). Phenotype was altered by staining nape feathers with a black mark. Following social exposure treatment, each group was halved to form 36 groups of 12 phenotypically homogeneous individuals, with 6 phenotypic treatment histories: 100A, 100NA, 84A, 84NA, 50A and 50NA. Remaining birds were penned together by phenotype for use as test stimuli. At 1, 6, 10 and 14 wk, 2 birds/pen (familiar F1 and F2) were tested (1x1 m open field; 348 tests) for 10 min with 2 unknown stimuli, one phenotypically identical to the focal pair (Si) and one different (Sd). Interindividual distance from each bird to F1 and total distance travelled by each bird were determined based on Euclidian distances. At 14 wk, all aggressive interactions were registered. Data were analysed with mixed model ANOVAs. Preliminary results revealed a clear familiarity effect, with minimum interindividual distance between familiar birds ($P<0.01$; 1, 10 and 14 wk) and maximum distance between stimulus birds and F1 ($P<0.001$; 1 wk). Stimulus birds also travelled longer total distances than familiars ($P\leq0.01$; 10 and 14 wk). However, by 14 wk, distance depended on both phenotype and familiarity ($P<0.001$), with Sd being furthest from F1. At 14 wk, altered Sd also received more aggressive pecks by familiars than non-altered Sd ($P<0.001$). A phenotype x exposure period interaction was also found at 14 wk, with altered birds being more aggressive if they were 1WK treatment birds than 6WK ($P=0.03$). Results suggest that social recognition of familiar birds was present already at 1 wk. Although there was no evidence of early phenotypic diversity, signs of active phenotypic discrimination indicated by longer interindividual distances and aggression

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towards Sd at 14 wk, suggest that a longer socialization period in homogeneous groups is necessary to discern the corresponding social archetype.

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Keywords: Pullets; Phenotypic appearance; Imprinting; Social recognition; Social aggression

3094 Bridging the gap: A comparative analysis of industry approaches and scientific perspectives on broiler health and welfare in Europe

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The European broiler sector, producing about 11 million tonnes of broiler meat annually, is under pressure due to society's call for environmental sustainability, improved animal welfare, and enhanced approaches to manage animal health concerns. In order to be prepared for a significant future change, farmers would need to adopt a series of innovative practices to maintain a social license to produce. The 4-year thematic network project BroilerNet funded by the European Commission aims to create a network to favour the adoption of ready-to-use innovative best practices on European broiler farms. BroilerNet brings together different stakeholders from science, industry and practice around Europe to focus on three main themes: animal welfare, animal health, and environmental sustainability. The 13 participating countries identified 16 sector priority challenges for animal health and welfare and submitted 232 good practices to tackle those. International experts evaluated those good practices objectively and selected 5 best practices per challenge, which are multilingually shared via videos, factsheets, and abstracts that are freely accessible through the project webpage and social media. In this case study, we aimed to assess whether the BroilerNet output aligns with the scientific opinion of the European Food and Safety Authority (EFSA). In 2023, EFSA published a report highlighting, among others, 16 welfare consequences of the current production systems (i.e. for broiler chickens) and 76 key recommendations to overcome those. We checked the BroilerNet and EFSA content for convergence and divergence on each topic. This comparative analysis revealed that 14 welfare consequences mentioned by EFSA (88%) related to a BroilerNet priority sector challenge. So, industry and scientific perspectives seem to largely align when it comes to animal health and welfare challenges. Preliminary results reveal that 46 key recommendations of EFSA (60%) were part of 22 out of 78 best practices in BroilerNet. Those results indicate that industry and scientific perspectives still diverge regarding animal health and welfare improving strategies. Even though, several best practices within the European broiler sector align with scientific recommendations (e.g. early feeding, on-farm hatching, elevated platforms). From this comparative analysis, it appears that the biggest gaps for broiler welfare improvement relate to decreasing stocking densities, providing covered verandas and/or outdoor access, and providing dark brooders to chicks. Reasons for the existing gap might include market demand and small margins that seem to leave little room for rather expensive or risky investments.

Keywords: thematic network; best practices; sector challenges; animal health; animal welfare

3095 Perceptions of laying hen mortality in non-cage housing systems: reflections of Danish farmers

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Mortality is an important indicator of laying hen health and welfare, with implications for productivity, economy and sustainability in the layer sector. The concept of 'normal mortality' has been described since at least the 1920's [1], but little is known about how farmers perceive mortality and its causes. In recent years, consumer demands have initiated a transition from cages to non-cage housing systems in many parts of the world. These transitions may impact mortality patterns negatively, which warrant investigating how mortality is perceived by farmers operating non-cage systems to improve support for farmers yet to make the change.

The objective of this study was to analyse Danish farmers' perceptions of laying hen mortality, including their reflections about normal mortality and its causes, as well as differences between farmers operating indoor multi-tiered aviaries (barn) and organic production. Semi-structured interviews were conducted with twelve farmers (seven barn and five organic). The transcribed interviews were analysed using reflexive thematic analysis.

Farmers perceived 'normal mortality' as natural, explainable and acceptable. Most farmers expressed limited concern about individual hens' death when flock mortality was considered normal. Mortality was often deemed uncontrollable due to systemic constraints (e.g. genetics, housing system, weather, financial stability). The perception of hens, and consequently their deaths, seemed linked to features of the housing system (e.g. stocking density, opportunities for natural behaviour). Infectious mortality causes were perceived as more important than non-infectious, contrary to a recent study of pathological causes of mortality in hens from five of these farms [2]. Cannibalism was underestimated as an important cause of mortality by most farmers. We interpret these observations as a form of desensitization or emotional detachment.

The normalization of mortality, underestimation and misinterpretation of causes of mortality pose a risk of overlooking early signs of health and welfare problems, which may delay effective preventative and control measures, including veterinary involvement. Negative consequences for mortality and unrealized potentials for improvement may be the result.

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Keywords: perception; mortality; cannibalism; laying hen; qualitative

3096 Evaluating CO2 gas stunning effectiveness in broilers using video analysis

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In the CasStunn project, the stunning effectiveness of electrical

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water bath and CO2 gas stunning systems in broilers was evaluated, aiming to characterize behavioral responses during stunning and assessing the effects of husbandry system on animal welfare at the slaughterhouse. Focusing on CO2 stunning, video recordings were used to analyze behavioral responses of broilers from different husbandry systems using a novel video-based behavioral analysis approach within the stunning chamber.
In 2022 and 2023, broilers from 29 flocks of conventional light fattening (LIT), 12 of conventional heavy fattening (HEV), 3 of outdoor climate rearing (OUT), and 9 of organic production (ORG) were observed. A custom frame equipped with a green flashlight and a GoPro camera was installed in transport crates to record birds during stunning. For each process, at least four videos at different drawer heights (1–4) and three positions within the stunning chamber (window, middle and door side) were recorded. This setup accounted for variation in CO2 concentration. In total, 69 stunning processes were recorded, resulting in 402 analyzable videos (LIT: 244, HEV: 105, OUT: 5 and ORG: 48).
Recorded behaviors included defensive behaviors (escape attempts, wing flapping, head shaking, jumping), CO2-related respiratory responses (swallowing, beak breathing), and other behaviors (deepened breathing, convulsions, sitting, loss of neck muscle tone, eye closure). Due to overdispersion and excess zeros, negative binomial regression models were applied in R. OUT data were evaluated descriptively only.
Defensive behaviors differed among chamber positions, while no significant differences were detected between drawer heights. Early CO2-related reactions such as swallowing, beak breathing, head shaking, and deepened breathing occurred within the first 30 seconds, while convulsions were mainly observed between 240 and 300 seconds. Duration of behaviors differed by husbandry system: ORG exhibited the longest rearing/jumping (29.1 s) and convulsions (20.3 s), whereas HEV showed the shortest durations (12.1 s and 13.1 s, respectively).
These results demonstrate that video-based behavioral analysis is a practical and precise tool for assessing CO2 stunning effectiveness and animal welfare in broilers and provide new insights into how husbandry type and chamber position influence behavioral responses. From an animal welfare perspective, this study suggests that adjusting CO2 stunning parameters according to husbandry system may further improve stunning effectiveness and welfare outcomes. The approach has potential for integration into routine welfare monitoring and for supporting refinement of controlled-atmosphere in commercial slaughterhouses.
Keywords: Stunning effectiveness; Animal Welfare; Broiler; Behavior; Carbon dioxide

Cargill Symposium: Follow your gut: microbiome intelligence integrated in broiler operations

Welcome

Decoding the Chicken Gut: The Science of Applied Microbiome Intelligence
Jean E. De Oliveira*1 1Cargill, Animal Nutrition and Health, Vilvoorde, Vlams-Brabant, Belgium

Galleon: From Microbiome Concepts to Action
Luisa Gene*1 1Cargill, , Brazil

Turning Microbiome Knowledge into Poultry Solutions Through Galleon Intelligence
Fernanda Castro*1 1Cargill, , Brazil

Round Table Q&A

Elanco Animal Health Symposium: Leading Intestinal Integrity with Consistency and data driven decisions

The Role of Ionophores for Future Poultry Sustainability (Economic, Environment and Health outlook)
George Tice*1 1Independent Scientific, Policy and Regulatory Consultant EPPA, , United Kingdom

How Intestinal Integrity Stability is Critical for Health and productivity
Ricardo Rauber*1 1Animal Health Consultant Vetinova, , Brazil

Data Driven decisions leading to an excellence in Cocci Control
philippe gelaude*1 1Elanco, Senior Technical Manager, , United Kingdom

Guangzhou Insighter Biotechnology Co. Ltd: Scientific Solution of Alternative to Antibiotic

Scientific Solution of Alternative to Antibiotic
Jing Yuan*1 1Guangzhou Insighter Biotechnology Co. Ltd, Senior Technical Manager, , China

Merck Animal Health Symposium: ILT Management from Hatchery to the Field

Guillermo Zavala
Daniel Maekawa

Novonesis Symposium: Savor the Science

Savor the Science
Christophe Bostvironnois*1 1Novonesis, Global Portfolio Manager at Novonesis., , Georgia, United States

Education/Youth Programming/Extension

4000 Academic Poultry Science Departments in the United States: Shared Opportunities and Challenges related to Workforce Development for Stakeholders
David Caldwell*1 1University of Arkansas, , Arkansas, United States
Six independent poultry science departments currently remain at land-grant universities in the United States. These include the University of Arkansas, Auburn University, the University of Georgia, Mississippi State University, North Carolina State University, and Texas A&M University. The department heads of these six academic departments are the authors of this presentation. With this presentation, we will collectively discuss historical perspectives that have impact the gradual decline of independent departments of poultry science over the past four to five decades in the United States. We will also speak to current challenges and shared opportunities facing independent poultry science departments as we strive to prepare graduates for careers in the commercial or allied industry, graduate school, professional school, governmental or regulatory agencies, or academia. Projected growth in poultry production on a global basis in the coming decades will provide consistent opportunities for students and graduates of our respective programs. How the three-tiered structure of the lang-grant mission-research, teaching, and extension-guides our academic departments will be the central foundation of this presentation.

4001 Youth Empowerment Through Small-Scale Poultry Farming: Pathways to Sustainable Livelihoods and Food Security in South Africa
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Youth unemployment in South Africa remains a critical socio-economic concern, particularly among rural populations. Small-scale poultry farming presents an accessible and scalable pathway for youth agripreneurship, income generation, and local food security. This study assessed the contribution of small-scale poultry enterprises to youth empowerment and identified key constraints limiting growth across Limpopo, Gauteng, and KwaZulu-Natal provinces. A mixed-methods approach was employed using a structured survey of 100 youth poultry farmers complemented by interviews with extension officers and agribusiness mentors. Quantitative data were analysed using descriptive statistics, while qualitative responses were examined through thematic analysis. Results showed that the average participant age was 26 years, with 57% female involvement. Broiler production was the most common enterprise (55%), followed by layers (30%) and mixed systems (15%). Poultry farming served as the primary income source for most respondents, with monthly earnings ranging from R3 000 to R25 000 depending on enterprise type. Although 61% of participants had received technical training, only 38% had access to mentorship, yet mentored farmers demonstrated significantly higher productivity (mean 4.2) compared to those without mentorship (mean 2.8). Access constraints were notable: 73% cited high feed costs as their main challenge, 59% faced market access difficulties, and only 28% had ever accessed formal credit. Digital innovations showed positive impact, with 52% of farmers using mobile tools for record-keeping, marketing, or input sourcing, leading to improved profitability for nearly half (46%) of users. Overall, the findings highlight strong potential for youth empowerment through small-scale poultry farming, particularly when supported with training, mentorship, financial access, and digital tools. The study recommends a collaborative model involving government, academia, and private sector actors to strengthen youth-led poultry enterprises, enhance market participation, and improve national food security outcomes.

Keywords: youth empowerment, small-scale poultry farming, agripreneurship, food security, South Africa
Keywords: youth empowerment; small-scale poultry farming; agripreneurship; food security, South Africa

4002 Youth Participation in Poultry Agribusiness Value Chains in Gauteng and KwaZulu-Natal Provinces of South Africa

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Objective: This study examined the extent, determinants, and challenges influencing youth participation in Poultry Agribusiness Value Chains (PAVC) in Gauteng and KwaZulu-Natal provinces of South Africa.
Experimental Design/Problem Description: Youth unemployment remains a critical challenge in South Africa, despite the agricultural sector's potential for job creation. The poultry industry, the country's largest agricultural subsector, offers multiple opportunities for youth inclusion. However, participation remains low due to economic, structural, and institutional constraints.
Materials and Methods: A structured questionnaire was administered to a randomly selected sample of youth engaged or interested in poultry-related enterprises. Data were collected on socio-economic characteristics, access to resources, and institutional support mechanisms.
Statistical Analysis: Descriptive statistics and multinomial logistic regression identified factors influencing youth participation across PAVC nodes.
Results: Although youth expressed strong entrepreneurial interest in poultry enterprises, their participation was limited by poor access to finance, high input costs, and inadequate business management skills. Age, education, access to credit, market information, and entrepreneurial training significantly influenced involvement ($p < 0.05$). Value addition and innovation present new opportunities for inclusion.
Conclusions: Youth-focused agribusiness policies, improved access to finance, and targeted capacity-building are crucial for sustainable participation. Strengthened mentorship and institutional support can accelerate youth-led transformation in the poultry industry.

Keywords: Youth employment; Poultry value chain; Agribusiness; Entrepreneurship; South Africa

4003 Empowering Youth through Poultry Education and Enterprise: Insights from Agricultural Colleges and Entrepreneurs in South Africa

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Hypothesis or Objective: To identify educational and enterprise strategies that enhance youth participation, leadership, and business success in South African poultry production.
Experimental Design / Problem Description: Youth involvement in poultry remains constrained by limited access to finance, training, mentorship, and markets. A multi-institutional initiative engaged agricultural colleges, youth entrepreneurs, and extension services to document best practices and scalable empowerment models linking education and production systems.
Materials and Methods: Data were collected from ≈ 100 participants, including students and facilitators from 11 colleges, youth operating small- and medium-scale poultry enterprises, and extension representatives. Structured interviews, focus groups, and participatory workshops were analyzed using thematic content analysis and descriptive statistics.
Statement of Statistical Analysis: Descriptive statistics summarized responses; one-way ANOVA tested differences in knowledge gain and enterprise performance between trained and untrained participants ($p < 0.05$).
Results: Participants with structured poultry training and mentorship reported significantly higher knowledge scores ($p < 0.05$) and improved enterprise outcomes—flock survival +19% and income +22% relative to untrained peers. Digital advisory tools and peer networks strengthened agribusiness confidence, while extension officers highlighted ongoing mentorship and institutional-market linkages as key success factors.
Conclusions: Aligning education, extension, and entrepreneurship enhances youth retention and productivity in poultry production. Strengthened institutional partnerships, targeted training, and improved access to finance and markets are essential for sustainable youth agripreneurship. Agricultural colleges remain pivotal in developing the next generation of poultry professionals and policy support is needed to institutionalize youth-focused enterprise models.

Keywords: Youth empowerment; Poultry education; Field report; Entrepreneurship; South Africa
Keywords: Youth empowerment; Poultry education; Extension services; Field report; South Africa

4004 Empowering Africa's Youth in Poultry Production:

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Status, Challenges, and Pathways from the PPC3 Youth Summit Case Study

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Hypothesis or Objective: To assess the status of youth participation in Africa's poultry sector and synthesize outcomes of the Third Pan African Poultry Conference (PPC3) Youth Summit (2025) discussions on challenges, opportunities, and interventions for enhancing youth integration along the poultry value chain.

Experimental Design / Description of the Problem: Poultry production remains central to food security and employment in sub-Saharan Africa. Yet, youth engagement is limited by barriers such as restricted finance, input shortages, weak extension, and limited leadership visibility. The PPC3 Youth Summit convened 200 delegates from 15 countries—students, farmers, agripreneurs, researchers, and policymakers—to evaluate the production landscape, identify constraints, and propose strategies for meaningful youth inclusion. The discussions provided a cross-continental perspective on challenges and emerging opportunities in poultry development.

Materials and Methods: Pre-conference focus sessions were held with selected respondents. Summit deliberations used a participatory workshop model combining thematic panels on empowerment, technology, resources, and policy. Discussions were guided by structured questions on sectoral challenges, innovation, and inclusion. Data from transcripts, polls, and plenary sessions were analyzed qualitatively to extract key themes and consensus recommendations.

Statement of Statistical Analysis: Qualitative data were coded and summarized using frequency counts and cross-theme co-occurrence to identify priority areas across discussion clusters.

Results: Respondents identified five main barriers to youth inclusion: finance (90 %), input access (82 %), inadequate mentorship (74 %), weak market linkages (69 %), and limited policy representation (63 %). Opportunities included small-scale hatchery development, agritech integration, and mentorship-driven incubation. Youth also called for stronger linkages between tertiary colleges, government, and private investors. Case discussions showed that combining incubation and market access improved youth enterprise viability by over 35 %.

Conclusions: The PPC3 Youth Summit highlighted that Africa's poultry sector faces intertwined challenges of youth unemployment and untapped enterprise potential. Empowerment requires coordinated action through targeted finance, digital extension, mentorship networks, and youth inclusion in policy processes. Scaling these interventions can shift youth from peripheral actors to central drivers of sustainable poultry growth in Africa.

Keywords: Youth empowerment; Poultry value chain; Policy support; Youth entrepreneurship; Africa

4005 The case study of the AgriFutures Chicken Meat Consortium (PRO-016111) in developing qualified Australia's chicken meat industry workforce

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The chicken meat production occurs mostly in rural and regional areas of Australia, presenting challenges to attract a young workforce. Engaging with the young workforce and promoting industry careers have been a priority for industry, academia and government. One of the strategic objectives of the AgriFutures Australia Chicken Meat Consortium (PRO-016111) in Australia is to foster an extension program that encourages PhD and Master's students to explore industry career potentials.

The AgriFutures Consortium is a multi-institutional research project aiming at improving the efficiency and sustainability of the Australian chicken meat industry with a strong focus on training the next generation of poultry scientists and connecting them to industry stakeholders. The extension program includes fostering relationships of industry-students through mentoring sessions with scientific events; an industry forum where students present research outcomes to industry stakeholders; and an industry placement program facilitating industry work experience for students. These programs were designed to facilitate the transition from academia to industry positions. They are also instrumental in translating novel innovations from academia to industry practice for potential adoption.

The results of the first two years showed that the mentoring program as part of the annual Australian Poultry Science Symposium connected >73 students with 59 industry mentors in 2024 and 2025, which has nurtured continued interactions beyond the mentoring sessions to learn career options with industry. The annual industry forum has achieved a milestone in gathering >80% of Australia's chicken meat production, showcasing the progress of the 18 PhD and 6 MPhil students. The industry placement offers industry partners to host PhD students for 3-month projects with industry exposure. Five placements have been confirmed and seven applications are in progress to date, accounting for 67% of the PhD students.

A mid-term review with all students and industry partners confirms the importance of incorporating extension programs throughout the duration of student training to provide continuous exposure to industry to attract a young workforce. The industry mentorship helps students with informed career paths which is difficult without industry connections. One of the challenges that needs continuous attention is the alignment of industry placements with university policies/programs which often require tailored approaches.

In conclusion, the extension programs of the AgriFutures Consortium show the collective commitment of industry and academia to support and sustain the development of a highly qualified chicken meat industry workforce in a coordinated effort.

Keywords: research; industry workforce; education; extension; professional development

4006 Acquisition and Maintenance of Competencies in Poultry Management

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The framework of the poultry production is constantly changing. New production systems and techniques, an increasing importance of animal welfare as well as management tools and the current level of scientific knowledge are challenging the competencies of people who work with poultry at different stages of production.

In order to assess the foundational knowledge acquired from agricultural vocational training, entrance exams were conducted anonymously in two summer semesters with a total of 258 second-semester students enrolled in the Bachelor's program in Agriculture at Osnabrück University of Applied Sciences. The majority of the students (83 percent) reported having completed agricultural vocational training prior to beginning their studies, thereby legally qualifying them as knowledgeable in the field. The examination focused on assessing the existing knowledge and competencies related to the fields of ethology, animal protection, and animal welfare within the context of livestock management. The results indicated that most of the surveyed students lacked fundamental knowledge required for these fields. Specifically, there were deficiencies in their understanding of potential indicators

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used to assess the adequacy of nutrition, care, movement, and housing for animals through animal welfare indicators (self-monitoring as per §11 (8) of the Animal Welfare Act) and in identifying signs of behavioral deviations or disorders as distinct from normal livestock behavior. Overall, only eleven (4.3 percent) of the 258 students successfully passed the entrance exam with at least 51 percent of the possible points. Consequently, it can be concluded that individuals in agricultural vocational training do not acquire sufficient competencies in the areas of animal protection, animal welfare, and ethology.
Within the context of poultry studies at Osnabrück University of Applied Sciences, students can assess themselves to determine the effort required during the module, the difficulty level, and thereby the maximum attainable grade for the oral examination (Levels A to C). Over a period of 10 years, it was observed that the proportion of students in Level C has increased from 5 percent to over 60 percent. It is expected that the initial acquisition of competencies through fundamental qualification measures may not be sufficient to meet the complex demands placed on employees throughout the poultry value chain. Relevant, continuing professional development and training programs are necessary to address these deficiencies; the Osnabrück Poultry Academy (OPA) targets this area with various offerings for further education and training in the poultry industry.
Keywords: welfare; lifelong learning; poultry production; continuing professional development; education gaps

4007 Development of an international certification program for animal health professionals
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Objective: We aim to create an internationally recognized certification program for animal health professionals

Description of the problem: In many regions of the world, there is a severe lack of animal health expertise in major animal productions, mainly in poultry, swine and aquaculture. This deficit contributes to heavy losses during production, hereby limiting productivity and impacting food security. At the same time, there is an absence of international standards regarding the status of expert in production animal health.

Materials and methods: At the request of the World Organization for Animal Health (WOAH), the European Association of Veterinary Specialization has developed an educational platform (World Veterinary Education in Production Animal Health (WVEPAH) with advanced training available for all animal health professionals (vets and non-vets). To ensure the quality of the courses, WVEPAH has partnered with the Université de Montréal. Hence, a tripartite agreement was signed between WVEPAH, the University, and WOAH.

The next logical step is the creation of a certification authority affiliated with WOAH, yet independent in its structure and decision processes. This independence is desirable so that all major international organizations, such as the FAO, may recognize the value of this certification. Although the examination process and level would be the same worldwide, adjustments due to species and disease prevalences are necessary depending on the targeted region (e.g., Latin America, Africa, Asia, and Russian speaking countries). Each region will have an examining board whose members will be renowned health professionals from the given region.

There are 3 scenarios and requirements to get certified:
1) You are already a board-certified veterinarians (American or European colleges) and you pass the WOAH course
2) or you have completed the WVEPAH training program in your field and have pass the certification exam
3) or you have substantial animal production health experience (recognized by the examining board) and you pass the WOAH course and the certification exam.

Results: This certification program will be presented as well as details of the wider program for each region of the world. The aim is to inform and to seek comments as adjustments can still be done to optimize its value.

Conclusions: In a world with emerging diseases, increased focus on animal welfare, and environmental conditions requiring constant adjustments, an animal health certification program will be a valuable tool in the development of a competent work force to meet today's and tomorrow's challenges in production animal health.

Five keywords for indexing: international, certification, animal health, expert recognition
Keywords: International; animal health; certification; expert; recognition

4008 Impact of scientific poultry interventions on livelihood security and income generation of rural women
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Hypothesis
It was hypothesized that capacity building through scientific training and the introduction of improved Rajasri birds would enable rural women to convert backyard poultry from a subsistence activity into a sustainable, income-generating livelihood.
Objective
To assess the impact of scientific backyard poultry interventions using improved Rajasri birds on income generation, livelihood security, and household nutrition among rural women.
Experimental Design
A pre-test–post-test experimental design was adopted to evaluate changes in knowledge, income, and livelihood outcomes before and after the intervention.
Materials and Methods
The study was conducted in Krishna district of Andhra Pradesh, India, covering five mandals with eight villages per mandal. A total of 400 rural women beneficiaries were selected based on a pre-structured questionnaire, baseline knowledge, and interest in poultry farming. Beneficiaries received structured entrepreneurial training on scientific Rajasri poultry management. Each participant was provided with 20 Rajasri chicks (eight weeks old) free of cost. The intervention spanned 42 months, during which 38 training programmes and 30 method demonstrations were conducted. Continuous technical support was provided through trainings, WhatsApp groups, and online FAQs.
Statistical Methods
Data were analysed using descriptive statistics (frequency, percentage, mean, and standard deviation). Pre- and post-intervention differences in income and livelihood indicators were assessed using paired t-tests, with significance declared at $P < 0.05$.
Results
Rajasri birds produced an average of 18–20 eggs per bird per

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month, of which 70–75% were marketed (12–15 eggs per bird per month). The mean annual income was Rs. 815 per bird, resulting in an average monthly income of Rs. 15,425 per beneficiary from poultry, in addition to household egg consumption. Egg incubation recorded a hatchability rate of 86%, while chick survivability up to marketing age was 81%. About 23% of beneficiaries marketed eggs collectively through Farmer Producer Organizations, selling 450–600 eggs per month. Additionally, 5% of beneficiaries adopted chick hatching as a micro-enterprise, producing 40–60 chicks per cycle. Paired t-test analysis indicated a significant ($P < 0.05$) improvement in income and livelihood indicators following the intervention.

Conclusion

Scientific backyard poultry farming with improved Rajasri birds proved to be a low-input, sustainable livelihood intervention that significantly enhanced income generation, nutritional security, and economic empowerment of rural women while requiring minimal labour and investment.

Keywords: rural women empowerment; sustainable livelihoods; rajasri birds; egg incubation; marketing

General Health/Disease/One Health IV

3097 Prevalence of avian pathogens and zoonotic risks in European backyard poultry: a multi-country evidence synthesis

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Backyard poultry farming is rapidly expanding across Europe, yet data on the health status of these flocks remain scarce and fragmented. These systems, often unregulated and lacking veterinary oversight, may act as reservoirs for pathogens with implications for both animal and public health.

This study aimed to identify and characterize the occurrence of avian pathogens in European non-commercial poultry systems, assess their reported prevalence, examine diagnostic approaches, and highlight associated risk factors.

A structured evidence synthesis was performed using PRISMA-ScR guidelines. Thirty-three eligible studies from 17 European countries were retrieved through systematic searches in PubMed, CAB Abstracts, and Scopus. Extracted data covered pathogen identification, prevalence, diagnostic methods, and risk factors.

The most frequently reported pathogens included parasitic agents (Eimeria spp., Dermanyssus gallinae), bacteria (Mycoplasma gallisepticum, Salmonella Enteritidis and Typhimurium), and viruses including infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILTV), Marek's disease virus (GaHV-2), and avian influenza virus (AIV). These agents were frequently detected, particularly in systems characterized by outdoor access, poor biosecurity, mixed-species flocks, and exposure to wild birds. Diagnostic methods varied widely (microscopy, culture, serology, RT-PCR), introducing methodological heterogeneity that complicates data comparability. Most studies were cross-sectional and geographically concentrated in Southern and Eastern Europe. Several pathogens identified, such as Salmonella spp. and AIV, have recognized zoonotic potential, underscoring the relevance of backyard poultry in the broader One Health context. These findings reveal a significant blind spot in Europe's animal health surveillance landscape. Harmonized diagnostic protocols and integrated monitoring strategies are needed to improve early detection, reduce pathogen circulation, and strengthen public health protection.

Keywords:
Backyard poultry, pathogen prevalence, zoonotic risk, disease surveillance, One Health
Keywords: Backyard poultry; pathogen prevalence; zoonotic risk; disease surveillance; One Health

3098 Seroprevalence of West Nile, Usutu, and Sindbis Viruses in Backyard Chickens in Romania

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Zoonotic arboviruses are increasingly detected across Europe as climate change, globalization, and shifting vector–host dynamics expand transmission niches. We assessed exposure to West Nile virus (WNV) and Usutu virus (USUV), both members of the Flaviviridae family (genus Flavivirus), and Sindbis virus (SINV) from the Togaviridae family (genus Alphavirus). The study focused on backyard chickens, considering their potential role as sentinel hosts for arbovirus circulation. From October 2017 to September 2018, a total of 197 blood samples were collected from backyard chicken flocks in 28 locations across ten Romanian counties. Sera were screened by ELISA, and reactive or doubtful results were confirmed by serum neutralization against WNV lineages I and II, USUV lineages Europe 3 and Africa 3, and SINV Edsbyn. Data were processed using EpiInfo™ 2000 software to calculate seroprevalence estimates. Overall, 13.71% of chickens were seropositive for WNV and 1.02% for USUV; one sample yielded a doubtful SINV result. Seropositivity was recorded in six counties, indicating widespread arbovirus exposure during the study period. Considering the recurrent reports of human West Nile neuroinvasive disease in Romania and neighbouring regions, serological detection of arbovirus activity in domestic poultry may serve as a valuable early indicator of virus circulation within a One Health framework. These findings provide the first serological evidence of WNV and USUV exposure in domestic chickens in Romania and support the integration of backyard flocks into national arbovirus early-warning system.

Keywords: backyard; chicken; West Nile virus; Usutu virus; Sindbis virus

3099 Mycotoxin occurrence in 2025

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Poultry species are particularly sensitive to mycotoxins. Therefore, it is imperative to monitor the prevalence of mycotoxins in agricultural commodities and the severity of mycotoxin contamination worldwide. To elucidate the threat of these toxic metabolites, dsm-firmenich has been conducting an annual mycotoxin survey for over 20 years, monitoring the incidences of different mycotoxins in finished feed and agriculture commodities used in animal feed.

In the first three quarters of 2025, 19.051 samples sourced worldwide from 89 different countries were analyzed for major mycotoxins, including aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin (T-2), fumonisins (FUM) and ochratoxin

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A (OTA). Samples were analyzed using liquid chromatography coupled to tandem mass spectrometry, high performance liquid chromatography or enzyme-linked immunosorbent assay.

In total, 95% of all samples contained at least one of the six main mycotoxins (based on samples for which at least three mycotoxins were analyzed). The Fusarium mycotoxin DON was the most common mycotoxin and was detected in 63% of all samples at a mean concentration of 731 ppb. FUM was present in 59% (mean of 1605 ppb) and ZEN in 62% (mean of 131 ppb) of samples. Aflatoxin (produced by Aspergillus species) and ochratoxin A (produced by Penicillium and Aspergillus species) occurrence are often related to storage conditions but also to grain damage in the field. Afla was present in 35% (mean of 21 ppb) and OTA in 22% (mean of 31 ppb) of samples.

Trends of mycotoxin occurrence in single commodities (corn, finished feed, etc.) were similar to the trend observed for the whole dataset. In corn samples, for example, FUM, ZEN and DON were the most common mycotoxins, detected in 81% (mean of 2267 ppb), 64% (mean of 119 ppb) and 63% (mean of 900 ppb) of samples, respectively. Furthermore, prevalence of Afla (36% positive tested samples) was rather high in corn worldwide.

The survey results indicate that mycotoxins remain a serious concern in agricultural production. An effective mycotoxin risk management program should be applied to protect poultry species from negative effects of mycotoxins.
Keywords: Mycotoxins; Survey; Global

3100 OCCURANCE OF MYCOTOXINS IN POULTRY FEED INGREDIENTS AND FEEDS

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The Pharmacovigilance Laboratory for Animal Feed and Food Safety, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India is involving various toxicological analysis such as mycotoxins, pesticides, antibiotics residue and other contaminants in livestock and poultry feeds and feed ingredients. The main objective of this study was to assess the contamination of mycotoxins namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), citrinin, ochratoxin A and T2-toxin in the tested samples. For that, 845 samples of poultry feeds (415) and feed ingredients (430) received from commercial poultry feed manufacturing units, poultry farms and academic institutes between October 2020 and October 2025 were investigated for the presence of mycotoxins at different seasons such as winter, summer and monsoon by High Performance Thin Layer Chromatography (HPTLC) method. The samples were initially screened by Thin Layer Chromatography (TLC) and the positive samples were confirmed and quantified by HPTLC. The HPTLC conditions were optimized to quantify mycotoxins using a TLC silica gel plate with a mobile phase of chloroform: acetone (9:1 v/v) for aflatoxins and toluene: ethyl acetate: formic acid (6:4:0.2 v/v/v) for citrinin, ochratoxin A and T2-toxin. Standards (certified reference material) and sample extracts were applied as 8 mm wide bands, 4 mm apart, using an automatic Linomat-5 TLC applicator with Nitrogen gas flow at 7 psi. The chromatographic chamber was saturated with the mobile phase for 20-30 minutes before the plate's linear ascending development, allowing a 70 mm migration distance. Densitometric scanning was performed using a CAMAG scanner 3 with fluorescence at 366 nm, a slit dimension of 6 x 0.45 mm, and a scanning speed of 20 mm/s. Among 845 tested samples, 48.99% samples were contaminated with AFB1 (2.4-1950 µg/kg), 24.97% with citrinin (9.8-947 µg/kg), 5.56% with T2-toxin (40-735 µg/kg), 4.62% with AFB2 (1.39-450 µg/kg), 3.79% with ochratoxin A (25-425 µg/kg), 0.59% with AFG1 (5-40 µg/kg) and 0.36% with AFG2 (5 µg/kg). Seasonal studies revealed that though the mycotoxins detected in all the seasons, the total number of positive samples (45.83-100%) as well as concentration of the mycotoxins were more in monsoon period (July-November) than winter (December-February) and summer period (March-June). One way ANOVA was performed. This study shows the importance of need for monitoring the mycotoxins in poultry feeds and feed ingredients to prevent the economic losses due to mycotoxicosis in poultry production and ensuring the food safety and security in human food-chain.
Keywords: Mycotoxins; Poultry Feed; Feed Ingredients; HPTLC; Season

3101 Comparative efficacy of dual chemical-based spray formulations against bed bug (Cimex hemipterus) infestation in caged poultry breeder units

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Cimex hemipterus (tropical bed bug) is a hematophagous ectoparasite widely distributed in tropical and subtropical regions and is increasingly reported in Indian layer and breeder poultry farms. Blood feeding causes irritation, reduced comfort, and production losses in affected birds. Heavy infestations result in repeated blood loss, anaemia and produce localized inflammation and scab formation around the vent, breast, and legs, predisposing birds to secondary bacterial infections and pecking injuries with compromised immunity. This study evaluated the efficacy of two dual-active insecticidal formulations against C. hemipterus under laboratory and field conditions over 30 days (D). Two severely infested poultry breeder cage houses (layer and broiler) and corresponding non-infested control houses were selected, with three cage units per house serving as replicates. In vitro knockdown assays were performed by exposing groups of ten bed bugs in Petri plates to insecticides using a glass atomizer, with mortality recorded at 5-m intervals up to 30 m. Field evaluations involved spraying insecticides at an application rate of 50 mL per sq. mt. Formulation 1 (Bifenthrin 2.5% + Acetamiprid 2.1%) was applied at 10 mL/L and Formulation 2 (Imidacloprid 21% + Beta-cyfluthrin 10.5%) at 4 mL/L on Day 1 and 14. Bed bug populations were monitored using glue-based trap cards, with eight traps installed on cages and four on adjacent walls. Visual inspections were conducted on D0, D2, D13, and D29, while trap retrieval and counts were performed on D1, D3, D14, and D30. Severe infestation was defined as counts exceeding ten bed bugs per trap. One-way ANOVA showed significant reductions in bed bug populations, with 82–87% efficacy by D3. Mean trap counts on D3 were 1.33 for Formulation 1 and 1.67 for Formulation 2, corresponding to reductions of 87% and 80%, respectively. From the second spray (D14) onward, bed bug counts dropped to zero and remained undetectable through D30, indicating effective and sustained control. Invitro trials demonstrated rapid knockdown, with Formulation 1 inducing mortality in an average of 8.2 m and Formulation 2 in 5.5 m. Both formulations act on the insect nervous system, causing rapid paralysis and death. The study demonstrates that dual-active neonicotinoid–pyrethroid

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formulations provide rapid and highly effective control of C. hemipterus. Repeat application after D14–15 effectively disrupts the bed bug life cycle and may help delay the development of insecticide resistance, supporting their use in integrated bed bug management in poultry production systems.
Keywords: Cimex; Imidacloprid; Acetamiprid ; Beta cyfluthrin ; Bifenthrin

3102 Molecular detection and characterisation of Cyptosporidium species in chickens from commercial farms and live bird markets in Ibadan, Nigeria

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Cryptosporidiosis is a serious intestinal infection in poultry with public health consequences. Despite its relevance, molecular monitoring has been restricted in Nigeria's commercial poultry systems. The purpose of this study was to detect and characterise Cryptosporidium species in commercial chickens and live bird markets in Ibadan municipality, Oyo State, Nigeria, using microscopy and molecular techniques. A total of 179 pooled fecal samples were collected from commercial farms and live bird markets across six Local Government Areas (LGAs) in Ibadan. Samples were examined microscopically using modified Ziehl–Neelsen staining and subjected to nested PCR targeting the 18S rRNA gene. Positive PCR products were sequenced, and phylogenetic analysis was performed. Data were analysed using SPSS version 23.0, with Fisher's exact test applied at $P < 0.05$.

An overall detection rate of 18.99% (34/179) was observed microscopically. Higher infection rates were recorded in commercial farms (21.13%) compared to live bird markets (10.81%). Layers had a higher detection rate (23.17%) than broilers (15.46%), and birds aged 21–40 weeks were most affected. Out of 34 faecal samples that were positive for Cryptosporidium species oocyst using microscopy, 5 (14.70%) were positive molecularly, with Cryptosporidium baileyi 4/34 (11.76%) and Cryptosporidium meleagridis 1/34 (2.94%). Phylogenetic analysis showed that local isolates clustered with global strains, suggesting conserved lineages and potential transboundary transmission. The presence of zoonotic C. meleagridis in poultry highlights a public health concern. The findings underscore the need for improved biosecurity, targeted management practices, molecular surveillance, and farmer education to control cryptosporidiosis in poultry production systems.
Keywords: Cryptosporidium; poultry; zoonosis; Nigeria; molecular detection

3103 Etiological study of egg peritonitis in commercial layer chicken in Namakkal region of South India

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Among various disease of poultry, egg peritonitis is one of the key problem in layer production. It is a common cause of sporadic death in layer hens. Since number of pathogens are associated with egg peritonitis, it is difficult to identify the causative agents based on the PM findings alone. Hence, this study was undertaken to identify the current status of egg peritonitis and etiologies. A comprehensive study was conducted on 25 layer chicken farms in the Namakkal region of India from March 2023 - February 2024. Namakkal is a major poultry-producing region in the world, housing ~80 million-layer chickens within 50 square kilometres. The clinical samples collected from 10 dead or moribund birds/farm were subjected for isolation and PCR detection of various etiological agents. Further, chi-squared statistical analysis was conducted to determine the significance of disease in different age groups (7 groups), combination of etiological agents associated with egg peritonitis-positive samples and egg production loss in different farms during the study period. The occurrence of egg peritonitis was found in 23 to 90 weeks old birds and majority of the cases were reported in 81-90 weeks age group (24%) followed by 41-50 and 61-70 weeks age group (20%) each, 21-30 weeks age group (16%), 31-40 weeks age group (12%) and 51-60 and 71-80 weeks of age group (4%) each and showed highly significant difference (x2 value=42.0, df=6) among the different age groups. The egg production drop was found to be significant (x2 value=62.0, df=24) due to egg peritonitis in commercial layer chickens ranged from 1.9 to 26.9% among different farms. In the present study, E. coli alone or combined with Newcastle disease virus (NDV), Infectious bronchitis virus (IBV), Salmonella Typhimurium, Gallibacterium anatis and Brachyspira pilosicoli were causing egg peritonitis. Combination of etiological agents associated with egg peritonitis differ significantly (x2 value=36, df=6) included E. coli alone (28%), E.coli + Salmonella Typhimurium (24%), E.coli + Gallibacterium anatis (12%), E.coli + Salmonella Typhimurium + Newcastle disease (12%), E.coli + Salmonella Typhimurium + Brachyspira pilosicoli (8%), E.coli + Newcastle disease (8%) and E.coli + Salmonella Typhimurium + Infectious bronchitis (8%). Egg peritonitis-affected birds exhibited dehydrated combs, somnolence, reduced feed intake, laying abnormal eggs, and drop in egg production. Grossly, the birds affected revealed fibrinous pericarditis, perihepatitis, adhering yolk with ovarian follicles and caseous yolk material in the peritoneal cavity. Combined infection of E. coli with other bacterial and viral etiologies showed the gross lesions in exacerbated manner.
Keywords: Egg Peritonitis; Poultry; Layer chicken; Namakkal; India

3104 The impact of antibiotics bans in Canada on antimicrobial resistance in Clostridium perfringens isolates from broiler chickens

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Necrotic enteritis, caused by Clostridium perfringens, poses a significant challenge to the poultry industry, resulting in high mortality and important economic losses worldwide. Although antibiotics have been widely used to manage this disease, concerns over antimicrobial resistance have led the Canadian poultry industry to ban the preventative use of category I antibiotics (very high importance in human medicine) in May 2014 and category II antibiotics (high importance) in December 2018. This study aimed to evaluate the impact of these bans on antimicrobial resistance patterns and to determine wild-type cutoffs for C. perfringens isolates from broiler chickens. We hypothesized that the bans decreased the prevalence of resistant strains to the withdrawn antibiotics but increased selective pressure on those still in use. A total of 111 isolates collected before May 2014 (n=55), between 2014 and 2018 (n=14), and after December 2018 (n=42) were tested for minimum inhibitory concentrations (MICs) against ten commonly used antimicrobials; Cat.I: ceftiofur, Cat.II: erythromycin, penicillin, tylosin and virginiamycin, Cat.III: bacitracin, and tetracycline, Cat.IV: monensin, narasin and salinomycin. MICs were determined using standardized agar dilution methods (CLSI). Statistical analyses involved Kruskal–Wallis tests and Dunn's multiple comparisons with Bonferroni correction (significance threshold: $p<0.05$). Cat.II antibiot-

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ics erythromycin, tylosin and virginiamycin showed a significant decrease in resistance as well as Cat.III antibiotic tetracycline after the bans. Penicillin, commonly used to treat Enterococcus cecorum infections even after the ban, exhibited a slight increase in resistance when comparing pre-ban and post-ban periods, though no significant difference was observed between the 2014–2018 and post-2018 periods. Bacitracin showed a significant increase in MICs after 2018, likely due to continued use and intensified selective pressure on this molecule. Before 2014, the wild-type cutoff was 16 µg/mL, while after the bans it dropped to 8 µg/mL, further supporting the rise in resistance. No significant changes in resistance were observed for Cat.IV antibiotics monensin, salinomycin, and narasin across all time periods. These findings reveal dynamic, antibiotic-specific shifts in resistance following regulatory changes. They also highlight the importance of rotating antimicrobial classes, as is done with anticoccidials, to limit the emergence of resistance. Continued surveillance is essential to guide the effective and responsible use of antimicrobials in poultry production.
Keywords: Antimicrobial resistance; Clostridium perfringens; Minimum inhibitory concentration; Broiler chickens; Antibiotics

3105 Dietary supplementation of endo-1,4-ß-D-mannanase improves turkey growth at day 21 through modulation of stress, inflammation, and gut integrity-associated genes
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Dietary supplementation of mannanase has been used for decades to improve gut health and growth performances in poultry, yet its underlying molecular mechanisms are not completely defined. The aim of the present study was to determine the effect of in-feed supplementation of a next generation endo-1,4-β-D-mannanase on growth performance and jejunal expression of stress-, inflammation-, and barrier integrity-markers in turkeys. A total of 640 one-day-old male Hybrid Converter poults were randomly assigned to one of two dietary treatments in a randomized complete design: Control (no enzyme, CON) and control diet supplemented with Natupulse® TS (800 TMU/kg feed, NPU). Each treatment had 16 replicate pens with 20 birds per pen. Birds were reared for 21 days with ad libitum access to diet and fresh water. Body weight (BW), BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR) and intestinal permeability using fluorescein isothiocyanate-dextran (FITC-d) were measured on days 21. Jejunal samples (12 birds/treatment) were analyzed for markers of stress, inflammation, and gut barrier integrity using real-time quantitative PCR and immunoblot. Data was analyzed by Student T using Graph Pad software and significant difference was set at $P < 0.05$. Supplementation of NPU significantly increased BW and improved FCR by 2 points, without affecting feed intake or serum FITC levels compared to the control diet. At the molecular levels, NPU significantly decreased mRNA abundances of HSP60 and 70, but increased their protein levels. The expression of proinflammatory cytokines (IL8 and IL18), and tight junction (CLDN1, CLDN4, and CLDN5), gap junctions (Gja1, Gjc1), and adherens junctions (AFDN1) were significantly down regulated by dietary NPU supplementation compared to the control group. Taken together, this study novel knowledge that NPU can improve young turkey's growth performance potentially through modulation of HSPs, cytokines, and gut integrity-associated genes.
Keywords: Turkey; mannanase; growth; stress markers; inflammation

Gut Microbiome and Health IV

3106 The gut microbiome as a driver of poultry health and disease resilience
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The gut microbiome plays a central role in poultry health, influencing nutrient utilization, immune function, and disease resistance. Despite considerable advances in sequencing technologies and bioinformatic tools, the mechanisms by which microbial communities mediate host health outcomes under different dietary, genetic, and production conditions remain incompletely understood. Microbial composition varies substantially across gastrointestinal tract segments, and responds differentially to a wide range of factors, including dietary mineral concentrations, enzyme supplementation, feed composition, host genetics, and housing systems. In laying hens, breed has been shown to have a stronger effect on the active gut microbiota than moderate dietary mineral reductions, demonstrating the importance of host genetics in shaping microbial community structure. A stable core active microbiota, consistently present across gut regions and nutritional conditions, has been identified in high-yielding layer breeds, suggesting that resilient microbial communities define a measurable baseline for assessing gut health. Beyond nutrition, production system design profoundly influences microbial diversity and stability; birds raised in organic or alternative systems exhibit distinct microbiome profiles compared to conventionally housed flocks, with associated differences in immune competence and susceptibility to enteric disease. Targeted modulation of the gut microbiome through probiotics, prebiotics, postbiotics, and feed enzymes represents a promising strategy to steer microbial communities toward improved health and performance outcomes. These interventions act through distinct mechanisms, and their efficacy depends on strain specificity and diet composition. At the host level, hologenomic prediction models combining host genomic and microbial data have further demonstrated improved accuracy for feed efficiency and mineral utilization traits, positioning the microbiome as a measurable biological marker complementary to classical breeding approaches. Overall, these findings show that integrating microbiome knowledge with precision nutrition and genetics offers a robust framework for developing targeted strategies to promote health, reduce disease burden, and support sustainable poultry production.

3107 Housing system and hemp products modulate the ileal microbiome and hepatic inflammatory gene expression in laying hens
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The transition from conventional housing can expose laying hens to greater microbial loads via litter contact and social interactions. Hemp seeds are high in protein and omega-3 polyunsaturated fatty acids, but their gut microbiome modulation and anti-inflammatory potentials remain unknown. This study evaluated the effects of housing system and dietary inclusion of hemp products on the ileal microbiome and liver inflammatory status of Lohmann LSL-Lite White hens in a 12-week trial, 2x3 factorial arrangement. Hens were assigned to either a three-tier conventional housing system (60 hens; 3 diets*4 housing units/diet*5 hens/unit) or a single-tier enriched housing system with wood-shavings bedded floor pens (120 hens; 3 diets*4 housing units/diet*10 hens/unit). The three diets included a control diet, a diet containing 20% hemp seed meal (HSM), and a diet containing 6% hemp seed oil (HSO). At the end of the trial, the ileal microbiome was assessed using 16S rRNA amplicon sequencing, with bioinformatics performed in QIIME2 and downstream analyses in R. Expression of hepatic

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immune genes (SAAL1, iNOS2, IL1β, TNF-α and IL-6) was quantified by reverse transcription quantitative PCR and analyzed using ANOVA in SAS. The ileal microbiome was dominated by Lactobacillus (>96%) within Firmicutes (>98%), yet was independently modulated by housing and diet. Enriched housing resulted in more unique amplicon sequence variants (ASVs) (167 vs 104 ASVs in the conventional housing; 96 shared), greater richness (observed, Chao1 and Faith's phylogenetic diversity, $P<0.05$), and distinct community structure (Bray-Curtis and unweighted UniFrac, $P<0.05$). HSM reshaped the abundance of dominant lineages already present (Bray-Curtis and weighted UniFrac, $P<0.05$; unweighted UniFrac, $P>0.05$) without impacting richness or evenness (observed, Chao1, Shannon, Inverse Simpson and Faith's phylogenetic diversity, $P>0.05$). HSO pruned rare taxa (observed and Chao1, $P<0.05$) without altering dominant taxa (Bray-Curtis and weighted UniFrac, $P>0.05$; unweighted UniFrac, $P<0.05$). Enriched housing reduced inflammatory gene expression (SAAL1, iNOS2, IL1β and TNF-α, $P<0.05$) compared to conventional housing. Dietary effects were only observed in conventional housing where HSM upregulated SAAL1 and iNOS2 expression ($P<0.05$), whereas HSO downregulated them to levels observed in enriched housing ($P>0.05$). Overall, enriched housing increased ileal microbial diversity and reduced hepatic inflammation. In conventional housing where the baseline inflammation was higher, HSO showed anti-inflammatory potential, whereas HSM might trigger inflammation. These results highlight the need for targeted nutrition tailored to the housing context in poultry production.

Keywords: alternative housing; laying hen; hemp; gut microbiome; gene expression

3108 Research progress on probiotic intervention for hyperuricemia

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Hyperuricemia prevalent in goose flocks poses significant challenges to the poultry industry, resulting in substantial economic losses. To address this issue, this study established a goose hyperuricemia (HUA) model and revealed intervention strategies for poultry hyperuricemia based on gut microbiota.

First, a hyperuricemia (HUA) model was established through 28 days of high-calcium, high-protein (HCP) feeding. Results showed that the HUA group exhibited significantly elevated serum uric acid (UA) and xanthine oxidase (XOD), accompanied by impaired liver and kidney function, gut microbiota dysbiosis, and abnormal levels of amino acid metabolites.

Further, antibiotic treatment and fecal microbiota transplantation (FMT) validated the close association between gut microbiota and hyperuricemia. Gut microbiota from HUA model geese induced hyperuricemia in recipient geese, including elevated serum UA and gut microbiota dysbiosis, further confirming that gut microbiota imbalance is a key driver in hyperuricemia development.

Building upon the model, continuous 14-day oral supplementation with three probiotic strains was administered. Results indicated that Lactobacillus rhamnosus GG (LGG) reversed proline deficiency, improved gut microbiota balance, and reduced serum UA and its precursor substances by regulating the gut-liver-kidney axis. Lactobacillus plantarum SQ001 (LP SQ001) significantly increased intestinal Lactobacillus abundance, downregulated hepatic XOD and renal UA reabsorption protein GLUT9, while upregulating renal excretion protein ABCG2. Akkermansia muciniphila K101 (AKK K101) directly degrades UA, activates intestinal ABCG2 to promote UA excretion, inhibits renal GLUT9 to reduce reabsorption, while suppressing renal inflammation and reshaping beneficial gut microbiota.

Further mechanisms of hyperuricemia alleviation by these strains were revealed through gene editing and genome-wide analysis. LGG degrades purine nucleosides via the ABCT transport system, iunH nucleoside hydrolase, and pbuX xanthine transporter. LP SQ001 efficiently hydrolyzes purine nucleosides via iunH, reducing UA production. AKK K101 directly degrades UA, stabilizing UA metabolism. The three strains synergistically achieve UA reduction by reshaping the gut microbiota.

In summary, LGG, LP SQ001, and AKK K101 collectively alleviate hyperuricemia by reshaping the microbiota, regulating proteins and enzyme activities associated with the gut-kidney or gut-liver-kidney axes, and directly degrading UA. This provides a novel probiotic-based intervention strategy for controlling hyperuricemia in poultry.

Keywords: Hyperuricemia; Gut-liver-kidney axis; Probiotics; Purine metabolism; Goose

3109 The Prophylactic Potential of Paromomycin against Histomoniasis in Turkeys

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Histomoniasis is a fulminating protozoal disease in turkeys characterized by high morbidity and mortality. Currently, no commercial vaccines or prophylactic/therapeutic measures are available to combat coccidiosis in the United States or Canada. To address this need, a prophylactic evaluation of paromomycin was conducted in an experimental turkey model. Poults were randomly divided into four groups (n=20/group): Group 1 (negative control), Group 2 (challenge control), Group 3 (paromomycin treatment-0.2 ml/kg) and Group 4 (paromomycin treatment-1ml/kg). From 21 to 37 days of age (DOA), poults in Groups 3 and 4 received paromomycin at their respective doses. At 23 DOA, Groups 2-4 were challenged via the cloacal route with 1x104 H. meleagridis/0.5 ml per bird. At 38 DOA (15 days post-challenge), all poults were euthanized, weighed, necropsied and evaluated for gross lesions in the liver and ceca. No mortalities or gross lesions were observed in Group 1. Group 2 exhibited 55% mortality (11/20) and 80% gross lesion prevalence (16/20). In contrast, Group 3 had 5% mortality (1/20) and 20% gross lesions (4/20), while Group 4 showed no mortality (0/20) and only 5% gross lesions (1/20). Statistical analyses were performed using SAS version 9.4. at significance levels of $P \leq 0.05$ and $P \leq 0.01$. Mortality in Group 2 was significantly higher than in all other groups. Group 3 demonstrated significantly reduced mortality compared to Group 2, while no mortality was reported in Group 4. Body weight was greater in Groups 3 (1141.95 grams ±285.22) and 4 (1237.35 grams ± 114.26) compared with Group 2 (933.11 grams ± 446.66). These findings indicate that prophylactic administration of paromomycin effectively reduces mortality, gross lesions and mitigates performance losses associated with histomoniasis in turkeys.

Keywords: Histomoniasis; Blackhead disease; Paromomycin; Prophylaxis; Turkeys

3110 Monitoring Eimeria Species in US Turkey Flocks: Prevalence and Trends

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Turkey coccidiosis causes significant economic losses to the poultry industry. The turkey industry employs multiple mitigation strategies to maintain control of this disease, and monitoring Eimeria species is essential for understanding strains prevalent in the

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field. In 2025 (May–October), 51 submissions were collected from commercial turkey operations across diverse geographic regions of the United States. Samples—including feces, litter, and/or intestinal tissues—were submitted from flocks exhibiting subclinical or clinical signs of coccidiosis, as well as from routine surveillance programs. Oocysts were isolated from fecal material using centrifugation and sucrose flotation. DNA was extracted from oocysts using glass beads for mechanical disruption followed by the QIAamp Fast DNA Stool Mini Kit (Qiagen), according to manufacturer's instructions. Species identification was performed via PCR targeting mitochondrial cytochrome c oxidase subunit I (mtCOI) locus of six turkey-specific Eimeria species: E. adenoeides, E. meleagrimitis, E. gallopavonis, E. meleagridis, E. dispersa, and E. innocua. PCR amplicons were purified and submitted for sequencing. Vaccinated flocks tested positive for the Eimeria species included in the vaccine. The overall prevalence of Eimeria species in unvaccinated US turkey flocks was as follows: E. meleagrimitis (90.9%), E. meleagridis (90.9%), E. adenoeides (72.7%), E. gallopavonis (54.5%), E. dispersa (27.3%), and E. innocua (18.2%). These findings highlight the complexity of turkey coccidiosis epidemiology and emphasize the value of ongoing molecular surveillance to characterize Eimeria species distribution in commercial turkey production.

Keywords: Coccidiosis; Turkeys; Eimeria species; Eimeria surveillance; Vaccination

3111 Impact of Low-Protein, High-Fiber Diets on Laying Hen Gut Microbiota

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This study investigated the effects of reducing dietary crude protein and increasing insoluble non-starch polysaccharides (NSP), while balancing diets for amino acids, on the cecal microbiome composition of laying hens. A total of 1,600 pullets (16 weeks old) were randomly assigned to one of four dietary treatments in a 2 × 2 factorial design, with crude protein (Low Crude Protein, LCP vs. Standard Crude Protein, SCP) and insoluble NSP (Standard Fiber, SF vs. High Fiber, HF) as factors. Crude protein level was 1.5% lower in LCP than SCP, and HF had 2% higher insoluble NSP than SF. Each dietary treatment had four replicate pens of 100 birds each in a randomized block design. At 50 weeks of age, three hens per pen (12 hens/treatment) were euthanized by intravenous injection of pentobarbital. Cecal contents were collected separately from the right and left ceca, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. DNA was extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN), and the 16S rRNA gene was sequenced on an Illumina MiSeq platform. Sequence data were processed using the DADA2 pipeline, and taxonomy was assigned using the SILVA rRNA database (release 138.1). All statistical analyses were performed in R. Alpha diversity (Shannon and Simpson indices), beta diversity (Bray–Curtis dissimilarities), and relative taxonomic abundances were assessed. Differences in microbial community composition were evaluated by PERMANOVA (adonis2, vegan package), and differentially abundant taxa were identified using the MicrobiotaProcess package (LDA threshold >= 2.0; Kruskal–Wallis followed by Wilcoxon test; $\alpha < 0.05$). Alpha diversity metrics did not differ significantly among treatments ($p > 0.05$). Clostridia were the dominant bacterial class across all diets. Beta diversity analyses (PCoA and NMDS) showed substantial overlap among treatments, with an NMDS stress value of 0.238, indicating that a two-dimensional projection captured only limited variation in community structure. Despite the visual overlap, PERMANOVA indicated significant but modest effects of diet on microbial composition (Fiber: $P = 0.003$, $R2 = 0.020$; Protein: $P < 0.0001$, $R2 = 0.027$). Linear discriminant analysis (LDA) revealed enrichment of Blautia, Marvinbryantia, and Butyricoccus in the HF group compared with SF. In contrast, Lactobacillus and Streptococcus were more abundant in SCP than in LCP, whereas Ruminococcaceae and Faecalibacterium were enriched in LCP compared with SCP. In conclusion, increasing insoluble NSP influenced the cecal microbiota of laying hens, enriching butyrate-producing taxa. Protein levels also affected microbial composition, but overall diversity was not significantly altered by either treatment.

Keywords: laying hens; dietary modulation; low crude protein; insoluble fiber; cecal microbiota

Incubation Technology and Physiology II

3112 Surface-Enhanced Raman Spectroscopy of allantois for in-ovo sex determination in chickens

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Objective

Male chicks from layer breeds are culled shortly after hatching, because they cannot lay eggs and are not economically viable for meat production. This presents a significant ethical dilemma, which can be solved through in-ovo sexing. In-ovo sexing through PCR is already implemented at commercial scale. Objective of our current research is to further increase throughput and reduce cost to enable global adoption. Spectroscopy techniques are promising candidates as they enable rapid and simultaneous detection.

Hypothesis

Surface-Enhanced Raman Spectroscopy (SERS) can be used to determine the sex of embryonated chicken eggs with an accuracy of >99%.

Experimental design

SERS was used to determine sex by treating allantois from embryonated eggs with silver nanocolloids, and analyzing Raman scattering.

Material and methods

Allantois was sampled on day 9 of incubation using an automated in-ovo sexing system (SELEGGT Circuit). For SERS, samples of allantois were prepared through protein denaturation and centrifugation, before mixing a 1 µL aliquot with 1 µL of silver colloid suspension via repeated aspiration and drying the spots to air. Conventional Raman spectroscopy was performed on dried samples using a Raman microscopy system with excitation at 532 or 785 nm. Sex was confirmed via PCR targeting the W chromosome.

Statistical analysis

Data analysis was performed in Python using open-source modules. Outliers were removed based on the distribution of the scores obtained from principal component analysis and the remaining 94 samples were split into training (n=74) and test (n=20) sets using stratified sampling for sex. Classification of embryo sex was performed using partial least squares – linear discriminant analysis after applying different preprocessing methods for baseline correction, smoothing and scaling.

Results

While conventional Raman spectroscopy yielded no detectable Raman bands, metabolites in the fluid were found to be susceptible to surface enhancement under both 532 and 785 nm excitation,

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with no autofluorescence and good signal stability at 785 nm. Sex classification accuracy of the training set ranged from 91.3% to 98.7%, depending on preprocessing methods, but dropped to 66.4% to 71.0% in cross-validation, falling short of the >99% benchmark achieved by PCR in SELEGGT's commercial practice. The observed discrepancy between training and test accuracy suggests that the model may be overfitting the training data and its ability to generalize to new samples is limited.
Conclusions
Despite the lower predictive performance, these findings may be promising for further investigation into the biomarkers driving sex classification, which may reveal new targets for in-ovo sex determination.
Keywords: SERS; Raman spectroscopy; in-ovo sexing; incubation; embryology

3113 Proteomic Profiling of the Chorioallantoic Membrane provides Insight for Pre-Hatch Sex Determination in Chicken Embryos.

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Hypothesis and Objective: This research aimed to develop an early, non-lethal method for sex determination in chick embryos by identifying W chromosome–encoded proteins in the chorioallantoic membrane (CAM). We hypothesized that accurate pre-hatch sexing could be achieved by immunochemically detecting W-encoded CAM proteins at early embryonic stages (ED6–10), while maintaining viability during sampling.
Experimental Design: The study was structured in two phases: (1) optimization of early-stage CAM manipulation and sampling protocols to ensure embryo viability, and (2) proteomic and molecular interrogation of CAM to identify W-linked biomarkers for female-specific protein expression. Embryos were sampled at ED6, 8, 10 and 12 to evaluate developmental and sex-linked proteomic changes.
Materials and Methods: Fertilized Gallus gallus domesticus eggs were incubated and subjected to different CAM manipulations varying in egg disinfection, eggshell (ES) erosion/piercing, eggshell membrane (ESM) removal, CAM sampling and ES sealing. Embryo viability was assessed at ED16. CAM samples were analyzed using LC/MS/MS-based proteomics and bioinformatics to characterize protein expression across developmental stages. Quantitative PCR was used to confirm the presence of the W-specific gene (SWIM locus). Statistical comparisons among treatment groups were performed using one-way ANOVA ($p < 0.05$).
Results: Embryo viability was significantly influenced by sampling day and method ($p < 0.05$). CAM manipulation at ED6 yielded low viability, whereas survival improved at ED8 and ED10. Proteomic analysis identified 2688 CAM proteins, with 1191 shared across stages, while 124, 47, 86, and 2 proteins were uniquely expressed at ED 6, 8, 10, and 12, respectively. Functional annotation (KEGG pathway analysis) revealed various functional pathways correlated with abundant CAM protein constituents involved in calcium mobilization (ATP6V1G1), intracellular transport (CAB39), vascular system (ANXA2), lymphatics (ACTG1), blood elements (HBA1) and protection against luminal toxic contents (TXN).
Conclusions: This research establishes the procedural and molecular framework for early, in-ovo sex determination using CAM proteomics. The findings confirm the feasibility of the CAM as a minimally invasive biological platform for detecting female-specific biomarkers, providing a path toward developing an immunoassay for accurate pre-hatch sexing. The project supports the long-term goal of eliminating post-hatch male chick culling, aligning with ethical and sustainability objectives in poultry production.
Keywords: Chorioallantoic membrane, Proteomics, W-encoded protein, Embryo sexing, Poultry ethics
Keywords: Chorioallantoic membrane; Proteomics; W-encoded protein; Embryo sexing; Poultry ethics

3114 Evaluating the interaction effect of egg weight and patio regimens on hatching traits and post-hatch performance of the broiler chickens

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Objective: The objective of the present study was to explore the impact of hatchery-based interventions on hatching traits and the post-hatch performance of the broiler chickens.
Experimental design: A completely randomized design was applied in this study to evaluate the effects of egg weight from the Hubbard strain and patio regimens on hatching traits and post-hatch performance of broiler chickens.
Materials and methods: A total of 3000 eggs of Hubbard broiler breeders were equally divided into three weight categories, i.e., Small (50-55g), Medium (56-61g), and Large (62-70g). From each category, eggs were distributed into 20 trays containing 50 eggs each. Each tray was considered a replicate. At the end of the 18th day of incubation, the eggs of each weight category were allocated to four distinct patio strategies. P0 (control), P3, P5, and P7, where feed and water were provided to the chicks for 0, 3, 5, and 7 days, respectively, in the hatcher, followed by conventional rearing.
Statistical analysis: Data on hatch windows, hatching traits, growth performance, physical asymmetry, welfare, and blood metabolites in broilers were subjected to statistical analysis using factorial ANOVA (two-way analysis of variance), with egg weight categories and patio strategies considered as the main effects, and significant means were compared through Duncan's Multiple Range test.
Results: Statistical analysis revealed that the egg weight affected ($P \leq 0.05$) the hatch window and hatching traits. The post-hatch performance was affected ($P \leq 0.05$) by egg weight and patio strategies, with superior outcomes observed in chicks from medium-sized eggs subjected to the P3 treatment. Feathers score, physical asymmetry, and gait were not affected by both treatments ($P > 0.05$). Blood metabolites profile was also influenced ($P \leq 0.05$) by egg weight and patio strategies, with birds from medium-sized eggs under the P3 treatment exhibiting the most favorable results.
Conclusions: Based on the findings of the study, it is concluded and recommended that the eggs of 56-61 grams should be selected for better hatchability, and the chicks should be provided with feed and water within the hatcher for at least three days for optimum performance in the rearing facility.
Keywords: Egg weight; patio; growth; welfare; blood biochemistry

3115 Study on Reproductive Traits and Embryonic Mortality patterns in relation to Hen age in Punjab Red breed of chicken

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In poultry, production efficiency and profitability depend largely on traits like fertility and hatchability. Embryonic mortality, due to various causes, is one of the main reasons for lower hatchability. The present study was conducted on the Punjab Red breed,

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categorized into five age groups with 5 weeks duration, viz., group I (28-32 weeks), group II (33-37 weeks), group III (38-42 weeks), group IV (43-47 weeks), and group V (48-52 weeks) at Hatchery Unit, GADVASU Ludhiana, India. Hen age relationship with reproductive and embryonic mortality traits were observed by dividing the total embryonic mortality (TEM) into three phases: early ≤7d (EEM), mid 8-17d (MEM), and late 18-21d (LEM), based on visual observation for developmental stages of the embryo. The frequency percentage was calculated for reproductive traits like fertility, hatchability, and embryonic mortality, including malpositions and malformations. The variables for each age group were compared using the one-way ANOVA statistic. The overall fertility was found to be ranging from 84.13±0.14% during 28-32 weeks of age and increasing significantly ($p<0.05$) up to peak fertility of 89.44±0.55% during 43-47 weeks of breeder's age. Higher hatchability estimates were observed at 38-42 weeks of age (78.38±0.55%), showing a significantly ($p<0.05$) increasing trend with the age of the breeder flock up to 42 weeks. The TEM was significantly affected by the age of the breeder, with higher mortality among the young and old breeders. About 32% of the TEM were found to be EEM, 7% MEM, and 40% LEM. About 21% of the TEM were observed to be live but not hatched embryos, which pose a greater loss to the hatchery. The overall distribution of malposition represents 19% of the malposition being foot over head, followed by head over the right wing (14%). The malpositions like upside down (12%), head between legs (11%), and head under left wing (11%) occurred with almost the same incidence. Only 9% of the malpositions occurred due to the beak being away from the air cell, whereas about 23% of the embryonic deaths could not be identified under either malposition or malformation. Only 1% of the total embryonic deaths were presented to be due to malpositions of the beak and head. The present study concluded the biphasic pattern of embryonic mortality, with higher death rates during the later phase of incubation followed by early embryonic mortality.

Keywords: Breeder age; Embryonic mortality; Fertility; Hatchability; Malpositions

3116 In-hatcher feeding and phase-specific dietary inclusion of potato peel meal in broiler chicken diets: Effects on gastrointestinal organ weight and growth performance

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The effects of in-hatcher feeding and phase-specific dietary inclusion of potato peel meal (PP; 14.67% CP, 2990 kcal/kg energy, 59.97% ADF and 73.79% NDF on DM basis) on productivity and gastrointestinal organ weight in broilers were evaluated. On d 18 of incubation, broiler hatching eggs were randomly allocated to eight hatching trays (88 eggs/tray) for two in-hatcher feeding treatments: a control group (n=4 hatching trays) without access to water and feed or a nutrient-access group (n=4 hatching trays) with access to water and 5% PP starter feed supplemented with a multi-carbohydrase (Superzyme CC, CBS Bio Platforms) (5PPE). Chicks hatched from the same in-hatcher feeding treatment were randomly placed into 24 pens (24 birds/pen) and fed either a control diet or 5PPE diet from d 1 to 25 of age. From d 25 to 33 of age, half of the birds from each of the treatment combinations were provided with either 10% PP or 10% PP supplemented with the multi-carbohydrase (10PPE). Production parameters were measured on d 7, 14, 25 and 33 and gastrointestinal organs were weighed on d 7 and 33. Data were analyzed using the Mixed procedure with a 2 x 2 x 2 factorial arrangement in SAS. No differences ($P>0.05$) in egg weight or rectal temperature at hatch were observed among the in-hatcher feeding treatments. Feed and water access in the hatcher increased ($P<0.05$) body weight at the end of incubation, and this weight advantage was sustained ($P<0.05$) for the entire production period. Birds from the nutrient-access group had higher ($P<0.05$) daily feed intake (FI) and body weight gain until d 25 of age and improved ($P<0.05$) feed conversion ratio (FCR) until d 14 of age. Birds provided with the control diet gained more weight ($P<0.05$) than those fed 5PPE from d 8 to 14 of age. No differences ($P>0.05$) in body weight, FI or FCR were observed among dietary treatments or their interaction with the in-hatcher feeding. The relative weights of gastrointestinal organs at d 7 of age were affected by in-hatcher feeding treatments. Chicks with nutrient access had lower ($P<0.05$) relative weights of emptied gizzard, jejunum, ileum and pancreas than unfed chicks. Regardless of in-hatcher feeding, the 5PPE diet resulted in a heavier ($P<0.05$) pancreas and lower ($P<0.05$) relative weight of liver and emptied ileum compared to those fed with the control diet. No differences ($P>0.05$) in gastrointestinal organ weight were observed on d 33 of age among treatments. These results demonstrate that PPE has potential as a sustainable broiler feed ingredient as its inclusion did not compromise body weight at market age. Although in-hatcher feeding practices improved overall growth, it did not enhance birds' capacity to utilize diets containing PP.

Keywords: In-hatcher feeding; Potato peel; Early feeding; Productivity; Gastrointestinal organ weight

Innovation and Technology IV

3117 Evaluating feed interventions in broiler chickens using methods that mimic realistic conditions

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Although animal trials remain necessary for the proper evaluation of efficacy and safety of new feed additives, and to assess raw materials and feeding strategies under experimental, commercial, or challenge conditions, efforts are needed to decrease animal number (Reduce), and minimize animal discomfort (Refine). Challenge models in the past often involved exposing birds to high levels of contaminants, long-term fasting periods, or acute bacterial infections, resulting in high mortality or severe symptoms. Today it is possible to evaluate feeding strategies and their effects on intestinal integrity without subjecting animals to such suffering conditions. Additionally, the advent of more advanced analytical methods, e.g., metabolomics, has enabled the assessment of animal health and intestinal function trough blood or excreta biomarkers. A gut leakage model that induces a mild increase in intestinal damage while simultaneously applying a practical dietary formulation exemplifies the potential to assess intestinal integrity under realistic conditions. In conjunction with a feed intervention study, markers for oxidative stress, inflammation, and nutrient utilization can be assessed by metabolomic analysis using a single excreta sample. These methods could be applied to evaluate alternative feed ingredients that do not compete with the food industry (circularity) and are gaining attention for broiler diets. Low-value raw materials can be subjected to fermentation processes to produce novel ingredients with nutritional value for animals. However, before these new raw materials can be evaluated in animal trials to determine their nutritional value or optimal inclusion levels, screening is necessary to identify promising products based on their nutrient digestibility. Therefore, in vitro approaches (Replace) that provide information on both the extent and rate (kinetics) of digestion play a critical role in this process.

Updated in vitro methodologies for crude protein and starch digestibility allow the evaluation not only of feed ingredients but also of complete (pelleted) diets, detecting differences due to processing

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conditions, including feed ingredient grinding and pelleting parameters (e.g., temperature in the conditioner and energy input in the expander). Thus, the digestibility improvement of feed ingredients resulting from pelleting under different conditions can be quantified, further contributing to greater accuracy in feed formulation.
A gas production test originally designed to use inoculum from cows has been adapted for swine and poultry, allowing to compare the fermentation kinetics among a variety of fiber-rich ingredients.
It is already possible to evaluate bacterial translocation using intestinal organoids. As a next step, in vitro systems, including intestinal cells to assess dietary interventions on nutrient uptake, appear as a method to screen ingredients before in vivo digestibility trials.
It is important to bear in mind that a cell, or a single organ, cannot replace the complexity of an animal and its interaction with its microbiome and the environment. The assessment of the intestinal microbiome should not be limited to its fermentation capacity but include its ability to send signs to other organs, including the brain. Additionally, the enteric glial cells, and not only the intestinal epithelial layer, play a role in animal health and welfare. Therefore, innovation will rely on the integration of intestinal function, focusing on the gut-microbiome-brain axis, which encompasses understanding how the microbiome influences not only digestion but also animal welfare.

3118 Assessment of the efficacy of cleaning and disinfection procedures in commercial broiler farms

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Proper cleaning and disinfection (C&D) procedures applied during the downtime represent a key biosecurity measure to decontaminate poultry houses between consecutive production rounds, thereby enhancing biosecurity and contributing to optimized flock performances.
The aim of this study was to evaluate the efficacy of a comprehensive C&D program in comparison with current C&D practices in commercial broiler farms, and to determine its impact on flock performance.
Eight commercial broiler farms located in Belgium were selected, and sampling was performed over three consecutive downtimes and production cycles (Control, Treatment 1, and Treatment 2). The sampling focused on the poultry barn environment, with samples collected both after cleaning and after disinfection. Environmental samples were taken from the floor, feeders, drinkers, walls, ceiling, air inlets, and feed hoppers. These samples were analysed for ATP concentration, total bacterial count (CFU), Enterococcus spp. count (CFU/ml), and E. coli and fungi presence. An additional sampling point was included at the start of each production cycle, consisting of cloacal swabs from 7-day-old chicks, which were tested for astrovirus, avian nephritis virus (ANV), reovirus, and rotavirus A. Sampling results obtained after cleaning procedures were compared with those obtained after disinfection (per treatment and per group). The effect of the whole C&D program in comparison to performance parameters and environmental viruses was also tested. Mixed Models and Generalized Linear Mixed Models with logistic regression were applied to account for farm effects and between-farm variability.
Results show that the proper application of a comprehensive C&D program led to an approximately 7% reduction in relative mortality compared to the control, as well as a significant increase in average daily gain (ADG). Specifically, after disinfection, the hygiene program significantly reduced ATP levels in drinkers compared to the control. Moreover, the complete C&D program alone resulted in an 89.5% reduction in total bacterial count between cleaning and disinfection ($p < 0.001$) and a 94.4% reduction in Enterococcus spp. levels ($p < 0.001$). A significant negative correlation was observed between ANV and FCR ($\rho = -0.78$, $p = 0.0215$), indicating that lower viral loads were associated with improved feed efficiency following C&D.
Overall, the findings of this study contribute to enriching the currently limited body of field-based biosecurity research. The results suggest that the adoption of proper C&D procedures supports the establishment of the new flock in a clean environment, which in turn improves overall flock performance.

Keywords: biosecurity; broiler farms; cleaning; disinfection; environment

3119 AI-Driven automated feather scoring for welfare monitoring in broiler breeders

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Feather cover loss is a key welfare indicator in broiler breeder hens, reflecting behaviors such as feather pecking, aggression, and mating-related damage. Poor feathering during the laying phase can cause decreased fertility, as increased scratches may reduce voluntary mating. However, current welfare assessments rely on subjective manual scoring. It is labor-intensive, inconsistent across observers, and impractical for large commercial flocks. Automated body-feather scoring systems are therefore needed to provide continuous, objective, and scalable welfare monitoring. This study developed and evaluated an artificial intelligence (AI)-based vision system to classify body-feather scores using affordable, farm-deployable cameras. Sixteen 2/3 slat pens with 68 broiler breeder hens and 7 roosters per pen were used for data collection. From each pen, 24 hens were randomly selected and manually scored by trained observers at 45, 50, 55, and 60 weeks of age. Scores ranged from 0 to 5 (S0 = fully feathered, and S5 = bald backs). After that, broiler breeders were placed individually into custom-built imaging pens for data collection. An RGB and depth camera was used to capture synchronized video of each broiler breeder. These videos were converted into images. A total of 1,200 RGB images were extracted and annotated for this study, with the dataset split into 70% for training, 20% for validation, and 10% for testing. Multiple training configurations were tested, including three optimizers (SGD, Adam, AdamW), different input resolutions, and various augmentation techniques. After identifying the best optimizer and augmentation strategy, lightweight YOLO classification model architectures were trained and evaluated. Performance was assessed using precision, recall, F1-score, and mAP_0.50–0.95. Statistical comparisons among optimizers and augmentation groups were performed using one-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$). The SGD achieved higher ($P<0.05$) precision (82.0%) and mAP_0.50–0.95 (59.7%) than Adam and AdamW. While AdamW produced the highest recall (86.2%). YOLOv8-cls yielded the best overall performance and achieved significantly higher F1-scores in feather-score classification (0–5) than other models. The S0 showed a higher mAP than the other scores. To enhance detection and classification performance, we plan to expand the dataset further and continue refining the model through additional training. These findings highlight the importance of selecting appropriate optimizers, augmentations, and model architecture for robust feather scoring. The optimized YOLOv8-cls system demonstrated strong potential as a real-time, farm-ready tool for precision welfare assessment in broiler breeders.

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Keywords: Broiler breeder; welfare; feather score; computer vision; precision livestock farming

3120 Hetasca: Serological detection of poultry roundworm infections and its practical applications.

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Roundworm infections in poultry are traditionally diagnosed through worm counts and faecal egg per gram (EPG) measurements. Although these methods are widely used, they may lack the sensitivity required for early detection, potentially delaying intervention and increasing the risk of pathogen spread. The newly developed Hetasca serologic test offers the potential for earlier and more sensitive identification of roundworm infections, improving flock health management.

To evaluate this diagnostic tool, an in vivo study was conducted comparing the sensitivity and specificity of the Hetasca test with conventional worm and EPG counts. The study included nine groups of 12-week-old SPF birds: one unchallenged control group, four groups challenged with Ascaridia galli, and four groups challenged with Heterakis gallinarum, each at varying infection doses and inoculated over nine consecutive weeks. Additionally, field samples collected from commercial operations in multiple countries were assessed using the Hetasca test to determine its applicability under practical farm conditions.

The Hetasca serologic test demonstrated markedly higher sensitivity than traditional diagnostic methods, consistently detecting infections earlier. It also identified helminth infections on farms previously considered negative based on EPG results and necropsy findings. Field testing in broiler breeders revealed substantial levels of roundworm exposure across all age groups, with infection prevalence increasing notably in birds older than eight weeks. The detection of maternal antibodies in day-old chicks further indicated vertical antibody transfer from grandparent flocks. A strong association was also observed between roundworm infections and Histomonas meleagridis, as all Histomonas-positive flocks also tested positive with the Hetasca assay.

Overall, this study demonstrates that the Hetasca serologic test is a highly sensitive tool for the early and accurate detection of roundworm infections in poultry. Its ability to reveal hidden infections, alongside the documented links to Histomonas meleagridis and potential implications for colisepticaemia, highlights the importance of robust worm control programs. These findings contribute valuable insights into the epidemiology of roundworm infections and support improved health management practices for commercial layer and breeder operations.

Keywords: Serology; Ascaridia galli; Heterakis gallinarum; Roundworm infections; Hetasca test

Nutrition: Amino Acids and Protein IV

3121 Reducing dietary crude protein in laying hens has a different effect on egg weight depending on the provision on amino acids

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This study evaluated the impact of the variation of crude protein (CP) level together with dietary digestible amino acids (AA) on laying performance through a meta-analysis. In practical diet formulation, there are several ways to reduce dietary CP: by maintaining or not the amount of digestible essential AA such as lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp), valine or isoleucine. The aim of this study was therefore to compile internal Mixscience experiments and to quantify the effect of the two different strategies of CP variation on laying hen performance considering egg mass, laying rate, feed intake, egg mass output, downgraded eggs and feed conversion ratio (FCR) at the beginning (under 30 weeks of age), intermediate and end (after 50 weeks of age) of the laying period. The database included 26 experiments conducted between 2005 and 2025, in white laying hen (20%) or brown (80%). Experiments took place in our research station in France, and they were equally distributed between cold and hot season and between the 3 different laying periods. The database was divided in two situations: when the level of digestible AA (Lys, Met, Thr, Trp…) varies together with the protein level; or when the level of digestible AA was control and didn't vary with the protein level. In all experiments, groups of crude protein variation were compared to the best treatment, and the laying performance were expressed as a relative percentage of this treatment. Data analysis involved assessment of variance using mixed-effects meta-analysis models. Results showed that the laying rate, the FCR or egg mass output were not significantly influenced by the variation of crude protein levels, and the percentage of downgraded eggs was significantly reduced (mostly dirty eggs), whatever the crude protein strategy. The reduction of crude protein by maintaining essential AA allows reduced feed cost and carbon footprint (by 7 points) without significant impact on laying performance. Contrary to the reduction of crude protein without control of Lys or Met led to a reduction of 2 percents of the egg weight for a crude protein reduction of 3 points. In conclusion, the level of egg weight depends more on level of dietary AA than on the level of dietary CP.

Keywords: Laying hen; Meta-analysis; Protein; Amino acid; Egg

3122 Effects of low-protein diet on production performance, egg quality and ovarian function of yellow-feathered broiler breeders

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Objective: This experiment aims to explore the effects and mechanisms of low-protein diet on the production performance, egg quality and ovarian function of yellow-feathered broiler breeders.

Experimental design: A single-factor design comprised two treatments: a normal protein (NP) diet and a low protein (LP) diet, with the LP diet containing 3% less crude protein phase-wise (growing: 12% vs. 15%; pre-lay: 13% vs. 16%; laying: 13% vs. 16%). The LP diet was supplemented with Lys, Met, Thr, and Trp to match NP levels.

Materials and methods: A total of 300 11-week-old yellow-feather broiler breeders were randomly divided into two treatment groups: control (NP) and the low protein group (LP), with 10 replicates per treatment and 15 birds per replicate, which lasted for 23 weeks.

Statistical analysis: data were analyzed using one-way ANOVA and Turkey's multiple comparison test in SAS 9.2.

Results: The LP group exhibited increased eggshell thickness, relative eggshell weight, and relative yolk weight ($P < 0.05$), while the relative albumen weight decreased ($P < 0.01$). In the albumen of the LP group, a decrease in the relative methionine (Met) content ($P < 0.01$) and an increase in proline (Pro) ($P < 0.01$) were observed. In the yolk, crude protein content increased, along with the relative levels of essential amino acids (Ile, Leu, Met, Phe, Thr, Val; $P < 0.001$) and non-essential amino acids (Gly, Ser, Ala, Asp, Cys, Glu, Pro, Tyr; $P < 0.01$). The yolk of the LP group also showed an increase in C18:1n9c ($P < 0.05$) and decreases in crude fat, C17:0, C18:1n9t, C18:3n3, and C22:6n3 ($P < 0.05$). The LP group exhibited decreased levels of progesterone ($P < 0.05$) and anti-Müllerian hormone (AMH) ($P < 0.01$), while Insulin-like

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Growth Factor 1 (IGF-1) was increased ($P < 0.05$). Furthermore, the LP group showed increases in histidine (His; $P < 0.01$), serine (Ser), and alanine (Ala) ($P < 0.05$), along with decreases in isoleucine (Ile) and valine (Val) ($P < 0.01$). Differential gene expression (DGE) analysis between the NP and LP groups revealed 146 significantly up-regulated and 396 significantly down-regulated genes in the ovarian tissue of the LP group. Low-protein diets have triggered ovarian oxidative stress in laying hens and have activated self-protective antioxidant mechanisms. Meanwhile, these diets have also diminished ovarian sensitivity to reproductive hormones. They have hindered normal follicular development and tissue remodeling. Consequently, these factors have led to impaired ovarian function. In conclusion, a low-protein diet has induced ovarian oxidative stress and reduced hormone sensitivity, hindering follicular development. This has impaired ovarian function and altered nutrient deposition in eggs.

Keywords: Low protein; Broiler breeder; Reproduction performance; Ovarian function

3123 Cage-Free Laying Hen responses to graded levels of dietary crude protein reduction

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The global demand for cage-free eggs has increased due to consumer concern for animal welfare, prompting the poultry industry to adapt housing and nutritional management systems. However, information on the nutritional requirements of layers, reared under alternative systems remains limited. This study aimed to determine the crude protein (CP) level required for maintenance and egg production in commercial laying hens from 33 to 49 weeks of age, housed in a cage-free system. A total of 540 Hy-Line Brown hens, were distributed in a completely randomized design with five dietary treatments (14, 15, 16, 17, and 18% CP), six replicates, and 18 birds per replicate. Diets were isoenergetic (2850 kcal/kg) corn–soybean meal based. Productive performance (egg production, feed intake, feed conversion ratio, egg mass), egg quality (weight, shell strenght, albumen height, Haugh unit, yolk color, shell thickness, yolk index), and hematological parameters (hematocrit, relative and absolute white blood cell count, heterophil:lymphocyte ratio, total plasma protein) were evaluated over four 28-day production cycles. Differences were tested by one-way ANOVA, followed by Tukey test, with the Statistical Software SAS 9.4. No significant differences ($p>0.05$) were observed among treatments for egg production, feed intake, feed conversion ratio, egg mass, or the proportion of saleable and unmarketable eggs. Among the quality parameters, only egg weight showed a significant effect ($p<0.05$), with the highest values obtained at 16 and 17% CP. Hematological variables were not significantly influenced by dietary protein level ($p>0.05$). Under the experimental conditions, it can be inferred that dietary CP can be reduced to 14% without adverse effects on performance or egg quality of cage-free layers. This finding supports strategies aimed at lowering feed costs and nitrogen excretion while maintaining productive efficiency and sustainability in alternative production systems.

Keywords: Laying hens; Cage-free; Crude protein; Egg quality; Welfare

3124 Improving Albumen Quality and Economic Efficiency in Laying Hens: The Role of Glycine and Serine in Low-Crude Protein Diets

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Reducing dietary crude protein (CP) in poultry nutrition is critical for sustainability, yet maintaining hen productivity remains a challenge. We hypothesized that supplementing glycine (Gly) and serine (Ser) in low-crude protein (LCP) diets would maintain albumen quality and production performance while reducing feed costs in laying hens. A completely randomized design was employed with 560 Hy-Line Brown hens (24 weeks-old) allocated to eight dietary treatments. The diets included one LCP diet (14.50% CP), a positive control (PC) diet (16.50% CP), and six LCP diets supplemented with varying levels of Gly or Ser (0.067%, 0.134%, and 0.20%). Data collected over 12 weeks included production performance, egg quality, amino acid (AA) profiles in eggs and blood, blood biochemistry, and histology and gene expression in ileum and magnum tissues. Data were analyzed using one-way ANOVA in SAS with Tukey's post hoc test. The LCP diets significantly decreased egg weight and egg mass ($P < 0.05$). However, supplementation with 0.20% Gly increased these parameters compared to the PC diet ($P < 0.05$). Albumen height and amino acid content were also improved in the LCP + 0.20% Gly group ($P < 0.05$). Furthermore, 0.20% Gly enhanced the ileal villus height to crypt depth ratio ($P < 0.05$), indicating improved gut health and nutrient absorption. Gene expression analysis showed that both 0.20% Gly and 0.20% Ser downregulated key genes and reduced phosphoserine phosphatase (PSPH) levels ($P < 0.05$). Metabolically, these supplements increased certain non-essential AAs in blood ($P < 0.05$), suggesting better nitrogen utilization. Economically, the LCP diet reduced feed cost by 40 RMB/ton, and adding 0.20% Gly to the LCP diet saved an additional 14 RMB/ton. Supplementing LCP diets with 0.20% glycine effectively restores egg production, improves egg quality and gut health, enhances nitrogen metabolism, and reduces feed costs in laying hens.

Keywords: Glycine; Serine; Low-crude-protein-diets; Albumen quality; Economic-efficiency

3125 Digestible valine requirement of laying hens in the peaking phase

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The present study aimed to determine the standardized ileal digestible valine (SID Val) requirement of laying hens during the peaking phase. Three hundred sixty Hy-Line W36 layers in the peak egg production phase (28 to 40 weeks of age) were used in a completely randomized design with six treatments and five replicates of 12 birds. The six dietary treatments were graded levels of SID Val (%): 0.64 (basal diet), 0.67, 0.70, 0.73, 0.76, and 0.79. During twelve weeks of the experiment, egg numbers and mortality of the hens were recorded daily, egg weight was determined twice weekly, and feed intake was measured weekly. Egg mass and feed conversion ratio (FCR) were then calculated.

The hens receiving the basal diet had the lowest performance, and the graded increase in the dietary SID Val up to the level of 0.76% improved egg weight ($p<0.01$), egg mass ($p<0.05$), and FCR ($p<0.01$). Based on regression analysis, the response curves for egg weight and egg mass showed the best fit with the linear broken line model (LBL), while the quadratic polynomial (QP) model showed the best fit for FCR. The estimated SID Val requirement based on egg weight ($R2=0.764$) and egg mass ($R2=0.801$) responses by the LBL model was 0.731%. Using FCR as a response, the SID Val requirement for minimum FCR was estimated ($R2=0.854$) to be

0.757%. In conclusion, the SID Val requirement of laying hens was estimated to be 0.73 to 0.76% in the peaking phase.
Keywords: Valine; Laying hen; Performance; Requirement; Peak

3126 Role of Branched-Chain Amino Acid (Leucine, Isoleucine and Valine) on Performance and Egg Quality in Peak and Late-lay Hy-Line W-36 Hens

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The objective of this study was to investigate the relationship among BCAA on the performance of Hy-Line W-36-laying hens during both peak and late production. The study was designed as a 23 full factorial circumscribed central composite design (CCD) with 20 treatments (14 treatments and 6 center points). For the peak age and late lay study, hens were utilized from 20 to 35 woa and 67-83 woa, respectively. In the peak phase, each treatment consisted of varying dig. Val:Lys (64 to 104), Ile:Lys (55 to 95), and Leu:Lys (120 to 220) ratios (dig. Lys was formulated at 0.8%). In late phase, the values were dig.Val:Lys 67 to 101; Ile:Lys 58 to 92 and Leu:Lys 120 to 220 (dig. Lys was formulated at 0.7%). Performance variables like hen-day egg production (HDEP), feed intake (FI), feed conversion ratio (FCR), egg mass and egg quality were analyzed. At the end of each study, tibia bone strength and tibia ash were analyzed. Data were analyzed as a CCD using the surface response option of JMP v. 18 with means considered significant at $P < 0.10$. In the peak phase, FI was significantly increased (103.63g/bird/day; P =0.0122; R2 =0.80) at moderate levels of Val:Lys (87.13) and Ile:Lys (74.84) and the lowest level of Leu:Lys (120) ratios. In the late lay phase, FI was significantly increased (115.2 g/bird/day; P=0.0981; R2 = 0.68); however, FI was decreased when Val:Lys (67) and Ile:Lys (58) were decreased while increasing Leu:Lys (220). Hen-day egg production in peak phase significantly increased (91.8%; P =0.0050; R2 =0.70) at high ratios of Val:Lys (91.05), moderate levels of Ile:Lys (75.07) and Leu:Lys (183.91). Also, the egg mass in peak phase was significantly increased (51.84 g; P =0.0030; R2 =0.85) at moderate levels of Val: Lys (84.07) and Ile: Lys (89.9) and the lowest Leu:Lys (120) ratios. There was a quadratic effect of Ile and Leu and an interaction of Ile and Leu on Haugh unit in late phase (89.69; P= 0.0159; R2 = 0.80). For the albumen weight, there was an interaction of Val and Ile, and Ile and Leu in the peak phase (67.3%; P=0.0230 and R2 = 0.77) and later (65.56%; P= 0.0142; R2 = 0.80). Tibial breaking strength was also significantly increased in the late-lay phase (217.97 N; P=0.0853; R2 =0.69) with a linear and quadratic effect of Val only. For both peaking and late-lay, FI was negatively influenced as Val:Lys decreased in the diet. When both Val:Lys and Ile:Lys decreased HDEP, HDEM, and egg weight were negatively influenced, but only in the peaking phase. Adjusting the Val:Lys and Ile:Lys ratios for both peaking and late-lay phases accordingly can help to maximize performance and egg quality throughout the life of the hen, particularly when Leu:Lys increases.
Keywords: branched-chain amino acids; central composite design; laying hens; performance

3127 Effect of algae meal inclusion on growth performance and meat quality of slow-growing Boschveld chickens

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This study aimed to investigate the effects of blue-green algae (Spirulina platensis) meal inclusion on the meat quality of Boschveld chickens aged 91 days. A total of 128 male indigenous Boschveld chickens were allocated to four dietary treatment levels (0, 10, 15 or 20 g per kg DM of diet) in a completely randomized design, replicated four times with eight chicks per replicate. The collected data were analysed using the Statistical Analysis System version 9.4. A quadratic equation was used to determine the optimal inclusion of dietary algae meal. Where there was a significant difference ($p<0.05$) of algae level as the main effect, Tukey's honestly significant difference test (HSD) was used for mean separation. The carcass weights and meat cut-up yields were assessed. Thereafter, carcass and meat parts' weights were measured using an electronic weighing scale. Meat parts' pH was measured using the digital pH meter (Crison, Basic 20 pH Meter). The shear force was determined using Warner-Bratzler Shear Force, meat colour was measured using HunterLab Test. For sensory attributes, meat samples were frozen at -20 °C and later allowed to be defrosted for 24 hours in a cooler room. A pre-heated oven was set to 160 °C and used to cook the breast meat. The meat sample of 1.5 cm thickness was boiled for approximately 50 minutes and turned over every 25 minutes. Tongs were used for turning to avoid piercing the meat, which could lead to moisture escaping. A taste panel of 25 assessors was used to evaluate the meat for tenderness, juiciness, flavour, and overall acceptability by using a 5-point scale. The findings revealed that algae meal inclusion levels have an effect ($p<0.05$) on the growth rate and feed conversion ratio (FCR) of Boschveld chickens aged 60 to 91 days. Algae inclusion levels had an effect ($P<0.05$) on carcass, breast, and abdominal fat weights of indigenous Boschveld chickens aged 91 days. Meat parts weights increased with an increase in algae inclusion levels. Algae inclusion levels had no effect ($P>0.05$) on meat colour, breast, drumstick, and thigh pH, meat sensory attributes, shear force, and cooking loss of Boschveld chickens. In conclusion, when formulating diets for Boschveld chickens, it is essential to consider specific parameters of interest and recognize that breed lines are continuously improved through efficient breeding practices.
Keywords: Growth; Meat quality; Algae; Indigenous chickens; Protein source

Nutrition: Feed Additives IV

3128 Effect of natural antioxidant supplemented within nano-sized vesicles in drinking water on Ross 308 broilers intestinal morphology, gut microbiota & biomarkers of adipocyte metabolism.

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Inflammation and oxidative stress are critical issues in Ross 308 as it can impact animal health & meat quality. This study evaluates the use of Nutrasome® nano-vesicle technology containing paprika, administered via drinking water, on gut morphology and microbiota using metagenomics and adipocyte metabolism biomarkers. 336 mixed-sex Ross 308 broilers were divided into 4 experimental groups of 84 birds with 12 replicates per treatment. From days 1 to 35, groups T1 (NC), T3, T4 received the same diet, while birds from T2 (PC) received a diet supplemented with 80 ppm of paprika extract without nano-vesicles. Birds in groups T3 and T4 received 80 ppm and 160 ppm of the extract in nano-vesicles through their drinking water, respectively. On day 35, the birds were weighted to evaluate their zootechnical performances. 144 animals, 36 broilers/treatment were randomly selected for the following measurements: Duodenum histology was examined in 10 animals per treatment, with 10 villi and 20 crypts per animal; Caecal microbiota were examined in 10 animals per treatment for sequencing and quantification on 16S rDNA; 30 adipose depots from T1 and T4 were

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collected: subcutaneous adipose tissue, adipose tissue from the neck and visceral adipose tissue. One piece of each tissue was cultured in a specific medium and analysed after 24h. Adipocytes and muscle tissues were observed in 3D with LFSM.
A significant effect on EBI was observed between T1 (478) and T2 (430) due to higher mortality (P<0.01). No significant effects on EBI were observed in the other treatments. Histology reveals that the villus surface area differs significantly between the treated groups and T1 or T2 (P<0.001). The crypt depth is different between the treated groups and T1 or T2 (P<0.001). Cecal microbiota analysis, is still under investigation. Adipocyte secretion analysis revealed that IL-6 and pro-collagen 1 concentrations in T4 were significantly lower than in T1 (P<0.01), indicating an improvement in fibroinflammation. Similarly, levels of released glycerol, which reflect lipolytic activity, were reduced in T4, particularly in subcutaneous adipose tissue. The decrease in basal lipolysis associated with the reduced fibroinflammation could contribute to a better control of free fatty acid efflux and favoring an adequate muscle glucose utilization and glycogen storage. LFSM approach enables high-resolution volumetric imaging of samples, and provide new insights into cellular structure and dynamics. These results confirm the potential of Nutrasome® to improve intestinal health & reduce systemic inflammation, which could explain the previously reported reduction in severe cases of myopathies (white stripping/ wooden breast) in Ross 308.
Keywords: Ross 308; Gut health; Inflammation; Antioxidant; Nano-vesicle

3129 Saponine: Same same but different! Comparative study of 3 saponin formulas in managing coccidial risk in broiler chicken

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The overuse of anticoccidial feed additives in poultry production has resulted in resistance development and the presence of residues in both animal-derived products and the environment. Plant-derived alternatives, particularly those rich in saponins, have emerged as promising tools to support coccidiosis control in broilers. Yet, because saponins differ across plant species in structure, concentration, and biological activity, proper characterization and standardization are essential to ensure reliable outcomes.
This work investigated the effect of different saponin-based feed additives on broiler performance under an experimental coccidiosis challenge.
A total of 1,020 day-old Ross 308 chicks were randomly assigned to six groups (6–7 replicates per group):
UUC (n=180): uninfected, untreated control;
IUC (n=210): infected, untreated control;
P1 (n=210), P2 (n=210), P3 (n=210): infected and fed one of three saponin-based formulations (1, 2, or 3) at 250 g/ton from day 7 to day 42.
The additives, derived from various botanical sources, were standardized to contain 6% total saponins. Birds were challenged orally at 14 days of age. Growth traits (body weight, feed intake, feed conversion ratio [FCR], and mortality) were recorded, and intestinal lesion scores (ILS) were assessed on day 21 (two birds/ replicate).
Analysis with GraphPad® Prism (v8.0.2; $p < 0.05$) confirmed the challenge model, with infected controls (IUC) showing reduced body weight (1407 g vs. 2267 g) and poorer FCR (1.8 vs. 1.4) compared with uninfected controls (UUC). At the intestinal level, significant reductions in ILS were observed only with P2 and P3. Regarding performance, group P3 achieved higher body weight than IUC and showed the best overall results (2241 g; 1.42) compared with P1 (1623 g; 1.50) and P2 (1641 g; 1.54). All three supplements improved FCR relative to IUC. No mortality differences were detected among groups.
These findings indicate that saponin-based additives can act as stand-alone tools for coccidiosis management in broilers. However, the efficacy varies with the botanical source, underlining the importance of standardization. Validation under field conditions is required to support these results.
Keywords: Coccidiosis; chicken; Eimeria; botanicals

3130 Phytogenic supplementation effects on live performance, intestinal permeability and metabolomics in broilers

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Currently, the ban of antibiotics used to enhance growth in poultry production, has driven the development of new potential alternatives in Antibiotic Free (ABF) programs. This study aimed to evaluate the effects of dietary supplementation with phytogenic additives (Bedgen® 40 Plus (Bedg): Chicory, artichoke and choline chloride and Cloxitam® (Clox): Cinnamon and oregano) compared to Zinc Bacitracin (ZnB) in broilers. A total of 840 one-day-old Cobb 500 male chicks, were allocated into 6 experimental treatments with 10 replicates of 14 birds each, in a completely randomized design. Birds were fed under three feeding phases: starter (1-21d), grower (22-35d) and finisher (36-41d). Dietary treatments were: Negative control, no additives (T1); Positive control (Basal diet + 55g/ton of ZnB) (T2); Bedg at 150 g/ton (1-21d) (T3); Bedg at 150 g/ton (1-21d) + 55g/ton of ZnB (22-41d) (T4); Bedg at 150 g/ton (1-41d) (T5); and Clox at 100 g/ton (1-41d) (T6). Live performance data (feed intake (FI), body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) was evaluated at 7, 14, 21, 35 and 41d of age. At 21 and 41d, 10 birds/treatment were euthanized by cervical dislocation to measure intestine length and weight, additionally, samples of jejunum and ileum, were collected to measure villi height (VH), crypt depth (CD), villi width (VW), VH/CD ratio. At the same days, 6 birds/treatment were orally gavaged with FITC-Dextran to asses intestinal permeability, and samples of cecum content were collected to measure volatile fatty acids (VFA), Enterobacteriaceae (EB)/lactic acid bacteria (LAB) ratio and metabolomics. At 42 days, 20 birds/treatment were slaughtered to obtain carcass, breast, drumsticks, thighs and wings weight. Differences were tested by one-way ANOVA, followed by Tukey test ($p<0.05$), while EB/LAB ratio were analyzed using Mann-Whitney/Kruskal-Wallis statistical methods with a significance threshold of ($p<0.05$). On 1-41d period, T5 showed the best overall performance (BW, BWG, and carcass yield) ($p<0.001$), while T3, T4 and T6 were better than T2 in all the performance parameters ($p<0.001$). At 41d, T4 and T1 had the higher VFA concentration, T2, T3 and T6 has an intermediate response and T5 has the lowest levels ($p<0.001$). In jejunum VW and VH/CD ratio at 21d and in ileum VH and VH/CD ratio at 42d, were better in T5 ($p<0.05$). T5 showed a positive correlation between EB/LAB at 21d. T3 and T6 exhibited metabolomic profiles similar to the antibiotic group at 21 and 41d, being the second one the most prominent. There were no statistical differences on intestine weight, length and permeability. In conclusion, Bedg and Clox, could be used as notorious alternatives to antibiotics in ABF programs.

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Keywords: Broiler life performance; Cecum metabolomics; Intestinal permeability; Phytogenic additives; Antibiotic free programs

3131 Bacteriophage supplementation on performance and myostatin gene expression in broilers

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The effect of dietary supplementation of two different level of bacteriophage cocktail on growth performance, nutrient utilization, carcass characteristics, real time expression profile of Myostatin (MSTN) gene, nutrient composition of meat, serum biochemical profile and economics of broiler production was investigated. 100, day old VENCOBB 400 broiler chicks were randomly distributed into 4 experimental groups, each consisted of 5 replicates of 5 chicks each. The standard broiler diets (T0) were formulated as per Cobb chick feed specification. Experiment was conducted for 6 weeks. Individual body weight and replicate-wise feed intake of broilers were recorded at weekly interval and feed to gain ratio was calculated. The nutrients utilization was studied by conducting a metabolic trial. On 35th day birds were sacrificed for performing carcass traits, nutrient composition of breast muscle, real time expression profile of Myostatin gene and serum biochemical parameters. Statistical analysis of the data was done by using of SPSS2022 software for analysis of varaiance using completly randomised design. Difference among the treatment were tested for significance by Duncan's Multiple Range Test.

The overall performance of broilers indicated that supplementation of bacteriophage cocktail with at 106 pfu/ml/bird in drinking water significantly ($p<0.05$) improved their overall performance (body weight, body weight gain, feed to gain ratio and production efficiency factor) during starter and finisher phase. Results indicated that digestibility of protein was improved significantly ($p<0.05$) in broilers supplemented with bacteriophage with at 106 pfu/ml/bird as compared to control group. However, feed intake, digestibility of nutrients (dry matter and ether extract), carcass traits (except for liver weight), nutrient composition of meat serum concentration of SGOT, SGPT, total cholesterol, LDL cholesterol and HDL cholesterol were not affected by bacteriophage supplementation in broilers. All the treatment groups showed more or less downregulation of myostatin gene after 30 days trial with maximum downregulation observed in broilers supplemented with bacteriophage with at 106 pfu/ml/bird (0.10 fold). However, in all the treatment groups this downregulation was statistically non-significant ($p<0.05$). Better feed cost/kg gain was observed in broilers supplemented with bacteriophage cocktail with at 106 pfu/ml/bird.

Thus, on the basis of the present study, it could be concluded that supplementation of bacteriophage cocktail with at 106 pfu/ml/bird in broilers significantly ($p<0.05$) improved their growth performance and thus can also be used as a substitute for antibiotic growth promoter in broiler diets.

Keywords: Bacteriophage; Performance; Nutrients utilization; Broiler; Gene Expression

3132 Growth performance and economic benefits of finisher broilers fed sundried false yam (Icacina tricantha) meal based diets.

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This study was conducted to evaluate the replacement value of sun-dried false yam meal (SFYM) for maize on the growth performance and economic returns of finisher broilers. A total of 150 four weeks old broiler chicks were randomly assigned to five dietary treatments (1,2, 3, 4 and 5) with 30 birds per treatment group. Each treatment was replicated three times in a completely randomized design (CRD) with 10 birds per replicate. Five experimental finisher diets (1, 2, 3, 4 and 5) were formulated and diet 1 served as the control which contained 100% maize and 0% SFYM, while in diets 2, 3, 4 and 5, SFYM was used to substitute maize at 4, 8, 12 and 16% inclusion levels respectively. All the diets were formulated to be isonitrogenous (21 and 19%) and isocaloric (2800 and 2800 Kcal/kg ME). Data generated were subjected to a one-way analysis of variance (ANOVA) and differences between means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of probability. The results indicated that the replacement of maize with SFYM significantly ($P<0.050$) affected the growth performance of broiler chickens with respect to the average live weight, daily weight and feed intake as well as cost of feed/kg weight gain (N/kg) and net profit (N/bird), while the feed conversion ratio (FCR) and protein efficiency ratio were not. Specifically, birds that consumed 4% SFYM based diets had better ($P<0.05$) average live weight, weight gain, feed intake, cost of feed/kg weight gain (N/kg) and net profit (N/bird) compared to other test diets. Therefore, sun-dried false yam meal can successfully be included in broiler ration up to 4% level without any adverse effect on growth performance and economic returns of broiler chickens.

Keywords: Broilers; false yam; growth; processing; substitution

3133 Extruded corn impacts performance and egg quality of Dekalb laying hens in peak laying phase

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An extruded corn (EC) product was evaluated in the peak laying phase of Dekalb white hens for performance and egg quality effects. Three corn-soybean meal diets were formulated with ground corn (CON), EC with 1000-micron particle size (E1000), and EC with 1200-micron particle size (E1200) to be isocaloric and meet or exceed nutritional requirements. Each diet was fed to 15 cages/treatment with 7 birds/cage for a total of 315 hens. Bird weight and egg quality were taken at 30-wks of age for a baseline measurement prior to a 1-wk adaptation to the diet. Measurements of BW, FI, and egg production were collected weekly for 4 wks and used to calculated HHEP, egg mass, and FCR. All eggs were collected one day/ wk for 4-wks for egg quality analysis on a DET6000: egg weight, Haugh units, yolk color, breaking strength, eggshell thickness, and eggshell weight. Bird BW was significantly lower for E1000 and E1200 compared to CON for all 4 wks ($P < 0.05$); overall, the CON BW of 2.06 kg was significantly greater than E1000 and E1200 BW of 1.92 and 1.92 kg ($P < 0.0001$). FI was significantly lower for E1000 and E1200 compared to CON in wk 1 and 2 ($P < 0.05$); overall, the CON FI of 0.12 kg/bird was significantly greater than E1000 and E1200 FI of 0.10 and 0.11 kg/bird, respectively ($P < 0.0001$). Overall HHEP was significantly lower for E1000 at 59.9% compared to CON with 81.4% ($P = 0.0099$), while E1200 was similar to other treatments at 68.2%. Egg mass was significantly lower for E1000 and E1200 at 35.2 and 39.4 kg, respectively, compared to CON at 50.8 kg overall ($P = 0.0018$). Overall FCR was significantly lower for CON at 2.33 compared to E1000 at 3.16 ($P = 0.0124$), with E1200 FCR similar to other treatments at 2.77. Egg weight was significantly heavier for CON at 64.5 g overall compared to E1000 and E1200 at 61.0 and 60.9 g ($P < 0.0001$). Yolk color was significantly lighter by approximately 1 unit for E1000 and E1200 compared to CON for wk 1-4 ($P < 0.05$). Eggshell thickness was significantly thicker for CON at 0.30 mm in wk 1 compared to E1000 and E1200 at 0.26 and 0.26 mm ($P = 0.0130$). Eggshell weight was significantly lighter for E1000 and E1200 at 5.12 and 5.43 g in wk 1 compared to CON at 6.28 g

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(P < 0.0001). In wk 3, eggshell weight of E1200 was significantly lighter than CON (6.03 g vs 6.54 g, respectively; P = 0.0058). There were no significant differences in Haugh units or breaking strength at any timepoint. Replacement of ground corn with EC decreased performance overall, which could be related to texture changes, however there was a recovery of performance by wk 4 without negatively affecting egg quality.
Keywords: extruded corn; gelatinization; particle size; peak laying phase; yolk color

3134 Supplementing broiler diets reduced in energy with a proprietary lysolecithin blend optimally balanced in poly- & mono-unsaturated fatty acids combined with a synthetic emulsifier and monoglycerides improves performance, flock uniformity, profitability, and digestibility of tryptophane and cysteine

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Increasing energy costs in broiler feed formulations are highlighting the need for nutritional interventions that lower feed cost and optimize profitability. Supplementing a proprietary lysolecithin blend optimally balanced in poly- & mono-unsaturated fatty acids combined with a synthetic emulsifier and monoglycerides (LEN) to broiler diets reduced in AME was investigated to determine its effect on performance, flock uniformity, and ileal digestibility of amino acids. A total of 320 one-day-old, male Ross 308 broiler chickens were randomly distributed to 4 dietary treatments for 42 days: a control basal diet (PC); PC reformulated to reduce AME 100 kcal/kg (NC); and NC + 250 mg/kg of LEN (NC+LEN250); and NC + 250 mg/kg of LEN (NC+LEN500). Each group consisted of 8 pens with 10 birds each. Birds were fed diets containing phytase (500 FTU/kg). Performance parameters were recorded per feeding phase and for the overall trial. Flock uniformity was assessed for each pen at 24 and 42 d. On day 42nd, 7 bird per pen (56 birds per group) were randomly selected to determine the ileal digestibility of amino acids. To assess the profitability of LEN supplementation, the income over feed cost (IOFC) for each treatment was calculated. Data were analysed using one-way ANOVA under the General Linear Model (GLM) framework, and means were separated using Tukey's HSD test. Birds from PC and NC+LEN500, compared to birds from NC showed better FCR (PC, 1.504 vs. NC, 1.547 vs. NC+LEN500, 1.52; p<0.0001), and flock uniformity (PC, 92.34 vs. NC, 88.69 vs. NC+LEN500, 91.82 %; p=0.003). Digestibility of tryptophan was higher for NC+LEN500 compared to NC (62.12 vs. 68.41 %; p=0.022) and cysteine was higher for PC and NC+LEN500 compared to NC (PC, 60.62 vs. NC, 53.82 vs. NC+LEN500 60.22; p=0.0191). The IOFC values for the NC+LEN250 and NC+LEN500 treatments exceeded those of the PC group by 19 and 51 €/1,000 birds, respectively, and were 76 and 108 €/1,000 birds higher than the NC group. In conclusion, birds receiving LEN at 500 mg/kg showed better FCR, enhanced flock uniformity, and increased ileal digestibility of tryptophan and cysteine compared to the negative control. Profitability was markedly higher, with IOFC gains up to 108 €/1,000 birds. These findings demonstrate that LEN supplementation is an effective nutritional strategy to offset energy reductions, optimize feed efficiency, and support profitability under rising energy costs.
Keywords: Broiler; Performance; Digestibility; Profitability; Uniformity

3135 Comparative Study on Oral Absolute Bioavailability and Intestinal Residues of Benzoic Acid Preparation versus Benzoic Acid in Broilers

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This study compared the pharmacokinetic profiles, bioavailability, and intestinal residues of a benzoic acid preparation and pure benzoic acid in broilers. The oral absolute bioavailability was evaluated in 28 healthy broilers randomly assigned to seven groups: low- (5 mg/kg BW), medium- (20 mg/kg BW), and high-dose (50 mg/kg BW) oral benzoic acid; low- (5 mg/kg BW), medium- (15 mg/kg BW), and high-dose (40 mg/kg BW) oral benzoic acid preparation; and intravenous benzoic acid (4 mg/kg BW). Blood samples were collected from 0.083h to 72h after administration. Pharmacokinetic parameters were derived using Winnolin 8.1.0. For oral benzoic acid, mean Cmax, Tmax, T1/2, and AUC0-∞ ranged 0.79-27.99 mg/L, 0.75 h, 3.8-10.67 h, and 4.9-48.7 h.mg/L, respectively. The AUC0-∞ of intravenous benzoic acid was 4.1 mg.h/L, yielding an absolute oral bioavailability of 92.02%-95.03%. For the benzoic acid preparation, mean Cmax, Tmax, T1/2, and AUC0-∞ were 0.09-0.39 mg/L, 0.75-1 h, 2.8-4.7 h, and 0.3-2.6 h.mg/L, respectively, with an absolute oral bioavailability of 5.69%-6.46%. Results indicated that benzoic acid was rapidly and extensively absorbed, whereas the benzoic acid preparation showed low systemic absorption, fast peak attainment, short residence time, and rapid elimination—characteristics offering significant advantages over conventional benzoic acid in feed applications.

In the residual study, benzoic acid in intestinal contents and tissues was quantified via HPLC using ethanol and HCl as extractants. The LOD and LOQ in broiler tissues were 0.1 μg/g and 0.5 μg/g, respectively, with a mean recovery of 87.9% ± 3.7%. A total of 110 50-day-old broilers were divided into two groups, fed either 1000 mg/kg benzoic acid (control) or 330 mg/kg benzoic acid preparation (treatment) for 26 days. Contents from the distal quarter of the small intestine and breast muscle were sampled on days 7, 14, 21-28 post-feeding, the data were processed using SPSS software. Intestinal benzoic acid levels were consistently higher in the benzoic acid preparation group, with statistically significant differences on days 14($p=0.042$) , 21($p=0.008$), and 21-28($p\leq0.05$) post feeding period. Withdrawal on days 27-28 led to declining residues in both benzoic preparation and benzoic groups[VG1] . No benzoic acid was detected in breast muscle at any timepoint. These findings confirm that the benzoic acid preparation effectively maintains high intestinal concentrations of benzoic acid without tissue accumulation. This targeted release characteristic offers dual benefits: enhanced animal safety through minimized toxicity potential and improved economic efficiency via reduced dosage requirements.
Keywords: benzoic acid; Bioavailability; Poultry; Small intestine; Feed additive

3136 Hibiscus sabdarifa L used as functional feed additives on laying hens' egg quality, fatty acid profile and oxidative stability

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Hibiscus sabdarifa L commonly known as 'Roselle' locally this plant recognized as 'Mesta', 'Chukur' or 'Tokfol'. Its leaves are consumed as vegetable and soup not only in Bangladesh even some developed country like Japan and Korea. Mesta is used as a folk medicine to the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis. Furthermore, special health promoting components like n-3 fatty acids and many non-nutrient components are presence on these medicinal plants for this reasons researcher pay attention to produce value added product from Mesta. Mesta

leaf can be utilized as feed additives for producing value added product in poultry sector, we must mitigate the scarcity of feed for livestock sector. Plants have provided nourishment and other health benefits to humans and animals for as long as they have existed. This study examined the effects of dietary incorporation of Hibiscus sabdarifa L (Mesta) leaf meal in the diet of commercial laying chickens on the quality, yolk cholesterol and fatty-acid profile of eggs. One hundred twenty-eight birds were split into four dietary groups, with 32 birds in each group, comprising four replications with eight birds per replication. The experiment lasted 16 weeks and was conducted at the open houses shed in the regional station Rajshahi of Bangladesh Livestock Research Institute. The four dietary treatments were formulated using basal feed as follows: control (T1), Mesta 1% (T2), Mesta 1.5% (T3) and Mesta 2.0% (T4). The analytical measurements were done one-way ANOVA with the SPSS statistical package (IBM Corp., 27.0). in triplicates, and the results were presented as the average of 3 analyses ± the standard deviation (SD). The quality, weight, length and width of eggs were not altered by the addition of Mesta leaf to the diet. Yolk color and eggshell breaking strength were significantly higher in the additive groups. The results showed that dietary addition of Mesta leaf meal significantly reduced ($p < 0.05$) serum, total cholesterol and triglyceride content compared to the control. Feeding this leaf at 1 and 1.5% increased omega-3 fatty-acid levels. Overall, the results indicate that the addition of 1.5~2.0% Mesta to layer feed could be an effective way to improve egg quality, fatty-acid profile, amino acid and reduce cholesterol in egg yolk.

Keywords: Laying hen; Jute; Functional feed; Cholesterol; Fatty acid

Waterfowl II

3137 Development Achievements and Technological Advances in China's Meat Duck Industry

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In 2025, China's meat duck slaughter volume exceeded 4.3 billion birds, duck meat production surpassed 10 million tons, making it the third-largest livestock and poultry meat industry in the country with an annual output value exceeding 120 billion yuan. Faced with the constraints hindering the healthy development of China's meat duck industry—including backward genetic breeding theories and technologies, particularly the monopolization of lean-type meat duck breeding stock by imported varieties, and the inability of single varieties to meet diverse market demands—significant breakthroughs have been achieved in genetic breeding theories, breeding technologies, and new variety cultivation through 13 years of research. A high-quality chromosome-level reference genome for ducks was constructed for the first time. Twenty-eight genes, including IGF2BP1, MITF, and TASP1, were found to be significantly associated with important economic traits such as body weight, feed conversion efficiency, feather color, breast muscle growth, and intramuscular fat deposition in meat ducks, among which five genes were applied in meat duck breeding. The project developed fully automatic equipment for recording free feeding and body weight development in ducks, established a duck phenotypic data acquisition system based on QR code identification and electronic tagging, and created a multiple regression model for accurately estimating traits that are difficult to measure in vivo, such as breast muscle weight, subcutaneous fat weight, and gizzard weight. Thirty-nine specialized meat duck lines with distinct characteristics were developed, and two new meat duck varieties featuring fast growth, high breast and leg meat rate, and low subcutaneous fat rate, as well as a non-force-fed Beijing roast duck special supporting line, were successfully bred, yielding remarkable economic and social benefits.

3138 Unfolding the diversity of indigenous Aseel chicken: From morphology to productivity under backyard and farm condition of Bangladesh

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Bangladesh has a rich heritage of indigenous chicken genetic resources including non-descript deshi, Aseel, Naked neck and Hilly chickens. Aseel chicken holds a significant cultural and economic value for many rural households in Bangladesh due to its morphological traits, behavioral characteristics, and resilience to adverse climatic conditions. But the population of Aseel chicken has drastically decreased in the last two decades and now at the risk of extinction in Bangladesh. Very few initiatives have been taken to explore their potentialities either backyard or farm system and conserve them in Bangladesh. So, the present study was conducted to explore the diversity of indigenous Aseel chicken from morphology to productivity under backyard and farm condition. Data on distribution pattern, management system, morphology, productive and reproductive performances were collected from backyard and farm condition of Bangladesh. A total of 100 households in backyard system and 25 farms were surveyed for this study. Qualitative data were categorized and expressed as percentage, quantitative data were expressed as mean±SE and students' 't' test was performed to compare the performances between backyard and farming system using GraphPad Prism 5.0 software. The results indicated that Aseel chicken was mainly concentrated in Brahmanbaria district of Bangladesh. Different plumage colors were observed in Aseel male and female chicken. Both sexes had yellowish eyes and reddish ear lobes, with no shank feathering and identical toe counts. Spurs were present on both legs of males, though less prominent but absent in females. Shank color was dark yellow (100%) in males, whereas females showed both dark yellow (57.15%) and yellow (42.85%) color. Comb type was pea in both sexes. Significant differences ($p<0.05$) were observed in productive and reproductive performances of Aseel chicken except the weight of male birds at 6 months (1384.62±51.25 vs 1460.56±37.45 g) and 12 months of age (2674.76±41.89 vs 2705.62±34.45 g, respectively) reared under backyard and farm condition. Age at first lay was 251.38±4.35 vs 203.85±5.58 days, average egg weight 40.92±0.62 vs 46.87±0.68 g, total number of eggs per year 36.22±1.23 vs 52.05±1.07, clutch size/year 3.58±0.03 vs 4.52±0.07, no. of eggs/clutch 9.81±0.36 vs 10.92±0.42, fertility (%) 84.36±1.18 vs 78.38±1.26, hatchability (%) 74.52±1.12 vs 69.18±1.06, hatch weight (g) 31.04 ± 0.08 vs 31.87±0.05 in backyard and farm condition, respectively. These results suggested that morphology of Aseel chicken were similar but the productive and reproductive performances of indigenous Aseel chicken significantly ($p<0.05$) increased in farm condition than that of backyard system in Bangladesh.

Keywords: Backyard poultry; rural households; clutch size; egg production; hatchability

3139 Molecular Regulatory Mechanisms of Feather Suppression Revealed by Transcriptome Analysis of the Naked Neck Trait in Chickens

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Objective

The naked neck phenotype is driven by the Na gene, which carries a 73-kb duplication. Although this structural variant has been confirmed, its regulatory impact on regional identity and develop-

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mental timing remains unresolved. This study used transcriptomics to clarify the molecular mechanism leading to feather suppression in naked neck chickens.
Experimental Design
This study conducted transcriptome analysis on 18 embryonic skin samples from naked neck chickens (Na) and wild-type (wt) chickens, covering two developmental stages (E7.5 and 9.5). A total of five comparisons were designed to assess regional (neck vs back), genetic (Na vs wt), and temporal (E7.5 vs 9.5) effects
Materials and Methods
Total RNA was extracted from embryonic skin and sequenced on an Illumina platform. Reads were aligned to the GRCg7b. DEGs was performed using DESeq2, with functional enrichment analyzed via clusterProfiler.
Statistical Analysis
Significant DEGs were defined as adjusted $p<0.05$ and $|log2FC|>1$. Enrichment analyses applied Fisher's test with Benjamini–Hochberg correction.
Results
Regional Effects
Comparisons between neck and dorsal skin showed altered positional identity, marked by reduced expression of HOX genes and other anterior–posterior regulators. Changes in ECM–receptor interaction and Hedgehog pathways suggested a cervical extracellular environment less supportive of feather placode initiation. These regional features contribute to the formation of featherless neck skin.
Genetic effects
Comparing Na and wt neck skin at E7.5 revealed only 84 DEGs, underscoring that regional identity is largely established by this stage. However, these changes were pivotal, showing enrichment for endogenous retroviral element activity and disruption of the core Hedgehog signaling pathway, directly linking the Na mutation to aberrant developmental programming.
Temoral effects
The transition from E7.5 to E9.5 in Na neck skin involved 1,076 DEGs, marking a shift from developmental patterning to skin maturation. This was characterized by the upregulation of structural genes and barrier function genes, alongside enriched pathways for cell adhesion and tight junctions. The data indicate E7.5 as the critical 'fate decision' window for the naked neck trait, while E9.5 executes the 'phenotypic construction' of a stable, featherless skin.
Conclusions
The Na mutation disrupts neck skin identity through HOX downregulation and modulates extracellular and morphogenetic signals, inhibiting feather development. Development progresses from a fate decision at E7.5 to structural execution at E9.5. This study elucidated the molecular mechanism of the naked neck trait, providing a foundation for breeding heat-tolerant poultry.
Keywords: naked neck chicken; Transcriptome; Skin Development; HOX Genes; Hedgehog Signaling

3140 Production and reproductive performance of indigenous chicken in the Western Ghats of Tamil Nadu, South India.

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Native chicken are well known for adapting to local agro-climatic conditions, hardiness, being able to use locally available feed, requiring minimal care and management with a unique flavor and taste. Peruvidai chicken is the non-descriptive native chicken of the Western Ghats of Tamil Nadu, India. The present research was undertaken to study the production and reproductive performance of Peruvidai chicken. A total of 600 day-old, unsexed Peruvidai chicken belonging to a single hatch were wing banded, weighed and reared. The birds were initially housed in a cage system until they were 4 weeks old, then they were placed in a deep litter management system for up to 72 weeks. All Peruvidai chicken were reared under standard management conditions. The body weight was significantly ($P<0.01$) higher in males compared to females. Day-old hatch weight, 6th, 8th and 10thweek body weight were 34.80 ± 1.08, 357.80 ± 13.85, 526.30 ± 21.66, 629.55 ± 21.42 g respectively. The mean body weight at 12 W, 14 W, 18 W, 20 W and 22W was 1010.86 ± 37.27, 1079.05 ± 31.93, 1821 ± 38.07, 1987.00 ± 97.63 and 2057.50 ± 105.82 g in males and the corresponding body weight in females were 794.23 ± 38.98, 873.47 ± 40.76, 1348 ± 41.27, 1442.39 ± 58.13 and 1552.06 ± 64.04 g respectively. The morphometry revealed a significant ($P<0.01$) difference between males and females in terms of full body length (58.20 ± 0.37 Vs 51.74 ± 0.73 cm), back length (15.29 ± 0.35 Vs 14.45 ± 0.28 cm), neck length
(23.75 ± 0.51 Vs 21.38 ± 0.43 cm), keel length (12.76 ± 0.11 Vs 12.27 ± 0.19 cm), shank length (10.83 ± 0.04 Vs 9.62 ± 0.23), drumstick length (16.63 ± 0.13 Vs 13.99 ± 0.43 cm), thigh length (10.72 ± 0.13 Vs 10.07 ± 0.15 cm) and breast angle (69.00 ± 0.67 Vs 62.94 ± 0.62 o) respectively. The age at first egg was 166 days. The mean hen day egg production and hen housed egg production were 17.22 ± 0.03 % and 17.00 ± 0.05 % respectively, with an average egg weight of 46.03 ± 0.13 g. A total of 500 hatching eggs were collected from the Poultry Research Station and stored at room temperature and set in an automatic setter cum hatcher. The mean fertility was 85.90 ± 1.32 %. The mean hatchability on total egg set and fertile egg set were 80.29 ± 1.20 and 69.11 ± 2.87 % respectively. Adult livability percentage was 98.90 ± 0.52. The present study provides basic information about Peruvidai chicken to develop suitable breeding programme for further improvement in economically important quantitative traits.
Keywords: Indigenous chicken; Body weight; Morphometry; Egg production; Hatchability

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3141 Maternal supplementation of guanadino acetic acid increased walking and pecking time, and decreased time sitting in broiler chicks early post hatch; with the magnitude of improvements dependent on hen age

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Broiler chicks have high energy demands to hatch, grow, and maintain body temperature in early life. Increased active behaviours are linked to better health and welfare, with reduced risk of leg injuries. Creatine supports energy storage and transport in high-demand tissues; its precursor, guanidinoacetic acid (GAA), is a stable feed supplement readily converted to creatine by hens.

This study investigated whether maternal GAA supplementation during early, peak, and late production improves energy and activity levels in chicks. We hypothesised that chicks from supplemented hens would show more active and fewer inactive behaviours due to enhanced energy availability.

Commercially housed Ross 308 broiler breeders were fed either a control diet (n=3 sheds) or control + 0.08% GAA (n=3 sheds) from

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15 weeks of age. Fertile eggs were collected and hatched from each shed at 28 (n=300), 38 (n=240), and 58 weeks (n=360). At hatch, 120 chicks (mixed sex) were allocated to 12 pens (10 birds/pen, 6 replicates/maternal diet).

Video recordings were analysed for the first 4 days post-placement using pinpoint scan sampling every 3 minutes for 30 minutes in the morning (0800–0830h) and afternoon (1600–1630h). Mutually exclusive behaviours were recorded daily and included, sitting, standing, walking, running, pecking the ground (while sitting or standing). Disturbance was recorded when a resting chick was disturbed by a walking or running chick bumping into it.

Data were analysed using mixed-effects logistic regression in R, comparing behaviour proportions by treatment. Pen was the random intercept. Hen age × GAA interaction effects were also assessed.

Maternal GAA supplementation increased active behaviours. Chicks from supplemented hens sat less (56.6% vs 59.9% p=0.002) and stood more (6.5% vs 5.4%,p=0.004). They also walked (12.5% vs 9.1%,p<0.001), ran (0.9% vs 0.5%,p=0.007), and pecked (7.5% vs 6.0%,p=0.021) more. Chicks from supplemented hens were also disturbed by other chicks less often (0.7% vs 1.2%,p=0.010) than controls.

Hen age influenced the magnitude of GAA effects. Walking increased most in chicks from 58-week-old hens (+7.0%,p<0.001) compared to 28-week (+1.3%) and 38-week (+1.6%) hens. Pecking increased in chicks from 38-week (+3.1%,p<0.001) and 58-week (+1.5%,p=0.014) hens, but not in 28-week chicks.

Maternal GAA supplementation enhanced early active behaviours, especially in chicks from older hens. These findings highlight the influence of hen age on chick health and welfare and suggest GAA may improve leg health by promoting activity and improve welfare by increasing natural pecking behaviours and decreasing disturbance behaviour.
Keywords: Broilers; Guanidinoacetic acid; Behaviour; Maternal supplementation; Hen age

3142 Broiler hocks, but not footpad health or gait improved with access to platform perches

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Platform perches allow broiler chickens to spend more time off the litter and may increase activity. Therefore, this could improve foot and leg health. The objective was to determine the impact of platform perches on foot and leg health in commercially housed broiler chickens.
Two commercial farms with two houses of 27,200 (Farm X) or 41,400 (Farm Y) Ross 708 broiler chickens were used. The treatment house contained gridded plastic platform perches providing ~12% additional floor space (target final stocking density 31.7 kg/m2 with platforms included), and the control house provided standard commercial conditions (36 kg/m2). In 6 flocks (3/treatment), 10 broilers from 10 locations were penned at 6 weeks old (300 broilers/treatment). Hock burn and footpad dermatitis were scored on a 0-4 scale; gait on a 0-5 scale. Hock burn, footpad, and gait scores were assessed using a CLMM, and GLMM (binomial) was used for proportion of gait scores ≥3. Fixed effects included farm and treatment, random effects included date and location. No random effects were included in the footpad model to improve convergence.
Flocks with platforms had more birds with healthy hocks (47±6% vs 30±5%; p=0.015), fewer birds with score 1 (47±5% vs 58±3%; p=0.018), and fewer birds with score 2 compared to control flocks (5±1% vs 9±2%; p=0.028). Scores ≥3 were rarely observed (platform: 5±1%, control: 10±3%).
Control flocks at Farm X had more birds with healthy footpads (95±2%) compared to both treatments at Farm Y (control: 76±3% and perch: 82±3%; p<0.001). Control flocks at Farm X also had a lower proportion of score 1 (Farm X control: 3±1%, Farm Y control: 12±1%, perch: 10±2%; p<0.003) and 2 (Farm X control: 2±1%) than at Farm Y (control: 9±2%, perch: 6±1%; p<0.005). Gait scores and risk of score ≥3 did not differ between farms and were not impacted by treatment (score ≥3: 20.5%; p>0.25).
Access to platform perches decreased the prevalence of hock burns only, perhaps by reducing contact with litter, while footpad dermatitis and gait issues were relatively infrequent in all flocks. Although footpad dermatitis appeared better in the control treatment at Farm X, all birds were at low risk for this condition, diminishing the potential impact of platforms. Other contributing factors besides platform access may need to be considered to improve gait.
Keywords: Commercial; Platform; Gait; Footpad; Enrichment

3143 Histopathologic characterization of toe pecking lesions in laying hens

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Toe pecking, a form of injurious pecking, has emerged as a flock problem in commercial laying hens within recent years with evident welfare consequences and economic losses [1]. From barn housed and organic flocks, it was recently reported that toe pecking may account for up to 40% of the normal mortality in layer flocks [2]. Approximately 9/10 birds with toe pecking lesions may die from blood loss from the injured toes and about 75% are in lay [2]. It has been observed that hens with toe pecking lesion both can be mutilated by flock mates and perform self-mutilation. The arteria and veins supplying the avian toe are very superficially located and are not protected by digital muscles or subcutaneous fat, which play an important role in the hemostasis. Furthermore, when the scaly skin on the dorsal aspect of the toe is compromised, there is no protection of the digital bones, which may cause lesions.
Despite the fatal consequences for hens with these lesions, very little is known about the pathogenesis, and if anatomical and physiological features play a role in continuous mutilation and/or contribute to the fatal blood loss.
The aim of this descriptive case study was to characterize the histopathological features of toes with toe pecking lesions in commercial laying hens.
From dead hens diagnosed with toe pecking (furnished cages), 20 toes with lesions and 10 unaffected toes were fixed in 4% neutral buffered formaldehyde. After demineralization with formic acid each toe was cut into 3-5 transverse sections, embedded in paraffin wax, cut into 4 µm thick sections, and stained with hematoxylin and eosin. All slides were systematically evaluated for presence of hemorrhage, inflammation, necrosis, fibrosis, and traumatic destruction of tissue architecture.
The dorsal epidermis and subcutis of the injured toes were severely damaged with varying degrees of ulceration, necrosis and tissue loss. In >50% of the injured toes the digital bone was exposed. The bone tissue had periosteal damage and showed varying degrees of necrosis and tissue loss. In one case the medullar cavity of the bone was exposed and filled with inflammatory cells.
Subcutaneous hemorrhages dominated toes with fresh lesions, whereas toes with chronic lesions had enlarged diameter and were

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dominated by necrosis, extensive fibrosis, loss of normal tissue architecture as well as congested vessels.
To our knowledge it is the first time the microstructure of toe pecking lesions has been characterized. The findings underline the traumatic origin of the lesions. Chronic lesions might be painful to the hens and could therefore play a role in cases of sustained self-mutilation. However, the underlying cause for the initial toe pecking remains unknown.
Keywords: Toe pecking; behavior; welfare; histopathology; Laying hens

3144 Bone-related blood parameters and keel bone health in laying hens across different housing systems

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Keel bone fractures in commercial laying hens are among the most significant animal welfare concerns in modern egg production systems. Vitamin D is essential for bone metabolism and repair, promoting the intestinal absorption of calcium and phosphorus. We therefore hypothesized that hens with keel bone fractures would exhibit higher serum calcium, phosphorous and 25-hydroxyvitamin D3 (25-OHD3) concentrations due to the ongoing bone healing process than hens with healthy keel bones. In this study, 360 Lohmann Brown laying hens aged 62 to 89 weeks from 18 Austrian farms were examined for keel bone fractures using radiography. Fractures were analysed dichotomously (fractured vs. intact) without determining their age. Blood samples were collected to assess calcium, phosphorous, and 25-OHD3 concentrations, after which hens were returned to their respective barn. Median (range) concentrations across all hens were 7.40 mmol/L (2.40–19.70) for calcium, 1.60 mmol/L (0.50–4.80) for phosphorus and 122.00 nmol/L (9.00–484.00) for 25-OHD3. Statistical analyses were performed using linear mixed models including prediction and farm type as fixed effects, farm as random intercept and prediction as random slope within farm; bird age and body condition were not included as fixed effects. Farms represented an even distribution across indoor, free-range, and organic housing systems. No significant associations were found between serum calcium or phosphorus levels and the presence of keel bone fractures. A tendency ($p = 0.077$) was observed for hens with intact keel bones to exhibit higher 25-OHD3 concentrations compared to hens with fractures. Although not statistically significant, this trend suggests that lower 25-OHD3 status may predispose hens to keel bone damage rather than result from the healing process itself. These findings underline the potential role of 25-OHD3 status in preventing keel bone damage in laying hens.
Keywords: keel bone; 25-hydroxyvitamin D□; minerals; poultry welfare; laying hens

3145 Assessment of the impact of bio-available silicium on gait scores in broilers

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Gait Score is a welfare assessment tool used to evaluate the walking ability or locomotion of broiler chickens. It provides an indication of leg health and overall mobility, which are often affected by rapid growth, poor housing conditions, or nutritional imbalances. A low gait score (near 0) signifies that the broiler chicken has normal, smooth, and unrestricted walking ability, indicating good leg health and welfare. Bio-available silicium supports bone, tendon, and skin health by promoting collagen synthesis, thereby improving the structural integrity of broiler legs and having potential benefit for lowering gait score. This study was conducted to assess the impact of bio-available Silicium (Poulsil®) on gait scores in broiler birds. A total of 250 broiler day-old chicks were randomly distributed into one control and four treatment groups. Each group included five replicate pens with ten chicks per pen, maintained under uniform management and nutritional conditions. The control group (T01) received no silicium supplementation, while the treatment groups (T02–T05) were provided with varying formulations and concentrations of silicium from day one to day 41 via drinking water. Birds in groups T02 and T03 received Silicium–orthosilicic acid–vanillin complex (Si-OSA VC) at dilution levels of 1/1000 and 1/200, respectively, whereas groups T04 and T05 were supplemented with Silicium–monomethylsilanetriol (Si-MMST) at the same respective dilutions. The resulting silicium concentrations in drinking water were 0 mg/L, 0.87 mg/L, 4.33 mg/L, 0.30 mg/L, and 1.48 mg/L for T01, T02, T03, T04, and T05 respectively. Moreover, birds in all groups were reared on humidified bedding material to simulate bad housing conditions and induce mild locomotive and skin complications. Gait scores for all groups were evaluated on day 41, with recorded values of 0.72, 0.70, 0.35, 0.39, and 0.46 for T01, T02, T03, T04, and T05, respectively. Data were analysed with R (version 3.2.5). The treatment groups were compared to the untreated challenged group as reference. Mean scores were added. Statistical significances were assessed at $P \leq 0.05$. Result showed that Poulsil® supplementation lowered the gait score for all treatment groups. The reduction in gait score was significant for the higher concentration of first silicium formulation (Si-OSA VC; $p = 0.001$) and both concentrations of second silicium formulation (Si-MMST; $p = 0.002$ for lower concentration, $p = 0.018$ for higher concentration). Thus, it can be concluded that supplementation with bio-available Silicium (Poulsil®) in broilers reduces gait scores, indicating a potential positive effect on their welfare conditions.
Keywords: bio-available silicium; broiler; gait score; welfare; poulsil

3146 Effect of very low density diets on performance and behaviour in breeder pullets

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Over the past 50 years, the growth potential of broilers, and consequently that of broiler breeders, has increased dramatically. As a result, feed intake of breeder pullets is restricted to approximately 50% of their ad libitum capacity. Previous attempts to mitigate feed restriction by diluting diets up to 30% were not sufficiently effective. Therefore, the present study investigated the effects of more strongly diluted diets (40% and 50%) during the rearing phase on the performance and behaviour of pullets. A total of 964 one-day-old Ross 308 breeder pullets were distributed across 24 identical pens and assigned to one of three treatments: (1) control diet once daily (CON), (2) 40% diluted diet twice daily (DIL-40%), and (3) 50% diluted diet twice daily (DIL-50%). Water was available throughout the 8-hour photoperiod. All treatments were fed according to the same body weight (BW) target profile. Pullets fed diluted diets (DIL-40% and DIL-50%) showed higher feed and water intake than CON birds ($P<0.05$). The water-to-feed ratio was lowest in DIL-50% pullets (1.7), highest in CON pullets (2.4), and intermediate in DIL-40% pullets (1.9) ($P<0.05$). BW uniformity was not affected by the feeding program. Total mortality was high-

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er in CON pullets than in DIL-40% and DIL-50% pullets, mainly due to reduced pecking-related mortality in the diluted diet groups. Feed clean-up time differed (P<0.001) markedly among treatments: DIL-50% pullets had feed available for the entire light period (8 hr), DIL-40% pullets for 7 hours, whereas CON pullets consumed all feed within 40 minutes. Pullets on diluted diets exhibited more feeding behaviour and less standing, sitting, foraging, comfort, and object pecking. Behavioural patterns during the light period were distinctly altered in DIL-40% and DIL-50% pullets due to the two daily feeding events. These pullets were also less motivated to approach a novel feeder and consumed less feed during the test (P<0.05). In conclusion, feeding breeder pullets 40% or 50% diluted diets provided near-continuous feed access during the light period, which improved welfare by reducing stereotypic pecking behaviour and decreasing feeding motivation.
Keywords: broiler breeder; diet dilution; performance; behaviour

3147 Physiological and behavioral responses to water deprivation in boilers selected for water conversion ratio
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Water management is essential for a sustainable poultry industry. Water conversion ratio (WCR) differs between broilers selected for fast growth. We aimed to explore the behavioral and physiological response of broilers selected for High (H) and Low (L) WCR during water deprivation (WD). Broilers were housed in 6 pens per line (15/pen). Feed and water intake and body weights were recorded weekly to calculate feed conversion ratio (FCR), WCR, and body weight gain (BWG, g/bird). On day (D)28-32, accelerometers were placed on 5 birds per pen (n=60) to measure activity during WD. On D32, 3 pens per line were water deprived for 12h (Dep), while the remaining 3 pens had ad libitum access (Con). After WD, thermal images were taken of the head as a non-invasive measure of stress, blood samples were analyzed for gas and chemistry, and the bursa of Fabricius (bursa) was collected from 5 birds/pen. Water intake (g/bird) was calculated at 0, 12, and 15 hours post WD. Data were analyzed using LMM and GAMM. On D28, H birds weighed more (1340g) than L (1237g) pre WD (p=0.03). On D32, H birds tended to remain heavier (1610g) than L post WD (1504g, p=0.09), and Dep birds weighed less (1487g) than Con (1627g, p=0.03). BWG was lower in Dep (13g/day) than Con (21g/day, p<0.001). WD increased D14-33 FCR (1.8) compared with Con (1.6, p=0.03). H and L broilers differed in WCR (4.4 vs 2.9, p=0.002), and this was not affected by WD. After WD (12-15hrs), Dep broilers drank more (116g/bird) than Con (37g/bird, p<0.001), and H Dep drank more (134g/bird) than L Dep broilers (98g/bird, p=0.007). There was no effect of WD or line on eye temperature, but Dep broilers had lower beak temperatures (33C) compared with Con (35C, p=0.004). L had lower activity (0.037m/s2) than H (0.41m/s2, p<0.001), and Dep had lower activity (0.034m/s2) than Con broilers (0.047m/s2, p<0.001). Con H and L broilers had stable activity over time (p=0.11, p=0.49). Dep H had a linear increase in activity (p=0.04), whereas L showed a variable temporal pattern (p<0.001). Dep broilers had lower blood HCO3 (p=0.01), BE ecf (p=0.02), TCO2 (p=0.02), PCO2 (p=0.06), and higher Na (p<0.001) than Con broilers. Relative bursa weight didn't differ between lines or deprivation time. Water deprivation induced physiological signs of dehydration stress and altered activity. H and L broilers differ in WCR and responded physiologically, but not behaviorally, alike during water deprivation.
Keywords: Broiler; Water Deprivation; Behavior; Physiology; Water conversion ratio

3148 Keel bone fracture in layer breeders: Relationship between fracture severities, hen body mass, physiology and welfare
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Studies on keel bone fractures and their welfare consequences have largely emanated from commercial layers. Given some differences in the management practices of layer breeders, this study assessed the association between keel fracture severities and layer breeder welfare (fear and anxiety), body mass (body, dead and muscle weights) and physiology (keel bone temperature, respiratory rate and bone strength). 120 Bovans Brown breeder hens (60 with palpated fracture and 60 suspected to be fracture-free) at 61 weeks were tagged and housed in 6 similar rooms (10 fractured and 10 suspected non-fractured per room). At week 64, all hens were weighed and subjected to tonic immobility and novel arena tests as measures of fear and anxiety. Respiratory rate and surface keel temperature of each hen was assessed once/week from weeks 65-67. At week 70, 111 hens were euthanized and their carcasses were weighed and dissected. Breast muscles were weighed, and keel and tibia bones were tested for breaking force. Severity of keel fractures was visually scored on a 4-point scale. Based on the scale, 21, 25, 29 and 36 hens were respectively classified as fracture-free, slightly fractured, moderately fractured and severely fractured. Healed keel bone status was confirmed in the fractured groups. Using the 4-level fracture severity as fixed factor, and room as random factor, data on tonic immobility and novel arena were analyzed with Kruskal-Wallis and logistic regression respectively. Other variables were analyzed with Linear mixed model. Bonferroni comparison showed that hens with severe fractures had lower body weight and dead weight than those with slight (P=0.027, P=0.020, respectively) and moderate fractures (P=0.028, P=0.039, respectively). Muscle weight of severely fractured hens (mean = 177g) was lower (P<0.05) than fracture-free and slightly fractured hens by 9.69 and 11.5% respectively. Breaking strength of keel bone manubial and tibia bones were lower in severely fractured hens compared to fracture-free (P<0.05) and slightly fractured hens (P<0.01). Fractured hens, regardless of fracture severity, had lower likelihood of performing ground scratching in novel arena (P=0.009). There was no association between fracture severity and tonic immobility duration, likelihood of freezing and escape, as well as hen respiration and surface keel temperature. In conclusion, while the direction of relationship cannot be ascertained, lower body mass and bone strength particularly in severely fractured hens suggest negative associations between severe fracture, some hen productive traits and skeletal integrity. Also, keel fracture, once healed is unlikely to be associated with hen respiration or long-term increased fear and anxiety
Keywords: Bone damage; Chicken; Skeletal health; Welfare; Physiology

3149 Associations between necropsy findings in non-caged laying hens indicate most common causes of death
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Monitoring of mortality causes is prerequisite to targeting control and preventative measures to the most relevant health and welfare

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challenges in laying hen flocks. Despite this, only few scientific studies have investigated causes of normal mortality in laying hens and with differences in terminology and reported diagnostic criteria. In the light of the transition to non-cage housing systems in many parts of the world, understanding mortality causes in these systems may help support farmers to alleviate health and welfare challenges arising when adapting to new management routines inherent to these systems.
The objective of this study was to investigate associations between necropsy findings in laying hens that died throughout the production period with an explorative approach applied to a previously obtained dataset with pathological findings of Danish laying hens from seven non-caged commercial flocks [1]. This approach may provide new insights compared to descriptive analysis of predefined diagnoses. Multiple correspondence analysis was applied to explore multidimensional associations between pathological findings across 49 variables recorded in 1,648 laying hens that would suggest clustering in tentative causes of death. The first four dimensions of the multiple correspondence analysis were retained, accounting for 20.2% of the variance. The results indicated clustering of hens with diagnoses of similar expected chronicity and general aetiology (infectious versus non-infectious). The most prominent clusters corresponded to the two most prevalent causes of mortality identified in previous descriptive studies: salpingitis-peritonitis and cannibalism [1]. At least two different clusters of hens that died due to cannibalism (acute or prolonged course with concurrent pathologies) were also identified by the multivariate approach. We suggest exploring relevant differences in aetiology and pathogenesis of these clusters in future studies. The results may inspire recommendations for time-efficient field necropsies and preventative and control measures to target the most common causes of mortality in laying hens. The results may be useful to farmers and their advisors to alleviate health, welfare, economic and environmental implications of mortality in non-caged laying hens.

1. Butler-Lund, V. P., Thøfner, I. C. N., Scharling, F. S., Nielsen, L. R., & Christensen, J. P. (2025). A longitudinal study of causes of mortality in Danish commercial laying hens in non-cage housing systems. Avian Pathology, 54(6), 775-791. https://doi.org/10.1080/03079457.2025.2527120.
Keywords: laying hen; mortality; pathology; multiple correspondence analysis; cannibalism

Egg Science, Quality, and Processing I

Fatty liver disease in humans and birds: Role of nutrients

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4010 Earlier age at first egg does not guarantee higher cumulative egg number at 100 weeks nor affect egg quality in layer

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Introduction Age at first egg (AFE) is a key selection criterion in layer breeding. With the laying cycle extended to 100 weeks, the relationship between sexual maturity and long-term productivity and egg quality should be evaluated. Moreover, modern breeding strategies focus on maintaining AFE within an optimal range. Therefore, understanding how AFE is associated with long-term egg production and egg quality is essential to ensure that selection for AFE aligns with current breeding objectives.
Materials and methods Beijing-You chickens and White Leghorns were used to generate purebreds (YY, WW) and crossbreds (WY, YW). Egg-laying performance was recorded including AFE, cumulative egg number and egg number at different stages from 28, till 100 weeks, and egg quality traits at 32, 54, 72, 86, and 100 weeks: egg shape index, eggshell color, eggshell strength, eggshell thickness, eggshell ratio, Haugh unit, yolk color and yolk ratio. Genetic and phenotypic correlations were estimated using mixed linear models including genetic group and rack as fixed effects and random animal effect using ASReml.
Results A positive genetic correlation (0.29) was observed between AFE and cumulative egg number till 100 weeks in the mixed population with purebred and crossbred animals, indicating that earlier sexual maturity does not guarantee higher egg production at late stage. Age-dependent genetic correlations between egg number at different stages and AFE further revealed that extremely early-maturing hens showed initial production advantages but exhibited weaker persistency at late stages. Importantly, the optimal timing of sexual maturity varied across genetic groups, WW reached peak egg production at 20-22 weeks, while this happened at 23-27 weeks for YY. We estimated phenotypic and genetic correlations between AFE and egg quality traits across five timepoints to determine whether early maturity compromises egg quality. Overall, the correlations were weak, ranging from -0.27 to 0.33, and were even lower for eggshell quality traits (-0.16 to 0.28). These findings demonstrate that AFE has negligible influence on egg quality, supporting the feasibility of optimizing maturity timing without risking deterioration in marketable egg quality traits.
Conclusions Earlier AFE does not ensure higher egg production at extended laying cycle. Optimal AFE differs by genetic background, and extremely early maturity reduces laying persistency at extended laying cycle. The weak genetic correlations with egg quality indicate that AFE can be optimized without compromising egg quality.
Keywords age at first egg, genetic correlation, egg number, egg quality
Keywords: age at first egg; genetic correlation; egg number; egg quality

4011 Effects of a high fiber diet on egg production and organ weight in hens in an extended laying cycle

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Extending laying cycle is increasingly becoming pivotal to the economics and sustainability of the egg industry. However, keeping hens longer is challenged by increased risks of declining health, metabolic issues, and reduced productivity. The objective of the study was to investigate the impact of manipulating dietary energy substrates by supplementing fibrous feedstuffs (FF) on egg production (EP), egg quality, body, and visceral organ weight in aged laying hens. Diets were a corn-soybean-based positive control (PC) meeting specification and a corn-wheat middlings-soybean meal-based negative control (NC) formulated with lower apparent metabolizable energy (100 kcal/kg) and amino acid content (3-4%), but higher fiber content (18% wheat middlings) than the PC. All diets had 400 FTU phytase and were prepared in mash form. A total of 320 Dekalb white hens at 75 weeks of age (WOA) were placed in enriched cages (4 birds/cage) and allocated to 8 diets (n=10) in a completely randomized design. Another set of 5 birds was sacrificed for baseline sampling. Birds had free access to water and feed for 28 weeks. EP was recorded daily, feed intake (FI) weekly, whereas egg weight (EW), eggshell thickness (EST), shell breaking strength (SBS), albumen height (AH), and haugh unit (HU) were measured on all eggs collected at the end of weeks

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5, 10, 15, 20, 25, and 28. Body, liver, and abdominal fat weights (AFW) were taken at the end of the experiment. The data were analyzed using PROC GLIMMIX in SAS with diet, period, and their interaction as fixed factors. There was a significant interaction between diet and period for EW ($P = 0.013$). The PC fed birds had heavier eggs than NC (64.3 vs. 63.7 g; $P = 0.01$). EW gradually increased (from 63.2 to 64.5 g; $P < 0.0001$) over time, peaking from week 16 to 20 (65.0 g). There was no diet effect on FI ($P > 0.05$). Birds fed PC diet had a higher Hen Day Egg Production (85.7 vs. 84.8%; $P = 0.03$) compared to NC. SBS was greater in PC hens (3.89 vs. 3.71 kilogram force; $P = 0.001$) than NC. Birds on the NC diet lost 11.5% of their BW (1828 vs. 1617 g; $P = 0.004$), whereas PC birds 2.4% (1822 vs. 1778 g). Additionally, birds on PC diet showed a 10% decrease in AFW (49.9 vs. 45.3 g), while NC birds, 28% (49.9 g vs. 36.3 g; $P = 0.02$). In conclusion, adding high FF reduces egg productivity, quality, BW, and AFW. Despite similar FI for PC and NC diets (104 vs.103g/bird/day), the reduced amino acid content in the NC diet limited protein synthesis for egg formation. Further research on the supplementation of fiber-degrading enzymes (FDE) in high-fiber diets is recommended to increase nutrient utilization, egg production, and egg quality, as birds lack the endogenous enzymes to break down fiber.

Keywords: Aged laying hens; Egg production; Egg quality; Fibrous feedstuff; Fiber degrading enzymes

4012 Effects of dietary fermented cottonseed meal supplementation on laying performance, egg quality, serum index and free gossypol residue in laying hens

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This study investigated the effects of cottonseed meal (CSM) or fermented cottonseed meal CSM (FCSM) as soybean meal (SBM) alternatives in laying hens. A total of 450 Hy-Line Brown laying hens (20-week-old) were randomly assigned to 5 treatments (6 replicates, 12 hens each) for 30 weeks. The control group was corn-SBM basal diet, designated as 0 group. The experimental diets-20CSM, 80CSM, 200CSM, and 80FCSM-contained 20, 80, 200, and 80 mg/kg of free gossypol (FG), respectively. These formulations were achieved by supplementing with 15.1, 60.0, or 150.0 g/kg of CSM, or 210 g/kg of FCSM. Compared to the control group, the 20CSM group showed the optimal feed conversion ratio. Compared to the 0 group, the 200CSM group significantly increased feed conversion ratio, body weight, serum ALT, AST, and UA levels, and significantly decreased egg albumen height and serum IgG level, resulting in abdominal fat accumulation and obvious inflammatory liver changes. The 80CSM group exhibited significantly higher serum AST level than the 0 group with mild liver inflammation. The 80FCSM group demonstrated significantly reduced average egg weight and increased feed conversion ratio compared to the 0 and 20CSM groups, while showing significantly elevated serum SOD, GSH-Px, IgG, HDL-C levels and jejunum V/C ratio compared to the 80 and 200 CSM groups, as well as significantly lower body weight, and reduced serum ALT, AST, UA, TG, LDL-C, VLDL-C levels compared to the 200CSM group. FG was only detected in feces, with the 200CSM and 80FCSM groups showing significantly higher levels than other groups. Overall, 20 mg/kg FG caused no adverse effects in laying hens. FCSM improved physiological indicators, alleviating fat accumulation and liver lesions, though its dosage requires appropriate reduction to optimize egg-laying performance.

Keywords: fermented cottonseed meal; egg quality; serum index; free gossypol residue; laying hens

4013 Studies on the Effect of Sacha Inchi Oil and Rosemary Oil on the Flavor of Eggs

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Egg flavor primarily originates from the yolk and is a key factor in determining egg quality. The objective of this study was to pinpoint the key volatile compounds in egg yolk, investigate the relationship between the hydrophilic and hydrophobic metabolites and flavors, and explore the regulation of egg flavor based on the change of flavor precursors and the deposition of essential oils through adding Sacha inchi oil and Rosemary oil to feeds. Based on the preliminary test results that characterization of flavor precursors and volatile compounds in egg yolks. Gas chromatography-olfactometry-mass spectrometry analysis showed that a total of 28 volatile compounds were identified. Among them, 8 volatile compounds contributed to the egg flavor. In the study on the effect of Sacha inchi oil (SIO) on volatile compounds in egg yolks. Fatty acid analysis results demonstrated that the addition of SIO (0.5% of diary) could increase the content of ω-3 polyunsaturated fatty acids in egg yolks. The introduction of SIO did not generate any off-flavor but improved the consumer preference. In the study on the effect of Rosemary essential oil (RO) on aroma compounds in egg yolks. The sensory evaluation results demonstrated that 100 mg/kg of rosemary oil eggs were the most acceptable. Notably, 1,8-Cineole, one of the key aroma compounds in RO, was directly retained in the egg yolk and made a significant contribution to the yolks' flavor. In conclusion, we conducted a multidimensional evaluation of the flavor and flavor precursors of egg yolks and screened and identified four different flavor substances, including 3-methyl-butanal and 48 lipid molecules with important contributions to the flavor. The effect of adding SIO on the flavor of eggs could increase the content of unsaturated fatty acids such as linolenic acid, and further influence the egg flavor through the oxidative degradation of fatty acids. The addition of RO to the feed could lead to the direct deposition of 1,8-cineole, a characteristic substance of the essential oil, in the egg yolk.

Keywords: Egg yolk; Multi-omics; Volatiles, Sacha inchi oil; Rosemary oil

4014 Olive Pomace Extract Supplementation Enhances Egg Quality in Laying Hens

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Olive pomace is a by-product generated during olive oil production, rich in bioactives compounds with immunomodulatory and antioxidant effects. The objective of the study was to evaluate the effect of including an olive pomace extract (OPE) in layer hens' diets on performance and egg-quality parameters associated with shelf-life. After a two-weeks acclimatization period, a total of 288 H&N Brown Nick layer hens, 59 weeks old, were randomly distributed in cages and assigned to 3 experimental groups with 8 replicates each (12 birds/cage). The dietary groups consisted in: a corn and soybean-meal-based diet (Control), Control + OPE at 600 ppm (OPE600), and Control + OPE at 1200 ppm (OPE1200). The animals were monitored over a 6-week feeding trial, and egg quality traits and antioxidant stability were assessed by sampling 3 eggs per replicate at the end of the trial, eggs were stored at room temperature (~26 oC, 52% RH). Egg quality traits were measured using a digital egg tester (storage time-points: 1, 7 and 14 days), while the oxidation marker (malonaldehyde, MDA) in the egg yolk was determined by TBARS method (storage time-points: 1 and 14

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days). Data was analyzed by one-way ANOVA and Duncan's Test was used to compare treatment means in case significant differences were observed ($P < 0.05$). No effects were associated with OPE addition on performance parameters (hen-day egg production, egg mass, feed intake, feed conversion, mortality and % of dirty and cracked eggs) during the period comprised between 59 to 65 weeks of life. However, eggs from OPE1200 showed higher albumen heigh and Haugh units compared to eggs from Control on day 7 and 14 of storage ($P < 0.05$). Furthermore, yolk index tended to increase with OPE1200 on day 7 and 14 of storage ($P < 0.10$). Regarding yolk oxidation stability, the inclusion of 600 and 1200 ppm of OPE significantly reduced the concentration of MDA on day 1 and 14 of storage ($P < 0.01$). In conclusion, incorporating olive oil by-products rich in bioactive compounds into laying hen diets offers an innovative strategy to enhance egg quality and shelf-life while supporting circular economy.

Keywords: olive pomace; egg quality; egg shelf-life; oxidation; circular economy

4015 Layer-Specific Glycosylation of Eggshell Matrix Proteins Drives Functional Divergence during Mineralization

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The eggshell provides antibacterial protection and serves as a vital calcium source for embryonic development. The mammillary layer supports shell mechanics and contains calcified bodies involved in calcium mobilization. Eggshell matrix proteins (EMPs) critically regulate eggshell mineralization, and identical EMPs can perform distinct functions through differential glycosylation, contributing to morphological differences among avian species.

We investigated EMP regulatory mechanisms during initial mineralization by analyzing glycosylation heterogeneity across shell layers. Proteomic and intact glycoproteomic profiling of cuticle, palisade, and mammillary layers was followed by differential analysis, functional enrichment, re-glycosylation simulation, and molecular docking. Intergroup comparisons used the t-test.

Proteomics identified 23135 peptides corresponding to 4262 proteins (3450 comparable). Differentially abundant proteins (DAPs) were mainly associated with protein binding, hydrolase activity, and organic cyclic compound binding. Enrichment analysis linked mineralization-specific DAPs to glycosylation-related terms, revealing divergent glycosylation programs. Serpin domains were most enriched; ovalbumin (OVA), rich in Serpin domains, is pivotal in mineralization initiation.

Intact glycoproteomics identified 7895 glycopeptides from 266 proteins (80 comparable) modified by 219 unique N-glycans. The cuticle had the fewest glycosites, whereas the mammillary layer showed the highest glycopeptide diversity, indicating pronounced microheterogeneity. Functional enrichment highlighted macromolecular binding. OVA exhibited 194 and 193 glycoforms at Asn293 and Asn312; ovocleidin-17 (OC-17) showed 81 at Asn59. Both influence mineralization initiation.

Quantitative analysis revealed opposite trends: from mammillary to palisade layers (initiation → progression), OVA glycosylation and heterogeneity declined, while OC-17 increased. In vitro studies report calcium-induced OVA unfolding and nucleation propensity. OC-17 modifies calcite morphology via its calcium-dependent C-type lectin domain. We propose that low OC-17 glycosylation enhances OVA binding to promote amorphous calcium carbonate nucleation, whereas higher OC-17 glycosylation later limits palisade growth. Docking simulations supported this model: fucosylated OVA bound OC-17 with greater complementarity (iPTM 0.57 vs 0.11 deglycosylated).

In conclusion, layer-specific EMP glycosylation modulates mineralization through spatiotemporal glycoform transitions that remodel protein interactions and confer stage-specific functions, providing mechanistic insight into avian eggshell formation.

Keywords: Eggshell; Eggshell matrix proteins; Biomineralization; Proteomics; Glycoproteomics

4016 Dietary vitamin D analogue supplementation could augment egg production and shell quality during long lay cycle

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With increase concerns over the welfare and the potential for higher egg production among the laying hens, studies are being carried out to extend the laying period to 100 weeks of age and achieving the production of 500 eggs per hen. The major challenge in this extended laying phase is the maintenance of egg shell quality. The quality of egg shell has been inversely related to the stage of the egg production. An experiment on extending the laying period to 100 weeks was carried out on 60 white leghorn hens (BV 300) and to assess the effect of dietary 25-(OH) cholecalciferol supplementation on the egg production and egg shell quality in laying hens. Initially, the birds were reared individually for 60 weeks under standard management conditions without grouping and subsequently, they were divided in to three groups (20 birds in each group), as control (basal diet with calcium 3.80% and vitamin D 2500 IU), T1 (basal diet + 35 ppb 25-(OH) cholecalciferol) and T2 (basal diet + 70 ppb 25-(OH) cholecalciferol) for the remaining 40 weeks of experiment. The individual bird's egg production and weekly egg shell quality were assessed during the entire study period. The data collected during the study were subjected to analysis of variance (single factor, repeated measures) and the means were compared using Tukey Range test. The total number of eggs produced during this 40 weeks period were higher ($P<0.05$) in T2 (250.93) group than the control (240.62) and T1 (239.81) groups. Similarly, the total number of eggs produced during the total 100 weeks laying period were 489.25, 487.68 and 499.24 in control, T1 and T2 groups, respectively. The supplementation of 25-(OH) cholecalciferol did not significantly ($P>0.05$) influence the egg shell weight and its thickness which are comparable among the groups. The supplementation (70 ppb) has resulted in higher ($P<0.05$) net calcium secretion to the eggs per bird compared to the control. The target of producing 500 eggs with acceptable egg shell quality in 100 weeks laying period is achievable.

Keywords: Egg production; Egg shell quality; Long lay cycle; Layers; Vitamin D analogue

4017 The effects of graded levels of Quillaja saponins (Clarity Q®) on productivity of commercial laying hens.

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Quillaja saponins have been shown in recent studies to promote better growth performance of broiler chickens. To determine whether commercial layers and resulting egg production variables are affected in a similar manner by Quillaja feeding, a 40-week study was conducted to evaluate the effects of Quillaja saponins (Clarity Q [CQ], Huvepharma, Antwerp, Belgium) on the perfor-

mance and productivity of commercial laying hens. At 20 weeks of age, 2688 H&N layers were equally allotted to 4 treatments arranged in a completely randomized block design. The treatments comprised graded levels of CQ (0, 125, 250 and 500 ppm) fed continuously from 20 to 60 weeks of age and utilized 8 blocks involving 672 layers per treatment. Daily measurements of percent egg production (EP), egg weight (EW), egg mass (EM) and feed consumption were determined. Totals of these variables and calculations of FCR (g of feed consumed/g of egg) were made weekly. Additionally, eggshell thickness (ST) was measured in 30 randomly selected eggs per treatment every other week for the entire test period. All data were summarized at 40 and 60 weeks of age and analyzed using one-way analysis of variance. Treatment means at each time interval were separated by the LSD procedure and statistical differences were established at $p<0.05$. At 40 weeks of age, CQ at 250 and 500 ppm significantly improved EP and FCR compared to lower doses, however, in each case, responses at 250 and 500 ppm were statistically equivalent. Significant improvements in EW and EM were recorded for each graded level of CQ. By 60 weeks of age, both 125 and 250 ppm CQ significantly improved EP and FCR, but for both variables, the response to 500 ppm was not significantly different from 250 ppm. EW was significantly improved at both 250 and 500 ppm and showed significant improvements compared to lower levels. EM was significantly greater at each level of CQ. ST was maximized by 250 ppm, and this response was significantly greater than the lower dose levels. In general, data indicate that the dose of 250 ppm produced the most consistent responses in performance and egg production parameters. Consistent with previous studies in broilers, results of this trial indicate that Quillaja saponins contribute to better layer productivity and enhanced egg characteristics.

Keywords: Quillaja saponins ; commercial layers; egg production; egg characteristics

4018 Systematic review and meta-analysis of the efficacy of non-antibiotic alternative strategies to reduce Salmonella population in cecum of different stages of laying-type poultry

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Intervention strategies in laying-type birds play a crucial role in minimizing the risk of Salmonella colonization in the ceca that can cause systemic infection and carry over into eggs and further processing facilities. While multiple non-antibiotic interventions (prebiotics, organic acids, bacteriophages, vaccines, or combinations) have been evaluated to reduce Salmonella colonization in laying-type birds, comparative effectiveness of these strategies remains unclear. The aim of this study was to evaluate the efficacy and consistency of non-antibiotic strategies against Salmonella colonization in laying-type poultry. The Web of Science, PubMed, and Google Scholar databases were used for searching each category, and the results were filtered according to pre-determined inclusion criteria. A total of 1,729 relevant publications were identified, out of which 40 trials from 20 research studies were included in the meta-analysis. Mean Salmonella concentration in ceca, standard deviation or standard error of the mean, and sample size of each trial were recorded and subjected to a random effect model using the metafor package in R. Mean differences (log CFU) between control and treatment were reported for ease of interpretation. Trials were further sub-grouped into pullet, laying, and molting stages to evaluate the efficacy of the intervention. The overall model indicated that interventions in laying-type poultry reduced Salmonella concentration in ceca by 1.28 log CFU ($P < 0.01$) with a high heterogeneity of 91.8%. The greatest effectiveness was observed during the pullet phase (MD = -1.50), followed by the molting period (MD = -1.16), with comparatively lower efficacy observed during the laying stage (MD = -0.46). Among all interventions at the pullet stage, the combination resulted in the greatest reduction (MD = -2.91), followed by vaccination (MD = -2.19), and prebiotics (MD = -0.56). Organic acids showed no reduction in Salmonella concentration (MD = 0.16). Additionally, interventions were more effective ($P < 0.01$) against non-Enteritidis serotypes (MD = -1.82) compared with those targeting Salmonella Enteritidis (MD = -1.38). Interventions are generally more effective at the pullet stage than in mature birds at the laying and molting stages, with combinations being more effective. Additionally, Salmonella Enteritidis is more difficult to control compared to other serovars at the pullet stage. Targeted interventions are necessary to control Salmonella Enteritidis by the layer industry to ensure the safety of shell eggs and egg products. However, caution should be used when incorporating the interventions during production and the optimal dosage and safety must be evaluated prior to implementation.

Keywords: Salmonella; laying; pullet; meta-analysis; intervention

4019 A multi-stage transcriptomic analysis reveals the molecular regulatory network underlying eggshell mineralization

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Objective

This study aimed to systematically identify candidate genes and pathways involved in eggshell calcification by comparing transcriptional profiles across key developmental stages to elucidate the molecular mechanisms and regulatory networks underlying avian eggshell mineralization.

Experimental Design

Rhode Island Red hens (300 days old) were selected, and eggshell/membrane samples along with uterine tissues and fluid were collected at precise post-ovulation time points. Samples were categorized into five stages: eggshell membrane (EM), initial mineralization (INI), and rapid growth stages (RG1/2/3). Uterine tissues were subjected to transcriptome sequencing.

Material and Methods

Total RNA of uterus was extracted and sequenced using Illumina platform. Bioinformatics analyses included identification of differentially expressed genes (DEGs), KEGG and GO pathway enrichment analysis, temporal gene expression clustering, and weighted gene co-expression network analysis (WGCNA). Calcium concentration of uterine fluid was measured by detection kit.

Statement of Statistical Analysis

Differential expression analysis was performed using DESeq2 with thresholds of $|\log2 \text{ fold change}| > 1$ and adjusted p-value < 0.05. Pathway enrichment analyses were conducted using clusterProfiler. WGCNA was implemented to identify co-expression modules, with module-trait correlations assessed using Pearson's correlation ($p < 0.05$ considered significant).

Results

Comparative analysis identified 403, 1095, 1316, and 1184 differentially expressed genes (DEGs) when comparing EM stage with the INI, RG1, RG2, and RG3 stages, respectively, including: VDR, CALB1, ATP2B1, ATP2B2, TRPA1, OC17, MEPE, SPP1, FN1, BPIFB3, ATP2A3, BCL2, HSPA5, COPE, SEC23B. Pathway analyses revealed significant enrichment in calcium signaling, protein processing in

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endoplasmic reticulum, and vesicle-mediated transport pathways during RG stages. WGCNA identified stage-specific co-expression modules, which were the green module (ATP2B1/CALB1) and the salmon module (FN1/COL4A1). No significant difference of calcium concentration was observed between EM and INI in uterine fluid. RG1 exhibited significantly higher Ca2+ than EM(p=0.014), while RG2 and RG3 showed extremely significantly higher Ca2+ than EM(p<0.01).

Conclusions

This study elucidates calcium uptake, ER-mediated calcium homeostasis, and protein secretion processes throughout calcification. The identified molecular network provides comprehensive insights into the precise regulation of eggshell formation and establishes a foundation for understanding the etiology of shell quality defects and reduced shell strength.

Keywords

Eggshell mineralization, Transcriptome, Calcium transport, Matrix proteins, WGCNA

Keywords: Eggshell biomineralization; Transcriptome profiling; Calcium transport; Matrix proteins; WGCNA

General Health/Disease/One Health V

From Hormones to Reproduction, Food Safety, Gut Integrity, Enteric Pathogenesis and Vaccinology: An Eclectic Forty-Four-Year Journey with Poultry, People and the Critters We Work

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4021 Effect of dietary supplementation with β-sitosterol on the health of Cobb 500 broiler chickens

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The study aimed to evaluate the effect of dietary β-sitosterol on metabolic substrate content, non-alcoholic fatty liver disease, oxidative stress, antioxidant status, and plasma surrogate markers of liver and kidney function of broiler chicken. Two hundred and forty-one-day-old, unsexed Cobb 500 broiler chicks were randomly allocated to four dietary treatments where β-sitosterol replaced oxytetracycline at 0 mg/kg (50 mg/kg oxytetracycline), 500, 1000, and 1500 mg/kg feed as a growth-promoting supplement. Chicks were reared in a deep-litter housing system, with 10 birds per pen measuring 1.7 m × 1.1 m × 1.3 m length, width and height, respectively. Each dietary treatment was replicated 6 times with 10 chicks per replicate. After six weeks, birds were fasted, weighed, and humanely slaughtered by decapitation with a small guillotine. Blood samples were collected, and plasma was harvested and stored at -20 °C for later assays. Blood glucose, triglyceride, calcium, and cholesterol concentrations were determined. The livers were dissected out, weighed, and preserved for lipid content and histology analyses. Oxidative stress, antioxidant status, and plasma surrogate markers of liver and kidney function were also determined. GraphPad Prism version 8.0.2 was used to analyze the data. Steatosis and inflammation were analyzed with Kruskal–Wallis and Dunn's test. All other data were analyzed using one-way ANOVA with Tukey's post hoc test. Broilers fed 1500 mg/kg β-sitosterol had significantly higher ($P < 0.0001$) cholesterol than those fed 500 mg/kg β-sitosterol and 50 mg/kg oxytetracycline. Dietary β-sitosterol at 1000 and 1500 mg/kg feed increased ($P < 0.05$) liver lipid content but did not affect hepatic microarchitecture. However, chickens supplemented with β-sitosterol at 1500 mg/kg feed exhibited micro- and macro hepatic steatosis, lobular inflammation, and higher ($P < 0.05$) non-alcoholic fatty liver disease activity scores. Dietary β-sitosterol at 1500 mg/kg significantly increased ($P < 0.0001$) catalase, superoxide dismutase, glutathione S-transferase, and glutathione peroxidase concentrations than other treatments. Broilers supplemented with β-sitosterol had significantly lower ($P < 0.05$) plasma malondialdehyde concentration than oxytetracycline. Plasma aspartate aminotransferase and gamma-glutamyl-amino transferase activities of broilers fed 1500 mg/kg feed β-sitosterol were significantly higher ($P < 0.0001$) than oxytetracycline. Dietary β-sitosterol enhances antioxidant enzyme activity and maintains metabolic homeostasis in broiler chickens at moderate inclusion levels. However, excessive supplementation may disrupt lipid metabolism, leading to hepatic steatosis and hypercholesterolemia.

Keywords: Broiler chicken; health; antioxidant status; antibiotic; liver

4022 Effects of dietary addition of encapsulated butyric acid on performance, egg quality, immunity, nutrient metabolizability, and faecal microbial counts in laying hens

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b) Hypothesis

Encapsulated butyric acid added to poultry diets enhances gut health, nutrient utilization, immune competence, and overall performance by ensuring sustained release of butyrate along the intestinal tract, thereby improving intestinal integrity, microbial balance, and mineral absorption.

Objective

To evaluate the effects of graded levels of micro-encapsulated butyric acid on production performance, egg quality, nutrient metabolizability, immune response, and faecal microbial counts in commercial White Leghorn laying hens.

c) Experimental Design

A completely randomized design was employed to assess encapsulated butyric acid as a non-antibiotic feed additive in White Leghorn layers.

d) Materials and Methods

A total of 288 White Leghorn (BV-300) laying hens (42 weeks old) were randomly assigned to four dietary treatments with six replicates of 12 birds each. Birds were fed iso-caloric and iso-nitrogenous diets under a 16 h light and 8 h dark lighting schedule. The control group received a basal diet, while treatment groups received encapsulated butyric acid at 125 (T2), 250 (T3), and 500 g/MT (T4) of feed. The trial was conducted from 43 to 58 weeks of age. Parameters evaluated included production performance, egg quality, humoral and cell-mediated immunity, nutrient utilization, and faecal microbial counts.

e) Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS (version 16.0), and differences among treatment means were considered significant at $P < 0.05$.

f) Results

Dietary addition of encapsulated butyric acid significantly improved productive performance. Birds in T4 showed higher egg production (93.35% vs. 88.91%) and better feed conversion ratio (1.92 vs. 2.10) compared to control. Egg mass increased significantly in T3 and T4 groups. Eggshell thickness and shell weight ratio improved with increasing inclusion levels. Salmonella spp. and Clostridium perfringens were not detected, while Escherichia coli counts were lower in treated birds. Both humoral and cell-mediated immune responses were significantly enhanced. Nutrient metabolizability of crude protein, calcium, and phosphorus was

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highest at 500 g/MT.
g) Conclusion
Encapsulated butyric acid at 500 g/MT improved productivity, egg quality, nutrient utilization, immune response, and gut microbial balance in White Leghorn layers during the mid to late laying phase.
Keywords: Encapsulated Butyric Acid; Layers performance ; egg quality; immunity; Faecal microbial counts

4023 Importance of dietary fiber on liver and reproductive function of laying hens at different production periods
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Dietary interventions targeting the gut-liver-reproductive tract axis in laying hens are needed to support intestinal health (of both host and microbiota), enhanced reproductive performance, and promote a proper hepatic fat metabolism to avoid fatty liver syndrome. In this study, such dietary interventions were focused on the use fiber sources. For this, 144 laying hens (18-wk-old) were distributed in a completely randomized design with 4 treatments consisting of diets with different fiber sources: control diet; diet with 10% oat hulls (OH), diet with 7% soybean hulls (SH), and a high crude fiber diet (HCF) containing oats, barley and wheat middlings. At 25, 55, and 85 weeks of age, 12 replicate hens per treatment (48 per time-point) were weighed for determination of body weight (BW) and euthanized for sampling. Each time-point was treated separately for statistical analysis. Liver and abdominal fat content were recorded, as well as their relative weight (as a percentage of BW). The relative weights of gizzard (plus thickness), caeca, ovary, eggshell gland and complete reproductive tract (RT) were also recorded. Liver color was scored from 1 to 6 (i.e. 1=fatty liver problems, 6=healthy liver) as indication of fatty liver occurrence. Gizzard, duodenum, liver, eggshell gland and kidney samples were collected for histological and gene expression analyses. Furthermore, serum samples were collected at these three time points to identify biomarkers of liver fat accumulation and intestinal and reproductive function. Data were submitted to one-way ANOVA, and liver score was analyzed via multinomial distribution and log transformation. At 25 weeks, no treatment effects were found for BW, liver color score, gizzard thickness or relative weights of liver, eggshell gland, ovary, reproductive tract and caeca. However, hens fed the SH diet had a lower ($P = 0.008$) relative abdominal fat weight (1.56 g/kg BW) compared with the control (2.03 g/kg BW). Relative empty gizzard weight was higher ($P<0.001$) in hens fed OH (1.91 g/kg BW) and HCF diets (1.81 g/kg BW) compared to the control (1.56 g/kg BW). At 55 weeks of age, hens fed the HCF diet showed a higher BW (1,539 g; $P=0.02$) than other treatments, and liver color score was higher in hens fed the HCF diet (5.6) compared with the control (4.5) and OH (4.5) diets, but similar to the SH diet (5.2). Early results suggest that dietary fiber source and content influenced gizzard development and abdominal fat deposition at 25 weeks, with potential effects on liver health and lipid metabolism. The additional data collected at 25, 55 and 85 weeks as described above are currently under preparation and will be available before the conference.
Keywords: Fatty liver syndrome; Laying hens; Lipid metabolism; Crude fiber; Fiber sources

4024 Effects of Black Soldier Fly Larvae Oil Supplementation on Growth Performance and Intestinal Health in Broiler Chickens Challenged with Mixed Eimeria Species
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A study was conducted to investigate the effects of replacing soybean oil with black soldier fly larvae oil (BSFLO) on growth performance and intestinal health of broiler chickens before and after challenge with mixed Eimeria species. It was hypothesized that dietary BSFLO, rich in lauric acid, would support growth and gut integrity during coccidial infection. A total of 800 one-day-old Cobb 500 broiler chicks were randomly assigned in a 2×5 factorial design with two challenge conditions (non-challenged or challenged with Eimeria maxima, E. tenella, and E. acervulina on day 14) and five dietary BSFLO inclusion levels (0%, 25%, 50%, 75%, and 100%) replacing soybean oil. Each treatment had six replicates with 13 birds per pen. Growth performance was recorded during the pre-challenge phase (0–14d) and post-challenge phase (14–26d). At 6 days post inoculation (dpi), gut permeability was measured using fluorescein isothiocyanate–dextran (FITC-d) assay, and intestinal lesion scores were evaluated in the duodenum, jejunum, and ceca. All data were analyzed using JMP Pro 18 by two-way ANOVA to determine main and interactive effects of Eimeria challenge and BSFLO level. Mean separation was performed using Tukey's test. Nonparametric data were analyzed by the Kruskal–Wallis test followed by Steel–Dwass multiple comparison ($P < 0.05$). Linear and quadratic orthogonal contrasts were applied to evaluate BSFLO dose responses. During the pre-challenge period, increasing BSFLO inclusion linearly improved body weight (BW) and body weight gain (BWG) ($P < 0.05$) and increased feed intake (FI), while feed conversion ratio (FCR) remained unaffected. Following Eimeria challenge, infection markedly reduced BW, BWG, and FI ($P < 0.0001$), confirming growth suppression. BSFLO inclusion did not significantly alter overall post-challenge performance; however, BW at 6 dpi ($P = 0.03$) and FI at 0–6 dpi ($P = 0.0098$) and 0–12 dpi ($P = 0.0014$) increased linearly with BSFLO level. No significant interaction was observed between Eimeria challenge and BSFLO inclusion ($P > 0.05$). Gut permeability increased in challenged birds ($P < 0.0001$) but was unaffected by BSFLO. Lesion scores decreased numerically with BSFLO supplementation, with a linear reduction observed in the ceca ($P = 0.0195$). Partial or complete replacement of soybean oil with BSFLO improved pre-challenge growth performance and tended to reduce intestinal lesions following Eimeria infection. These results indicate that BSFLO can be used as a sustainable lipid source to support broiler performance and intestinal health under enteric stress conditions.
Keywords: Black soldier fly larvae oil; Eimeria challenge; broiler chickens; growth performance; intestinal health

4025 Investigation of the combined effect of short- and medium-chain fatty acids on antibody titers in broiler chickens
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Short- and medium-chain fatty acids are known for their antibacterial, as well as immune-modulating properties. Previously we reported a stimulating effect of a combination of these acids (C-prove 80, Agrimprove) on peripheral blood mononuclear cells. Here we wanted to investigate whether this could result in an increased production of antibody titers after vaccination.
Day-old Ross 308 broilers (n = 48) received in ovo vaccination against IBD (Gumboro) and Newcastle Disease virus (NCDV) and were divided in two groups: a control group receiving un-supplemented drinking water and a group supplied with 0.1% C-prove 80 in the water from d0-d10 and d18-d21. Each treatment was further

divided in two groups of different sex. On d28 and d42 blood was taken from 10 birds from each group to analyze serum Gumboro (ELISA) and NCD (Hemagglutination Inhibition) titers and total IgY (ELISA). A 2-way ANOVA was performed using the General Linear Model in SPSS 22, which examined the main effects (sex and supplementation) as well as their interaction. Mean differences among different treatments were separated by Tukey multiple range tests, with significance set at $p < 0.05$ to indicate significant differences.

On d28, female chickens exhibited higher Gumboro and NCD titers compared to males ($p < 0.05$). Supplementation had no significant effect on Gumboro ($p > 0.05$) but significantly increased NCD titers ($p < 0.05$). For Gumboro, no interaction between supplementation and sex was observed ($p > 0.05$), while female chickens receiving fatty acids showed higher NCD titers compared to males ($p < 0.05$). No difference was observed in IgY concentrations between treatments (supplementation, sex nor interaction) ($p > 0.05$).

On d42, male chickens had higher Gumboro titers than females ($p < 0.05$), but there was no significant effect of supplementation ($p > 0.05$). An interaction between supplementation and sex was observed, with male chickens receiving the product showing the highest Gumboro titers ($p < 0.05$). Supplementation significantly decreased NCD titers ($p < 0.05$) and males had higher titers than females ($p < 0.05$). There was a significant interaction between supplementation and sex, with male chickens of the control group showing the highest NCD titers ($p < 0.05$). There was no significant difference in IgY concentration between treatments ($p > 0.05$).

In conclusion, a sex-specific IgY response against the Gumboro and NCD in ovo vaccines was shown. Furthermore, C-prove 80 significantly influenced specific IgY titers against NCD. These results show the potential of combining short- and medium-chain fatty acids as vaccine booster, but further investigation of their immune-modulating effects is needed to validate this.

Keywords: short- and medium-chain fatty acid; antibody titer; vaccination; broiler chicken

4026 SUPPLEMENTAL EFFECT OF LACTOBACILLUS PLANTARUM DERIVED POSTBIOTICS ON BROILERS GUT HEALTH UNDER COCCIDIOSIS CHALLENGE

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Coccidiosis, caused by protozoa of the genus Eimeria, remains a major challenge to the poultry industry due to intestinal damage, impaired performance, and dysbiosis. Increasing concerns regarding resistance, drug residues, and regulatory restrictions on ionophores and vaccines have intensified the search for safer alternatives. Postbiotics non-viable bioactive metabolites derived from probiotic fermentation have emerged as a promising strategy. This study evaluated Lactobacillus plantarum derived postbiotics (LPPB) as a potential replacement for antibiotic growth promoters (AGPs) and coccidiostats by assessing their effects on growth, immunity, and gut health in broilers. A total of 192 straight-run coloured broiler chicks were randomly assigned to six treatment groups comprising negative control (−CONT; no coccidiostat), positive control (+CONT; 0.1% diclazuril), and LPPB (0.6% v/w), each under infected and non-infected conditions. Birds were reared in cages for 42 days and fed ad libitum diets formulated according to ICAR (2013) nutrient requirements. Postbiotics were isolated from a pure single colony of L. plantarum as described by Kareem et al. (2016). On day 21, birds in the infection groups were orally challenged with mixed Eimeria oocysts (20,000 oocysts/bird). Growth performance, survivability, immune responses, gut health parameters, histopathology, oocyst shedding, lesion scores, and anticoccidial index (ACI) were assessed. Data were arcsine-transformed and analyzed using two-way ANOVA in SPSS with Tukey's post-hoc test. LPPB supplementation improved ($p<0.05$) body weight gain, feed efficiency, survivability, and economic indices ($p<0.01$). It also enhanced immune organ indices and vaccinal immunity. Regarding gut health, LPPB reduced ($p<0.05$) villus shortening in coccidiosis-challenged broilers compared to controls. Postbiotics significantly elevated interferon-γ levels (178.24 pg/mL vs. 151.88 pg/mL in -CONT) and reduced tumor necrosis factor-α from 1–9 days post-infection ($p=0.001$). LPPB (0.6%) decreased oocyst shedding by 27.2% from 6–11 days post-infection and lowered lesion scores (1.67) versus -CONT. Cecal lesion scores dropped by 50% in the LPPB group, comparable to +CONT. Intestinal damage, enteritis index, and ACI were similarly reduced in LPPB-supplemented groups versus -CONT. Overall, supplementation with L. plantarum-derived postbiotics (0.6%) enhanced growth performance, immune competence, and economic efficiency while mitigating the severity of mixed Eimeria infection in broiler chickens. These findings suggest postbiotics as a safe and effective alternative to conventional coccidiostats for coccidiosis control in broiler production.

Keywords: POULTRY; EIMERIA; COCCIDIOSTAT; POSTBIOTIC; LACTOBACILLUS

4027 Implementation and Evaluation of a Dynamic Model for Feed Intake Reduction in Broilers During a Health Challenge Condition

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This study aimed to implement and evaluate a dynamic model to describe the reduction in feed intake of broiler chickens subjected to a health challenge, assessing its robustness using complete (Trial 1) and incomplete (Trial 2) datasets. The dynamic model, originally developed by Nguyen-Ba et al. (2020) for swine, was adapted to quantify resistance (k1) and resilience (k2) in broiler chickens. The model describes the target cumulative feed intake (CFI) trajectory in non-challenged birds using a reparametrized polynomial function, with parameters: CFI=0 (t0), final CFI (CFIf) at final age (tf), mid-point CFI (CFImp), and the age of transitions from quadratic to linear (ts). The perturbation module, fitted to challenged birds, estimates the challenge period (tstart, tstop) and the key rates k1 and k2. It was evaluated with data from two trials: birds challenged with E. maxima (Trail 1 and 2, 17 d) and C. perfringens (Trial 1, 18-20 d). In Trial 1, 612 one-day-old male Cobb 500 broilers were randomly allocated to one of two treatments (non-challenged or challenged), housed in 18 floor pens (34 birds/pen). CFI was recorded daily from 1 to 42-d-old. In Trial 2, 96 one-d-old male Cobb 500 broilers were randomly assigned to the same two treatments, housed in eight cages (12 birds/cage), with CFI recorded from 7 to 20-d-old. Model parameters were estimated by fitting the target CFI of non-challenged groups, while the challenge-related parameters (tstop, k1, k2) were estimated from DFI of challenged groups. The model was successfully reparametrized, with all parameters being statistically significant ($P<0.01$). In Trial 1 (complete data), the target CFI was accurately described, yielding parameter estimates of t0=0.01 d, tf=41.6 d, and CFIf=4921 g. A transition from quadratic to linear growth was identified at ts=32.3 d. For the challenged birds, DFI recovered by tstop=23.75 d. The model estimated a resistance k1=37.7%, representing the maxi-

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mum DFI reduction, and a resilience k2=2%, indicating the recovery rate. In Trial 2 (incomplete data), the target CFI exhibited only quadratic increase, with consistent estimates for t0 (1.01 d) and tf (21.31 d); no transition point (ts) was identified. The challenge duration remained approximately 4 days (tstop=20 d). Notably, the parameter estimates were highly consistent with Trial 1, showing a resistance k1=30.7% and resilience k2=2.1%. The dynamic model demonstrated high sensitivity in quantifying DFI reduction under a health challenge and exhibited considerable robustness across both complete and incomplete datasets. The consistent physiological responses, primarily driven by the E. maxima challenge, validate the model's ability to reliably characterize DFI behavior.
Keywords: Stressor; Feed intake behavior; Resistance; Resilience

4028 Characterization of Missense Mutations in Acute Phase Proteins in a Cellular Model for Chicken Amyloid Arthropathy

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Amyloid arthropathy (AA) in laying hens is a form of AA amyloidosis with serum amyloid A (SAA) deposition predominantly in joints. Among bacteria inducing AA, E. faecalis is the most frequent bacterial isolate in field cases and is a major multi-resistant pathogen in poultry. Brown-egg-laying hens (BL) are affected, whereas white-egg-laying hens (WL) appear resistant. Line-specific immune differences indicate cell-mediated responses in WL versus humoral responses in BL. Since these line differences suggested a genetic predisposition, we analysed sequence data and identified a fixed missense mutation in SAA in WL in a previous study, and six associated missense variants in the fibrinogen alpha chain (FGA) gene in BL. SAA is a key driver of AA, whereas fibrinogen has not been implicated despite being among a few known amyloidogenic proteins in animals. Free SAA directly binds fibrinogen and promotes amyloid formation. We therefore investigated whether these SAA and FGA variants influence cellular programs relevant to AA. Stable cell lines overexpressing SAA. WT and SAA.R90S were co-transfected with fibrinogen subunits (FGA.WT or FGA.MUT, FGB and FGG) that co-expressed distinct fluorophores to allow fluorescence-activated cell sorting (FACS) of triple-positive cells for RNA-seq. After read alignment to the reference genome, differential gene expression and gene set enrichment analysis (GSEA) were performed using R (DESeq2, clusterProfiler). For further statistical analyses, t-tests were performed. Transfection with FGA, both WT and MUT, significantly increased SAA transcript expression compared with the control plasmid ($p<0.001$). Differential gene expression analysis further indicated that FGA.MUT broadly reshaped transcription relative to FGA.WT. While many signals were shared between conditions (padj < 0.01; log2 FC >1), 551 were unique to FGA.MUT and 209 to FGA.WT. Calcium Homeostasis Modulator 1 (Calhm1) was strongly upregulated in FGA.MUT (baseMean = 1.49; log2FC = 3.11; padj = 0.0004). Calcium mediates SAP binding to amyloid fibrils, stabilizing them. Accordingly, the upregulation of Calhm1 in the FGA.MUT condition may reflect altered regulation of calcium homoeostasis with potential consequences for SAP-amyloid interactions. GSEA further revealed distinct effects on signalling pathways involved in biotic interactions and defence mechanisms. Taken together, these findings support the assumption that the differing susceptibility to AA can be attributed to genetic differences. To substantiate our findings, complementary investigations at the proteome level are planned.
Keywords: avian amyloid arthropathy; laying hens; serum amyloid A; fibrinogen; RNA sequencing

4029 Characterization of Lytic Bacteriophages as Biocontrol Agents Against Pathogenic Enterococcus faecalis in Poultry

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OBJECTIVE: Given the high antimicrobial resistance rates of Enterococcus faecalis and its capacity for biofilm formation, combined with the limited effectiveness of conventional antimicrobials, this study aimed to evaluate the diversity of E. faecalis-targeting bacteriophages in avian environments and assess their potential for designing effective cocktails against pathogenic strains in poultry production.

EXPERIMENTAL DESIGN: A phage isolation and characterization study was performed using 54 E. faecalis strains from three poultry production systems (breeders, broilers, layers). Environmental samples from poultry farms were screened for bacteriophage isolation. Isolated phages were characterized for host range, efficiency of plating, cocktail formulation and stability under different conditions.

MATERIALS AND METHODS: Environmental samples (feces, swabs, litter) were pooled and screened using spot-on-lawn after 0.22 μm filtration. Isolated phages underwent plaque purification and amplification at 37.5°C. Host range and EOP were assessed via double-layer agar. Cocktail design used computational exhaustive search (maximum seven phages) to maximize strain coverage. Thermal stability was evaluated from -80°C to 80°C and pH stability across pH 2–12. Viable particles were enumerated by drop-plate technique.

STATISTICAL ANALYSIS: Phage viability expressed as percentage survival relative to controls (4°C, pH 7). Efficiency of plating values normalized and visualized via heat maps.

RESULTS: Forty bacteriophages were isolated, showing lytic activity against 80% of strains (44/54). Among assays, 210 showed lytic activity (9.7%): 151 complete lysis and 59 turbid. Phages F5, F41 and F36 showed broad host range (>11 strains). A seven-phage cocktail (F2, F9, F10, F18, F41, F29, F33) achieved 40% overall coverage. Alternative cocktail combinations demonstrated markedly higher coverage within specific systems: 100% lysis of laying hen strains, 80% from breeders, 70% from broilers. All tested strains were multidrug-resistant, rendering conventional antimicrobials ineffective. Thermal stability was optimal between -80°C and 40°C with progressive inactivation above 50°C. pH stability was optimal between pH 4 and 9.

CONCLUSIONS: This study isolated and characterized diverse E. faecalis bacteriophages from poultry environments and identified phage cocktails with relevant coverage against multidrug-resistant strains compatible with poultry production conditions. While in vivo validation, biofilm assays and resistance assessments are required, the host range and stability profiles support further investigation of phage-based approaches as a complementary tool to reduce antibiotic use in poultry.
Keywords: Bacteriophage-therapy; E. faecalis; Antimicrobial-resistance; Phage-cocktail; Poultry-biocontrol

4030 Delivery of oligodeoxynucleotides containing CpG motifs twice in neonatal broiler chickens induced trained immunity led to decreased mortality and increased body weight gain under field conditions.

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Synthetic oligodeoxynucleotides containing CpG motifs (CpG-ODN) containing unmethylated cytosine and guanosine with a phosphorothioate backbone mimic bacterial DNA act as pathogen associated molecular pattens and stimulate the innate immune system through toll like receptor 21in chickens. It has been demonstrated that in ovo delivery of CpG-ODN can protect neonatal broiler chickens against Escherichia coli and Salmonella Typhimurium septicemia. Recently, we have demonstrated that delivery of two doses of CpG ODN at the day of hatch and 3 days later protected broiler chicken against E. coli septicemia and necrotic enteritis later in the production cycle by inducing trained immunity under laboratory conditions. Induction of trained immunity in broiler chickens led to switch in cellular energy metabolism of immune cells from glycolysis to mitochondrial OXPHOS (cellular respiration). The objective of this study was to investigate immunomodulatory activities of CpG-ODN mediated trained immunity under field conditions in broiler chickens. In a Ross 308 broiler chicken flock (n=4080), 200 broiler chicks (5%) received CpG ODN (50µg/bird) intramuscularly at day 1 and day 4 of age. Body weight of each bird was recorded at days 7, 14, 21 and 28 of age and first week mortality and total flock mortality were obtained. The body weight of birds that received two doses of CpG-ODN were significantly high compared to birds that did not receive CpG-ODN ($P < 0.05$), particularly the top 100- 150 birds received CpG-ODN. Moreover, birds that received CpG-ODN had lower first week mortality and total mortality (2.5%) compared to birds that not received CpG-ODN (4.09%). This study demonstrated that delivery of CpG-ODN induced trained immunity which led to better growth and reduced mortality in broiler chickens under field conditions.

Keywords: CpG-ODN; Trained Immunity; Mortality rate; Body Weight; Broiler chicken

Genetics, Genomics & Epigenomics III

4031 From Selection to Regulation: The Next Era of Poultry Genomics

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Over the past seventy-five years, poultry breeding has represented one of the most remarkable examples of genetic improvement in agriculture. Through quantitative genetics and selective breeding, modern poultry populations have been optimized for growth rate, feed efficiency, and production traits at a considerable rate. While these achievements demonstrate the effectiveness of classical breeding, they have also revealed biological constraints and trade-offs, including metabolic stress, breast muscle myopathies, and challenges related to immune competence and environmental resilience. Addressing these complex traits now requires a deeper understanding of the complex biological mechanisms that connect genotype to phenotype. Recent advances in genomics are transforming our ability to explore these mechanisms. High-quality reference genomes, pangenomes, and telomere-to-telomere assemblies are providing increasingly comprehensive maps of avian genetic variation and the diversity of local breeds. Simultaneously, functional genomics approaches, including transcriptomics, single-cell profiling, and integrative multi-omics, are allowing poultry scientists to investigate how genes are regulated across tissues, developmental stages, and environmental conditions. These tools are revealing the importance of regulatory networks, 3D chromatin architecture, and gene-by-environment interactions in shaping economically important traits. Beyond describing genomic variation, emerging technologies are enabling functional validation and direct intervention. Genome editing platforms, such as CRISPR-Cas systems, provide new opportunities to test causal relationships between genetic variants and phenotypes, while epigenetic and developmental programming studies highlight the role of early-life environments in shaping long-term performance. Together, these approaches signal a transition from traditional selection toward a more integrated framework that combines genetic diversity, functional genomics, and targeted perturbation to better understand and guide fundamental biological processes. This talk introduces the conceptual framework connecting the diverse presentations within the Genetics, Genomics, and Epigenetics section of the World's Poultry Congress 2026. By highlighting the progression from genome resources and population diversity to functional genomics and emerging technologies, it aims to place individual presentations within the broader field of poultry science. Collectively, these contributions illustrate how modern genomic tools are reshaping our understanding of poultry biology and opening new pathways toward sustainable, resilient, and precision breeding strategies for the global poultry industry.

4032 Chicken Interleukin-6 polymorphism imparts better humoral immunity and resistance against Newcastle disease virus challenge

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Interleukin-6 (IL-6) is a multifunctional cytokine that plays a critical role in modulating innate and adaptive immune responses. Genetic polymorphisms within the IL-6 gene may influence immune competence and susceptibility to infectious diseases, including viral pathogens such as Newcastle disease virus (NDV). The present study investigated the association between IL-6 promoter polymorphism and immune response as well as resistance to NDV challenge in broiler chickens. Genomic DNA (n = 106) was isolated from 200 µL of whole blood collected from 3-day-old broiler chicks using a conventional phenol-chloroform method. DNA integrity and purity were assessed by spectrophotometry and agarose gel electrophoresis. A 799 bp fragment of the IL-6 promoter region was amplified by PCR and subsequently digested with Eco130I, yielding two fragments of 530 bp and 269 bp. The genotypic distribution based on PCR-RFLP analysis revealed three genotypes: AA (41.5%), AB (51.0%), and BB (7.5%). Allele A was found at a higher frequency than allele B, and the population adhered to Hardy–Weinberg equilibrium.

Experimental birds were reared under standard management up to 42 days of age and challenged with NDV (0.1 mL of 10^7.4 ELD50) on day 43. Immune responses were assessed by measuring delayed-type hypersensitivity (foot-web index, mm) and humoral response (SRBC antibody titre, log2) at 28 days of age. Birds with the AA genotype exhibited significantly higher humoral immune response ($P < 0.01$) compared to the AB genotype, whereas BB birds showed significantly higher ($P < 0.05$) cell-mediated response than AA and AB genotypes. Post-challenge evaluation showed similar mean death time in AA and AB birds, while BB birds demonstrated the lowest survival duration. Gross pathological lesions were most severe in AB birds across all post-inoculation days, whereas AA and BB genotypes exhibited comparatively milder lesions.

Overall, the IL-6 promoter polymorphism appears to influence immune competence, and the AA genotype may confer enhanced resistance to NDV, indicating its potential utility in selective breeding programs aimed at improving disease resilience in commercial broiler populations.

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Keywords: Interleukin-6; Polymorphism; Humoral immunity; Newcastle disease virus; Chickens

4033 Integrative Genome-Wide Association Study and Transcriptomic Analysis to Identify Candidate Genes and Pathways for Heterophil and Lymphocyte Traits in White-Feathered Broiler

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Objective

Heterophils and lymphocytes are the two primary leukocytes mediating inflammatory responses in birds. The heterophil-to-lymphocyte ratio (H/L ratio) serves as a key indicator of stress and immune status in chickens. This study integrated genomic and transcriptomic data to identify functional variants and candidate genes affecting H/L ratio in white-feathered broilers.

Experimental Design

A total of 218 one-day-old broilers were collected for H/L ratio and H count. Genome-wide association studies (GWAS) were performed using whole-genome sequencing (WGS) data, followed by fine-mapping analysis. RNA-seq was conducted on bursa and thymus tissues from 15 broilers each in high and low H/L ratio groups. Weighted gene co-expression network analysis (WGCNA) integrated transcriptomic data with H/L ratio and serum immunoglobulin (IgA, IgG) levels.

Materials and Methods

The H/L ratio was determined from blood smears stained with Wright–Giemsa. The WGS from whole blood and RNA-seq were used for genomic and transcriptomic profiling. GWAS was performed using GEMMA under a mixed linear model, with variants filtered by minor allele frequency ≥ 5% and call rate ≥ 90%. Differentially expressed genes(DEGs) were detected using DESeq2. WGCNA identified hub genes associated with immune traits, and KEGG pathway enrichment was assessed for GWAS and RNA-seq candidate genes.

Statement of Statistical Analysis

DEGs were identified using the criteria of FDR < 0.05 and |log-2FC| > 1.

Results

GWAS identified 25 and 35 significant SNPs associated with H/L ratio and H count, respectively. Fine-mapping prioritized a causal variant in MYO16 (chr1:138395826) for H/L ratio and three linked variants in AKT3 (chr3:34663003, 34663053, 34663073) for H count, explaining 2.12% and 2.68% of phenotypic variance. RNA-seq revealed 123 and 136 DEGs in bursa and thymus, respectively. Integrated analysis highlighted enrichment in mTOR, FoxO, and MAPK signaling pathways, suggesting their coordinated role in immune cell homeostasis.

Conclusions

AKT3 and MYO16 are potential causal genes influencing H/L ratio in broilers. Critical immune pathways (mTOR, FoxO and MAPK) coordinate H/L balance. These findings provide genetic insights for improving disease resistance in poultry breeding.

Keywords: Broiler; H/L Ratio; GWAS; RNA-seq; Immune Pathways

4034 Multi-omics-based analysis of the molecular mechanisms underlying chickens' resistance to Salmonella infection

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Objective:

This study aimed to elucidate the molecular mechanisms underlying variation in chicken resistance to Salmonella Enteritidis (SE) infection using an integrated multi-omics approach.

Experimental Design:

Forty 6-day-old Guangming No. 2 broilers were randomly divided into SE-challenged and control groups (n=20 each).

Materials and Methods:

Peripheral blood was collected 72 hours post-challenge for PBMC and serum isolation. Resistance phenotypes were classified based primarily on the heterophil/lymphocyte (H/L) ratio, which was further validated by significant correlations with cecal bacterial load. LC-MS/MS-based proteomics (PBMC and serum) and serum non-targeted metabolomics were performed. Data integration enabled screening of key molecules and networks associated with resistance.

Statistical Analysis:

Data were analyzed using SPSS 26 (Student's t-test; significance: $P < 0.05$, highly significant: $P < 0.01$) and visualized with GraphPad Prism 6.0.

Results:

A valid infection model with distinct resistance phenotypes was confirmed by cecal bacterial load and serum cytokines (IL-6, IL-8). PBMC proteomics identified PDK4 as a key differentially expressed protein across comparisons. Infected vs. control comparisons showed significant enrichment ($P < 0.05$) in immune pathways (Toll-like receptor, IL-17, TH17 differentiation, ferroptosis). High vs. low H/L ratio comparisons revealed enrichment ($P < 0.01$) in ether metabolism linked to ferroptosis. WGCNA integration of multi-omics data identified core proteins (e.g., FTH) and the metabolite Dyclonine, significantly correlated with protein expression.

Conclusions:

Multi-omics integration identified PDK4 and the ferroptosis pathway as central to resistance variation. PDK4 is associated with the regulation of cellular energy metabolism via inhibiting PDC, which may impact glycolysis, fatty acid oxidation, and oxidative stress, and shows correlation with ferroptosis activation. Key regulators (FTH, GPX4) and the biomarker Dyclonine were identified. These findings suggest a novel PDK4-ferroptosis axis that may influence Salmonella resistance and provide potential targets for breeding disease-resistant poultry.

Keywords:

Chicken, Salmonella infection, Multi-omics, PDK4, Ferroptosis

Keywords: Chicken; Salmonella infection; Multi-omics; PDK4; Ferroptosis

4035 Genetic variation in hepatic response and CYP1A1 regulation in chickens exposed to hydrocarbon inhalation: implications for breeding pollution-resilient poultry

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Breed-specific genetic architecture may govern detoxification efficiency under pollutant stress. This study evaluated the hepatotoxic effects of crude-oil fume inhalation and the modulatory role of antioxidants on CYP1A1 expression across different chicken breeds. 512 five-week-old chickens from four genetic groups - FUNAAB Alpha (FAC), Ross 308 Broilers (BRO), two Nigerian light ecotypes from Hydrocarbon-Polluted (HP) and Non-Hydrocarbon-Polluted (NHP) regions, were reared under controlled exposure to 1L of Bonny Light crude oil combustion fumes on alternate days for 35 days. They were assigned to four dietary treatments - in a 4 × 4 factorial design - unexposed control without antioxidant (CON), exposed without antioxidant (EXPO), exposed + vitamin C (0.05g/L water, EXV), and exposure + aqueous ginger extract (50 mL/L water, EXG - Oleforuh-Okoleh et al., 2015). Each treatment was replicated four times with eight birds per replicate. Serum alanine (ALT), aspartate (AST), and alkaline phosphatase (ALP)

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activities and total protein were evaluated, while CYP1A1 mRNA expression was quantified by qRT-PCR. Data were analyzed using 2-way ANOVA, and significant means separated using Tukey's post-hoc test. Hydrocarbon exposure significantly ($P < 0.01$) elevated ALT and AST, indicating hepatocellular injury most severe in BRO (+63 %) and HP (+57 %). Reduced enzyme activities and improved total protein levels ($P<0.05$) were observed due to antioxidant intervention - especially ginger, confirming amelioration of hepatocellular stress. Significant variations ($P<0.01$) were found in xenobiotic regulation among the breeds: CYP1A1 transcription was markedly up-regulated in HP (2.36-fold) and FAC (1.87-fold) under exposure, but was downregulated (0.40-fold) in BRO and minimally induced in NHP (1.1-fold). Vitamin C down-regulated expression (0.85-fold in HP), whereas ginger induced further CYP1A1 activation in FAC (15.78-fold). This distinct hepatic and molecular responses observed among the chicken breeds demonstrate a breed-dependent hepatic adaptation patterns, and identify ginger as a potent nutrigenomic antioxidant capable of modulating CYP1A1-mediated pathways. Crosses such as BRO × HP and FAC × NHP are recommended to integrate metabolic efficiency with pollutant tolerance and physiological stability. Incorporating these crosses into multi-generational selection schemes, supported by marker-assisted screening for moderate CYP1A1 activation and robust antioxidant capacity, could yield high-performing, environmentally resilient hybrids suitable for sustainable poultry production in hydrocarbon-affected ecosystems.
Keywords: hydrocarbon; hepatotoxicity; CYP1A1; antioxidant modulation; marker-assisted selection

4036 Multi-evidence driven triage of candidate genes for feather pecking behaviour in chickens
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Hypothesis or objective: Feather pecking in laying hens is a complex, multifactorial behavioural trait with a genetic component that significantly impairs animal welfare and increases energy requirements, resulting in lower resource efficiency. Since divergent selection for high and low feather pecking behaviour led to the establishment of two distinct lines with very high and no propensity for the behaviour, respectively, a causative connection between genetics and the behaviour is evident. Hence, we hypothesise that functional genetic variants that alter the structure, stability, or expression of proteins in key neurobiological pathways are likely to influence individual variation in feather-pecking behaviour in laying hens. Our objective is to prioritise a short list of candidate haplotypes for experimental validation, recognising that many protein-altering variants may be phenotypically silent.

Experimental design: Whole-genome sequences from hens of high and low feather-pecking lines (>15 generations of selection; n=49) were contrasted using population differentiation to identify strongly diverged loci; haplotype-aware protein sequences were reconstructed and candidates ranked using an integrated evidence score.

Materials and methods: Previously called SNPs, small indels (<50 bp), structural variants and tandem repeats were analysed. Fixation index (FST) was computed in PLINK; haplotype-aware protein prediction used Haplosaurus (Ensembl VEP). Variant consequences were annotated with VEP/snpEff; missense impact with SIFT 4G and PROVEAN; splice effects with SpliceAI; disordered regions with IUPred3 and motif mapping with ELM. Protein structures/complexes were modelled with AlphaFold2/3 and stability/interface effects estimated with FoldX; interaction partners were obtained from STRING; tissue expression context from Bgee and Expression Atlas. Evidence signals were standardised and combined as a weighted score.

Statement of statistical analysis: Differentiation was quantified by per-variant FST; highly differentiated variants were defined as FST>0.9 and summarised descriptively.

Results: Across 49 sequenced hens, 125,000 highly differentiated variants (FST>0.9) were detected between lines, including 1,362 protein sequence–altering haplotypes (up to 15 missense variants per haplotype as well as indels). SpliceAI predicted 66 variants with potential splice disruption.

Conclusions: This multi-evidence, tool-based triage integrates sequence, structure, regulatory and biological-context evidence to prioritise a manageable set of neurobiologically plausible candidates for functional testing, explicitly addressing that structural changes alone may not translate into behavioural phenotypes.
Keywords: Feather pecking; Genomics; Artificial Inteligence; Alphafold; Animal welfare

4037 Intergenerational effects of Paternal Stress and Associated Epigenetic Mechanisms in Birds
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Domestic birds are increasingly exposed to environmental stressors as poultry production systems evolve. Changes in the environment experienced by parents—or even by hens alone—can influence offspring development, health, and behavior through intergenerational effects. In mammals, such effects can also occur when only the father is exposed, mediated by modifications in the sperm epigenome, particularly involving small non-coding RNAs (sncRNAs) and DNA methylation. However, this phenomenon has yet to be demonstrated in birds. Therefore, this study aims to investigate paternal intergenerational effects in birds and to identify the underlying sperm epigenetic mechanisms.

Male Japanese quails were exposed, or not, to unpredictable stressors (predator sounds, overcrowding, isolation, novel objects, transport) for three weeks before reproduction. Offspring development, exploratory behavior, and gamete quality were then assessed through body and egg weights, sperm mobility, and integrity. Paternal sperm epigenomes were analyzed for small non-coding RNAs (sncRNAs) and DNA methylation. RNA-seq libraries (QIAseq miRNA Library Kit) were sequenced on Illumina NextSeq, mapped to the quail genome (GCF_001577835.2) using Bowtie2, quantified with Samtools, and analyzed for differential expression using edgeR. sncRNA identities were predicted with miRDeep2, PICB, and MINTmap, and their potential targets with IntaRNA. DNA methylation was analyzed using EM-seq (NEBNext Enzymatic Methyl-seq Kit), processed via Nextflow, and differentially methylated cytosines (DMCs) and regions (DMRs) were identified. Two-way ANOVA tests were used to explore the impacts of individual sex and paternal treatment on all physiological traits. The Likelihood Ratio Test ($|Log2FoldChange|>1$) was applied for sncRNAs and Wald test for DMCs. p-value<0.05 was considered significant.

Offspring physiology was affected, with heavier males at hatching (p-value=0.0012), enhanced exploratory behavior in 3-week-old males (p-value=0.0135), and larger eggs laid by adult females

derived from stressed fathers (p-value=0.0087). Six sncRNAs were differentially expressed in the sperm of stressed males (p-value<0.0001), all predicted to be siRNAs with targets involved in diverse biological functions. In parallel, 915 differentially methylated regions (DMRs) were identified (p-value<0.0001) across 1,204 genes, mainly associated with organism and brain development. These results demonstrate for the first time the existence of intergenerational effects mediated exclusively by fathers in birds. These effects appear to be mainly mediated by sperm DNA methylation changes in genes associated with biological functions consistent with the affected physiological traits.

Keywords: Sperm cells; sncRNAs; DNA methylation; intergenerational effects; birds

4038 Green Light During Late Embryogenesis Epigenetically Programs Growth and Metabolism in Broilers

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Research in last years demonstrated that the incubation environment strongly influences chick quality and long-term productivity. In this study, we show that green monochromatic illumination (GMI) during incubation reprograms hypothalamic pathways controlling growth, appetite, and metabolism in broiler chickens. In our experiment fertile Ross 308 eggs (n=150) were incubated under dark (Control), white (White), continuous GMI (Green), or GMI applied only during the last three days before hatch (G3D). Results, using RNA-seq, ChIP-qPCR, and immunofluorescence methodologies, revealed that only G3D treatment induced pronounced hypothalamic transcriptional and epigenetic responses: More than 500 genes, including those involved in growth, appetite, and metabolism, were differentially expressed in the G3D group, compared to Control, White and Green treatment groups. This was accompanied by significantly ($p<0.05$) elevated phosphorylated CREB1 (pCREB1) binding at the promoters of Agrp by 4 fold, Vip by 2.5, Pacap by 2 fold, Gal by 2 fold, Ghrh by 3.5 fold, and Leprot by 3.5 fold which are a part of the somatotropic and metabolic regulatory networks and H3K27 acetylation binding levels at the same promotors were elevated by 1.5, 1.6, 1.5, 1.8,1.5, 1.7 fold, respectively . Those results confirm epigenetic reprogramming by in-ovo GMI.

To examine neuronal responsiveness after hatch, all treatment groups were exposed to a 5 min GMI pulse at day of hatch, and weekly 60 hours of GMI during growth (at days 8-9, 15-16, 22-23, 29-30). Results showed significantly elevated cFOS activation levels in the hypothalamus in the G3D group by 2.5-fold at day of hatch. Moreover, on day 16, significantly higher body weight by 47 grams, and improved feed conversion ratio (FCR) by 0.2 FCR score was observed in the G3D compared to Control group. All statistical analysis was conducted with ONE WAY ANOVA tests. This indicates that embryonic light imprinting remains active within the post-hatch hypothalamus and may represent a form of molecular memory.

In conclusion, green light exposure during the last three days of incubation, reinforced after hatch, improves growth efficiency through hypothalamic epigenetic mechanisms and provides a simple, non-invasive means to enhance performance in broiler production.

Keywords: neuroepigenetics; green monochromatic illumination; hypothalamus; in-ovo

4039 Construction of a Gene-Perturbation Atlas Model in Chicken via the CRISPR–Cas9 System

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Introduction

Gene editing shifts breeding from random selection to targeted optimization. In poultry, traits like growth rate and feed efficiency impact both economics and public health. However, many of these traits are controlled by genes with unknown functions. Traditional gene-editing methods face challenges such as low throughput and cellular interference. To overcome these, we developed the Inducible Gene-Perturbation Model of Chicken (IGPMC), a transgenic model enabling simultaneous expression of multiple sgRNAs, allowing efficient gene knockout and the creation of chimeric chickens. This system facilitates rapid gene function evaluation and accelerates genetic improvement.

Materials and Methods

Based on the research accumulated and multi-omics data analysis, we selected 31 key genes related to growth traits. Using the PiggyBac vector, we introduced an sgRNA library into PGCs and achieved controlled expression of gRNAs through the Cre-LoxP system and drug induction. After obtaining stable PGC cell lines, we directly microinjected them into recipient embryos and cultured them in vitro, yielding chickens that expressed the gene-editing elements. Drug induction further generated chimeric chickens in which each cell exhibited different editing effects. High-throughput sequencing was used to track dynamic changes in sgRNAs. Combined with phenotypic and transcriptomic data, we created the chicken functional gene perturbation atlas.

Results

We have completed the construction of all elements and the system setup for the perturbation map, including: 1) successfully optimizing the isolation, culture, and efficient transfection methods for PGCs, and constructing a PGC cell line; 2) designing sgRNAs for the 31 selected genes, along with the construction of expression vectors and the identification of highly efficient candidates via DF-1 cell screening, culminating in tandem vector assemblies; 3) successfully verifying the single recombination property of the Cre-LoxP system in DF1 cells. As a proof of concept, we constructed a tandem vector targeting five genes and conducted a preliminary functional assessment in DF-1 cells.

Discussion

This study aims to build a high-throughput gene research platform based on CRISPR-Cas9 gene editing technology and the Cre-LoxP regulated expression system to map the functional gene perturbation atlas in chickens. Furthermore, IGPMC allows for the rapid generation of single-gene edited chicken, which can be used to verify and further explore candidate genes identified in preliminary studies. In conclusion, IGPMC offers an efficient technical pathway and data support for high-throughput poultry functional gene research and breed improvement.

Keywords: Chicken; CRISPR-Cas9; Functional Gene Editing; Perturbation Atlas

4040 In-vitro Cleavage validation assay of Myostatin gene by CRISPR-Cas9 Ribonucleoprotein Complex: From PCR Amplification to Cleavage Assay

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The primary experimental objective of the present investigation is to study the targeted genome editing of the myostatin (MSTN) gene using ribonucleoproein (RNP) complex composed of Cas9 protein and guide RNA (gRNA). Muscle tissue from thigh region of control broiler male line (PB-1) of chicken that is being maintained at ICAR-Directorate of Poultry Research, Hyderabad, India was used to clone the myostatin gene into a plasmid (pGEM-T vector), sequenced using oxford nanopore technology to identify genetic variants, and then a sgRNA was designed for further validation by CRISPR/Cas9 Ribonucleoprotein Complex. The Cas9 protein was incubated with sgRNA at a 2:1 ratio for 30 minutes at room temperature to form the RNP complex. The PCR amplification of myostatin gene consistently produced clear, distinct bands of 1128 base pairs in size, confirming targeted and specific amplification of the gene region of interest. Analysis of the recombinant clone sequence confirmed the total size of the recombinant plasmid to be 4128 bp and the insert length of 1128 bp encoding a protein of 375 amino acids. Within this coding sequence of myostatin gene, two single nucleotide polymorphisms (SNPs) were detected, a cytosine-to-thymine substitution at position 672 bp and a thymine-to-guanine substitution at position 699 bp of the coding region of the gene. Both of the SNPs identified were synonymous mutations that did not alter the amino acid sequence relative to the reference MSTN sequence (GenBank accession NM_001001461.2). Incubation of PCR products with the assembled RNP complex resulted in three distinct bands, corresponding to sizes of 1128 bp, 764 bp, and 364 bp, respectively. In control samples that contain only the PCR product of myostatin gene without RNP complex, a band of approximately 1128 bp was observed. This pattern indicates specific cleavage by the RNP complex resulting in digestion of the PCR products of the Myostatin gene. This strategy enables identification of potent guide RNAs and serves as a critical step toward generating genome-edited chicken tailored for improved muscle development and sustainable agriculture.

Keywords: Chicken; Myostatin; Gene editing; Cas9; RNP complex

4041 Genetically sterile roosters as a platform for efficient generation of transgenic chickens

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Objective:

The aim of this study was to develop a genetically defined and reproducible model of male sterility in chickens to improve the efficiency of germline transmission from modified primordial germ cells (PGCs) and to overcome the limitations associated with irradiation-based recipient preparation.

Experimental design:

Primordial germ cells were genetically edited by introducing a frameshift deletion into a meiosis-initiating factor using the CRISPR/Cas9 system and expanded in vitro. Heterozygous edited PGCs were transplanted into irradiated adult roosters to generate founder animals, and the line was maintained through heterozygous parent flocks. Homozygous knockout progeny were characterized for fertility and chromosomal constitution. Subsequently, embryos from the parent flocks were used as hosts for embryonic injection of GFP-expressing donor PGCs, and germline transmission was assessed in the resulting adults.

Materials and methods:

Edited PGCs were used to generate founder animals by transplantation into irradiated adult roosters. Progeny of the knockout line were genotyped, and homozygous sterile individuals were evaluated for gonadal morphology and fertility. Embryos derived from homozygous knockout hens were analyzed for ploidy by metaphase karyotyping of embryonic fibroblasts. GFP-positive donor PGCs were subsequently injected into embryos of heterozygous parent flocks. Hatched chicks were genotyped, and homozygous knockout males were raised to maturity with donor-derived gametogenesis evaluated through GFP screening of their progeny.

Results:

Homozygous knockout males developed morphologically normal testes but produced no endogenous functional sperm. Homozygous knockout females laid fertilized eggs at frequencies comparable to heterozygous controls, yet only a subset of these embryos initiated development. All developing embryos were triploid with a uniform ZZ constitution, suggesting fertilization involving diploid oocytes. Chicks hatched from embryos injected with GFP-expressing donor PGCs were genotyped, and homozygous knockout males matured into adult roosters producing exclusively donor-derived sperm. All fertile eggs from matings with these males yielded offspring entirely derived from the donor PGC population, consistent with complete germline replacement.

Conclusions:

Genetic sterility combined with embryonic PGC transplantation provides a genetically defined, irradiation-free platform for producing non-chimeric transgenic poultry. This approach complements existing germline ablation systems by enabling stable male sterility and exclusive donor-derived spermatogenesis, thereby improving the predictability and efficiency of avian genome engineering.

Keywords: primordial germ cells; rooster sterility; avian genetics; germline replacement; triploidy

Marketing I

4128 Consumer, retail and out-of-home preferences for sustainable eggs and poultry meat in the European Union

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In the second half of the 20th century cost price minimalization was leading in the European poultry chain. Since 1990, ethical values gained attention and broadened the product assortment from bulk towards animal welfare based sustainability levels and brands. For a very long time, consumers egg choices for home use used to be routine and price elasticity was measured nearly zero. NGO's had a very strong role in Europe for consumer awareness for more sustainable animal friendly eggs. Normally new product introductions need lots of marketing and publicity support for market acceptance, but for eggs the change went smoothly due to the low product price(elasticity).

For poultry meat price elasticity has always been very high and market penetration of sustainable products is relatively low. For poultry meat supermarkets and short food chains were very important for market acceptance of new products. Several countries used a different approach for market development of sustainable poultry meat.

In the out-of-home market price elasticity is much higher and the use of sustainable products much lower, since sustainability is dif-

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ficult to control and hardly visible communicated. Other consumer choice motivations are more leading in this market: price, taste and location.
At this moment EU faces supply drops, due to Avian Influenza outbreaks and decrease of animal production, and total food prices increase. This immediately led to other consumer choice behavior and assortment changes.
Wageningen UR explored various transition and intervention paths to maintain consumer engagement on animal friendly sustainable food products. This presentation will give an overview of the learnings of several studies that concluded that knowledge transfer and marketing are not sufficient for real consumer engagement with sustainable food products on the long run.

4129 Economic potential of newly selected chicken genotypes for dual-purpose use under organic conditions

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Dual-purpose chickens could help pose a solution to the ethical dilemma of in-ovo sexing and the uneconomic production of chicken meat from males of layer lines. However, to be a real alternative, the animals must also be economically viable for farms (Hörning, 2024). The use of robust and productive dual-purpose genotypes could have the potential as an adaptation strategy to end the practice of chick culling in the egg sector. The PPILOW project investigated the performance of three new dual-purpose genotypes to improve welfare and sustainability in organic farming (Hammershoj et al., 2021). Trials in France, Denmark, and Germany identified the most promising types for each country, with Germany selecting the laying-type (DPG Dual purpose genotype).
This study assessed the economic impacts of the on-farm trials of males and females in Germany. The on-farm analysis focuses on how the performance and product characteristic of the males and females of this genetic differ compared to the control genotype and how this will be reflected in the economic efficiency. The technology impact policy impact calculations (TIPI-CAL) model was used to analyze the farm data from trials conducted using two genotypes (JA757 and DPG laying type). TIPI-CAL (Chibanda et al., 2020) is a production and accounting model used for farm economic analysis allowing a detailed examination of farm-level variables.
Dual-purpose males of the laying type (DPG) reared under local farm conditions showed additional costs of roughly €194 per 100 kg live weight (LW; +38%) compared with the control genotype. This increase is mainly due to higher feed costs resulting from lower feed efficiency. The daily weight gain of the laying-type (DPG) was comparable to previously reported results for other dual-purpose males (Hörning, 2023). Total costs, at €703 per 100 kg LW, were higher than those reported by Hörning (2023). The total revenues for the dual-purpose genotype (€5,90 per kg LW) were not sufficient to cover the total costs.
Over a 71-week production period, the additional cost of dual-purpose hens compared with the control genotype was approximately €0,06 per egg. Their economic performance indicators were comparable to those reported by Pieper (2024). Feed consumption was similar to that of other dual-purpose genotypes (KTBL, 2024), but significantly higher than in the control group. The laying performance of the tested dual-purpose hens matched the data from Hörning (2023). To cover full costs, a price of €0,42 per egg would have been required.
Economic evaluations clearly demonstrate that efficient resource utilisation and effective marketing are pivotal for the economic and environmental sustainability of the dual-purpose chicken concept.
Keywords: Dual purpose genotype; Economic efficiency; Performance; Cost benefit analysis; Organic farming

4130 Developing a shadow price calculator for faba beans in phase-specific rations for layers and broilers using a stepwise regression analysis.

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Increasing feed costs are driving interest among poultry producers in alternative feedstuffs. Faba bean (Vicia faba L.) is an attractive ingredient because of its positive environmental impact, reasonable protein (~26%), amino acid and energy content (~35% starch). Increasing usage of faba bean for poultry could therefore have mutual benefits for both pulse and poultry sectors. The objective was to develop a practical and robust decision-making tool for poultry producers to assess the economic feasibility of faba bean inclusion in specific feed cost and formulation contexts. Monthly historical Western Canadian ingredient price data (March 2018 to April 2025; Agriculture and Agri-Food Canada) were obtained for wheat, corn, barley, peas, corn distillers dried grains with solubles, soybean meal, canola meal, canola oil, and synthetic Lys, Met, Thr and Trp. Historical prices were combined with a standardized ingredient composition matrix (including faba bean) to formulate least-cost, phase-specific diets for broilers (starter, grower finisher) and laying hens (early, mid, late lay) for each month using least cost feed formulation (New Century™ Format Solution Product software v. 2403.1.1.0; Datacor; Pittsburgh, PA). The shadow price (the maximum price at which an ingredient will be drawn into a least-cost formula without increasing formula cost) of faba beans in each formulation context was recorded. Optimized regression equations relating the historical shadow price of faba bean to historical prices of the other ingredients were generated using the GLMSELECT procedure of SAS v. 9.4 (SAS Institute; Cary, NC) in each formulation context. For laying hens, the optimized models included the price of corn, peas and D, L-methionine; with the same equation applicable to early, mid and late lay phases. The optimized broiler models included the costs of corn, wheat and soybean meal; with minor differences among starter, grower and finisher phases. The resulting models had a corrected $R2 > 0.97$. A Microsoft Excel™ (Microsoft; Redmond, WA) calculator was developed for laying hen and broiler diets, in which the relevant ingredient prices can be input, and the maximum price at which faba beans could be used in the ration without increasing the cost of the feed is the output. The retrospective shadow price analysis allowed a simple yet robust calculator to assess the economic feasibility of faba bean inclusion in laying hen and broiler diets. These equations could also serve as the basis for establishing contract prices between poultry producers and faba bean suppliers to develop a market for faba beans. This method is a novel approach of determining the relative economic value alternative of feedstuffs.
Keywords: Faba beans (Vicia faba L.); Feed formulation; Economics; Broiler; laying hen

4131 Big data analytics and decision-making effectiveness in feed processing firms: evidence from Ghana using the Information Systems Success Model

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Background: This study examines the role of big data analytics in enhancing decision-making effectiveness within Ghana's feed

processing firms, a critical but operationally constrained segment of the poultry value chain. Despite the strategic importance of feed mills—most of which are small-to-medium enterprises facing high input costs, seasonal supply volatility, quality compliance requirements, and production scheduling challenges—data-driven decision-making remains limited. Unlike feed industries in more advanced economies that leverage enterprise systems and predictive analytics, Ghanaian firms largely depend on spreadsheets and managerial intuition, restricting strategic and operational efficiency.

Anchored in the Information Systems Success Model, the study investigates how big data analytics utilization influences decision-making effectiveness across three management domains: strategic procurement, operational production, and tactical inventory management. Specifically, it evaluates how system quality, information quality, and service quality affect user satisfaction among key decision-makers, and how this satisfaction translates into improved decision accuracy, timeliness, and organizational performance outcomes.

Using a quantitative cross-sectional design, data are collected through structured surveys administered to 180 managers from registered feed processing firms in Ghana's major poultry-producing regions. Partial least squares structural equation modeling is employed to test the hypothesized relationships while controlling for firm size, technology maturity, and managerial experience. The study anticipates that information quality will exert the strongest influence on decision-making effectiveness, particularly for procurement and formulation decisions requiring accurate, timely, and integrated data. System quality is expected to significantly enhance user satisfaction, while service quality is projected to moderate sustained analytics use among less digitally experienced managers. The findings aim to provide practical guidance for feed processors' analytics investments, inform technology vendors serving agri-processing sectors, and support policymakers advancing Ghana's agricultural digitalization agenda.

Keywords: Big Data Analytics, Decision-Making Effectiveness, Feed Processing, Information Systems Success Model, Ghana, Data Quality, Agribusiness Management
Keywords: Big Data Analytics and Decision-Making Effectiveness; Feed Processing ; Information Systems Success Model; Data Quality; Agribusiness Management

4132 Integrated Assessment of Growth Performance, Carcass Characteristics, and Economic Efficiency of Three Broiler Breeds Raised Under Humid-Tropical Conditions

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This study evaluated the growth performance, carcass characteristics and economic efficiency of three broiler breeds—Hubbard, Cobb 500, and Ross 308—under humid-tropical conditions. A total of 300-day-old chicks (100 per breed) were randomly assigned in a completely randomized design (CRD) with five replicates of 20 birds per breed. All birds were fed a standard broiler diet over six weeks. Key parameters assessed included growth performance, carcass and internal organ traits, and economic efficiency indices. Growth was modeled using the Gompertz function, and correlations between performance traits were analyzed. Growth, carcass and economic efficiency data were analyzed using one-way ANOVA. Cobb 500 exhibited the highest ($p<0.05$) asymptotic body weight (4.30 kg) and growth rate (0.057/day), indicating superior growth potential. Ross 308 recorded significantly higher full and empty intestinal weights as well as liver weight, suggesting enhanced metabolic activity. Feed conversion ratio (FCR) and feed cost per kilogram of weight gain differed significantly among strains, with Cobb 500 and Ross 308 demonstrating greater cost-efficiency ($p<0.05$). Significant positive correlations were observed between body weight, dressing percentage, liver weight, and heart weight. Mortality rates varied significantly ($p < 0.001$), with Ross 308 showing the lowest mortality (0.25%), compared to Cobb 500 (2.16%) and Hubbard (2.67%). Morbidity did not differ significantly among strains ($p >0.05$). These findings suggest that Cobb 500 is genetically predisposed to rapid growth and economic efficiency, while Ross 308 offers advantages in survivability and gut development. The integration of biological performance, economic analysis, and growth modeling offers a comprehensive framework for optimizing broiler production strategies in humid-tropical conditions.

Keywords: growth modeling ; broilers; Cobb 500; Ross 308; Hubbard

4133 Economic Analysis of Integrated Fish-Poultry Farming Systems performance in Southwest Nigeria

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Integrated fish-poultry farming systems involve the simultaneous production of fish and poultry, where the waste products from one enterprise are utilized as inputs for the other. This symbiotic relationship promotes efficient resource use, reduces waste, and increases overall farm productivity and profitability. The study analyses and compares the economics of integrated fish-poultry farming systems performance in southwest Nigeria. A multistage sampling procedure was used to select a total of 381 respondents dis-aggregated into 112 poultry farmers, 121 fish farmers and 148 fish-poultry farmers using copies of structured questionnaire administered by the trained enumerators. Among the specific objectives stated for the study are to describe the socioeconomic characteristics(-Descriptive Statistics), estimation of enterprise profitability (Gross Margin Analysis), analysis of efficiencies (Stochatic Frontier Production Function), quantifying the economic value of waste recycling in the integrated system(Budgetary Analysis) and examining the constraints to fish-poultry farming system (Likert Scale).The result of the socioeconomic characteristics indicated that farmers that are into integrated fish-poultry enterprise are found to be most experienced (mean=12years), relatively young(mean=25years), mostly males and have more years of education(mean=15). The results of the farmers performances in terms of of technical and allocative efficiency shows that farmers operating integrated fish-poultry system are most economically efficient efficient when compared to their counterparts in lone poultry or lone fishery enterprises. Also, the results showed further that farmers in integrated enterprise of fisheries and poultry realized best profit margin probably because of the lesser input mobilized as a result of the use of waste from poultry to support the fishery section most especially in the area of feed that attracts about 70% of the production cost. Credit constraints, high cost of feed, climate change effect among others are the prominent constraints adversely affecting the enterprise performance. It was concluded that integrated farming system is an opportunity to mobilize optimal resource and realize more than proportionate output. It is recommended that government, institutions and other stakeholders in agriculture should, as a mat-

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ter of urgency, create awareness on fish-poultry farming systems through extension services and provide sufficient credit facilities for farmers to invest in the enterprise.
Keywords: Economics; Farming System; Fish-Poultry; Integrated; Economic Efficiency

4134 Economic Valuation of Live Poultry Markets in Lomé-Togo (West Africa)
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Abstract

This study offers a comprehensive economic valuation of the live poultry trade in Lomé, the capital of Togo, focusing on urban market dynamics. Drawing on field data from 100 traders across four principal marketplaces, the research investigates supply chains, species-specific sales frequencies, input costs, pricing strategies, and profit margins throughout the value chain.

The analysis reveals a strong predominance of local free-range chickens, sold by 89% of surveyed traders, followed by guinea fowls (45%) and pigeons (44%). Supply strategies differ by trader type: wholesalers typically source poultry from northern regions, while retailers rely on local procurement. Profitability is shaped by species type, transaction volume, and trader category, with local chickens and guinea fowls demonstrating the highest daily turnover.

The study identifies several structural constraints affecting market performance, including competition from imported frozen poultry, price instability, and elevated logistical expenses. Employing a robust margin analysis framework—encompassing markup ratios, profit margin rates, and daily gross margins—the research generates key indicators for assessing the sector's economic viability.

Findings underscore the strategic significance of live poultry markets for Togo's urban economy. They provide an evidence-based foundation for public policy interventions aimed at enhancing domestic production, optimizing distribution networks, and fostering long-term sustainability within the poultry sector.
Keywords: Live poultry trade; Urban markets; Lome; Togo; West Africa

4135 Commercial poultry production in Bangladesh: current trend, challenges and opportunities
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Current scenario:
Commercial poultry production in Bangladesh has rapidly accelerated since the early 1990s, driven by numerous beneficial factors, including improved genetics, advanced nutritional biotechnology, sophisticated equipment, and innovative management strategies. Approximately 16 GP farms and 206 breeder farms produce 17.5 to 20.0 million DOCs weekly (WPSA-BB, 2025). In Bangladesh, commercial poultry is conventionally raised by almost 80,000 small- and medium-scale farmers (SMFs) in open-sided gable-type houses, accounting for almost 60 % of total meat and egg production.
Challenges: Over the past four decades, the poultry sector has seen substantial growth in meat and egg production; however, numerous challenges have emerged one after another. The primary challenges include rising poultry feed costs, volatile market prices for DOCs) broiler meat, and eggs, disease outbreaks, and quality concerns about poultry meat and eggs; collectively, these factors pose significant obstacles for small and medium-sized farms (SMFs). Additional issues involve inadequate supply chain infrastructure, poor waste management, and a lack of effective policy incentives and regulatory frameworks.
Opportunities:
Despite the country's notable economic growth in recent years, the consumption of animal protein among the majority of marginal families remains inadequate. Poultry meat and eggs represent the 'cheapest' source of animal protein; hence referred to as the 'protein of the poor.' Furthermore, there is potential to increase per capita consumption of chicken meat and eggs from 10.3 kg and 137 eggs to 37.0 kg and 167 eggs by 2031, as established by our poultry industry's objectives.
Strategic recommendations:
In addition to production potential, it is imperative to focus on each phase of harvesting, processing, and marketing live broilers to ensure the safety and quality of the chicken meat and eggs. The transition from wet markets to processed and further processed poultry meats and egg products is a crucial issue that should be addressed urgently. An inequitable competition persists between giants and SMEs, in which the latter consistently emerge as the losers. The government must implement essential policies and support to ensure the survival of small-scale farmers in a favourable environment.
Conclusion:
The poultry sector in Bangladesh is vital for providing low-cost animal protein to its large population, hence enhancing food security, nutrition, and rural livelihoods. A coordinated effort among diverse stakeholders, including government, the business sector, and development partners, seems crucial to unlock the potential and sustainability of the sector.
Keywords: Poultry, sustainability, small-scale
Keywords: Commercial poultry; Live broilers; sustainability; Current scenario; Small-scale farmers

4136 Resilience and Innovation in the African Smallholder Poultry Sector: Pathways to Food Security and Sustainability
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The smallholder poultry sector plays a pivotal role in Africa's economic and food systems, contributing to livelihoods, poverty alleviation, and food security. Smallholders raise approximately 80% of poultry flocks on the continent, integrating local resources with modern technologies to sustain household nutrition and income. The sector still has problems, though, such as technical inefficiencies, managerial and socio-cultural barriers, weak governance structures, and being more vulnerable to global crises like the COVID-19 pandemic and the Russia-Ukraine conflict. This study evaluated the resilience strategies and innovations employed by actors in the African smallholder poultry sector and examined how these can be strengthened to foster sustainable economic growth and long-term contributions to Africa's food security.
A systematic review and meta-analysis were used to synthesise real-world evidence from empirical data. This approach helps to identify gaps and trends from data sourced from AJOL, Google Scholar, PubMed, ResearchGate, Scopus, and Web of Science databases, using specific inclusive and exclusive criteria. The findings indicated that literacy levels, stakeholder experience, cooperative society participation, and access to support links are critical for developing resilience strategies and ensuring that innovation thrives in sustained success. The establishment of smallholder poultry innovation platforms (SPIPs) is recommended to foster an inclusive approach and to promote a participatory market system among stakeholders. Systemic challenges continue to exist, stem-

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ming from limited technical capacity, governance weaknesses, and external shocks that disrupt production and trade. Consequently, SPIPs are expected to create a supportive policy environment that facilitates collaboration among stakeholders in the sector, thereby enhancing sustainability and improving food security.
Keywords: African economy; Innovation platform; Resilience; Smallholder poultry; Sustainability

Meat Processing Technology I

4042 Broiler breast myopathies: causative mechanisms and connective tissue involvement

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A remarkable post-hatch hypertrophic growth of the fibers represents a hallmark of the Pectoralis major muscles of broiler chickens belonging to fast-growing and high breast yield genotypes. This increase in myofibers diameter is negatively correlated to the connective tissue spacing both at perimysial and endomysial level which however are essential for proper muscle functioning and vascularization. Therefore, considering the importance of the extracellular matrix environment, the abovementioned diminished connective tissue spaces together with an enlargement of the myofibers have been associated with a progressive overproduction of fibrillar collagens and proteoglycans resulting in a fibrotic organization of the connective tissue and, ultimately, to the development of myopathic conditions in broilers including the wooden breast and white striping abnormalities. The worldwide emergence of these myopathies paved the way for a wealth of studies performed over the past fifteen years with the aim of characterizing the affected breasts as well as to identify the putative etiologies and molecular mechanisms underlying their occurrence. Despite the comprehensive knowledge gained concerning these conditions, which have multifaced and interconnected origins, their exact causative mechanisms remain not fully understood. Therefore, this speech will provide an update of the putative phenomena playing a key role in the pathogenesis of the growth-related myopathies in broilers by considering the structural arrangement of the connective tissue as the root for describing the microscopic alterations associated to their onset.

4043 Longitudinal study of the persistent presence of Salmonella Infantis in broiler houses

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Objective: Over the last decade, Salmonella Infantis became a persistent problem in the broiler sector, posing a threat to human health. In the European Union, S. Infantis is currently the fourth most common serovar in human cases, mainly caused by the consumption of improperly cooked poultry meat. In Belgium, S. Infantis was the most prevalent serovar since 2015. While the contamination on some farms is a one-time occurrence, the majority of farms struggle with a persistent presence. Therefore, it is crucial to understand the source and persistence of the S. Infantis contaminations in order to remediate these farms.
Methods: Five broiler farms (14 broiler houses) with a history of persistent S. Infantis contamination were monitored for two consecutive production rounds. Swab samples of the broiler houses and their environment were taken after cleaning and disinfection (C&D), during delivery of the one-day old chickens, during one thinning event and every week during production until slaughter or until the broiler houses were positive for S. Infantis. The samples were analyzed using the ISO 6579-1:2017 method.
Results: A first important observation is that, although rare (on average 2.9% of the 1243 samples), S. Infantis was still detected after C&D at all the farms, with only one exception. Frequently found contaminated areas were the floor (remaining loose dirt, drain, seams and cracks), cleaning materials (mop, broom, squeegee) and external samples (cadaver container). This shows current C&D protocols are insufficient to completely remove S. Infantis. This is a possible reason for the swift colonization of the birds during production. Based on overshoe samples 13 of the 28 broiler houses were contaminated already after one week, observed on four of the five farms. During delivery of one-day old chicks, Salmonella was never detected on the crates or paper inlays, indicating delivery is not the main contamination route. However, during 3 of the 9 delivery moments, tires of the truck were found contaminated probably indicating that the direct environment of the broiler houses was contaminated. During thinning, contamination of crates (n=320), loading surfaces of the trucks (n=22) and/or boots (n=52) was observed in three out of the four events. At that moment Salmonella was found in 21.7%, 50% and 5,7% of the samples, S. Infantis respectively in 14.1%, 13.6% and 3.8%,
Conclusion: The results of these monitoring underscore the importance of a thorough C&D, biosecurity and thinning as a risk factor.
Keywords: Salmonella Infantis; broiler houses; biosecurity; cleaning and disinfection

4044 Salmonella prevalence among various stages of broiler production and processing complex: A systematic review and meta-analysis

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Salmonella is a leading cause of foodborne illness in the U.S., often linked to poultry products. Vertically integrated broiler systems improve efficiency but may also enable Salmonella introduction and spread across production stages. To assess Salmonella prevalence in different stages of integrated broiler complexes and to determine the risk of bacterial spread, a systematic review and meta-analysis were conducted. A total of 34 trials from 27 different relevant studies were included from four different databases: Web of Science, Google Scholar, PubMed, and ProQuest. The keywords used were: (poultry OR bird OR chick* OR “Gallus domesticus” OR broiler OR avian) AND (pullet OR breeder OR hatchery OR broiler OR transport* OR processing plant OR slaughterhouse) AND (Salmonella OR “Salmonella enterica” OR “Non typhoidal Salmonella”) AND (commercial OR integrated OR organic OR pasture-based OR conventional) AND (strains OR serotypes OR variants) NOT (layers OR laying hens). The studies that incorporated at least two stages were included, while studies that involved nutritional studies, pathogen challenge, or vaccination trials were excluded. A generalized linear mixed model approach combined with logit transformation was used to stabilize the variance. Data was analyzed using metafor packages in R based on Salmonella prevalence in various stages and their changes from one stage to the subsequent. Significance level was set at 0.1. Due to high heterogeneity across studies for both prevalence and odds ratio (I2>90%), a random-effects model was used. The overall Salmonella prevalence in breeder house, hatchery, broiler house, transportation, pre-chill, and post-chill were 10%, 21%, 15%, 30%, 31% and 14%, respectively. In the U.S., the Salmonella prevalence was lowest in parent flocks at 6% (95% CI; 2-13%) and was significantly lower than hatchery, broiler houses, transportation, and pre-chill stage ($p<0.1$). The transportation stage had the highest Salmonella recovery of 62% (95% CI; 30-86%) in the

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U.S. Similar results were observed in the studies from outside the U.S., except slightly higher (42% and 23%) prevalence rates in pre-chill and post-chill rinsates, respectively. The odds of Salmonella detection were significantly increased ($p<0.01$) moving from parent flock to hatchery and from broilers to transportation. The odds of Salmonella detection were significantly decreased ($p<0.01$) when moving from the hatchery to the broiler house and after the chilling process. The results from this meta-analysis increased our understanding of Salmonella dynamics along the broiler production systems, therefore, potentially allowing for precise interventions to reduce Salmonella contamination in chicken products.
Keywords: Salmonella; Broiler production systems; meta-analysis; processing facilities; foodborne infections

4045 Walking a thin line: thinning broiler houses with Salmonella contaminated material

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Chicken meat and derivative products are one of the most important sources for human Salmonella infection. An important aspect is that certain serotypes of this pathogen continue to circulate persistently on broiler farms notwithstanding different intervention measures. In search for the role of certain contamination routes in the sector, equipment used during partial depopulation or thinning was sampled at ten different broiler farms in Belgium. All thinning materials, such as the trucks, crates and workwear of the catching crew were sampled before entering the broiler houses. The broiler houses were sampled using overshoes immediately before thinning and approximately one week after thinning, immediately before final depopulation, to evaluate if the remaining birds became colonized by Salmonella strains introduced during thinning. The results show that during nine of the ten thinning events Salmonella was present on the equipment used. Especially the crates and loading surfaces of the trucks were frequently contaminated, 19.3 % (62/320) of the sampled crates and 50 % (11/22) of the sampled trucks were found contaminated with S. Infantis, Paratyphi B var. Java or Livingstone. The relative prevalences of S. Infantis, Java and Livingstone were, respectively, 31.3 %, 67.5 % and 1.3 %. Despite the presence of Salmonella during thinning, no cross-contamination to the remaining chickens was observed. A low abundance of Salmonella bacteria, a higher resistance of the older (five weeks), remaining chickens or a small sampling size can explain these results. Nevertheless, the results of this study underscore the importance of appropriate biosecurity during thinning.
Keywords: Salmonella Infantis; Salmonella Paratyphi B var. Java ; broiler; thinning; biosecurity

4046 Gut instincts: Salmonella contamination based on monitoring systems in the broiler sector

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Salmonellosis is the second most important zoonosis in Europe, with consumption of contaminated broiler meat and its derivative products as an important source of infection. One of the many measures to prevent Salmonella contamination of poultry meat at the slaughterhouses is logistic slaughter, this means that per day the Salmonella contaminated flocks are slaughtered after the Salmonella free flocks. However, this principle relies on a monitoring system at farm level that is prone to false negatives. Therefore, this study evaluated the Salmonella status determined at farm level three weeks before slaughter using minimum 2 pairs of overshoes by comparing it with the Salmonella status at slaughter determined by testing the duodena and ceca contents of 153 flocks having an age of 5 or 6 weeks and mainly originating from the Flemish region of Belgium. Per flock, 30 gastro-intestinal packages were analyzed. This showed that despite the negative Salmonella status of all flocks, based on the overshoe samples, 20.9 % tested positive when analyzing intestinal samples at slaughter. The most abundant serovar was Infantis (68.8 %), followed by Paratyphi B var. Java (25.0 %). Other notable observations were that no differences in Salmonella prevalence were found between the different slaughter ages of 5 and 6 weeks ($P = 0.431$) and that Salmonella was more frequently isolated from the duodena than from the ceca (90.6 % and 53.1 % respectively; $P = 0.001$). In conclusion, more in-depth research is needed to understand the different reasons for the discrepancies in monitoring results, resulting in a re-evaluation of the current monitoring system to guarantee more correct logistic slaughter.
Keywords: Salmonella status monitoring; broiler; overshoe sampling; gastro-intestinal packages ; logistic slaughter

4047 Bio-preservative efficacy of lactic acid bacteria postbiotics for improving microbiological safety in broiler meat

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Postbiotics derived from lactic acid bacteria (LAB) are promising natural antimicrobials due to their bioactive metabolites. This study evaluated the chemical composition and antimicrobial efficacy of the product both in vitro and in vacuum-packaged broiler breast meat inoculated with meatborne pathogens during 10 days of storage. Postbiotics from Lactiplantibacillus plantarum (LP), Lactobacillus acidophilus (LA), and Lacticaseibacillus rhamnosus GG (LGG) were prepared by culturing LAB in MRS broth, followed by centrifugation and filtration, and subjected to GC-MS analysis. Neutralized postbiotics were used to obtain bacteriocin-like substances (BLS). Both postbiotics and BLS were tested against Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, and Bacillus cereus in an in vitro assay, while postbiotics were applied to the meat in a 1:5 ratio in the meat model. Principal component analysis revealed distinct strain-specific fatty acid profiles, with oleic acid being the primary driver of separation among LAB-postbiotics. In vitro, BLS achieved peak inhibition within 4-12 h, reducing the OD600nm of E. coli and Salm. Typhimurium by 40-50% at 12 h, whereas postbiotics provided stronger early suppression, lowering these pathogens by 80-86%, with the highest activity observed with LGG postbiotics (86%). In the model, untreated samples exhibited an increase in total mesophilic aerobic bacteria, as well as Gram-negative and Gram-positive pathogens, from 6.91 to 9.26 log10 CFU/g. In contrast, postbiotic treatments reduced counts to 1.06-3.64, 1.41-4.36, and 0.93-3.30 log10 CFU/g, respectively, with higher concentrations being more effective. LGG-postbiotics reduced Salm. Typhimurium in broiler meat compared to LA and LP. In conclusion, LAB-derived postbiotics, particularly LGG, can be utilized as natural preservative agents to enhance microbiological safety in the broiler industry.
Keywords: Bio-preservation; meat-borne pathogens; microbiological safety; lactic acid bacteria; postbiotics

4048 Effect of Booster Route on a Live-

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Modified Salmonella Typhimurium Vaccine in Challenged Broilers: Impact on Cecal Colonization, Organ Prevalence, and Carcass Contamination

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Vaccination serves as a key pre-harvest strategy to reduce Salmonella contamination in broilers. This study evaluated the effect of a live-modified Salmonella enterica serovar Typhimurium (ST) vaccine in broilers challenged with ST, comparing two booster routes (spray vs. drinking water) on cecal colonization, organ prevalence (liver and spleen), and carcass contamination. Six hundred broilers were randomly assigned to three treatments: T1 (vaccine spray + water booster), T2 (vaccine spray + spray booster), and T3 (sham spray + sham booster [spray + water]), with 8 replicate pens of 25 birds per treatment. All birds were orally challenged on day (d) 10 with 105 CFU/bird of nalidixic acid-resistant ST. Ceca, liver, and spleen samples were collected aseptically on d18, d28, and d35, with carcass rinses on d41. Cecal counts were log-transformed and analyzed using one-way ANOVA, while liver, spleen, and carcass prevalence were analyzed using Fisher's exact test or chi-square, as appropriate, in R ($P<0.05$ or $P\leq0.1$ as a tendency). On d18, cecal Salmonella log10 counts did not differ among treatments (T1: 0.63 ± 0.26, T2: 1.28 ± 0.34, T3: 0.68 ± 0.25). Counts increased by d28 and then declined by d35 compared with the previous sampling point, with no significant differences among treatments at any time point. Vaccinated birds showed a tendency towards lower liver Salmonella prevalence on d18 (T1: 4.17%, T2: 8.33%) compared with unvaccinated birds (T3: 29.2%; $P = 0.06$). On d28, overall liver prevalence differed significantly among treatments (T1: 20.8%, T2: 0%, T3: 25%; $P = 0.03$), although pairwise comparisons were not different between individual treatments. By d35, prevalence decreased in T1 and T3 and increased in T2 compared with d28, with no significant differences among treatments. On d18, spleen Salmonella prevalence did not differ among treatments (T1: 4.17%, T2: 8.33%, T3: 12.5%; P=0.86). By d28, prevalence increased in all groups relative to d18, with no significant differences. By d35, prevalence declined across all treatments compared with d28, again with no significant differences among treatments. Carcass Salmonella prevalence on d41 was similar across all treatments (T1: 87.5%, T2: 100%, T3: 68.8%). Under the conditions of this study, the live-modified ST vaccine tended to reduce Salmonella prevalence in the liver on d18 and showed a significant overall treatment effect on d28, suggesting potential protection against systemic infection; however, no measurable impact was observed on cecal colonization, spleen prevalence, or carcass rinses. These findings underscore the need for a comprehensive, multi-hurdle approach for Salmonella control in commercial poultry production.

Keywords: Salmonella Typhimurium; broiler; vaccine; booster route; prevalence

4049 Cost-utility analysis of measures against Campylobacter on broiler meat in Germany

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Campylobacteriosis enteritis is the most frequently reported foodborne zoonosis in the EU, with more than 246,000 confirmed cases annually and an estimated societal cost of €2.4 billion. Raw poultry meat is often contaminated because Campylobacter colonizes chickens asymptomatically making undercooked broiler meat and cross contaminated foods the main sources of infection. Since Campylobacter does not harm flock productivity, farmers and slaughterhouses have little incentive to invest in control, while society bears the health and productivity costs.

This study assessed on farm, slaughterhouse and consumer level interventions in Germany using cost utility analysis. For the cost estimate, guided multi stage expert interviews with 8 experts from slaughterhouses (around 75% of German production), slaughterhouse equipment supplier and banking were held, an actual offer for each intervention was obtained and a profit-maximizing farm model and the AGMEMOD market model were used. The utility of the measures was determined using literature, German health insurance data and a case-specific quantitative risk assessment model. Utility was measured in disability adjusted life years (DALY) Banning thinning (partial depopulation) reduces contamination by avoiding introduction of catching crews and equipment and delivered a positive cost utility ratio (6,000€/DALY), when slaughter age is not restricted additionally (-114,000€/DALY). Vaccination (-28,000€/DALY) remained uneconomical because no licensed vaccine exists today and its efficacy is uncertain. Chemical decontamination with peracetic acid (PAA) was cost effective via spray (21,000€/DALY and dip (13,000€/DALY) methods. Hot steam treatments (25,000€/DALY) also produced net societal benefits, with additional ultrasound measures (20,000€/DALY) reducing cost utility. Crust Freezing (-107,000€/DALY) shows inferior cost utility. Consumer education programs (25,000€/DALY) proved valuable since poor kitchen hygiene and cross contamination remain important risk factors, so improved hygiene reduces Campylobacter exposure and other food borne infections.

Broiler chickens raised in "open climate" barns, which German food retailers want to establish nationwide by 2030 for animal welfare reasons, are exposed to higher pathogen exposure, which would further enhance the benefits of these measures (+50%). This represents a significant tradeoff between biosecurity, food safety and animal welfare. Overall, thinning bans, PAA decontamination, hot steam treatments and consumer education are recommended, with compensation to producers for added costs.

Keywords: Broiler Chicken; Campylobacter; Food Safety; Meat Quality; Cost-Utility Analysis

4050 Optimization of PMAxx Viability RT-qPCR for Selective Detection of Viable Avian Influenza Virus in Animal Food Matrices

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Accurate detection of infectious avian influenza virus (AIV) in food matrices is essential for disease surveillance and public health. Conventional RT-qPCR cannot differentiate viable from non-viable virus, leading to potential false-positive results. This study aimed to optimize a PMAxx-based viability RT-qPCR (v-PCR) assay for selective detection of viable AIV in animal food matrices, using low-pathogenic avian influenza virus (LPAI) as a biosafety level 2 surrogate for highly pathogenic AIV (HPAI). LPAI was propagated in Madin–Darby canine kidney (MDCK) cells and divided into viable and non-viable groups. Viral inactivation was achieved exclusively by heat treatment at 95 °C for 6 minutes, and complete loss of infectivity was confirmed by cell culture passage. To evaluate assay performance in complex food matrices relevant to dry cat food, viable and heat-inactivated virus were spiked into liquid milk samples, which were used as a surrogate high-protein model to mimic matrix-associated inhibition encountered in dry pet food.

Viral suspensions were treated with 50 µM PMAxx prior to RNA extraction. AVL buffer was incorporated to minimize protein coagulation and enhance dye penetration, while sodium dodecyl sulfate

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(SDS) and RNase inhibitors were added to preserve RNA integrity. Treated and untreated samples were analyzed by RT-qPCR targeting the influenza A matrix (M) gene. Statistical analysis was performed using one-way ANOVA ($p < 0.05$).
PMAxx-vPCR significantly reduced detection of non-viable viral RNA following heat inactivation, with a mean ΔCt of 5.9, confirming effective discrimination between viable and non-viable virus. In milk-based model assays, PMAxx efficiently suppressed amplification of non-viable RNA at low viral loads but showed reduced suppression at higher RNA concentrations, highlighting matrix-associated limitations. This optimized PMAxx-vPCR protocol improves selective detection of viable AIV in dry cat food–relevant matrices and provides a practical framework for developing HPAI viability assays under standard biosafety conditions. Further optimization of dye concentration and buffer composition may enhance performance at high viral loads.
Keywords: Avian influenza; PMAxx; viability PCR; RT-qPCR; food safety

4051 Using integrated model systems to study the effects of partial meat replacement with four pulse proteins on the rheological, textural, and water-binding (T2 NMR) properties
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This study presents a systematic framework combining dynamic rheology, texture analysis, T2 NMR, and cooking loss to evaluate the functionality of pulse proteins as partial chicken meat replacers. Two lean ground meat systems were examined: one without added water (S1) and another with 50% added water and further finely chopped (S2). Both systems were prepared at three salt levels (0, 1.5, and 3%) and partially replaced (0, 25, or 50%) with chickpea protein concentrate (CP), faba bean protein isolate (FB), and low- and high-gelling pea protein isolates (PA and PB), hydrated to match the protein content of the meat. Samples were mixed well cooked to 72°C. Three-way ANOVA evaluated the effects of protein type, salt, and replacement level, with significant interactions further examined using two-way ANOVA and Tukey's HSD test. At 0% salt, all pulse proteins significantly reduced cooking loss in S1, whereas in S2, only 50% PB showed a significant reduction; other treatments increased loss. At 1.5% and 3% salt, cooking losses in S1 were minimal; in S2, FB and PB significantly reduced cooking losses at 50% replacement, while CP increased it. NMR T2 data (25% replacement) revealed that PB retained the highest proportion of immobilized water (T21: 30-70 ms) within its gel matrix (at 3% salt, 51% in S1 and 65% in S2). Although hybrid systems generally showed lower maximum penetration force than their respective controls (CL), PB matched the CL in S1 (25% replacement) and ranked highest among all protein types in both systems. Gel hardness followed a similar pattern; although no hybrids matched the CL, PB replacements consistently produced the firmest gels. Rheological measurements (25% replacement) showed that PB's storage modulus (G′) most closely aligned with the CL, particularly at 3% salt (PB: 100 kPa vs. S2's CL: 115 kPa). Overall, PB demonstrated the greatest water-binding capacity and most closely replicated the texture of the control model meat systems at 25% replacement, suggesting that high-gelling pea protein isolates can effectively replace up to 25% of meat under both reduced- and regular-salt conditions. This screening framework successfully differentiated the performance of pulse proteins across lean meat systems differing in moisture, particle size, and salt level.
Keywords: Chicken; Plant proteins; Hybrid meat; Texture; NMR

4052 Integrated multi-omics profiling identifies energy metabolism dysfunction and RHOA/ROCK pathway activation in wooden breast
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Wooden breast (WB) is a severe myopathy affecting broiler Pectoralis major muscles, resulting in significant economic losses. To elucidate the molecular mechanism, we conducted an integrated multi-omics analysis on 42-day-old male Arbor Acres broilers. Birds were classified via manual palpation into Normal (NM) and Severe WB groups. Muscle samples were analyzed using untargeted metabolomics (n = 10) and data-independent acquisition (DIA) proteomics and phosphoproteomics (n = 4). Statistical analyses employed Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) for metabolomics and the Limma package for proteomic data. Significance was defined as an FDR-adjusted $q < 0.05$. Metabolomic analysis revealed distinct group separation ($Q2Y = 0.647$), identifying 24 differentially expressed metabolites ($q < 0.05$). Specifically, energy carriers (ATP, ADP) were significantly downregulated, while degradation products (inosine) and acylcarnitines were upregulated in WB, indicating a severe energy crisis. Proteomic profiling identified 1,036 differentially expressed proteins ($q < 0.05$; fold change □1.2). Key enzymes in glycolysis (e.g., ENO3, PKM), the TCA cycle (e.g., IDH, SDH), and oxidative phosphorylation were significantly downregulated. Conversely, fibrosis-related proteins (Collagen I/III) were upregulated. Machine learning (SVM-RFE/Lasso) identified five candidate biomarkers (GOLGA2, AHCYL2, L1CAM, PTRH2, and GOLPH3). Phosphoproteomic analysis detected 126 differentially phosphorylated sites ($q < 0.05$) and highlighted significant activation of the RHOA/ROCK signaling pathway, linking calcium dysregulation to cytoskeletal remodeling. These findings demonstrate that WB is driven by an energy metabolic collapse leading to fibrosis via RhoA/ROCK activation.
Keywords: Wooden Breast; Multi-Omics Analysis; Energy dysfunction; RHOA/ROCK signaling; Proteomics

Nutrition: Enzymes I

4053 The past, the present and the possible future of feed enzymes
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Feed enzymes have been used commercially for more than 40 years and are now included in the majority of monogastric feeds globally. Their value is in their ability to target anti-nutrients or poorly digested nutrients thus allowing the animal to extract more value from its diet. Nevertheless, there is still debate regarding the exact mechanism by which these benefits are achieved and the lack of consensus has probably limited the rate of advance in their design and thus functionality. This is especially true for NSPases which were the first enzymes employed commercially. Three major mechanisms have been debated since their introduction; Viscosity reduction, cell wall destruction and prebiotic provision. Authors tended to focus on their favoured mechanism and as a result regularly failed to collect data relevant to the competing mechanisms. Thus, there was a lack of consistency in reporting not only the materials and methods, but also the outcomes which has limited the rate of advance in understanding the mode of action of these enzymes. The greatest limitation has been a clear explanation of the identity of the enzyme, the quantity employed and most importantly its purity. Contaminant activities which are neither listed or assayed could play a significant role in the responses observed. In the cases where mixtures of two separate enzymes are blended it is possible that the activity(s) responsible for the response noted

are not aligned with the activities declared. Lack of dose optimisation data for some constituent enzymes coupled with lack of full factorial designs questions the choice of inclusion levels for components of such multi-sourced enzymes. This is especially relevant given the value of adding more enzyme classes to the diet follows the law of diminishing returns. The state of the literature is such that retrospective analysis (meta- and holo-analysis) is hampered by inconsistently reported characteristics of the enzymes, diets and animals employed along with variable reporting of critical information.

The three proposed mechanisms for NSPases (viscosity, cell wall and prebiotic) each have their strengths and weaknesses and it is likely that all three play a role in the responses observed, but in modern diets it is likely a modification of the prebiotic mechanism (ie stimbiotic and prebiotic combined) which likely plays the greatest part. Current use and future development of new and better enzymes would clearly benefit from a more complete understanding of the mechanisms by which they function which would allow for more strategic and targeted use of current enzymes and for the design of better future products. The same is true for phytases where it is clear that significantly more benefit can be derived from this enzyme if it were able to completely dephosphorylate phytate before it exits the gastric phase. An understanding of the mechanism of action is essential if the bottlenecks are to be identified, allowing for design of superior products.

4054 Effect of Protease Type and Dosage on Growth Performance in Broilers: A Dose-Response Trial

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This study evaluated the dose-response effects of three commercially available protease types alkaline, acidic, and a combination of acidic + alkaline on broiler growth performance. A total of 6,000 mixed-sex broiler chicks were assigned to a 3 × 4 factorial arrangements comprising three enzyme types (Acidic protease 20000 units per/g, Alkaline protease 50000 units/g) and four dosages (0, 0.02%, 0.04%, 0.06%). Each treatment had 10 replicates of 50 birds each. Birds were fed a standard commercial diet as per Nutrient standards set by Ross 308, containing a mix of vegetable and animal protein sources with assigned protease treatments. All the enzyme products were used over the top, without using any matrix value. Growth parameters including feed intake (FI), body weight (BW), feed conversion ratio (FCR), and mortality (%) were recorded on day 35. Data were analyzed using a completely randomized design (CRD) applying two-way ANOVA. Statistical significance was set at $P < 0.05$.

At the 5th week, protease treatment showed no significant effect on any parameter. Feed intake was the only parameter significantly affected by dosage. The effect of protease supplementation on broiler growth varies by age and treatment interaction. While certain protease types improved body weight and FCR during the 3rd week, their effects diminished by the 5th week. Dosage alone had limited influence, with significant impact observed only on feed intake at later stages. Notably, the interaction between enzyme type and dosage played a critical role in determining performance outcomes. These results emphasize the importance of timing, enzyme selection, and appropriate dosage combinations when formulating enzyme-supplemented poultry diets.

Keywords: Protease; Dose Response; Broiler; Growth performance; feed conversion ratio

4055 Multi-component protease supplementation mitigates the negative effects of low crude protein diets in broilers

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Severe crude protein (CP) reduction can impair growth, but supplementation with a multi-component protease (PRO, Jefo Nutrition) may help maintain performance and reduce nitrogen (N) excretion in low-CP diets balanced with essential amino acids. This study evaluated the effects of PRO on growth performance, gut health, metabolic responses, and N excretion in broilers fed low-CP diets. A total of 720 male Ross 308 broilers were assigned to five treatments: (1) Positive Control (PC) with standard CP (23%, 21.5%, and 19.5% in starter, grower, and finisher phases); (2) Negative Control 1 (NC1) with a 1.5-point CP reduction; (3) Negative Control 2 (NC2) with a 3.0-point CP reduction; (4) NC1 supplemented with PRO (NC1+PRO); and (5) NC2 supplemented with PRO (NC2+PRO). Diets were balanced with synthetic methionine, lysine, threonine, tryptophan, valine, arginine, and isoleucine. Electrolyte balance was maintained using sodium bicarbonate and potassium chloride. Each treatment had 12 replicates of 12 birds per pen. Performance was measured on days 1, 10, 24, and 42. Cecal contents and jejunal tissues were collected on day 28, and fecal N was measured on day 42. Data were analyzed by ANOVA with Tukey's test ($P \leq 0.05$). By day 42, body weight (BW) was higher in PC (3,271 g), NC1+PRO (3,263 g), and NC2+PRO (3,257 g) than NC2 (3,143 g; $P<0.05$), while NC1 was intermediate (3,198 g). Average daily gain (ADG) was also higher in PC (76.9 g), NC1+PRO (76.7 g), and NC2+PRO (76.5 g) vs. NC2 (73.8 g; $P<0.05$), with NC1 intermediate (75.1 g). Mortality-adjusted feed conversion ratio (FCR) was better in NC1+PRO (1.487) vs. NC2 (1.556; $P<0.05$) and comparable to PC (1.501), NC1 (1.528), and NC2+PRO (1.499). Cadaverine in the hindgut was higher in NC1 (2.23 μmol/g) and NC2 (2.27 μmol/g) than in PC (1.36 μmol/g; $P<0.05$), while NC1+PRO (2.02 μmol/g) and NC2+PRO (1.82 μmol/g) were intermediate. Tyramine was higher in PC (0.22 μmol/g) and NC1 (0.21 μmol/g) than in NC1+PRO (0.15 μmol/g) and NC2+PRO (0.14 μmol/g; $P<0.05$), with NC2 (0.18 μmol/g) intermediate. Nutrient transporter EAAT3 expression was higher in NC2+PRO (2.55; $P<0.05$ vs. NC2 [0.83]), while PEPT1 and BOAT1 were also elevated in NC2+PRO (1.33 and 1.43) vs. NC2 (0.54 and 0.71; $P<0.05$), with other treatments intermediate. PRO increased IGF-1 expression, which was higher ($P<0.05$) in NC1+PRO (2.56) and NC2+PRO (2.12) vs. PC (1.01), NC1 (0.97), and NC2 (1.00). Fecal N was lower in NC1+PRO and NC2+PRO (1.79%) vs. PC (1.95%; $P<0.05$), with NC1 (1.81%) and NC2 (1.82%) intermediate. In conclusion, protease supplementation in low-CP diets mitigated the negative effects of CP reduction by restoring performance, enhancing gut health and metabolic responses, and reducing N excretion.

Keywords: Protease; Broilers; Growth performance; Nitrogen excretion; Gut health

4056 Enhancing laying performance and sustainability with glucose oxidase and protease during peak production in brown layer hens

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Energy and protein are the most expensive nutrients in poultry diets, and the cost of energy is higher in layer feeds because rations

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are diluted with 8–9% limestone or shell grit to meet calcium requirements. Any feeding strategies that improve nutrient utilisation can reduce feed costs and enhance production efficiency. Exogenous proteases improve protein digestibility, lower feed costs, and reduce nitrogen excretion. Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide, providing antimicrobial activity and reducing intestinal pH to promote the proliferation of beneficial microbiota. Therefore, this study was designed to evaluate the effects of protease and GOD supplementation on laying performance and egg quality in Hy-Line Brown hens during peak production.

The experiment included four dietary treatments: a positive control (PC: 2,800 kcal/kg ME, 0.84% digestible lysine), a negative control (NC: ME and lysine reduced by 30 kcal/kg and 5% relative to PC), and two NC diets supplemented with either YIDUOZYME protease (200 g/t, VTR Biotech, Zhuhai, China) or YIBEIKANG GOD (200 g/t, VTR Biotech, Zhuhai, China). All diets were corn–soybean meal based with canola meal and wheat DDGS and contained 1,000 FTU/kg of Microtech phytase (VTR Biotech, Zhuhai, China). A total of 384 hens (20 weeks old) were randomly allocated to the four treatments, each with eight replicates of eight birds. Hens were fed for 15 weeks following a 2-week adaptation. Egg production was recorded daily and egg weight weekly. Egg quality was assessed at 35 weeks using 25% of eggs per replicate. Data were analysed by one-way ANOVA in JMP® with replicate means as the experimental unit (n = 48).

Diets significantly affected egg production rate (P = 0.009), egg weight (P = 0.034), egg mass (P = 0.001), and FCR (P = 0.013), while feed intake and body weight were unaffected (P > 0.05). The PC birds achieved the highest production (98.2%) and egg mass (64.3 g egg/bird/day) with the most efficient FCR (1.919 g/g). The NC birds showed lower production rate (95.1%) and poorer FCR (2.020 g/g). Protease (97.4%, 63.1 g egg/bird/day, FCR = 1.955) and GOD (97.5%, 63.6 g egg/bird/day, FCR = 1.938) improved laying performance and FCR to levels statistically similar to the PC. Egg quality parameters, including eggshell strength, thickness, Haugh unit, and yolk colour, were not significantly affected by dietary treatments (P > 0.05).

Supplementing nutrient reduced layer diets with protease or glucose oxidase restored production and feed efficiency to that of the control diet, demonstrating their potential to maintain performance under lower nutrient density diets and improve cost efficiency.

Keywords: Laying hen; Protease; Glucose oxidase; Feed efficiency; Sustainability

4057 Effect of a multi-carbohydrase complex produced by Talaromyces versatilis on growth performance of broiler chickens fed multi-ingredient diets from day 1 to 21

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Non-starch polysaccharides (NSPs) are well-known anti-nutritional factors in cereal-based poultry diets, as they reduce nutrient digestibility and compromise intestinal health. The inclusion of exogenous carbohydrases can help to mitigate these adverse effects, particularly when using non-conventional feed ingredients such as rice polishings, rapeseed meal, wheat bran, and sunflower meal. This study investigated the impact of a multi-carbohydrase enzyme complex derived from Talaromyces versatilis on the growth performance of broiler chickens fed multi-ingredient diets.

A total of 1,500 one-day-old Ross 308 mixed-sex broilers were randomly allocated to three treatments, each with ten replicates of 50 birds per pen. The dietary treatments included a Positive Control (PC) group fed a standard broiler diet formulated according to commercial nutrient specifications as per industry practice; a Negative Control (NC) group fed a diet reduced in apparent metabolizable energy (AME; -80 and -79 kcal/kg) and digestible amino acids (DAA; -2.35 and -2.57%) in starter (day 0–10) and grower (day 11–21) phases, respectively. The third treatment consisted of NC supplemented with a multi-carbohydrase enzyme complex (Rovabio® Advance T-Flex) at 50 g per metric ton of feed. All diets were formulated using multiple ingredients, including maize, soybean meal, rice polishings, canola meal, poultry by-product meal, rapeseed meal, fish meal, wheat bran, and sunflower meal. Data were analyzed using GLM Procedures of Minitab Statistical Software 18. Means were compared using Tukey's Test.

Compared to those fed the PC diet, broilers fed the NC diet exhibited inferior performance (P<0.05) in terms of body weight (BW; 1001 vs. 943 g/bird), feed conversion ratio (FCR; 1.383 vs.1.307), and European production efficiency factor (EPEF; 351 vs. 306), indicating that reductions in AME and DAA levels adversely affected growth performance. Feed intake and livability were not different (P>0.05) among treatments. Supplementation of multi-carbohydrase enzyme complex to the NC diet improved (P<0.05) birds' performance (FCR, 1.278 vs. 1.307; EPEF, 350 vs. 306). The multi-carbohydrase enzyme complex compensated for these deficiencies, restoring performance parameters (FCR and EPEF) to levels comparable to the PC group. This improvement is potentially attributed to enhanced nutrient digestibility by enzyme supplementation, resulting in greater availability and utilization of metabolizable energy and amino acids. In conclusion, inclusion of a multi-enzyme complex produced by Talaromyces versatilis in multi-ingredient diets effectively alleviates and compensates for the impact of reduced AME and DAA levels on the growth performance of broilers.

Keywords: Broiler; multi-carbohydrase enzyme; Talaromyces versatilis; non-starch polysaccharides; growth

4058 Combination of β-mannanase and xylanase enhances growth performance, nutrient digestibility, and intestinal integrity of broilers fed low-energy diets containing high soluble non-starch polysaccharides

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Exogenous carbohydrases are effective in mitigating the anti-nutritional effects of non-starch polysaccharides (NSP) and enhancing the release of entrapped nutrients. Copra meal and palm kernel meal are cost-effective alternative feed ingredients for poultry diets; however, they contain high levels of NSP, such as β-mannans and arabinoxylans, which can reduce nutrient digestibility and limit bird performance. A total of 744 one-day-old male Ross 308 broiler chicks were used in two feeding trials to evaluate the effects of β-mannanase and xylanase supplementation on growth performance, nutrient digestibility, and gut morphology in broilers fed diets containing high levels of soluble NSP. In Experiment 1, 600 chicks were distributed into 24 replicates of 25 birds each and assigned to one of the following treatments: a control diet (CON; corn–soybean meal with copra meal and palm kernel meal), a negative control with reduced energy (NC; −90 ME kcal/kg), and a reduced-energy diet supplemented with 140 ppm β-mannanase and xylanase (NCMX; Hemicell W-L, Elanco Animal Health, USA) in a completely randomized design (CRD). In Experiment 2, 144 birds were housed in metabolic cages to assess nutrient digestibility using the same dietary treatments (8 replicates of 6 birds each). Titanium dioxide at the level of 0.3% was used as indigest-

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ible marker. All birds were housed in a curtain-sided, evaporative cooling system house with rice husks as a bedding material. Feed and water were provided Ad-libitum from day 0 until day 35. All zootechnical data were subjected to one-way ANOVA. The NC diet increased feed intake (FI), decreased body weight gain (BWG), thereby, increased feed conversion ratio (FCR) during the grower, finisher, and overall phases ($P < 0.05$). Birds received NC diet had lower apparent ileal crude fiber digestibility, apparent total tract crude fiber digestibility and apparent metabolizable energy (AME) when compared with the CON group ($P<0.05$). However, the NCMX diet restored FCR, the digestibility crude fiber and AME to levels comparable with the CON group. On day 35, the NC birds exhibited higher crypt depth and lower villus height-to-crypt depth ratios in the duodenum, jejunum, and ileum when compared with the CON and NCMX birds ($P < 0.05$). Overall, combination of β-mannanase and xylanase supplementation in reduced-energy broiler diets containing high soluble NSP improved feed efficiency, nutrient digestibility, and intestinal morphology, thereby enhancing overall production performance.

Keywords: β-mannanase; copra meal; crypt depth; palm kernel meal; xylanase

4059 Effect of ahiflower seed and ahiflower presscake with or without multi-enzyme supplementation on growth performance, gizzard weight, bone weight, and bone breaking strength in broiler chickens

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Ahiflower seed (AS) is a recently explored oilseed recognized for its high concentration and unique fatty acid profile. In Canada, ahiflower oil is extracted via an expeller-pressing method, generating a substantial amount of presscake (APC) as a by-product. The APC is rich in Ca, P, protein, and energy and has potential as a cost-effective ingredient in poultry nutrition. Due to their high fiber content, AS and APC could negatively affect nutrient utilization and growth performance. The objective of this study was to determine the effect of including 10%AS and 10%APC in broiler chickens' diets with or without the supplementation of a multi-enzyme (ENZ) consisting of xylanase, beta-glucanase, phytase, protease, and cellulase on growth performance, gizzard weight, and bone weight and breaking strength in broiler chickens.

One-day-old Ross 308 broiler chicks were housed in 3 rooms and assigned to 6 treatments (2x3 factorial design), with 2 levels of ENZ (with or without) and 3 diets:1)Control (CON); 2) CON+10%AS; and 3)CON+10%APC. There were 6 replicate floor pens/treatment, each housing 15 birds. Feed intake (FI) and body weight (BW) were determined on d 14, 24, and 35, and BW gain (BWG) and feed conversion ratio (FCR) were calculated. On d35, 8 birds/treatment were euthanized to determine tibia weight and breaking strength, and gizzard weight. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS

Room effect was not significant in all the parameters measured. 10%AS and 10%APC resulted in lower FI ($P=0.0004$), lower BWG ($P<0.0001$), and higher FCR ($P<0.0001$), than CON, in the starter phase. During the grower phase, 10%APC resulted in lower BWG ($P<0.0001$) and higher FCR ($P=0.0075$) than CON and 10%AS. In the finisher phase, both 10%AS and 10%APC reduced ($P=0.0001$) BWG while only 10%APC increased ($P=0.0008$) FCR, compared to CON.

There was no effect of ENZ or diet x ENZ on gizzard weight, right and left tibia weight or right and left tibia breaking strength. However, birds fed 10%APC with ENZ had higher ($P=0.0020$) gizzard weight (19.3g/kgBW) compared to CON with ENZ (15.3g/kgBW) or without ENZ (15.2g/kgBW). Irrespective of ENZ, the left and right tibia weights were lower ($P<0.05$) in the birds fed 10%APC compared to those fed CON. Furthermore, the right tibia of birds fed 10%APC without ENZ were easier ($P=0.0211$) to break (377N), compared to CON (478N) and 10%AS (480N). In conclusion, including 10%APC in broiler chickens' diet reduced growth performance and tibia weight. However, ENZ may improve the utilization of APC by increasing gizzard weight and bone strength.

Keywords: ahiflower; multi-enzyme; bone breaking strength; gizzard weight; growth performance

4060 Enriching broiler chickens' breast and thigh meats with fatty acids by feeding ahiflower seed and ahiflower presscake with or without multi-enzyme supplementation

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Ahiflower seed (AS) is a novel oilseed known for its high and distinctive composition of fatty acids (FA). Ahiflower presscake (APC), the product left after oil extraction from AS also contains moderate amounts of FA. While AS and APC have been successfully utilized to enrich eggs with FA and support laying hen nutrition, their application in enriching broiler meats with FA remains unexplored. Given their high fiber content, which may impair the capacity to enrich meats with FA, this study evaluated the effects of dietary inclusion of 10%AS and APC, with or without a multi-enzyme supplement (ENZ) containing xylanase, β-glucanase, phytase, protease, and cellulase, on breast and thigh FA contents.

One-day-old Ross 308 broiler chicks were housed in 3 rooms and assigned to 6 treatments in a 2x3 factorial design, with 2 levels of ENZ (with or without) and 3 diets: 1)Control (CON); 2) CON+10%AS; and 3)CON+10%APC. There were 6 replicate floor pens/treatment, each housing 15 birds. On d35, 8 birds/treatment were euthanized to collect breast and thigh muscles for FA analysis. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS.

Room effect was not significant in all the parameters measured. There was no effect of ENZ or ENZ x diet for all FA in the breast meat. However, the concentration of stearidonic acid (SDA) was significantly higher ($P=0.0467$) in the breast of chickens fed 10%AS (0.14 mg/g), compared to those fed CON (0.06 mg/g) and 10%APC (0.06 mg/g). Omega6:Omega3 (n6:n3) was significantly lower ($P<.0001$) in the breast of chickens fed 10%AS (2.89), compared to those fed CON (5.00) and 10%APC (3.71).

In the thigh meat, there was no effect of ENZ or interaction between ENZ and diets, except for n6:n3, which showed a significant interaction ($P=0.0002$). ENZ reduced n6:n3 in the CON (5.86 vs. 6.73) but not in the other treatments. The Inclusion of 10%AS significantly increased the contents of total polyunsaturated FA (PUFA; $P=0.0100$), total omega3 FA ($P<.0001$), gamma linolenic acid (GLA; $P=0.0002$), alpha linolenic acid (ALA; $P<.0001$), SDA ($P<.0001$), eicosapentaenoic acid (EPA; $P<.0001$), and docosahexaenoic acid (DHA; $P<.0001$) in the thigh muscle. Furthermore, n6:n3 was significantly reduced ($P<.0001$) by 10%AS (3.00), compared to the CON (6.30) and 10%APC (4.16).

In conclusion, inclusion of 10%AS in broiler chickens' diets significantly increased the concentrations of total omega3 FA, total PUFA, ALA, GLA, SDA, EPA, and DHA in the thigh, reduced n6:n3, and increased SDA in the thigh and breast meats. Addition of ENZ did not affect the enrichment of broiler meat with FA but increased n6:n3 in the CON.

Keywords: ahiflower; multi-enzyme; thigh and breast meat; polyunsaturated fatty acids; omega 3 fatty acids

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4061 Egg production and apparent retention of components in 48-week-old Lohmann Brown-Classic hens fed a corn-soybean meal-wheat middlings-based diet supplemented with multiple doses of fiber-degrading enzymes

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The effects of multiple doses of FDE on egg production, quality traits, and apparent retention (AR) of dietary components were investigated in 48-week-old Lohmann Brown-Classic hens placed in enriched cages (4 hens/cage). A corn-and soybean meal basal diet was formulated with 19% wheat middlings and fed without or with 50, 100, or 200 g FDE/ton. The FDE was a blend of endo-1,4-β-xylanase and endo-1,4-β-glucanase, at 5600 and 2500 U/g, respectively. The mash diets contained titanium dioxide as an indigestible marker and 2,000 FTU of phytase/kg. Diets were allocated using a completely randomized design (n = 10). Birds had free access to feed and water for 16 weeks. Hen-day egg production (HDEP), feed intake (FI), egg weight, egg mass, FCR, eggshell thickness, breaking strength, Haugh unit, albumen height, and yolk color were reported at 4-week periods. Excreta were collected for three consecutive days at the end of weeks 4 and 12 of feeding to assess the AR nutrients and energy. Data were analyzed using the Proc GLIMMIX in SAS Studio learning Edition (v9.4). The cage served as the experimental unit, and the model included diet, period/sampling time point, and their interactions as fixed factors. HDEP responded to FDE in a cubic increment pattern ($P < 0.001$), peaking at FDE100 before declining. Egg mass also showed a quadratic response ($P < 0.001$), peaking at FDE100. An FDE × period interactions ($P < 0.001$) produced cubic decrement patterns for eggshell thickness and yolk color, with the greatest shell thickness at FDE50 and FDE100 in period 4 and the highest yolk color in period 1. Albumen height exhibited quadratic-to-cubic increases across periods, with FDE100 producing the highest values ($P \leq 0.043$). The AR of dry matter and neutral detergent fiber increased linearly ($P < 0.001$) while the AR of CP increased quadratically ($P = 0.002$), with the greatest retention observed at FDE100. Nitrogen-corrected apparent metabolizable energy increased cubically from 2,530 to 2,608 kcal/kg, peaking at FDE100 ($P < 0.05$). In conclusion, supplementing a high fiber laying hen diet with the FDE blend at 100 g/ton optimized egg production, improved egg quality, and enhanced nutrient and energy utilization, demonstrating its efficacy as a nutritional strategy for modern laying hens.

Keywords: Fiber-degrading enzymes; Lohmann Brown-Classic laying hens; egg production; Apparent retention; High phytase dose

4062 Transforming dietary fiber of feed ingredients into bioactive prebiotic components for poultry

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Ingredients rich in dietary fiber contain substantial amounts of non-starch polysaccharides (NSP), including galactomannans, mannans, arabinoxylans, β-glucans, or pectic polysaccharides. Hydrolysis of NSP by multi-carbohydrase enzymes releases a range of degradation products that can exert prebiotic potential. When metabolized by the gut microbiota, these compounds can modulate microbial composition and activity in the gut, ultimately improving intestinal development, health, and function. The aim of metabolic studies in broiler chickens and laying hens was to evaluate the effects of novel prebiotic products derived from enzymatically modified canola meal (CM), i.e., ECM and its water-soluble fraction, ECMS, as well as products generated by enzymatic yeast cell wall hydrolysis, on performance, microbial populations, short-chain fatty acid (SCFA) production, intestinal health, and nutrient digestibility. All statistical analyses were performed using the MIXED procedure in SAS.

Ross 308 broilers were assigned to three dietary treatments, each consisting of eight pens of eight birds. Birds were fed starter (4-14 d; 100 g/kg CM) and grower (14-21 d; 200 g/kg of CM) Control (C) diets, C with ECM replacing 50 g/kg of CM, or C with ECM replacing all CM. Excreta were collected to determine NSP disappearance and AMEn of the diets. Cecal digesta was analyzed for SCFA concentrations and bacterial community diversity. Overall, ECM increased NSP digestibility from 5.3% to 18.9% and AMEn of the diets from 2,943 kcal/kg to 3,119 kcal/kg ($P<0.01$). Replacing CM with ECM increased ileal and cecal populations of Lactobacillus, reduced E. coli counts, elevated SCFA levels, and decreased intestinal sialic acid production, which reflects mucin secretion ($P<0.01$).

ECMS included at 10g/kg were evaluated in a Salmonella Enteritidis (SE) challenge study with 24-week-old Hy-Line hens (twelve hens per treatment). SE counts were measured at 0 and 3 days post-infection (dpi). ECMS reduced ($P<0.01$) SE cecal counts from 1.52 to 0.0 log10 cfu/g and fecal shedding from 1.96 to 0.82 log10 cfu/g. In a separate SE challenge study with White Leghorns (eight hens per treatment), yeast-derived prebiotics included at 0.5g/kg decreased SE cecal counts from 4.3 to 1.9 log10 cfu/g and reduced the incidence of SE colonization of the spleen and liver by 62.5% ($P<0.01$) at 7dpi, compared to the control group.

Overall, these results demonstrate that enzymatic release of bioactive components from dietary fiber can enhance its prebiotic properties, improve gut function, and support antibiotic-free feeding strategies for monogastric animals.

Keywords: non-starch polysaccharides; multi-carbohydrase; enzyme hydrolysis products; prebiotic; dietary fiber

Nutrition: Feed Additives V

4063 Dietary fiber-derived glycobolites as prebiotic components for poultry

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Advances in glycobolomics, a field focused on understanding the roles of carbohydrates and their derivatives in biological systems, have enhanced our understanding of dietary fiber (DF) and its metabolites in poultry nutrition. Traditionally viewed as an anti-nutritional factor due to its limited digestibility and potential to reduce nutrient absorption, DF is now being recognized for its functional benefits, such as improving gut health and enhancing digestive efficiency. These benefits can be achieved through the use of target-specific enzymes, as the application of carbohydrases extends beyond improving nutrient utilization and animal performance; it also plays a crucial role in generating the fiber-derived glycobolites, which can be considered novel feed additives. Although DF is structurally complex and diverse, the ability to quantify and evaluate its various fractions allows for a better understanding and realization of its functional properties. Ingredients high in DF contain significant amounts of non-starch polysaccharides (NSP), such as galactomannans, mannans, arabinoxylans, β-glucans, cellulose or pectic polysaccharides, including rhamnogalacturonans. Galactooligosaccharides (GOS), such as raffinose and stachyose, found mainly in legumes, may constitute 2.0-2.5% of poultry diets. When NSP or GOS are hydrolyzed by carbohydrase enzymes, various functional glycobolites are released.

In our research, in vitro evaluation of NSP and GOS depolymerization across a range of feed ingredients enabled the identification of optimal combinations of ingredient-specific carbohydrases and

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their ability to release bioactive glycobolites. Achieving substantial depolymerization of NSP led to the development of technologies to upcycle high-fiber biomass into novel prebiotic feed additives. For example, significant enzymatic depolymerization of yeast cell wall β-1,3-1,6-glucan was achieved, resulting in the development of a prebiotic containing water-soluble, low-molecular-weight β-1,3-glucan along with soluble mannans. Additionally, prebiotic bioactive components were also generated from canola meal NSP. Metabolic studies conducted with broiler chickens, turkeys, and laying hens demonstrated that enzymatic release of bioactive components from DF can enhance its prebiotic properties. These improvements contribute to better gut function, support antibiotic-free feeding strategies, and positively affect animal performance, gut microbiota composition, short-chain fatty acid production, reduction in Salmonella and E. coli counts, and nutrient digestibility. Other feed ingredients are currently being evaluated for their potential to be upcycled into prebiotic feed additives containing bioactive glycobolites.

Keywords: prebiotic, glycobolites, non-starch polysaccharides, galactooligosaccharides, carbohydrases

4064 Effects of brown seaweed meal on blood glucose, growth, meat quality, and intestinal health of Ross 308 broiler chickens

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The effects of dietary brown seaweed meal on blood glucose, growth performance, feed intake, meat quality, and intestinal lesion scores of Ross 308 broilers were evaluated. The study was a completely randomized design with a 3 (in-hatcher diet) x 2 (grow-out diet) factorial arrangement of treatments. On day 18 of incubation, 792 viable embryos were randomly distributed into nine hatching trays and assigned to one of three in-hatcher feeding treatments: no feed (control), a starter diet and water, and a starter diet containing 2% brown seaweed and water (3 hatching trays/treatment). Diets were provided at hatch until the end of incubation. After hatch, 576 chicks from each treatment were placed into 24 floor pens (24 birds/pen) and fed one of two diets from day 1 to 35 of age: a standard broiler diet (D1) or a standard diet containing 2% brown seaweed (D2). On day 7, 26 and 35, one bird/pen was humanely euthanized to test blood glucose levels, as well as jejunal lesion scores on day 26 and 35, and meat quality analysis on day 35. Data were analyzed using the mixed model procedure in SAS. At day 33, the broilers fed the starter diet containing 2% brown seaweed and water at hatch gained significantly ($p=0.02$) more weight (2544.86g/bird) than those on the control treatment (2403.54 g/bird). There was no significant effect of post-hatch dietary treatment on day 33 broiler weight. However, feed conversion ratio was higher ($p<0.05$) in birds fed the 2% brown seaweed diet over the entirety of the growth trial. In-hatcher feeding did not affect ($p>0.05$) blood glucose or jejunal lesion score. Broilers fed D2 exhibited higher necrotic lesion scores (2.51) on day 35 ($p=0.02$) as compared with D1 (0.91), with scores ranging from 1 (thin or brittle walls with removable fibri) to 4 (localized necrosis or ulceration). There was no significant difference ($p>0.05$) among treatments for white striping, muscle pH or cooking loss. There was no significant difference ($p>0.05$) among the grow-out dietary treatments for L* score. However, broilers fed 2% brown seaweed in the hatcher had significantly darker ($p=0.002$) breast meat on day 35 (58.28) than the other two treatments, irrespective of post-hatch treatment. There were no significant interactions ($p>0.05$) between the in-hatcher feeding and grow-out dietary treatments for any of the parameters tested. In conclusion, in-hatcher inclusion of 2% brown seaweed improved early growth. However, long-term feeding throughout the production cycle was linked with reduced feed efficiency and impaired the intestinal integrity of the broiler chickens in this trial. We recommend investigating ingredient processing for future use of this brown seaweed in broiler diets.

Keywords: brown seaweed; broiler chicken; blood glucose; growth; intestinal health

4065 Evaluation of the pre- and probiotic effects of bacteria-derived cellulose on egg production and laying hens' gut microbiome

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The growing interest in utilizing byproducts from the agri-food industry and the search for novel bioactive feed additives for poultry prompted us to explore whether bacteria-derived cellulose (BDC), a byproduct of tea fermentation during kombucha production, could exhibit probiotic and prebiotic properties, thereby supporting gastrointestinal health and function in laying hens. Kombucha, a fermented tea beverage produced using a symbiotic culture of bacteria and yeast (SCOBY), is recognized for its potential health benefits. BDC is a biopolymer synthesized by SCOBY during fermentation. The objective of this study was to characterize the physicochemical properties of BDC and evaluate whether its dietary inclusion affects the composition and diversity of the gut microbiome in laying hens, as well as its impact on egg production. Raw BDC, obtained from a commercial kombucha producer (Prism Kombucha, Winnipeg, Canada), was blended and freeze-dried. Metagenomic analysis revealed that Komagataeibacter and Acetobacter dominated the raw BDC microbiome, together accounting for 90% of the total bacterial population, with lactic acid quantified at 2.33 mg/g. The three-week laying hen study followed a completely randomized design. A total of 45 twenty-four-week-old Dekalb White hens were allocated to three dietary treatments: a control diet (C) and two experimental diets supplemented with dry BDC at 1 g/kg (T1) and 5 g/kg (T2), with 15 cages per treatment and 1 hen per cage. At the end of the study, eight hens per treatment were euthanized, and cecal digesta were collected. The DNA was isolated from cecal microbiota and then analyzed using high-throughput sequencing on the Illumina NovaSeq platform. The performance data were analyzed using the MIXED procedure of SAS (9.4), while the microbiota data were analyzed using R packages.

Egg production and quality parameters were not impaired by BDC. Supplementation with 1 g of BDC/kg of diet (T1) enriched the relative abundance of Lactobacillaceae compared to the C and T2 diets ($P < 0.05$). In conclusion, although dietary inclusion of dry bacteria-derived cellulose did not affect laying hens' performance, it tended to enhance the relative abundance of beneficial cecal bacteria. However, given that the raw kombucha fermentation byproduct contains approximately 95% moisture, drying and milling may pose practical challenges for its use as a feed additive in commercial egg production.

Keywords: bacteria-derived cellulose; laying hen; prebiotic; probiotic; symbiotic culture of bacteria and yeast

4066 Supplementation of Telfairia occidentalis leaf extract as natural feed additives on growth performance, biochemical indices and immune status of Cobb 500 broiler chickens

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The current research aimed to assess the effects of Telfairia occidentalis leaf extract as potential alternative to antibiotic growth promoters on growth performance, hemato-biochemical indices and immune status in Cobb 500 chickens.
A total of 600 one-day-old chicks were allotted in four experimental groups: a control (C) receiving the basal diet, an antibiotic group receiving 0.5 g/L of Tetracolivit (T), and two groups receiving either 1 mL (TOE-1) or 2 mL (TOE-2) of hydroethanolic Telfairia occidentalis leaf extractin one liter of drinking water everyday throughout the experiment. Each group had five replications of thirty chicks.
The data was analyzed using Graph Pad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA), which implemented a one-way analysis of variance (ANOVA). The statistical model that was applied was as follows:
$Yij = \mu + tj + eij$, where eij is the random error term, t is the treatment effect, and μ is the overall mean. A mean ± standard error of the mean (SEM) is used to present the results. The Tukey post hoc test was used to evaluate differences in treatment means at a significance level of 5% ($p < 0.05$). Additionally, orthogonal polynomial contrasts were used to assess the linear and quadratic impacts of treatments.
Cobb 500 broiler chickens in TOE-2 group experienced significant ($P < 0.05$) improvements than the control group in the average daily gain (48.49 g ± 0.04 g vs 41.54 ± 0.04 g), final body weight (1992 ± 0.05 g vs 1867 ± 0.02 g) and Feed Conversion Ratio (1.52 ± 0.01 vs 1.86 ± 0.02). Furthermore, on day 21, Telfairia occidentalis leaf extract significantly reduced serum total cholesterol (195,7 ± 0.91 mg/dL vs 231,0 ± 0,33 mg/dL), triglycerides levels (65.3 ± 0,2 mg/dL vs 127,6 ± 0,16 mg/dL), while significantly elevated serum calcium (10.74 ± 0.32 mg/dL vs 8.83 ± 0.51 mg/dL), total protein (4.18 ± 0.01 g/dL vs 3.36 ± 0.05 g/dL) and albumin (3,32 ± 0,02 vs 2,35 ± 0,05) than both control and antibiotic groups. Chickens in TOE-2 group and antibiotic group had a higher ($P < 0.05$) IgA (244.0 ± 0.01 mg/mL vs 132.13 ± 0.01 mg/mL) and IgM (1.1 ± 0.01 ng/mL vs 0.82 ± 0.01 ng/mL) antibody titer than control.
In summary, supplementing with 2 mL of hydroethanolic Telfairia occidentalis leaf extract could be beneficial for safe broiler production due to its role in enhancing growth performance, improving biochemical indices and immune status.
Key words: Telfairia occidentalis; Cobb 500 broiler; growth performance, biochemical indices; immunity.
Keywords: Telfairia occidentalis; Cobb 500 broiler; growth performance; biochemical indices; immunity

4067 Hydrolysed Kluyveromyces marxianus yeast improves growth performance of broilers under coccidiosis and Clostridium challenge.

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Various yeasts and their derivatives are used in animal feeds, due to the presence of bioactive components such as mannan oligosaccharides and beta-glucans, which have been shown to support animal health and growth performance. However, yeast products vary by species and processing (e.g. full yeast, hydrolysed yeast, yeast extracts), making it important to evaluate their nutritional value and contribution the immune response and hence performance under different conditions. The objective of this study was to evaluate the effect of an enzymatic hydrolysed yeast product of the species Kluyveromyces marxianus (KM) in broilers. A total of 608 Ross 308 male broilers from a commercial hatchery were allocated to 2 barns: 1) non-challenged and 2) challenged with coccidiosis via overdose Paracox vaccine followed by Clostridium perfringens inoculation. Each barn consisted of 16 floor pens with 17 birds per pen (starting with 19), randomly assigned to 2 treatments (8 replicates): control and 0.05% yeast KM. Broilers were fed ad libitum with a corn, wheat and soybean meal-based diet in 3 phases: starter (day 0-14), grower (day 15-28) and finisher (day 29-35). Anticoccidials (Maxiban, Sacox) were only included in the non-challenged barn during the starter and grower phase. Broilers followed a standard lighting and temperature schedule and vaccination program. Growth performance (BW, ADG, ADFI, FCR) was recorded at multiple time intervals and corrected for mortality. Footpad lesions (0-4 scale), litter quality (days 14, 21, 28 and 35) and oocyst counts (day 21, 23 and 26) were evaluated as health indicators. Necropsies were performed on two birds per pen (n=64) on day 20 to assess intestinal lesions and pathology. Statistical analysis for growth performance was done using independent t-tests. Differences were regarded as significant when $p \leq 0.05$ and trends when $p < 0.10$. In challenged birds, 0.05% yeast KM improved BW ($p=0.041$) and tended to improve FCR corrected ($p=0.055$). Under non-challenged conditions, yeast supplementation did not significantly affect growth performance, except for a slight increase in feed intake during the starter phase ($p=0.049$). Descriptive observations indicated similar litter quality and mortality between the two groups, while oocyst counts reflected the expected challenge pattern. These finding suggest that supplementation with an enzymatically hydrolysed Kluyveromyces marxianus yeast improved broiler performance under enteric challenge, highlighting its potential role in supporting broiler resilience during gut health stress.
Keywords: Broiler; Yeast; Coccidiosis; Clostridium; Performance

4068 How breed and production context can help to predict the efficacy of a vectorized blend of essential oils and organic acid on broiler performance — a comprehensive meta-analytic approach

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This study evaluated the impact of Lumigard TCB (LTCB) on growth performance of broilers through a meta-analysis. LTCB is a blend of vectorized standardized essential oils and organic acid known to improve gut health, and feed efficiency, improving also broilers growth performance. Its efficacy has been demonstrated in various trial contexts. The aim of this study was to compile all these trials and quantify the effect of LTCB on broiler performance considering average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) for the overall period from 0 to 35 days. The database included 14 experiments conducted between 2014 and 2024, in male Cobb 500 (60%) or Ross 308 (40%). Experiments took place in USA, Brazil and France. Part of those experiments were conducted in optimal breeding conditions for 65%, the remaining were conducted in challenge conditions (necrotic enteritis challenge, sub-optimal breeding conditions). Also, the database contained data about feed composition, with standard diets (corn + SBM; 65%) or diversified diets (wheat + sunflower meal + rapeseed meal; 35%). In all experiments, groups with LTCB were compared to a control without supplementation. Data analysis involved forest plots and assessment of variance using mixed-effects meta-analysis models. Results showed that LTCB improved growth performance in all tested conditions ($P<0.05$). Further analysis showed that Cobb 500 showed higher response to LTCB (LTCB×Strain; $P<0.05$) than Ross 308. Thus, in Ross 308, results showed that inclusion of LTCB improved ADG by 0.65 g/d ($P=0.009$), decreased FCR by 1.8 points ($P = 0.003$) and showed no effect on ADFI ($P=0.399$). In Cobb 500, inclusion of LTCB improved ADG by 1.09 g/d ($P<0.001$), decreased

FCR by 3.5 points (P=0.007) and showed no effect on ADFI (P = 0.799). Results also showed that in optimal breeding conditions (LTCB×conditions), LTCB improved ADG by 0.89 g/d (P<0.001), and decreased FCR by 3.1 points (P <0.001). While, in challenged conditions, LTCB increased ADG by 0.63 g/d (P=0.012) without effect on ADFI and FCR. Finally, there was no interaction between the effect of LTCB and the feed formulation context (LTCB×formulation; P>0.05), and overall LTCB improved ADG by 0.73g/d and reduced FCR by 2.5 points. In conclusion, results indicate that Lumigard TCB improves growth performance of broilers under a wide range of breeds, environments, and dietary conditions.
Keywords: Broiler; Performance; Gut health; meta-analysis

4069 Growth performance of E. coli-challenged broilers receiving different levels of an organic acid blend in water

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Organic acids have been increasingly explored as functional additives in poultry nutrition for their antimicrobial, acidifying, and buffering properties. Their use through drinking water allows continuous modulation of gut environment, supporting feed efficiency and animal resilience during enteric stress. This experiment evaluated the effect of graded levels (0.2–1.0 L/1000L; Trouw Nutrition, Netherlands) of a synergistic blend of free and highly buffered organic acids including copper for drinking water application on performance and survivability of broilers challenged with Escherichia coli up to 42 days of age. The study was conducted at the BITA Experimental Center (Brazil) using 2,772 male broilers distributed in a completely randomized design (7 treatments × 18 replicates × 22 birds). Birds were challenged orally on day 14 with E. coli ID 19501 (3.3 × 10^9 CFU/mL) for three consecutive days. The acidifier was supplied ad libitum in drinking water throughout the growing period. Diets were corn–soybean meal–based and offered in five feeding phases. Zootechnical parameters including body weight (BW), feed intake (FI), feed conversion ratio (FCR), and mortality were measured on days 21, 35, and 42. Statistical analyses were conducted by ANOVA ($p < 0.05$). Linear regressions were also performed to evaluate dose–response trends. Regression analysis confirmed a linear improvement in FCR from 0.0 to 0.8 L/1000L, and no negative effect on FI, indicating stable intake across effective inclusion levels. The 1.0 L/1000L treatment was excluded from the regression because it exhibited atypical, biologically inconsistent behavior, acting as an outlier and distorting the linear model. The E. coli challenge effectively impaired growth performance ($p < 0.001$), reducing final BW and increasing mortality (PC: 8.3%; NC: 1.8%). The 0.6 L/1000L treatment consistently delivered the best FCR and growth results, matching the NC group and outperforming all other challenged groups ($p < 0.05$). Analysis of water titration parameters (pH ≈ 7.8, moderate hardness and low alkalinity) confirmed that 0.6 L/1000L provided the most effective acid-base balance. In conclusion, supplementation with 0.6 L/1000L of organic acid blend in drinking water ensured optimal growth, feed efficiency, and survival of E. coli-challenged broilers, providing a practical and scalable strategy for maintaining performance under moderate enteric pressure.
Keywords: Organic acids; Broilers performance; E coli challenge

4070 Phytocee™ as an Adaptogen for Heat Stress: Systems-Level Analysis of Longevity and Resilience Pathways in Poultry

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Objective: To evaluate whether Phytocee™, a phytogenic blend, enhances cellular resilience and activates conserved longevity pathways in broiler chickens under cyclic heat stress using in vivo transcriptomics and in silico predictive modeling.

Experimental design: 1-day old broilers were assigned to thermoneutral or cyclic heat stress (34-36°C, 8 h/day, Days 8-42) groups, fed basal or Phytocee™ (200 g/ton) supplemented diet (6 pens/group, 120 birds/group). Liver, blood, and gut tissues were collected for molecular analyses, and major Phytocee™ phytochemicals, gallic acid, ellagic acid, ursolic acid, withaferin A, were assessed in silico using DIGEP-Pred 2.0 for predicted modulation of longevity-related gene expression.

Materials and methods: Phytocee™, rich in hydrolysable tannins, withanolides, and triterpenoids, was characterized by chromatography techniques. Total RNA was profiled on Agilent 4×44K chicken microarrays, 75th-percentile normalized and log2 transformed; differentially expressed genes were defined at fold change ≥1.2 and FDR <0.05. The four phytochemicals were submitted to DIGEP-Pred 2.0 for predicted up/down regulated genes, followed by KEGG/ShinyGO enrichment and compound-gene-pathway network construction.

Statistical analysis: Gene level comparisons employed linear model microarray statistics with Benjamini-Hochberg FDR correction. Pathway enrichment utilized hypergeometric testing with FDR adjustment. Comparison of selected markers utilized t-test.

Results: Heat stress upregulated DNA repair and cell cycle genes (FANCF, BRCA1, EXO1, MAD2L1, NDC80) and HSP25, while suppressing antioxidant (HSPA2, SOD3), metabolic (ME1, FADS2, ALDH1L2, DHCR7), and antigen-presentation genes (MHC II, LECT2), indicating genomic stress, oxidative imbalance, and weakened adaptive immunity. Phytocee™ supplementation improved resilience by further upregulating DNA repair genes such as FANCF, BRCA1, and EXO1; chaperone and metabolic genes; and restoring heat suppressed protectors of HSPA2, SOD3, ME1, FADS2, MHC II, and LECT2. In silico analysis confirmed the synergy of its phytochemicals to engage with longevity pathways, significantly enriching FoxO, PI3K-AKT, autophagy, and mTOR signaling, thereby jointly modulating FOXO, SIRT1, AMPK, mTOR, and DNA repair networks.

Conclusions: Phytocee™ improves DNA repair capacity, antioxidant defense, metabolic flexibility, and immune regulation in heat-stressed broilers. In silico modeling confirms synergistic targeting of conserved longevity hubs, supporting Phytocee™ as a promising adaptogenic strategy to improve resilience and cellular longevity under heat stress.

Keywords: Phytocee™; heat stress; longevity pathways; gene expression ; broilers, adaptogens

Keywords: Phytocee; Longetivity; gene expresssion; heat stress; broilers

4071 Effects of neem cake with enzyme supplementation on growth performance and health parameters in Broiler Chickens

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In the context of rising feed costs and restrictions on synthetic antimicrobials, local, sustainable, and functional alternatives in poultry nutrition are important. Neem cake (Azadirachta indica), a by-product of neem seed oil extraction, is a promising local resource due to its high protein content and bioactive compounds. This study evaluated the nutritional potential, health benefits, and zootechnical effects of incorporating neem cake as an alternative protein source to soybean, in broiler diets, with multi-enzyme sup-

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plementation (Maridav, 1 g per 4 kg of feed) containing cellulases, xylanases, β-glucanases and amylases to enhance fiber digestion and nutrient availability. Six hundred one-day-old Sasso chicks were randomly allocated to five treatments with six replicates of 20 birds: T0- (negative control diet without medication), T0_med (positive control, with antibiotics, anticoccidials, and antiparasitic), T5- (5% neem cake without enzyme or medication), T5+ (5% neem cake with enzyme without medication), and T10+ (10% neem cake with enzyme without medication). All diets were isonitrogenous and isoenergetic. From 0-3 weeks, all birds received a standard starter diet without medication; from 4-12 weeks, experimental diets were fed. Parameters assessed included body weight, feed intake, feed conversion ratio (FCR), mortality, hematological indices, and oocyst counts. Data were analyzed using one-way ANOVA (GraphPad Prism 8) with Tukey's test ($p < 0.05$). Feed intake did not differ among treatments. Weight gain was higher in T5+ (32.93±0.63) and T0_med (32.76±0.53) than in T0- (31.41±0.68), T5- (30.08±0.56), and T10+ (30.93±0.58). FCR was lower in T5+ (2.12±0.04), T0_med (2.10±0.04), and T10+ (2.14±0.03) compared with T0- (2.238±0.05) and T5- (2.318±0.05). Mortality was higher in T0- (19.79±1.85%) and T10+ (19.56±0.95%), compared with T0_med (15.03±1.22%), T5- (14.21±1.79%), and T5+ (14.67±0.76%). Hematology showed T0- had the highest leukocyte (73.58±1.82 g/L) and lymphocyte (64.49±4.18%) levels, whereas RBC count (1000/mm3) was higher in T5+ (2570±116) and T10+ (2563±61.7) than in T0- (2137±39.3) and T0_med (2157±23.3). Hemoglobin and hematocrit were also higher in T5+ (Hb 13.91±0.13; Ht 31.37±1.46) and T10+ (Hb 13.83±0.19; Ht 30.83±0.43) compared with T0- (Hb 12.30±0.16; Ht 25.30±0.64) and T0_med (Hb 13.33±0.19; Ht 30.50±0.10). Oocyst counts were similar at 4 weeks but markedly higher in T0- (1533±8.70) than in T0_med (800±30.90) and neem cake treatments (66.67±3.33) at 12 weeks. Overall, 5% neem cake with enzyme improved growth, FCR, and hematological status while reducing coccidial load. It can be used as a viable alternative to soybean meal and antimicrobial drugs in broiler production.

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Keywords: Neem cake; Enzyme supplementation; Zootechnical performance; Hematological parameters; Broiler chickens

4072 Quercetagetin modulates gut-reproductive microbiome interactions, mineral metabolism, and immune responses to mitigate salpingitis in laying hens: A multi-omics approach

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Aging in laying hens is accompanied by reduced laying rate and egg quality and increased inflammation and dysbiosis along the gut–oviduct axis, yet mechanisms distinguishing healthy hens from those with salpingitis remain unclear. Quercetagetin (Que), a flavonoid with antioxidant and microbiota-modulating properties, has been little studied in laying hens. This study investigated aging-related phenotypic, microbial, and metabolic alterations and evaluated Que using feeding and LPS-induced salpingitis models. Healthy (High-Per) and salpingitis-affected (Low-Per) hens during the extended laying period were compared using integrated phenomics, spatial metabolomics, and microbiomics. Low-Per hens exhibited a markedly reduced laying rate (22.92% vs. 83.86%), impaired eggshell ultrastructure with thickened mammillae layers and reduced palisade and crystalline layers, and disrupted calcium–phosphorus homeostasis, reflected by decreased serum and uterine Ca, P, PTH, CaBP, ALP, and vitamin D metabolites. Microbiome analysis showed enrichment of Flavonifractor and Lachnospiraceae_UCG-002 in the cecum and increased Fusobacterium, Clostridium_sensu_stricto_1, and Treponema in the shell gland of Low-Per hens, whereas High-Per hens exhibited higher abundances of Enterococcus, Papillibacter, and Intestinimonas. Spatial metabolomics revealed higher levels of choline, succinate, riboflavin, nicotinate, D-arginine, and lysyl-serine in High-Per hens, while toxic metabolites including Toxin B1 and picric acid accumulated in Low-Per hens; KEGG analysis highlighted amino acid metabolism and cytochrome P450 pathways in High-Per hens versus ABC transporter enrichment in Low-Per hens. Dietary Que significantly improved laying rate, egg mass, albumen height, eggshell ultrastructure, and intestinal morphology, increasing villus height-to-crypt depth ratio. In an LPS-induced model, Que reduced serum and uterine IL-1β, IL-6, IL-8, and TNF-α, enhanced antioxidant defenses (T-SOD, CAT, GSH-PX), and upregulated CaBP-D28k, NPt2b, and PMCA1B expression in the shell gland. Integrated multi-omics analyses further demonstrated that Que reshaped gut and shell gland microbiota (including Lactobacillus, Bacillus, and Rothia), increased short-chain fatty acid production (acetate, propionate, and butyrate), enriched anti-inflammatory metabolites such as LysoPCs and hydroxyethyl retinamide, and activated immune-, calcium signaling-, PPAR-, and tryptophan metabolism–related pathways. Overall, this study delineates microbial and metabolic distinctions between healthy and salpingitis hens and demonstrates that Que enhances egg production, egg quality, and gut–reproductive tract health during late laying.

Keywords: Laying hens; Salpingitis; Quercetagetin; Gut-reproductive tract axis; Gut-reproductive tract axis

4073 Evaluation of an In-Feed Multi-Species Probiotic (Enterococcus, Clostridium, Bacillus) on Performance in Commercial Layer Farms

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Probiotics are increasingly adopted as alternatives to antibiotics in poultry diets to support health and production under commercial conditions. This study evaluated the efficacy of an in-feed multi-species probiotic containing Enterococcus, Clostridium, and Bacillus compared with a single-species Bacillus control, representing the existing in-feed program on a commercial farm. The trial aimed to determine the effects of multi-species probiotic inclusion on layer performance, including egg production, egg quality, and mortality across production periods (60–85 weeks and 85–100 weeks until depopulation). Conducted at a commercial operation involving two farms, each with selected six houses divided into three control and three treatment houses of similar age, the study encompassed a total population of 135,100 birds over trial duration of 140–180 days. Production parameters, including feed intake, egg production, eggs' feed conversion ratio (FCR), Hen House and Hen Day%, mortality, and second-grade eggs%, were analysed using the Mann–Whitney U test, while egg quality parameters (Haugh unit, albumen height, shell strength, yolk colour, and shell thickness) were analysed using the T-test. Across both farms, treatment groups demonstrated up to 1.95% lower in average daily feed intake ($P < 0.05$) and up to 0.62% improvement in eggs' FCR ($P < 0.05$), with egg quality remaining comparable to the control. The reduction in feed intake, averaging 2 g per bird daily, has significant economic implications; extrapolated to a population of 5 million layers at an average feed cost of RM1.50/kg, annual feed savings could exceed RM 5 million. From a veterinary and poultry health perspective, improved feed efficiency enhances

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nutrient utilization, promotes gut health, and strengthens flock resilience. Replacing antibiotic growth promoters with multi-species probiotics also reduces antimicrobial selection pressure, reinforcing responsible antibiotic stewardship and aligning with One Health objectives by decreasing the environmental impact of feed use while supporting more sustainable poultry productions. These findings underscore the potential of multi-species probiotics as effective alternatives to antibiotic-based programs, improving feed efficiency without compromising egg quality. Long-term studies across different production systems are recommended to validate these findings and explore broader benefits on intestinal health, immunity, and overall flock performance.
Keywords: probiotics; Enterococcus; Clostridium; Bacillus; layer performance

Nutrition: Vitamins & Minerals I

4074 Research Developments in Vitamins, Macro and Trace Minerals for Poultry

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Advances in poultry nutrition over the past two decades have shifted from requirement–driven formulation toward precision nutrition frameworks that integrate nutrient bioavailability, inter–nutrient interactions, environmental sustainability, and functional physiological outcomes. Latest research has redefined the utilization of macro minerals, particularly calcium (Ca) and phosphorus (P), emphasizing digestible and interaction–corrected values that reflect modern genetics and the widespread use of exogenous enzymes. Genetic progress and enhanced phytase application have substantially altered Ca and P requirements in broilers, enabling significant reductions in inorganic P inputs without compromising skeletal integrity and performance.

Phytase technology has evolved from simple P release to comprehensive phytate deconstruction. Next–generation phytases exhibit improved thermal and pH stability, and super–dosing strategies (≥1,500–2,000 FTU/kg) enhance the liberation of Ca, amino acids, and trace minerals. These effects extend beyond nutrient release, contributing to improved gut barrier function, immune modulation, and feed efficiency.

Parallel advancements in trace mineral nutrition highlight the superior relative bioavailability of organic trace minerals (OTMs), including amino acid complexes and chelated forms of Zn, Mn, Cu, Se, and Fe. The OTMs reduce antagonistic interactions with macro and trace minerals, enhance gut integrity, immune competence, bone quality, reproductive performance, and significantly decrease mineral excretion, supporting sustainability objectives. Evidence indicates that reduced inclusion rates of OTMs can maintain or enhance zootechnical performance, accelerating their integration into precision mineral strategies.

Vitamin research has focused primarily on vitamins D, E, A, and C, with an emphasis on optimizing physiological function rather than merely preventing deficiency. High–performance poultry genetics exhibit elevated metabolic demands, requiring vitamin strategies that support immune resilience, oxidative stress mitigation, gut integrity, and reproductive efficiency under conditions of heat stress and immune challenge. The recent global vitamin shortage underscored the vulnerability of current production systems, revealing that marginal reductions in vitamin supply can impair performance, eggshell quality, and reproduction. These findings highlight the economic and biological consequences of suboptimal vitamin nutrition and reinforce the need for updated requirement models aligned with contemporary production intensity.

Advances in poultry nutrition now center on precision strategies that optimize the bioavailability and interactions of vitamins and minerals, supported by improved phytase technologies and highly bioavailable organic trace minerals. Modern genetics and recent supply challenges highlight the need for updated vitamin and mineral requirement models, as even small deficiencies can impair performance, gut health, skeletal integrity, and reproductive outcomes.

4075 Phytase supplementation in experimental diets increases true ileal digestibility of phosphorus in soybean meal irrespective of the calcium and phosphorus level in pre-experimental diets

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The study aimed to investigate the effects of (1) dietary calcium (Ca) & phosphorus (P) levels in pre-experimental (pre-EXP) diet and (2) phytase supplementation in experimental (EXP) diet on growth performance and true ileal digestibility (TID) of P in soybean meal (SBM) determined by the regression method in 21-d-old broiler chickens. A total of 756 male broilers was assigned to a 3×2×3 factorial arrangement. Upon arrival, birds were allocated to 3 pre-EXP diets in a randomized complete block design, then divided into 6 EXP diets with 6 blocks (7 birds/pen) in a split-plot design on d14. From d 0-14, birds received corn-SBM-based pre-EXP diets: (1) standard diet−adequate Ca & P level (9 & 4.5 g/kg, respectively), (2) deficient diet−deficient Ca & P level (7.4 & 3 g/kg, respectively), and (3) phytase-supplemented deficient diet (500 FTU/kg phytase added to deficient diet to match the Ca & P levels of the standard diet). From d 14-21, six semi-purified EXP diets were provided with 2 factors: (1) phytase (0 or 500 FTU/kg), and (2) SBM (400, 510, and 620 g/kg). Titanium dioxide was included as an indigestible marker, and ileal digesta from the distal half of the ileum were collected on d 21 to determine the ileal P digestibility. Data were analyzed using PROC MIXED in SAS. For growth performance and apparent ileal digestibility (AID) of P, fixed effects included pre-EXP diet, phytase in EXP diet, SBM levels in EXP diet, and their interactions (3-way ANOVA). For TID of P in SBM, fixed variables were pre-EXP diet, phytase in EXP diet, and their interaction (2-way ANOVA). The random effect was pre-EXP diet within block in both models. During the pre-EXP period, birds fed the standard and the phytase-supplemented deficient diet had higher ($P<0.05$) weight gain (WG) and feed intake than those on the deficient diet. In the EXP period, WG and feed conversion ratio (FCR) were unaffected by pre-EXP or interactions. Birds receiving phytase in EXP diet had improved ($P<0.05$) WG and FCR. WG and FCR improved linearly ($P<0.05$) with higher SBM inclusion. The AID of P in EXP diets showed a phytase×SBM interaction: without phytase, AID increased linearly ($P<0.05$) as SBM increased, but with phytase, AID remained similar across levels. Coefficients of TID of P in SBM from pre-EXP diet 1-3 were 0.356, 0.403, and 0.377 without phytase in EXP diet, and 0.844, 0.847, and 0.851 with phytase in EXP diet, respectively. No pre-EXP or interaction effects were observed, but phytase supplementation in the EXP diet increased ($P<0.05$) TID of P. In conclusion, phytase supplementation in EXP diets increased TID of P in SBM, whereas Ca and P deficiency in pre-EXP diets had minimal effects on growth performance and TID of P on d 21.
Keywords: true ileal digestibility; Phosphorus; Soybean meal; Phytase; Broiler

4076 Effects of Varying Levels of Vitamin E in the Starter Phase Diet in Male Cobb 500 Broilers

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An eight-level vitamin E titration was administered to 480 male Cobb 500 broilers from d 1 to 18 to determine the impact of vitamin E level on live performance at d 18, 35 and 45, as well as both 36 d and 46 d processing traits. Breast tissue samples were also collected in order to assess vitamin E levels in muscle tissue. The experiment was a random complete block design with each titration level having 6 pen replicates of 10 birds/pen. Experimental diets with added vitamin E levels of 0, 10, 20, 30, 40, 50, 60, or 70 IU were fed from placement to 18 d of age. Common grower and finisher diets with surfeit vitamin E were fed for the duration of rearing (45 d of age). All birds placed were processed to access processing yields, with 5 birds from each pen processed on d 36 and the remaining birds processed on d 46. All data was analyzed using both linear and quadratic regression models in JMP pro18 software. A significant ($P < 0.05$) difference in body weight gain (BWG) was seen at 18 d when analyzed linearly and quadratically, with birds fed 70 IU having the highest BWG. A significant difference was also found in 18 d feed conversion ratio (FCR) when analyzed quadratically, with birds fed 70 IU having the lowest FCR and those fed 30 IU having the highest. No other differences in BWG or FCR were seen at either 35 d or 45 d assessments. Carcass yield percentages differed significantly in the birds processed on d 36, with birds fed 0 IU having the lowest yield. No other differences in processing traits were seen in d 36 processing. Final processing (d 46) showed significant differences in Pectoralis major and Pectoralis minor, when analyzed both linearly and quadratically. Both yields were lowest in the birds fed 50 IU and highest in those fed either 0 or 70 IU. Feeding high levels of vitamin E (70 IU) in the starter phase improved both BWG and FCR at the end of the feeding phase. However, these improvements when compared to the lower level of vitamin E fed were lost at termination. Carcass yields at d 36 are detrimentally affected when birds are fed 0 IU of vitamin E but are recovered by d 46. The impact of vitamin E in the starter phase on breast meat yield at d 46 showed a U-shaped response, with the highest yields appearing in birds that are fed either the lowest (0 IU) or highest (70 IU) levels of vitamin E. In conclusion, while live performance may be improved by feeding high levels of vitamin E in the starter phase, they are masked once the birds are offered common grower and finisher diets.
Keywords: broiler; fat soluble vitamin; vitamin E; feed conversion

4077 Use of organic trace minerals and prebiotics to maintain broiler health and performance in low soy broiler diets

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The aim of this study was to determine whether the performance deterioration commonly associated with non-soy plant based protein broiler diets could be mitigated through a combination of organic trace mineral and prebiotic feed additive.
336 1-d old male Ross 308 chicks were randomly allocated in pens of 7 chicks to 1 of 4 dietary treatments, with 12 replications per treatment for 35 days. A novel blend of organic trace minerals and mannan-rich-fraction (APK; Advantage PakTM; Alltech, UK) and two soy levels were arranged in a 2 × 2 factorial design with 0 or 0.075% APK supplementation and moderate or low soy. Diets were wheat/soy based, with 50% of the soy being replaced by peas, field beans, lupin and sunflower extract in low soy diets,. Mash feed was offered ad libitum as starter (d0-14), grower (d15-28) and finisher (d28-35). Effects on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), carcass yield (CY), and litter nitrogen (LN) and phosphorus (LP) were analyzed using SPSS v.28 (IBM) in a randomized block design.
Soya level had no effect on Day 0-35 BWG but there was a significant increase in BWG (2,136 vs 1,887g [P<0.001]) with APK supplementation. APK had no effect on FI but low soy diets resulted in a significant increase in cumulative FI (3057 versus 3324g [P<0.001]). FCR was significantly improved in diets containing APK supplementation (1.53 vs 1.66 [P<0.001 and a trend was seen towards poorer FCR (1.63 vs 1.53 [P=0.061]) associated with the low soy diets, but adding APK to the low soy diet mitigated this effect. Soy level had no effect on CY but APK supplementation improved CY (394 vs 379 g/kg BW [P=0.007]). For litter, low soy diets significantly reduced LN (3.4% vs 3.2% [P=0.001]) and LP (7.9 vs 7.5g P /kg [P=0.043]) contents of the litter. There was no effect of APK on LN or LP, but LCa was reduced (16.9 vs 23.8g/kg [P<0.001]). In terms bird health, low soy and APK both significantly increased absolute bone weight but when strength was corrected for bird size no effect on bone strength was seen (43.0 vs 44.0 N/kg bird [P=0.714].
These data suggest APK supplementation could improve broiler BWG, FCR, and CY irrespective of soy level and that broilers are able to compensate for the possible reduction in nutrient digestibility associated with partially replacing soy with other plant protein sources by increasing FI. This indicates that, where barriers exist to the use of soy as protein source in broiler diets, low protein diets fed in combination with organic trace minerals and a prebiotic source are a viable route to maintaining similar performance and health to that seen in soy-fed broilers but with lower nitrogen output in manure.
Keywords: broiler, ; environment, ; organic trace minerals; soy, ; mannan-rich fraction

4078 Influence of calcium pidolate on bone traits, egg quality and performance of layers fed diets with low calcium and phosphorus levels

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Lower levels of phosphorus in layers diets could help the egg production sector to save resources and reduce environment contamination. Calcium pidolate (PID) has been studied in broilers in both conventional field diets and in contexts of reduced calcium (Ca) and phosphorus (P) diets. These trials have shown that it is possible to formulate with lower levels of these minerals while maintaining good performance thanks to PID. So far, PID has only been studied in layers in conventional diets. The objective of this trial was to assess whether PID also enables nutritionists to formulate layer diets with lower levels of Ca and P without impacting performance, egg quality and bone quality traits. A total of 256 Hy-line laying hens of 50 weeks old were distributed following a completely randomized design for 4 treatments (8 replicates of 8 birds) for 84 days. Control group was fed following industrial Ca and P levels (CTRL) and PID group received the CTRL feed supplemented with 300g/MT of PID (Pidolin PCa, Dietaxion SAS). VLCaP group was fed with a diet reduced in CaT and Pdig (10% and 30%, respectively). VLCaP-PID was supplemented with 300 g/MT of PID. Performance and egg quality traits were weekly measured: feed intake, egg production, feed conversion ratio (FCR), rate of fragile eggs (RFE), eggshell thickness (ETH), eggshell strength (EST) and eggshell specific gravity (ESG). At the end of the experiment one bird per replication was slaughtered to measure the following traits in tibia and femur bones: strength, mineral density, mineral %, Ca % and P%. Data were analyzed using ANOVA followed by Tukey tests. All the analyzed bone traits of tibia and femur were improved

using PID (P<0.05) and were deteriorated with a diet reduced in Ca and P (P<0.05). There were no significant differences between groups for feed intake, egg production, FCR and RFE (P>0.05). Some of the egg quality traits were significantly improved with PID: ESG and ETH (P<0.05). Reduced levels of Ca and P in diets reduced significantly the same parameters of egg quality (P<0.05). Supplementing 50-72-week-old laying hens with 300 g/MT of PID improved bone traits, eggshell thickness and eggshell specific gravity when they fed an industrial diet as well with a diet with reduced Ca and P levels. PID then could be a good strategy to improve egg quality and bone quality.

Keywords: calcium pidolate; laying hens; phosphorus; bone strength; eggshell quality

4079 Vitamin K as a functional nutrient to support calcium metabolism in pullets for extended laying period.

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Genetic selection has increased the productivity and longevity of laying hens, but aging is associated with calcium (Ca) dysregulation and compromised bone integrity. Vitamin K, acting through its dependent proteins, contributes to Ca homeostasis and may influence the establishment of Ca reserves during puberty. This study evaluated the impact vitamin K forms, inclusion level, and its interaction with vitamin D on pullet performances and bone mineralization. One-day old Lohmann White pullets (n=360; 36.9 g ± 0.95) were randomly placed in 72 pens (n=5/pen) and assigned to 6 treatments: 1) Control (normal VitK3, 3 mg/kg), 2) VitK1 (12 mg/kg of vit K1), 3) VitK2-mk4 (12 mg/kg of vit K2-MK4), 4) VitK2-mk-7 (12 mg/kg of vit K2-MK-7), 5) VitK3 (12 mg/kg of vit K3), and 6) VitK3+D (12 mg/kg of vit K3 + 3000 IU of Vit D). Animals and feed were weighed every two weeks and at the end of each feeding phase to determine body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). On week 15, 36 pullets were scanned using DXA to evaluate bone mineralization content (BMC). A mixed model ANOVA (SAS) was used with the treatments set as fixed effects and a pen of 5 pullets as the experimental unit. Pre-planned orthogonal contrasts were used to assess the effects of Vit K levels (Trt 1 vs 2,3,4,5), alternative K forms (Trt 2,3,4 vs 5), vitamin K2 vitamers (Trt 3 vs 4) and additive effect of vitamin D (Trt 5 vs 6). During the starter phase (1-3 weeks), ADFI was lower in pullets receiving VitK3 compared to pullets receiving either vitamin K1 or K2 ($P < 0.05$), and their FCR was also lower ($P < 0.05$). For the grower phase (4-8 weeks), ADG and ADFI were lower for VitK3 pullets compared to pullets receiving either vitamin K1 or K2 ($P < 0.05$), with a tendency to have a lower BW ($P = 0.08$). Adding vitamin D to K3 tended to increase the ADG ($P = 0.08$) and BW ($P = 0.10$). However, the FCR was lower for Control pullets compared to all treatments ($P < 0.01$), and lower for K2-mk7 pullets compared to K2-mk4 ($P < 0.01$). During the developer phase (9–17 weeks), Control pullets maintained a lower FCR ($P < 0.01$), but also a lower ADFI ($P < 0.05$) compared to the other treatments. No difference was observed for BMC and BMC/BW. Overall, this study provides novel insights into vitamin K supplementation in modern pullets. Although vitamin K forms modulated early growth performance and FCR, the lack of effects on BMC at week 15 suggests either vitamin K levels are adequate or that skeletal benefits may only emerge once pullets begin to actively accumulate Ca reserves later in development.

Keywords: Pullets; vitamin K; bone; calcium

4080 Responses of Broilers to Digestible Phosphorus in Terms of Growth Performance and Bone Mineralization in Broilers from 0 to 21 days of age

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Optimizing apparent digestible phosphorus (Pdig) utilization is essential for supporting skeletal development and growth in broilers while reducing P excretion. This experiment was originally designed to validate a prediction model of Pdig of broiler diets obtained from a meta-analysis. This validation step offered an opportunity to estimate the Pdig response from directly measured digestibility values, rather than depending on total or assumed estimates. The optimal dietary Pdig level (g/kg) for performance (average daily gain (ADG), feed conversion ratio (FCR)) and bone mineralization (tibia ash, %) was thus determined for broiler chickens from 0 to 21 days of age. To generate a wide range of Pdig values, eight diets were formulated with varying combinations of non-phytate phosphorus (NPP), total calcium, and microbial phytase levels. Dietary NPP ranged from 2.0 to 4.0 g/kg, total calcium from 5.5 to 8.0 g/kg, and phytase supplementation from 0 to 1,000 FTU/kg. Average daily feed intake and ADG were calculated per cage, and phosphorus digestibility was determined via prececal ileal digesta collection at 21 days of age to estimate the real Pdig value. Linear-Plateau (LP) and Quadratic-Plateau (QP) regression models were evaluated for ADG, FCR, and tibia ash. For ADG, the LP model revealed the best fit and showed a linear increase up to a plateau estimated at 3.62 g/kg of dietary Pdig ($P < 0.001$), with no additional performance improvement observed beyond it. For tibia ash, the LP model also provided the best fit, estimating the optimal response at 3.58 g/kg of Pdig ($P < 0.001$), suggesting that optimal bone mineralization was achieved at a similar, though slightly lower, level of digestible phosphorus. Interestingly, for FCR, the best-fitting model was the QP model, with a predicted minimum FCR at 3.30 g/kg of dietary Pdig. Although it is often reported that P requirements for optimal bone mineralization are higher than those for growth performance, the present study found a striking convergence between the two. However, it is important to note that, because the experimental design simultaneously varied calcium and phytase levels to induce variation in Pdig, it was not possible to isolate the specific effect of Pdig from Ca–P interactions and their impact on the evaluated responses. It is concluded that, under the experimental conditions of this study, the response of growth performance and bone mineralization showed an increase and then a plateau which shows optimal level of dietary Pdig around 3.3 to 3.6 g/kg.

Keywords: broilers; digestible phosphorus; performance; bone mineralization; calcium

4081 Achieving Gut Harmony Through Jefo Matrix Technology: Optimizing Nutrient Utilization and Performance in Poultry

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Gut Harmony (GH) is a concept that involves the microencapsulation of vitamin and trace mineral (VTM) premix using Jefo Matrix Technology (JMT), a triglyceride-based matrix. The JMT was designed to stabilize sensitive micronutrients during feed manufacturing and storage, while enabling progressive nutrient release along the intestinal tract for better absorption. The objective of this work was to evaluate the impact of JMT protected VTM premix on

growth efficiency, intestinal morphology, and productivity across diverse poultry systems. Three independent trials were conducted under commercial like conditions in broilers and broiler breeders. Microencapsulated GH replaced conventional free premixes at levels ranging from 100% to 25% relative to the control diets. Data were analyzed using one-way or two-way ANOVA, with significance declared at $P \leq 0.05$. In experimental conditions in the Middle East, Ross 308 broilers that received GH supplementation at 25% and 50% showed increased final body weight by 8.5 and 11.8% ($P<0.0001$), improved cumulative feed conversion ratio (FCR) from 1.46 to 1.40 and 1.39 ($P=0.012$), and raised European Production Efficiency Factor from 393 to 444 and 456 ($P<0.0001$), respectively. Under controlled North American experimental conditions with Cobb 500 and Ross 708 broilers, GH maintained or improved performance at reduced inclusion rates, with the 50% inclusion group achieving a significantly ($P=0.014$) lower FCR (1.57 vs. 1.59) compared to all other treatments from 0-42 days, and improved intestinal morphology characterized by higher villi and increased villus area. Research conducted with breeder hens in a North American University setting, GH inclusion at 100% and 300% significantly improved hen-day ($P=0.0019$) and hen-housed ($P=0.0014$) egg production, while reducing feed per dozen eggs ($P=0.014$), indicating enhanced nutrient bioavailability and metabolic utilization. Collectively, these results demonstrate that Jefo Matrix Technology delivers measurable biological advantages by synchronizing nutrient release and protecting active compounds from early degradation, allowing for better vitamin and trace mineral delivery, which allows for better growth, gut integrity, and performance. The Gut Harmony concept offers an efficient and sustainable nutritional solution that optimizes nutrient delivery and performance outcomes in modern poultry production systems.
Keywords: Gut Harmony; Broiler; Nutrition; Vitamins and Trace Minerals; Broiler Breeder

4082 Evaluation of Phase-Specific Calcium and Phosphorus Feeding Strategies on Growth Performance and Body Composition in Broiler Chickens

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Optimal supplementation of Ca and P in broiler diets is essential for supporting growth, bone development, and nutrient utilization, while also influencing feed costs. This study evaluated the effects of different depletion and repletion feeding strategies of dietary retainable Ca (rCa) and retainable P (rP) on growth performance and body composition of broiler chickens. A total of 4320-day-old Ross 708 male broilers were randomly assigned to one of four treatments in a randomized complete block design, with 18 replicate pens per treatment (60 birds/pen; final stocking density: 34.4 kg/m2). The birds were fed in four feeding phases, with feed transitions determined based on pen-level feed intake, resulting in varying durations: Starter (S) feed was offered for 12–13 days (~450 g), Starter-Grower (SG) for 8–10 days (~800 g), Grower (G) for 6–8 days (~1000 g), and Finisher (F) for 6–8 days (~1400 g). Diets were formulated to contain standard or control (C; 100%), moderate (M; ~85% of standard), or low (L; 66.7–81.3% of standard) rCa levels. For the moderate and low rCa, rP was maintained at 85–89% of standard to minimize potential effects on feed intake. Standard rCa and rP levels reflected commercial broiler diets used in Quebec, Canada. Treatments were: T1 (Control; CCCC)—standard rCa and rP in all phases; T2 (LCCL)—low rCa and rP in S and F, standard in SG and G; T3 (LCML)—low in S and F, standard in SG, moderate in G; T4 (LMML)—low in S and F, moderate in SG and G. Diets included antibiotics, coccidiostats, and 500 FTU/kg of phytase to represent a commercial broiler diet in Canada. Meat and bone meal were not included to reduce Ca and P variability. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were measured at 21 d and 35 d. At 35 d, one bird per pen was euthanized for body composition analysis via dual-energy X-ray absorptiometry (DEXA). Data were analyzed using PROC GLIMMIX in SAS, with treatment as a fixed effect and block as a random effect. From 0–21 d, T4 had the highest ADG (41.1 g/d), significantly greater than T2 and T3 (both 40.2 g/d; $P < 0.05$), and the lowest FCR (1.324 g/g; 2.2–3.8 points lower than other treatments, $P < 0.05$). No significant differences were observed from 22–35 d ($P > 0.19$), nor in d0–d35 performance ($P > 0.22$), survivability ($P = 0.35$), bone mineral content ($P = 0.72$), bone density ($P = 0.34$), lean mass ($P = 0.75$), or fat mass ($P = 0.34$). These results suggest that strategic reductions in rCa and rP can be implemented without compromising growth or bone health. The most depleted strategy (T4) warrants further investigation as a cost-effective alternative for commercial broiler production.
Keywords: Calcium; Phosphorus; Nutritional strategies; Growth performance; Skeletal integrity

4083 Impact of varying calcium and digestible phosphorus levels on growth, skeletal development and nutrient digestibility during the complete rearing pullet phase.

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This study assessed the effects of varying dietary calcium (Ca) and digestible phosphorus (dP) on performance, bone quality and apparent total tract retention (ATTR) of minerals in Lohmann Brown pullets during the rearing period (0 to16 wk). A total of 1,632 birds were assigned to eight treatments in a 4×2 factorial design, with three levels of Ca reduction relative to the reference Ca level (CON) throughout the entire period: moderate (-15%; MOD), intermediate (-30%; INT) and severe (-50%; SEV); and two levels of dP: the reference dP level (dP-I) and a reduction of 15% (dP-II). Each treatment started with 12 replicates of 17 pullets and concluded with 7 pullets per replicate. The pullets were fed using a 3-phase feeding program (0 to 3 wk, 4 to 10 wk, and 11 to 16 wk). The reference levels included 9.0, 8.0, and 7.0 g/kg of Ca, and 3.7, 3.3, and 2.9 g/kg of dP for the different feeding phases, respectively. None of the diets contained phytase. During the starter phase, MOD, INT and SEV Ca reductions improved daily weight gain by 2.8% ($p=0.003$), and feed intake by 3.6% ($p<0.001$), on average compared to CON, but these differences disappeared after 6 wk. The reduction in dP did not affect the performance during the complete period. Tibia weight, length and mean diameter were similar among treatments during rearing ($p>0.05$); however, tibia strength and ash content declined with SEV Ca reduction from the beginning to the end of the rearing ($p<0.001$). At 16 wk, cortical, medullary and epiphyseal tibial traits were not negatively affected by Ca or dP reduction. At 6 wk, a trend toward higher serum P concentration by 8.9 % was observed in pullets fed the SEV Ca reduction compared to CON ($p=0.058$), suggesting that dietary Ca level exerts a stronger influence on gastrointestinal P absorption than dietary dP levels themselves. Treatment including CON and dP-I levels resulted in the lowest ATTR of dry matter ($p=0.005$) and energy ($p=0.006$). Reductions in dietary Ca increased linearly the ATTR of Ca from 15.1 to 18.9, 24.8 and 30.5 % ($p<0.001$) and ATTR of P from 12.6 to 19.0, 21.8 and 22.8 % ($p=0.050$). Phosphorus retention was also enhanced by reducing dietary dP (16.8 vs. 21.3 %; $p=0.042$). Overall, dP reduction did not impair growth nor bone development at the levels studied, validating the

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use of dP-II levels independently of Ca level in diet formulation. These findings support the optimization of Ca and dP levels during the rearing to enhance growth and nutrient digestibility without compromising skeletal development, while also reducing environmental impact by decreasing P excretion. Nonetheless, more research is needed to understand the long-term effects on both egg production and bone health.
Keywords: Calcium; Digestible phosphorus; Bone development; Nutrient digestibility; Pullets

4084 A model to determine the dynamics of digestible phosphorus utilization in growing pullets and laying hens
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This study aimed to develop and parameterize a deterministic factorial model to dynamically describe the utilization of digestible phosphorus (digP) in growing pullets and laying hens. The model was designed to determine digP requirements for maintenance, growth, and egg production to optimize bone mineralization and egg performance while identifying opportunities to reduce P excretion. The dynamic model was implemented in Vensim® and structured to simulate the main productive functions of hens. Body weight (BW) was described by a reparameterized Gompertz function, and cumulative feed intake (CFI) was fitted by a quadratic polynomial driven by BW. Body weight gain (BWG) and daily feed intake (DFI) were obtained as the first derivatives of the BW and CFI functions, respectively. For laying hens, egg mass (EM) was modeled using a non-linear plus-exponential function of age. DigP utilization was expressed as: P (mg/d) = a*BW0.75 + b*BWG + c*EM, where a, b, and c represent the coefficients for maintenance, growth, and egg production, respectively. The coefficients were determined experimentally. The maintenance coefficient (a) was estimated from an experiment with 1,320 Bovans pullets (16-week-old) fed graded levels of non-phytic phosphorus (NPP: 0.15–0.60%). Endogenous P losses, obtained by regressing P excretion on P intake, were used as the maintenance requirement, yielding 44.7 ± 2.45 mg P/kg BW0.75 (R2= 0.89; P<0.01). The growth coefficient (b) was determined using body P content derived from DXA scans and published equations, yielding 8.25 ± 0.6 mg P/g BWG (R2 = 0.76; P < 0.01). The egg production coefficient (c) was based on the analytical determination of P content in eggs from Bovans White hens (24–56 weeks), with a mean value of 1.95 mg P/g EM. Model integration and simulations were performed in Excel. During the growing phase (9–16 weeks), estimated P requirements ranged from 152 to 161 mg/d, values lower than standard recommendations, indicating a potential excess of dietary P at advanced ages. During the laying phase, the model predicted a constant digP requirement of approximately 222–227 mg/d, equivalent to 0.21% absorbed P, which is nearly half of the current genetic guideline (0.42% avP). Consequently, the model suggests that almost 50% of the non-phytic P supplied in layer diets is excreted. In conclusion, the developed factorial model successfully quantifies the dynamic partitioning of digP among maintenance, growth, and egg production. The results reveal a wide safety margin in current dietary digP recommendations and demonstrate that significant reductions can be achieved without compromising skeletal integrity or egg production.
Keywords: Factorial model; Phosphorus excresion; Mechanicistic model

Physiology I

4085 Advances in the Physiology of Stress in Poultry: Towards a New Synthesis
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The “classical” model for the stress response in poultry is that “stressors” act via the hypothalamus. This is releases corticotropin releasing hormone (CRH) that stimulate synthesis and release of adrenocorticotropin (ACTH) with ACTH being the result of peptidases cleaving proopiomelanocortin (POMC). In turn, ACTH stimulates release of corticosterone from adrenocortical cells. It is now clear that there are multiple hypothalamic neuropeptides that affect ACTH release and synthesis; these include neuropeptides W (inhibitory) and arginine vasotocin (stimulatory)., it is now clear that there are at least two adrenocortical glucocorticoids; respectively cortisol and corticosterone. There are also effects of stress on other pituitary hormones and both hypothalamic and pituitary neuropeptides. Not only is POMC cleaved to produce ACTH but so also is the opioid peptide, β-endorphin. Circulating concentrations of β-endorphin are increased in stressed poultry. There are other opioid peptides in the hypothalamus, pituitary and adrenal glands including Met-enkephalin, Leu-enkephalin and Dynorphins. Both Met-enkephalin and Leu-enkephalin are generated by peptidase acting on pro-enkephalin (PENK) while dynorphins originate from prodynorphin (PDYN). Circulating concentrations of these opioid peptides are influenced by stressors with different stressors exerting either stimulatory or inhibitory effects on plasma concentrations of β-endorphin, Met-enkephalin, Leu-enkephalin and Dynorphins. Furthermore, circulating concentrations of growth hormone are also decreased by multiple stressors. It is cautioned that because a stressor affects chickens, does not per se indicate they will exert the same effect in turkeys and vice versa. For instance, shackling is accompanied by increased plasma concentrations of corticosterone and wing flapping in broiler chickens. However, there are no such effects in turkeys. Rather than there being the physiological response to stress, are multiple responses

4086 Gastrointestinal pH profile of heavy broiler breeders
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Gastrointestinal pH is a critical determinant for nutrient solubility and enzyme activation. While extensive data exist for broilers, literature regarding heavy breeders is scarce. This study aimed to characterize the pH of the crop and proventriculus in 62-week-old heavy broiler breeders, hypothesizing that high dietary limestone inclusion results in a distinct gastric environment compared to broiler chickens. This study utilized a descriptive experimental design to characterize the physiological pH profile of a flock of heavy breeders. The experimental unit was the individual bird, with a total sample size of 36, Cobb 500®, heavy breeder (62 weeks old). The birds were housed in individual boxes and fed a corn-soybean meal diet containing 3.30% calcium (fine and coarse limestone) and 0.39% available phosphorus under a restricted feeding program (fed once daily). After a 10-day adaptation period, all birds were euthanized for the collection of digesta from the crop and the proventriculus/gizzard complex. The pH was measured immediately using a digital pH meter (Starter 300, OHAUS). Data from all 36 birds were pooled, and descriptive statistics (mean, standard deviation, and range) were calculated to characterize the gastric profile. Normality was verified using the Shapiro-Wilk test. The analysis revealed an elevated gastric pH profile. The mean pH observed in the crop was 5.03 ± 0.42, with values ranging from 4.39 to 5.95. For the proventriculus, the mean pH was 4.12 ± 0.33, with individual values ranging from 3.29 to 4.89. A significant portion of the flock exhibited a proventricular pH above 4.0, which

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is considerably higher than the physiological reference typically observed in broilers (2.5–3.5). Heavy broiler breeders at 62 weeks of age present a gastric environment less acidic than standard broiler references. The elevated proventricular pH (>4.0) suggests that the buffering capacity of high dietary calcium creates a condition that may limit the efficacy of acid-dependent enzymes and nutrient digestibility. Furthermore, this insufficient acidification could weaken the gastric barrier function, potentially predisposing senescent breeders to pathogenic colonization and dysbiosis.
Keywords: Digestion; Gastric acidity; Heavy breeders; Limestone; Proventriculus

4087 Characterization of Intestinal Permeability Assessed Through the FITC-Dextran Marker in Commercial Poultry Operations

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This study aimed to characterize intestinal permeability in commercial poultry flocks in Colombia using fluorescein isothiocyanate–dextran (FITC-d) as a marker and to establish permeability severity profiles. A multicenter, cross-sectional study was performed with 394 observations from 12 companies: broilers (n = 102; 3 companies), laying hens (n = 237; 7 companies), and female broiler breeders (n = 47; 2 companies). Age ranges were: broilers 2–6 weeks; layers 12–81 weeks; female breeders 10–59 weeks; and male breeders 20–30 weeks. Among layers, 126 birds were cage free (53.0%) and 111 caged (46.8%). Intestinal permeability was quantified as serum FITC-d concentration (ng/mL). Variable records included age, production purpose, housing system, and company. Because data were non-normal, medians, quintiles, and non-parametric tests were used. Permeability severity profiles were derived by production type using hierarchical clustering to determine k, followed by Partitioning Around Medoids (PAM, k-medoids) with a Gower dissimilarity matrix based solely on FITC-d data. Differences between housing systems in layers were assessed with the Wilcoxon–Mann–Whitney test ($\alpha = 0.05$). In laying hens, FITC-d values ranged from 4.07 to 436.77 ng/mL (geometric mean = 31.52; median = 27.30). In broilers, values ranged from 2.25 to 72.58 ng/mL (geometric mean = 21.23; median = 22.21), and in breeders from 4.07 to 679.03 ng/mL (geometric mean = 31.84; median = 21.31). For layers, three clusters (n = 138, 68, 31) with median FITC-d values of 17.5, 52.4, and 228 ng/mL defined severity thresholds: low ≤ 52.2 ng/mL, moderate = 52.4–227 ng/mL, high ≥ 228 ng/mL. Cage-free housed hens showed higher FITC-d values than caged hens (W = 5690; p = 0.0134). In broilers, four clusters (n = 33, 36, 25, 8) with medians of 8.59, 21.7, 33.0, and 54.8 ng/mL established thresholds of low ≤ 33 ng/mL, moderate = 33–53 ng/mL, and high ≥ 54 ng/mL. In heavy breeders, three clusters (n = 27, 9, 11) with medians of 19, 96.4, and 300 ng/mL yielded thresholds of low ≤ 63 ng/mL, moderate = 63–256 ng/mL, and high ≥ 257 ng/mL (based on Q60 ≈ 63.0; Q80 ≈ 257.8). This study provides baseline values for intestinal permeability measured via FITC-dextran and proposes quantitative thresholds to classify permeability severity in commercial poultry. These cutoffs offer a practical framework for monitoring gut integrity and guiding sanitary and nutritional interventions at the farm level.
Keywords: Intestinal Permeability; broiler; layers; health; Gut

4088 Effect of heat stress on jejunal epithelial barrier integrity in broilers divergently selected for high- and low-water efficiency

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Water scarcity is a significant and growing threat to worldwide food security and sustainability that is only exacerbated by concurrent rising global temperatures. Poultry, in particular, are highly and negatively impacted by adverse environmental conditions. As nutrient absorption and intestinal integrity are critical for growth and performance, understanding the impact of these stressors on the broiler gastrointestinal tract is highly relevant. Recently, we divergently selected broilers for low (LWE)- and high-water efficiency (HWE), where the HWE line consumes less water, while maintaining growth and improving FCR as compared to the LWE line. Here, we examined the effect of chronic cyclic heat stress (HS) on the jejunal expression profile of tight-junction, gap-junction, adherens, and desmosome genes in the 4th generation of these two lines. Day-old chicks (240/line) were placed in 12 environmentally controlled chambers (2 pens/chamber, 20 birds/pen). On D29, daily HS (36°C, 9h/day) was applied to half of the chambers while the rest remained at 25°C. On D49, samples were collected for measures of gut integrity and related jejunal gene and protein expression. Data were analyzed by two-way ANOVA with line and environment as factors. When line by environment interactions was significant, individual means were compared using Tukey's multiple range test. Data were analyzed with GraphPad Prism, with significance set at $P \leq 0.05$. Jejunal gene expression of multiple gut integrity factors was higher in the HWE as compared to the LWE lines, including claudins (CLDN22 and 34), occludin (OCDN), zona-occludin-2 (ZO-2), gap junctions (GJA1, GJA3, GJC1), and cadherin 1 (CDH1). CLDN8, -20, -25, -4, GJC2, and GJD2 were also greater in HWE, but were additionally downregulated during HS. Conversely PALS1-associated tight junction protein (PATJ) and desmocollin 1 (DSC1) mRNAs were downregulated in the HWE as compared to the LWE broilers. An interaction between the line and environment was seen in CLDN1, where expression was decreased in the LWE but increased in the HWE in HS. Additionally, CLDN15 and -16 genes were greatest in the HWE under TN conditions, while catenin alpha 2 (CTNNA2) was highest in the HWE during HS. Overall, the jejunal expression profile of key genes associated with intestinal barrier integrity likely contributes to the water efficiency phenotype and the response of these birds to HS. These results could have significant implications for breeding strategies improving water efficiency, while simultaneously enhancing gut health and resilience to HS.
Keywords: broiler; gut integrity; heat stress; water efficiency; jejunum

4089 Dynamic Single-cell Resolution Atlas of Skeletal Muscle Development Across Chicken Breeds

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Skeletal muscle consists of diverse cell types whose development is governed by temporally regulated events. Artificial selection has strongly shaped the genetic architecture of chickens, especially for muscle growth. Here, we generated a single-cell/single-nucleus transcriptomic atlas of chicken skeletal muscle from embryonic day 3.5 to 84 weeks of age, encompassing over 1,902,250 cells/nuclei. We identified 16 major cell types and mapped their dynamic changes across development. Muscle stem cells progressively decreased in proportion, whereas immune cells increased with age and then declined. Fibro/adipogenic progenitors (FAPs) represented a predominant population. Subclustering of FAPs uncovered two previously unrecognized subpopulations: myogenic-like FAPs and myogenic-differentiated FAPs. Pseudotime trajectory

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analysis revealed key transcription factors (TFs) regulating FAP fate: TWIST1 and HES1 were enriched along the tenocyte branch, whereas ETS1 and KLF6 were associated with the myogenic branch. Cell–cell communication analysis indicated that FAPs occupy a central position in the interaction network and engage in the most extensive signaling. We also detected strong crosstalk among muscle stem cells, myogenic cells, and myocytes, reflecting intensive signaling during muscle development and regeneration. Within this atlas, we further examined three representative breeds—commercial broilers, layers, and gamefowl—from embryonic day 11 to post-hatch day 1. Histology revealed breed-specific myofiber formation: broilers had established myofibers by E14; layers initiated myofiber fusion at E14 and formed mature myofibers by E15; gamefowl formed myofibers at E15. Broilers exhibited significantly larger myofiber diameters than the other breeds at E16 and E19. Analysis of myogenic populations showed broadly similar patterns in broilers and gamefowl, whereas layers displayed the highest proportion of myofibers in late embryogenesis. Pseudotime analysis further identified ATF3, EGR1, CREB5, FOS, and others as key TFs driving the transition of muscle stem cells toward differentiated myofibers. Overall, this study establishes a high-resolution atlas of chicken skeletal muscle development, reveals marked cellular heterogeneity and diversity, and defines core TFs governing lineage differentiation, providing a genetic framework for understanding breed-specific muscle growth.
Keywords: Chicken; scRNA-seq; Skeletal muscle; FAPs; Transcription factors

4090 In Ovo their heads: Can behavioral and physiological stress responses from heat stress be replicated without a parental component in Pekin ducks?

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Episodic heat stress (HS) costs the poultry industry millions of dollars each year. Previous studies in our lab have shown that HS in breeder ducks leads to an increased deposition of glucocorticoids in the egg. Furthermore, hatchlings from these eggs exhibit enhanced physiological responses to stress and increased fear. We tested the hypothesis that increased glucocorticoids cause exaggerated stress and fear responses. We utilized an in ovo injection of glucocorticoids to simulate this stress response, but without the parental component. Eggs were injected with either dexamethasone (DEX; 0.1ml of 1µg/µl in saline), or with 0.1ml saline control (n=100/treatment), and incubated. Ducklings were separated into their respective groups (n=50/treatment/pen). At 3 weeks of age, ducks were given an ACTH stim (cosyntropin, 0.04mg/kg) in saline, vs saline control challenge (0.1ml/kg saline) with .01% mannitol (n=6/treatment). Blood was collected at 0,1,2, and 4 hours. An isolation fear test was also performed at 4 weeks of age. Heterophil to Lymphocyte ratio, ELISA for glucocorticoids, FCR, and weekly body weights were all calculated and recorded. All physiological data were normally distributed and therefore analyzed by Student's t-test. Behavior data were analyzed by Wilcoxon Rank test. Our results show that the in ovo dexamethasone injection increases the glucocorticoid response to the ACTH challenge in the DEX-ACTH group compared to controls. DEX ducklings showed an increased ($p<0.05$) corticosterone response to ACTH compared to controls. However, no differences were observed in the isolation fear test. Our results suggest a mechanism for how epigenetic changes may occur in physiological responses but perhaps not behavioral.
Keywords: HPA Axis; Transgenerational; cortisol; vocalization; Novel Environment

4091 Feeding live black soldier fly larvae (Hermetia illucens) to free-range Lohmann Brown laying hens: Effects on performance, organ development and ileal histomorphology.

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This study investigated the effects of feeding live black soldier fly larvae (BSFL, Hermetia illucens) as a substitute for soybean meal (SBM) and soybean oil (SO) on the organ development and ileal histomorphology in Lohmann Brown laying hens.

A total of 144 18-week-old hens were allocated equally to two dietary treatments (4 pens/treatment, 18 hens/pen, free-range system). Birds in the control group (SOY) received a commercial layer diet containing 13.5% SBM and 1.5% SO during phase 1 (P1, weeks 18-39). In the BSFL group (LAR), SBM and SO were completely replaced with BSFL. During phase 2 (P2, weeks 40-50), the inclusion level of SBM+SO (SOY) and BSFL (LAR) was reduced to 10%. BSFL were provided twice a day, with quantities adjusted according to expected dry matter intake. Body weight and feed intake were recorded weekly, and laying performance was measured daily.

At birds 50 weeks of age, 8 hens per treatment were slaughtered for carcass and organ analysis. Carcasses were weighed to calculate percentage dressing, and the organs (heart, liver, proventriculus, and gizzard) were weighed to calculate relative organ weights. Tissue samples of the last ileum section were taken for histomorphological analysis. Data were analyzed using one-way ANOVA ($p<0.05$).

Feeding live BSFL did not affect hens growth and laying performance (SOY=88 vs. LAR=89%; $p>0.05$). During the whole trial period, BSFL-fed hens showed a significantly lower average dry matter intake (SOY=105 vs. LAR=101g/hen/day; $p<0.05$) and a higher individual egg weight (SOY=60.1 vs. LAR=61.3g/egg; $p<0.05$), resulting in a tendency toward improved feed conversion ratio (SOY=2.5 vs. LAR=2.3g Feed/g Egg mass; $p<0.1$).

Ileal histomorphology parameters, including villus height (VH), villus width, and villus surface area, were not affected ($p>0.05$). However, crypt depth (CD) tended to be lower in LAR-fed hens (SOY=0.21 vs. LAR=0.03mm; $p<0.1$), leading to a tendency for a higher VH/CD ratio (SOY=2.84 vs. LAR=3.50mm/mm; $p<0.1$). No significant differences were found in percentage dressing (SOY=60.2 vs. LAR=60.8%; $p>0.05$) or relative organ weights (g/kg BW) of heart, liver, proventriculus, and gizzard.

In conclusion, replacing SBM and SO with live BSFL represents a suitable and sustainable alternative protein and energy source for laying hens, maintaining production performance without affecting intestinal histomorphology or organ development.
Keywords: Insects; Sustainability; Physiology; Egg quality; Alternative feed

4092 The parasympathetic and sympathetic nervous system play a role in tonic immobility recovery.

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Tonic immobility is a temporary state where a chicken will remain immobile after restraint. It is commonly induced and used as a behavior-based test for welfare, where a longer latency to right is equated with more fear. Despite its commonality, there is very lim-

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ited research done on the physiological systems that underlie tonic immobility. The objective of our study is to examine the roles of the sympathetic and parasympathetic nervous systems on tonic immobility. Sixteen Dekalb White hens (43 weeks of age) were given 0.1 ml/kg of either saline (0.9% NaCl) as a control (CON), labetalol (5 mg/ml) to inhibit the sympathetic nervous system (SNS), or glycopyrrolate (0.2 mg/ml) to inhibit the parasympathetic nervous system (PNS). The study utilized a crossover design over 3 days where each hen got a different treatment each day. Immediately before and after injection of the drug, respiratory rate (RR) was measured and a thermal image was taken of the hen that included the comb, beak, and open eye. Tonic immobility was then induced and another thermal image was taken. Respiratory rate was taken every minute until 10 minutes or the hen righted itself. Number of attempts to induce tonic immobility, latency to first leg movement, latency to first alert head movement, and latency to righting were also measured. An ANOVA was used for all statistical analysis. A p-value less than or equal to 0.05 was considered significant. After injection and before induction, the PNS had a lower respiratory rate ($p = 0.019$) compared to the SNS and CON. This trend continued through the first minute ($p = 0.020$) and the average of the time under tonic immobility ($p = 0.010$). There were no differences in average eye or maxilla thermal temperature. For comb thermal temperature, SNS pre-injection was higher than CON post-injection ($p = 0.0173$), CON post-induction ($p = 0.0004$), and SNS post-induction ($p = 0.0208$). PNS pre-injection was higher than CON post-induction ($p = 0.0066$). The CON group was quicker to right than the PNS ($p = 0.0047$) and SNS ($p = 0.0745$) and there were no differences for latency to first leg movement or alert head movement. These results indicate that both the parasympathetic and sympathetic nervous systems play a role in recovery from tonic immobility, however further studies need to be undertaken to better understand the neurobiology of tonic immobility.
Keywords: tonic immobility; parasympathetic; sympathetic; laying hen; neuroendocrinology

4093 Are laying hens left-handed? Investigating lateralization in laying hen chicks following a 10-week exercise routine

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In commercial aviary housing, coordinated flapping flight is essential for safe navigation and collision avoidance for skeletally fragile laying hens. Domestic pullets show consistent lateralized behaviors, right-foot preferences and left-turning bias, indicating right hemisphere dominance (controlling the left body side). These asymmetries imply coordinated lateralization across motor systems, potentially influencing flight mechanics and muscle development. Early-life exercise may enhance musculoskeletal development, but its effects on motor lateralization are unclear. Thus, this study investigates whether flapping descent training (FDT) in pullets induces asymmetric flight muscle development (indicating lateralization), and improved keel bone characteristics (total keel area and percentage ossified).

Day-old female chicks (n=168, 84 white-feathered, 84 brown-feathered) were randomly placed into 12 pens (14 chicks/pen), with randomization stratified by strain to ensure equal numbers of brown and white birds per pen. Seven chicks/pen were assigned to FDT, the rest to a control group (CON). FDT involved placing each chick atop a 1.2 m tower to flap down to a 20 cm high soft-landing mat. Training occurred 5 days/week from 1-10 weeks of age. Descents began at 1/day, adding 1 descent every 2 days. At 11 weeks of age, bodyweight, flight muscle thickness (ultrasound), keel bone ossification (%) and total keel area (X-ray) were recorded. Data were analyzed using a generalized linear mixed model (SAS), with treatment, strain and their interaction as fixed effects, pen as a random effect, and bodyweight as covariate.

At 11 weeks, FDT pullets had significantly thicker left pectoralis (PEC) ($p=0.0005$), higher left PEC:SUPR (supracoracoideus) ratio ($p=0.0005$), and greater left PEC thickness relative to bodyweight ($p=0.0002$) than CON chicks. FDT primarily activated the PEC (downstroke), which SUPR (upstroke) was less engaged. Left PEC hypertrophy may reflect coordinated motor lateralization, where known right-leg dominance complements increased left-wing activation. White-feathered pullets had thicker PEC and SUPR muscles (both sides), larger keel area, and greater keel ossification (all $p<0.0001$) than brown-feathered pullets, indicating superior flight muscle and skeletal development.

This data suggests lateralized flight muscle development, specifically left PEC hypertrophy in pullets, complementing known right-footedness and left-turning bias. Motor lateralization may thus extend to wing use during flapping descent. In aviary systems, understanding lateralization can inform perch placement, ramp orientation, and flight path design to support natural movement patterns.
Keywords: Muscle Physiology; Development; Lateralization; Laying Hen; Aviary System

4094 PHYSIOLOGICAL MONITORING OF BROILER CHICKENS USING IMPLANTABLE LOGGERS

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Continuous monitoring of physiological parameters in chickens can provide valuable insights into their health and welfare. This study tested the use of Star-Oddi Micro-HRT data storage tags for measuring body temperature (BT), heart rate (HR), and heart rate variability (HRV) in broiler chickens under control and corticosterone (CORT) treated conditions. A total of eight male Ross 308 chicks were used, consisting of four sham-gavaged control birds and four CORT-treated birds given 5 mg/kg orally each day between 09-10 h. All chicks were implanted with intracelomic Micro-HRT loggers and intra-muscular thermal microchips at one week of age, and data was collected until day 41. HR and BT were recorded every 10 minutes between 07 and 13 hour, and every 30 minutes for the remainder of the day. Analyses were conducted over age and across four periods of the day: morning (04:00–10:00 h), midday (10:00–16:00 h), evening (16:00–22:00 h), and night (22:00–04:00 h). HRV (RMSSD and SDNN) was calculated based on 3-second continuous ECG. Data was analyzed in R Studio

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using a linear mixed-effects model, where treatment, age, and time of day were included as fixed effects, and individual chickens were treated as a random effect. Out of 17,588 total HR readings, 80.2% (14,112) were classified as Quality Index 0 (QI0; highest quality), representing all birds, and were subsequently analyzed. Excluded measurements graded as QI1,QI2 and QI3 accounted for 4.9%,8.4% and 6.5% respectively. Temperatures of control birds recorded by the Star-Oddi Micro-HRT were significantly higher than those obtained via thermal microchips (median difference = 0.23 °C; $p < 0.001$). Overall, BT decline significantly after the third week of age. HR demonstrated a consistent decrease ($p<0.05$) with age, transitioning from over 450 beats per minute (bpm) during the second week to 320–340 bpm by the sixth week. A clear circadian rhythm was observed where HR of the birds at night was lower ($p<0.05$) when compared to other times of the day. A significant CORT × Time of the day interaction was observed for HR ($p<0.05$), with CORT treatment significantly affecting HR during the morning and evening.For HRV, a significant Treatment × Age interaction ($p<0.05$) revealed that CORT treatment reduced HRV in young birds up to 3 weeks of age.The loggers recorded physiological parameters in broiler chickens, identifying clear age and time-related changes in HR and BT. BT measured by the loggers was higher than that recorded by thermal microchips, likely due to differences in sensor placement sites. The interaction of CORT treatment with time of the day affected HR, while its interaction with age influenced HRV, highlighting complex physiological effects of CORT.

Keywords: Poultry; implantable loggers; animal-based sensor; physiological indicators; precision livestock farming

4095 Mitochondrial dynamics and their implication in the physiology of hen ovarian cells

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Mitochondria are essential organelles that regulate energy production, cell proliferation, apoptosis, oxidative stress, and steroidogenesis. In mammals, mitochondrial dysfunction has been associated with ovarian aging, characterized by a decrease in both oocyte number and quality. In hens, ovarian aging leads to reduced laying rates, prolonged ovulatory intervals, and increasing cycle irregularity after 60–70 weeks of age. Mitochondria regulate their shape and distribution through cycles of fission and fusion, with the balance shifting toward fusion in senescent cells. However, the role of mitochondrial dynamics in hens remains largely unexplored.

The present study aimed to evaluate in vitro impact of mitochondrial fusion on primary chicken ovarian cells (granulosa and theca cells) from 35 weeks old hens. Mitochondrial hyperfusion was induced using MDIVi1, a selective inhibitor of the fission-related protein Drp1, or leflunomide, a compound known to promote mitochondrial elongation. Following treatment, cell viability was assessed using a CCK-8 assay, cell proliferation was measured by BrdU incorporation, and progesterone and testosterone secretions were quantified by ELISA. The results were evaluated by one-way analysis using Graphpad Prism 10.5.0 and the p-value <0.05 was considered statistically
significant.

Mitochondrial elongation was confirmed by electron microscopy and RT-qPCR, which revealed a significant upregulation of genes encoding mitofusins and OPA1, both involved in mitochondrial fusion. Induced fusion resulted in a decreased number of mitochondrial DNA copies. Using a ROS assay, we further demonstrated that leflunomide reduced oxidative stress in granulosa cells. Functionally, mitochondrial hyperfusion led to reduced cell viability and proliferation, increased progesterone production in granulosa cells, and decreased testosterone production in theca cells.

In summary, mitochondrial hyperfusion in ovarian cells impairs cell viability and proliferation while altering steroid hormone secretion, potentially disrupting the hormonal balance required for normal follicular development. These findings highlight the pivotal role of mitochondrial dynamics in regulating ovarian cell function, and consequently the egg-laying performance and their potential involvement in ovarian aging.

Keywords: mitochondria; ovarian cells; ovarian aging; hen

Olmix Symposium: Advanced Poultry Immunity Through Algae-Based Solutions and Yeast Extracts

Raquel Pereira, Post Doctoral, PhD, MSc, Product Manager – Feed Additives

Mariel Neves Tavares, DVM, MSc, MBA Technical Sales Manager US

Zoetis Symposium: Immunity Starts Here: The Hatchery Health Team

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Babak Sanei, DVM, Associate Director Poultry & Aquaculture, Canada, Zoetis

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Bone Health and Quality I

4096 Bone Health and Skeletal Integrity in Modern Poultry Production: Emerging

Challenges, Welfare Implications, and Future Solutions

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Rapid genetic progress, intensive production systems, and increasing demand for poultry meat have dramatically improved growth rates and production efficiency in modern broiler chickens. However, these advances have also intensified skeletal health pressure that threatens bird welfare, production sustainability, and economic efficiency across the global poultry industry. Bone disorders remain among the most significant
causes of lameness, reduced mobility, culling, and carcass condemnation in commercial flocks worldwide.A wide spectrum of skeletal diseases affects modern poultry systems. Developmental and metabolic disorders such as tibial dyschondroplasia, rickets, valgus-varus deformities, and vertebral deformities arise from complex interactions among genetics, nutrition, incubation conditions, and rapid growth rates. Infectious and degenerative conditions such as Bacterial Chondronecrosis with Osteomyelitis (BCO) have become increasingly important in high-performance broiler strains. Additional skeletal challenges, such as osteoporosis, bone fractures, avian osteopetrosis, and joint-associated disorders, including viral arthritis and infectious synovitis, further complicate the landscape of bone health management in poultry production. Collectively, these
disorders compromise locomotion, reduce production efficiency,

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and represent a major animal welfare concern in modern poultry production systems. Rapid growth, skeletal immaturity, nutritional imbalances, microbial infections, and environmental stressors all contribute to the multifactorial nature of poultry skeletal disease. This opening lecture will provide an integrated overview of the major skeletal health challenges facing the poultry industry today. The presentation will explore the biological mechanisms underlying key bone disorders, examine their impacts on welfare and production, and highlight emerging advances in nutrition, microbiology, management practices, and precision monitoring technologies that offer promising strategies for improving skeletal integrity in modern poultry systems worldwide.

4105 Radiographic evaluation of keel bone damage and bone mineralization in laying Japanese quails (Coturnix japonica) at different ages

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Since keel bone damage can affect an entire flock of laying hens and is associated with pain, it constitutes a serious animal welfare issue. The occurrence of keel bone damage is a multifactorial problem; however, laying performance seems to play a key role. Laying quails show a similarly high laying performance as laying hens and could therefore also suffer from keel bone damage. The objective of these studies was therefore to investigate whether keel bone damage can also be found in Japanese quails. In a first study, a group of 51 female quails were radiographed at 8, 10, 15, 19, and 23 weeks of age to obtain more detailed information about the development of their keel bone during the production cycle and to assess keel bone fractures and deviations. The X-rays were used to detect fractures and deviations and to measure the lateral surface area, length, and radiographic density of the keel bone. In addition, the length of the caudal cartilaginous part of the keel bone was measured to learn more about the progress of ossification. The length of the cartilaginous part of the keel bone was calculated as the percentage of the total keel bone length. For the length of the cartilaginous part (log-transformed), body weight, radiographic density, keel bone length, keel bone lateral surface area, and the score of the deviation (log-transformed), mixed models with week of age as fixed effect were created using the nlme package and an ANOVA was performed. The animal was included as a random factor to take the repeated measures into account. The residuals were controlled for normal distribution by visual examination using q-q-plots and histograms. Post-hoc analysis was performed using Tukey's HSD tests.

In a second study, 20 quails at 16 weeks of age and 63 quails at 41 to 43 weeks of age were euthanized and plasma was collected for the analysis of estradiol-17ß. After death, all animals were x-rayed, and the keel bone and the tibiae were removed for histology and for analyzing the bone mass using the inductively coupled plasma optical emission spectrometry (ICP-OES).

The results of the first study show that in the 23rd week of age, 82 % of the quails had a deviated keel bone as assessed after maceration. Furthermore, there was a decrease in radiographic density, lateral surface area, and length of the keel bone between weeks of age 8 and 19. This indicates a general loss of bone substance and/or demineralization of the keel bone as can also be observed in laying hens. The findings of the second study are still pending, but it can already be concluded that female laying quails also suffer from keel bone damage.

Keywords: japanese quails; keel bone damage; fracture; deviation; mineralization

4097 Bone quality of broiler breeders derived from a divergent selection for breast meat ultimate pH

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Objective

This study aimed to assess the bone status of broiler breeders from the 18th generation of two lines divergently selected for the ultimate pH (pHu) of the Pectoralis major muscle.

Experimental Design

Two broiler chicken lines, pHu-: low pHu; pHu+: high pHu, representing opposite muscle glycogen levels, were compared. Both lines were reared under identical conditions to evaluate the effects of selection for muscle pHu on bone development. The study focused on bone morphology, mineralization, and strength, as well as laying performance.

Materials and Methods

Birds were floor-reared until 18 weeks of age and then individually housed in cages until 46 weeks. Egg production was recorded daily. After slaughter, 60 left tibiae (15 per line and sex) were collected. Bone samples were analyzed using computed tomography for structural assessment, followed by morphometric, mechanical (TBS), and chemical evaluations.

Statistical Analysis

A one-way ANOVA was performed to evaluate the effect of the pHu selection on bone and laying traits. When significant differences were found ($P < 0.05$), Duncan's multiple range test was used for mean comparisons. Pearson's correlations were calculated between bone and reproductive parameters, with significance set at $P < 0.05$.

Results

Significant effects of both line and sex were observed. The pHu- birds exhibited larger and more voluminous bones, whereas the pHu+ line showed higher mineralization (+6% ash content), greater fracture resistance (+19.5% TBS), and an increased TBS-to-length ratio (+21.9%), reflecting more efficient mineralization. Computed tomography analyses confirmed a denser and more homogeneous cortical bone structure in pHu+ birds, while pHu- individuals displayed greater total, trabecular, and marrow space volumes. Positive correlations between bone mass, mineralization, and mechanical strength supported the internal coherence of the bone phenotype. Regarding reproductive performance, pHu+ females produced fewer hatchable eggs and showed lower laying intensity than pHu- females, which may have limited calcium mobilization and thus helped maintain slightly higher bone mineral content.

Conclusions

Selection based on muscle pHu significantly influenced bone growth, composition, and mechanical strength, as well as reproductive performance. Higher muscle glycogen reserves (in the pHu- line) were associated with higher bone development, while lower reserves (in the pHu+) were associated with denser and stronger bones but a lower reproductive efficiency. These findings underscore the importance of integrating muscle energy metabolism, skeletal development, and reproduction into breeding strategies for producing robust, welfare-compatible broiler breeders.

Keywords: broiler breeder; bone quality; muscle metabolism; ultimate pH; mineralization.

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4098 RORA Gene Variant Modulates Bone Metabolism through the Wnt/β-Catenin Pathway and Influences Leg Health in Broilers

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Leg diseases pose a major constraint on the sustainable development of the broiler industry, causing substantial economic losses and serious animal-welfare concerns. The etiology of leg problems is multifactorial, involving interactions between genetic, metabolic, and environmental factors. However, the underlying metabolic mechanisms are poorly understood, hindering genetic improvement. To address this, we performed an integrative study on the commercial broiler line Guangming No. 2 using a case-control design, combining phenomic, genomic, and metabolomic analyses to uncover the metabolic and genetic basis of leg health. At 42 days of age, X-ray imaging classified 54 healthy and 54 leg-disorder birds. Leg-affected broilers showed significantly reduced bone strength, density, ash content, and morphological indices, indicating severe skeletal impairment. Metabolomic profiling with weighted correlation network analysis (WGCNA) identified 135 metabolites linked to leg health, including isobutyryl-L-carnitine, terpyridine, and fluroxene. Notably, 41% of these metabolites exhibited moderate heritability ($h2 > 0.2$), implying strong genetic influence. Metabolome-wide genome-wide association study (mGWAS) revealed 1,704 significant metabolite quantitative trait loci (mQTLs), with associated genes enriched in lipid metabolism pathways. Of these mQTLs, 1,541 (90.4%) were specific to one metabolite, while 163 (9.6%) had pleiotropic effects on multiple traits. The intronic variant rs738261170 in RORA showed strong pleiotropy, affecting 12 metabolites related to immune modulation (Resolvin E3), sphingolipid metabolism (Sphingofungin B), and anti-inflammatory activity (Kuwanon E). Functional assays indicated the TT genotype increased RORA expression and these metabolite levels. RORA overexpression in murine osteoblasts reduced β-catenin levels, alkaline phosphatase activity, and Axin2/TCF7 expression, suggesting RORA inhibits bone formation via the Wnt/β-catenin pathway. Cross-species analyses confirmed conservation of RORA and metabolite-associated genes across chickens, mice, and humans, with links to skeletal disorders. These findings identify key metabolic biomarkers and candidate genes for leg health assessment in broilers, and elucidate how RORA variants influence bone metabolism through Wnt/β-catenin signaling, offering a foundation for genetic improvement of leg disorders in poultry.

Keywords: leg health; metabolomics; biomarkers; RORA; Wnt/β-catenin pathway

4099 Effects of dietary 25-OH-D3 in normal and reduced calcium and phosphorus diets on performance and bone characteristics of broilers

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This study evaluated the effect of supplementing 25-hydroxycholecalciferol (25-OH-D3) in diets with reduced calcium (-0.16% Ca) and available phosphorus (-0.08% avP) on the performance and bone characteristics of broiler from 1 to 40 days of age. A total of 1,000 male Cobb 500 chicks were randomly allocated to four dietary treatments with 10 replicate pens of 25 birds each in a commercial poultry farm in southern Brazil. The dietary treatments were as follows: (1) Positive Control (PC), formulated to meet commercial nutrient requirements); (2) PC + 25-OH-D3 (PC25OHD3), supplemented with 25-OH-D3 at a dose of 69 µg/kg feed (equivalent to 2,760 IU of vitamin D3 activity); (3) Negative Control (NC), reduced Ca (-0.16%) and avP (-0.08%) compared with PC; and (4) NC + 25-OH-D3 (NC25OHD3), NC diet supplemented with 25-OH-D3 at the same dose as treatment 2. All diets contained 1,500 FYT/kg of phytase with a nutrient matrix equivalent to 0.19% Ca and avP, so that NC diets were reduced both by formulation and phytase contribution. Basal diets provided 5,160; 5,160; 4,300; 4,300; and 3,440 IU of vitamin D3 per kg of feed in the pre-starter, starter, grower 1, grower 2, and finisher phases, respectively. Feed intake (FI), body weight (BW), BW gain, and feed conversion ratio (FCR) were recorded weekly from 1 to 40 days of age. On day 40, tibia samples were collected from two birds per pen for the evaluation of tibia breaking strength, color, and ash content. Data were analyzed using one-way ANOVA, and means were compared using Tukey's test ($P \leq 0.05$), with additional contrasts performed between control and 25-OH-D3-supplemented diets. The FI did not differ among dietary treatments; however, final BW was 2.2% higher in the PC25OHD3 group compared with the NC. Broilers fed the PC25OHD3 diet had the best FCR (1.517), whereas the NC group had the poorest (1.557; $P \leq 0.05$). Supplementation with 25-OH-D3 improved tibia breaking strength and ash content, with tibia ash increasing by 1.1% in the NC25OHD3 group compared with the NC. Additionally, birds fed the PC25OHD3 diet showed lower muscle a* values (reduced redness: 0.30) compared with those on the PC diet (1.07: $P \leq 0.05$) indicating an improved bone density (reduced bone marrow pigment leakage). It was concluded that dietary PC25OHD3 improved growth performance, feed efficiency, and bone quality. These effects were particularly evident in diets with reduced Ca and P levels, indicating that PC25OHD3 supports optimal mineral metabolism. By enhancing Ca and P utilization, PC25OHD3 enables the formulation of more efficient and cost-optimized diets with lower mineral inclusion levels and lower environmental impact.

Keywords: 25-hydroxyvitamin D; broiler; feed efficiency ; mineral; bone ash

4100 Effects of photoperiod and protein content in feed on keel bone status and productivity in layer hens

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Keel bone fracture (KBF) is a major welfare problem in commercial layer hens with high prevalences (36-97%). KBF can reduce welfare due to pain and can cause economic losses due to lower feed efficiency and poor egg quality. Several factors may influence KBF occurrence, such as late ossification of keel bone, early onset of lay and high egg productivity.

The aim of the study was to investigate if different managements can reduce the risk of developing KBF. The KBF development and productivity were compared between layer hens with different treatments which can delay the onset of lay (short day and lower protein). The hypothesis was that delaying onset of lay could strengthen bone development thus reducing the prevalence of KBF.

This study has a 2×2 factorial design, with 2 main factors, photoperiod (short-day: 8h, normal-day: 14h) and protein content in feed from onset until post-peak of lay (low protein feed: 16%, regular feed: 17%, commercial feeds). In total 320 Lohman LSL lite hens were split into 4 groups and kept in enriched cages of 10 birds each. The number of eggs and egg weight were recorded at cage

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level daily. All hens were weighed and their keel bone was X-rayed biweekly. To evaluate egg traits (e.g. size, shell weight and thickness), eggs from 2 cages per group were collected biweekly. Egg shell strength was measured every 3 months on 30 eggs/group. The data was collected from first egg to end of production. The continuous data was analyzed by ANOVA and the status of KBF was analyzed by logistic regression.

The egg weight was lowest in short-day/low protein group from onset to peak of lay ($p<0.05$). Body weight and laying rate were lower from onset to peak of lay in short-day/low protein group than normal-day/low protein group, as well as short-day/regular feed group ($p<0.05$). Shell strength was lower during peak of lay and shell weight was lower from onset to post peak in short-day group than normal-day group within the hens fed low protein feed ($p<0.05$). There was no significant difference in the age of the onset of lay.

The prevalence of KBF was higher in short-day than normal-day groups in 39 and 41 weeks old (wo) ($p<0.05$), and tends to increase more rapidly in normal-day groups than short-day groups. The short-day groups showed more severe callus formation than normal-day groups in 39 and 41 wo ($p<0.05$).

Despite no significant delay in onset of lay, these results indicate that lower protein content in feed during peak of production does not affect productivity but have negative effects on KBF occurrence. Short photoperiod might be effective to slow the increase of KBF prevalence, but combination of short day and low protein feed negatively affected the productivity.
Keywords: keel bone fracture; productivity; photoperiod; protein content; welfare

4101 Outdoor access impact on bone traits in slow-growing broilers

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Free-range rearing is widely endorsed by consumers, who associate it with a higher level of animal welfare. To fully benefit from access to the outdoor area, birds must maintain a high level of bone quality. As terminal birds are raised with outdoor access but grandparent lines are selected indoors for sanitary reasons, it is important to determine to what extent the production environment can affect bone quality traits.
To address this question, we compared bone quality traits of 300 male and female slow-growing broilers used as sire of Label Rouge chickens reared either in confinement or with access to a grassy range from 4 to 12 wk. At 12 wk, tarsus and tibia length and diameter were measured, along with tibia weight, breaking strength, and mineralization. To estimate their correlations with bone quality traits, production traits (feed efficiency, body weight, feed intake) were measured individually with an electronic feed station from 2 to 12 wk, and breast yield and thigh yield were recorded at 12 wk.
A principal component analysis (PCA) was performed on all bone and production traits, using rearing environment and sex as illustrative variables. First 4 axes explained 81% of variance (Kaiser criterion), with eigenvalues 9.4, 2.3, 1.8, 1.1. The second axis accounted for 12.5% of the total variability, was mainly driven by bone mineralization parameters and discriminated between indoor and outdoor environments ($P<0.001$). Low PCA scores were associated with high ash percentage, high ash weight relative to body weight, low organic matter percentage, and a low organic-to-inorganic content ratio. These low scores were linked to outdoor-reared birds, reflecting their higher ash content and mineral density, together with lower proportions of organic matrix.
The first principal axis accounted for 52% of the total variance and primarily discriminated between sexes ($P<0.001$). Males were characterized by higher body weight, improved feed efficiency, greater thigh yield, reduced breast yield, and smaller but more mineralized and mechanically stronger tarsi and tibiae.
Correlations between bone and production traits indicated that heavier, more efficient birds with higher feed intake had smaller but heavier, denser, and stronger bones ($P<0.001$). Better mineralization was also associated with higher thigh yield ($P<0.001$). Correlations between bone quality traits and breast yield were weaker and opposite in direction to those with thigh yield ($P<0.01$).
Overall, these results highlight that the rearing environment and sex significantly influence bone quality and production traits, and that outdoor rearing tends to promote stronger and more mineralized bones without compromising growth performance.
Keywords: bone quality; free-range rearing; mineralization; broilers; production traits

4102 Multi-strain eBeam-inactivated Staphylococcus vaccine successfully reduced lameness due to Bacterial Chondronecrosis with Osteomyelitis in broiler chickens

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Bacterial Chondronecrosis with Osteomyelitis (BCO) is a significant issue affecting the commercial broiler industry, resulting in annual revenue losses of tens of millions of dollars. The disease is frequently associated with Staphylococcus spp. infections and leg and vertebral bone-necrosis. Neither have the etiology been understood, nor have effective, commercially available preventive measures been identified. Electron beam (eBeam) technology is renowned for producing efficient whole-cell vaccines by preventing bacterial multiplication through irreversible DNA shredding while preserving the integrity of membrane proteins (immunogenic epitopes). The aim was to reduce BCO lameness in broiler chickens via immunization using an eBeam-inactivated multi-strain Staphylococcus vaccine, expecting an effective immune response in chickens against Staphylococcus, thereby reducing lameness. A total of 1080 birds (240/treatment) were assigned to four in ovo vaccination groups: eBeam-inactivated, formalin-inactivated, combination of eBeam and formalin-inactivated, and sham (vehicle). Birds were placed at 60 birds/pen in 4 litter-floor pens/treatment, distributed randomly. Birds were directly exposed to aerosolized, natural BCO challenge until 56 days by placing 2 pens of sham-vaccinated birds on wire-floor, in front of the barn, against the aerosol exhaust. Daily cumulative lameness was assessed from day 22 until 56 by walking the birds. Resistant birds to walk/stand were identified as clinically lame, humanely euthanized, and necropsied to visualize the BCO lesions of the proximal femoral and tibial head bones. Blood and tracheal mucus were sampled on days 16, 32 and 56 from 3 birds/pen to compare plasma antibody profiles and leucocyte responses against Staphylococcus via ELISA and flow cytometry, respectively. On day 56, bacteria were isolated from the BCO lesions of 4 birds/treatment, for DNA extraction, amplification and sequencing for species identification. Two-way-ANOVA, followed by Tukey's post hoc tests were used

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to compare the treatment vs time effects of antibody profiles and leucocyte responses. A logistic regression model was used to compare the impact of treatments on lameness reduction. Statistical significance was considered at P<0.05. Birds vaccinated with only the eBeam-inactivated Staphylococcus vaccine showed a significant reduction (>50%) in daily cumulative lameness compared to other groups and an absence of Staphylococcus colonization in the leg joints of treated birds. eBeam and combination-vaccinated birds showed higher mucosal IgA levels, early in the time-course. Hence, the eBeam vaccine successfully reduced broiler BCO lameness by generating a targeted and robust immune response.
Keywords: osteomyelitis; lameness; vaccine; broilers; electron

4103 Tibia density increases at puberty and through laying and keel bone damage slowly accumulates from onset of lay
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Background: Bone quality and particularly keel bone damage have become an important focus of laying hen welfare. There is a widespread belief that bone quality declines as hens age which is frequently referred to as osteoporosis.

Aim: This study aimed to follow individual hens through a laying period to document the changes in tibia density and keel bone damage using live hen x-ray and bone density quantification.

Materials and methods: One-day-old Hy-line brown chicks (n=60) were reared in four pens. Hens were radiographed and weighed at 10, 14, 18, 20, 24, 28, 36, 48 and 60 weeks of age. Tibia density was assessed from the living hens x-ray images using a published protocol and a keel bone quality score was made from the same image. Estimates of cortical and medullary bone were made from the tibia in the image (see accompanying abstract).

Statistical analysis: Repeated measures ANOVA in Genstat.
Results: Tibia bone density increased or remained stable after puberty rather than declining. The differences were most significant between the values prior to lay (weeks 10-18) and those after (P<0.001). The values at 48 and 60 weeks of age were greater and significantly different from those at all earlier ages (P<0.001). Estimates of medullary bone suggest that it was responsible for most of the effect, being significantly different between pre- and post-puberty (P<0.001). In general, medullary bone estimates increased from 20 weeks of age to 60 weeks of age (P<0.01-P<0.001). Keel bone damage increased slowly as measured from the x-rays, not surpassing 3 for any bird out of the 5-maximum score, most damage rated a score of 1 with 20 birds in total being affected.

Conclusions: The results suggest, as have independent studies, that bone quality post-puberty requires reconsideration and the use of osteoporosis in hens may be inappropriate. Tibia density increases significantly at puberty, primarily due to medullary bone accretion, and remains consistently high through the laying period. Keel bone damage occurs gradually over time, with no sudden increase with the majority of birds having a low or no score. We hope this will ensure steps to ameliorate bone issues are correctly targeted.

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Keywords: Welfare; eggs; sternum; keel; tibia

4104 It's not magic, it's science. Dry grape extract supplementation positively influences bone health in pullets.
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In laying hens, maintaining optimal bone health and development from the early stages is crucial, as it directly affects their egg-laying efficiency, overall welfare and productivity. Dietary intervention with a standardized dry grape extract (NG), rich in antioxidant polyphenols, have already proven beneficial in poultry production for antioxidant protection and immune modulation, and studies have shown that grape polyphenols from Vitis vinifera enhance bone health in both humans and animals. Still, the mechanisms behind this effect remain unclear, given the diversity of grape polyphenols and their varying mechanisms of action. The objective of this study was to investigate the effect of NG on bone quality and metabolome of future laying hens reared in commercial conditions. Therefore, 36300 day-old pullets were randomly divided into two barns on the same farm site. Both received the same diet, with the addition of 30 mg/ kg of NG (Nor-Grape®, Nor-Feed, France) from day 1 until week 17 in the supplemented group (NG). At the end of the supplementation period, several bone quality parameters were analyzed on 50 individuals per group. Additionally, non-targeted metabolomics on plasma and bones were performed to uncover the impact of NG supplementation on pullet's metabolome. Results demonstrated that keel bone deformity tended to improve following NG supplementation (-14%, P = 0.10). Moreover, NG intake significantly increased bone dry and mineral content, compared to the control group (+1.1%, P = 0.007 and +0.9% respectively, P = 0.016) and tended to increase calcium (Ca) (+0.3%, P = 0.074) and phosphorus (P) content (+0.2%, P = 0.055). Furthermore, non-targeted metabolomics on plasma samples revealed an impact of NG supplementation on fatty acids and glycerophospholipid metabolisms while the same analysis on bone samples uncovered pathways related to sphingolipid and estrogen-mediated signaling pathways, underlining tissue-specific modulation. Overall, this study suggests novel mechanisms related not only to direct antioxidant effect, but also to modulation of estrogen signaling through lipid metabolism in NG supplemented pullets and highlights that nutritional intervention with NG in early developmental stage of future laying hens improves their bone health prior to entry into the laying phase, thus proving a useful tool in bone health management.
Keywords: Mineralisation; Grape extract; Polyphenols; Pullet; Metabolomics

4106 Enterococci and amyloid arthropathy: an in-vitro host-pathogen interaction model
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Amyloid arthropathy (AA) is a disease in laying hens characterised by the accumulation and deposition of serum amyloid A (SAA), mainly in joints. Since this secondary amyloidosis is usually observed in brown egg-laying hens (BL), a genetic predisposition to the disease may be inferred. We identified a dysfunctional point mutation in white egg-laying hens through whole genome sequencing, which causes an arginine-to-serine substitution at position 90 of the SAA protein (SAA.R90S), that may confer disease resistance. To further validate the functionality of the mutation, a reliable in-vitro model is warranted. AA is triggered by bacteria; in field cases, Enterococcus faecalis emerges as the most frequently isolated AA-inducing bacterium. However, current in-vitro models utilize lipopolysaccharide stimulation to mimic bacterial exposure. Given the limited understanding of the pathogen-specific

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mechanisms and host cellular responses that drive AA, it is critical to establish and validate a robust in-vitro model to enable mechanistic dissection of pathogenesis and systematic investigation of infection-induced cellular responses. For this purpose, chicken hepatocellular carcinoma cell lines (LMH) are co-cultured for 6, 24, and 48 hours with either single-strain Enterococcus isolates (inter alia E. faecalis), LPS or negative control. RT-qPCR quantified SAA induction at each time point are used to identify the optimal incubation time. Total RNA is extracted for RNA-seq to profile the host responses on the transcriptomic level. Differential expression and pathway analyses are performed to evaluate the effect of four different treatments: E. faecalis, other Enterococcus spp., LPS (positive control), as well as a negative control. The results provide high-resolution information about the suitability of LMH cells overexpressing SAA as an in-vitro infection model and lay the groundwork for an in-vitro approach to model host-pathogen interactions for the mechanistic dissection of AA. Thus, the approach could serve as a valuable tool for pathogen-induced amyloid diseases and may guide the development of preventive and treatment strategies.

Keywords: Avian amyloid arthropathy; Laying hens; Enterococcus spp.; In-vitro model; Transcriptomic analysis

Egg Science, Quality, and Processing II

4107 Modern Advances in Egg Science, Quality, and Processing

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This review explores transformative innovation trends in egg science that are setting new benchmarks for quality, safety, and animal welfare. The global egg industry is undergoing a seismic shift as it transitions from traditional, labor-intensive production models to a high-tech ecosystem defined by digitalization, molecular precision, and ethical transparency. Innovation at the intersection of food science and cutting-edge technology is redefining every stage of the egg's journey—from the poultry barn to the retail shelf.

The increasing industrial adoption of in-ovo sexing represents an "ethical revolution" in poultry science. Among other methods, low-field Magnetic Resonance Imaging (MRI) and hyperspectral imaging, leveraged with advanced AI, allow hatcheries to determine embryo gender with high accuracy by Day 12 of incubation. This advancement will effectively end the practice of male chick culling, and align commercial operations with rigorous animal welfare mandates now standard across Europe and expanding rapidly into North American markets.

Changes in the egg surface cuticle affect food safety with respect to the risk of contamination. Current advances in table egg cuticle coverage in-line evaluation focus on non-destructive, rapid, and automated techniques designed to measure the quality of the eggshell's protective organic layer during commercial grading. Recent developments prioritize optical technologies, specifically spectral reflectance and fluorescence, to provide real-time assessment of cuticle coverage, allowing for the segregation of eggs based on their resistance to bacterial penetration.

The limitations of manual candling can be overcome in modern processing lines, which identify microscopic shell fissures and internal defects at speeds exceeding 250,000 eggs per hour, using high-resolution optical imaging. Additionally, "Electronic Noses" can detect volatile organic compounds (VOCs), such as hydrogen sulfide and ammonia, at parts-per-billion levels, allowing for a chemical "freshness fingerprint" that exceeds the accuracy of traditional expiration dating.

Thus, the global egg industry is transitioning toward a more sustainable, transparent, and humane production model. These advancements ensure that future egg production aligns with both consumer demands and rigorous ethical guidelines.

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Keywords: in-ovo sexing, cuticle quality, crack-detection, volatile organic compounds

4108 Assessment of post-harvest losses of egg through supply chain in different cities of Bangladesh

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Post-harvest losses of egg have become an increasingly important issue in agricultural development and reduction of post-harvest egg loss is a critical component of ensuring future global food security. The aim of this study was designed to investigate assessment of quantitative and qualitative post-harvest losses of chicken egg along supply chain in some selected cities of Bangladesh. For this investigation, a survey was conducted with the pretested questionnaire in different egg producing areas of Mymensingh sadar and Dhaka Metropolitan areas. A total of 100 respondents (producer-25, middleman-25, wholesaler-25, retailer-25) were interviewed and the data was analysed with Delgado models. There was a specific age group (31-50 years) that constituted a majority of the producers and middlemen. Most producers and middlemen in Mymensingh were uneducated and most wholesalers and retailers were well educated at the secondary level. The majority of respondents possessed an experience of up to 10 years. The average number of losses of egg in producer level was more than 3% in Mymensingh city. The number of egg loss (%) and value loss (Tk.) in Mymensingh city were found highest during egg transportation followed by handling, washing, grading and packaging. In wholesaler level, the number of egg loss (%) and value losses (Tk.) in Kaptan bazar, Dhaka was found highest during egg marketing followed by transportation, handling and storing. At retailer level, the number of egg loss (%) and value losses (Tk.) in Mirpur bazar, Dhaka was found highest during egg handling followed by transportation, marketing and storing. Majority of the egg producer, middleman, wholesaler and retailer thought that broken egg, improper collection, improper handling, during storage, transportation, marketing, packaging and loading-unloading were the main causes of post-harvest losses of egg. The suggested possible solutions to reduce the losses of egg were proper overall preparation, proper collection, proper storing and proper transportation and marketing. The egg producer thought that higher price of feed, lack of quality feed, lack of high good quality layer strain, disease infestation, lack of capital, higher labor cost were the main problems and limitations of rearing layer hen. These problems and limitations should be taken into consideration by the proper authority to enhance the improvement of egg production sector and reduce post-harvest losses of egg in Bangladesh.

Keywords: Post-harvest loss; quantitative; qualitative; supply chain; Bangladesh

4109 Impact of the Implementation of a Novel Rotational-Spray Egg Washing System on Microbial Reduction and Egg Quality during Extended Refrigerated Storage

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Hypothesis: We hypothesized that implementing a novel rotation-

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al-spray egg washing system using 150 ppm free available chlorine would significantly reduce microbial loads on eggs without negatively affecting shell or internal quality during extended refrigerated storage.

Experimental Design: Eggs were stored for 0, 7, 14, or 21 days post-lay before being washed in the new rotational-spray system, which combines mechanical scrubbing with sanitizer application. Unwashed eggs served as controls. After washing, initial microbial load was tested. The remaining eggs were stored under refrigeration and evaluated for microbial and physical quality at 45 days post treatment.

Materials and Methods: Aerobic plate counts, yeast and mold, and Salmonella after enrichment were determined. Egg quality measurements included Haugh unit, albumen height, yolk and shell weights, shell thickness, yolk color, and shell and vitelline membrane strength. Analyses were performed using a texture analyzer and USDA egg-quality standards. Data were collected over three timepoints.

Statement of Statistical Analysis: A mixed-model ANOVA was used with treatment as a fixed effect and date as a random effect. Tukey's HSD was applied for mean separation at alpha = 0.05.

Results: Washing with the rotational-spray system reduced aerobic bacteria by more than 1.7 to 3.9 log CFU per gram and yeast and mold by more than 1.3 to 2.3 log CFU per gram, with counts below detection limits. Salmonella was not detected in any washed samples. After 45 days of refrigeration, microbial loads did not differ significantly among washed groups ($P > 0.05$). Haugh units (≥ 80) indicated maintenance of AA-grade quality. Shell thickness, albumen height, and vitelline membrane and shell strength were not negatively affected by washing or pre-wash storage duration.

Conclusions: The rotational-spray washing system using 150 ppm free available chlorine effectively improves microbial safety while maintaining shell and internal egg quality during extended refrigerated storage. This technology offers a promising option for commercial egg processors seeking to enhance hygiene and improve safety without compromising egg quality.

Keywords: egg washing, rotational-spray system, microbial reduction, egg quality, refrigerated storage
Keywords: egg washing; rotational-spray system; microbial reduction; egg quality; refrigerated storage

4110 Development and optimization of a TXRF protocol for elemental analysis of chicken eggs using reference materials and internal standards

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Total reflection X-ray Fluorescence spectroscopy (TXRF) is a well-known technique for tracing elements in food samples, requiring minimal sample preparation. This has been considered as a fast and cost-effective, verifiable approach to evaluate food authenticity, extensively used for both qualitative and quantitative elemental analysis of plant-based samples, oils and milk. However, studies on the elemental composition of chicken eggs using TXRF are limited and previous studies revealed the need for calibration in order to obtain reliable results. Therefore, the aim of this study is to develop and optimize a TXRF analysis method specifically for chicken eggs, including the development of sample preparation protocols tailored for egg analysis and calibration of the measurements against the reference method. Dried powders of egg white, egg yolk and homogenised whole egg were prepared in digested form in 70% nitric acid in a microwave oven and analysed using TXRF. The calibration of the TXRF was carried out by analysing five plant certified reference materials (CRM) to obtain relative sensitivity values. Subsequently, an egg powder CRM was used to verify the calibration values specifically for egg sample matrix. For all samples, two internal standards of vanadium (1 g/L V in nitric acid) and gallium (1 g/L Ga in nitric acid) were added to improve analytical accuracy and ensure quantification reliability. TXRF measurements were carried out in Bruker S4 T-STAR using both molybdenum (Mo-K) and tungsten (W-L) as excitation source. The measurement results were used to construct calibration curves using linear regression and to determine the calibration factors for various elements of egg samples. The TXRF recovery values based on the CRMs were 85.55±6.48% for Phosphor, 87.53±20.52% for Sulfur, 112.93±9.49% for Potassium, 98.39±6.62% for Calcium, 112.79±3.75% for Manganese, 86.99±3.05% for Iron, 89.23±7.03% for Copper, 109.67±2.07% for Zinc, 99.80±4.11% for Selenium and 115.95±4.01% for Strontium. All samples were additionally analysed using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES) as reference method. The calibrated TXRF results from acid digestion showed comparable values to those obtained by ICP-OES for Potassium, Iron, Copper, Zink, Selenium and Strontium concentrations. Manganese and Calcium measurements even demonstrated better performance with TXRF analysis compared to ICP-OES. The findings indicate that the TXRF protocol developed in this study is capable of analysing the main elements in egg samples in a verifiable, faster, simpler, cheaper and environmentally friendly manner. The method can be further used for elemental fingerprinting of eggs from different origins.

Keywords: Egg quality; X-ray Fluorescence analysis; rapid elemental quantification; Egg elemental composition

4111 An innovative intervention to remove and inactivate Salmonella Typhimurium cells in eggshells

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Eggs are often involved in foodborne outbreaks dealing with contamination because of the presence of Salmonella. The cell attachment to the unique porous surface of eggshells and the viability of this pathogen in the hatchery represents a constant food safety risk. In some countries, eggs are washed and sanitized before they reach the consumer, using chlorine solutions with the concern of carcinogenic compounds. The search for green alternatives in sanitizers has taken scientists to explore other natural options. This research studied the use of a novel intervention known as ultrasound alone and in combination with caffeine as a natural sanitizer to remove and inactivate S. Typhimurium from eggshells. The study was conducted with large white eggs (58 g ± 1) inoculated in the surface with S. Typhimurium 53647 cells (103 cfu/egg) and allowed dry overnight for cell attachment. An ultrasonic processor (Fisherbrand, 700 W, 20 kHz) was used together with a double-wall reactor (1000 ml), using water as the sonication medium. The temperature of the experiment was set at 40°C, and the constant sonication times were 3- and 5-min. Additional experiments were conducted using sonication plus 1.5% of caffeine in the water and the same temperature and processing times. Temperature was kept constant and recorded during processing. Egg quality was tested after processing, evaluating weight, Haugh unit, yolk index, thickness and strength of the eggshell (digital egg tester Nabel, DET 6500), yolk color, pH of yolk and albumen, and albumen absorbance. A one-way ANOVA ($\alpha = 0.05$) was conducted to find

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significant differences. Results showed that ultrasound at the longest processing time (25.52 J/ml, 5 min) reduced 2 log of microbial cells in the eggshell but affected significantly the albumen absorbance ($p \leq 0.05$). However, shorter processing time (14.92 J/ml, 3 min) together with caffeine fully decontaminate the eggshell (3 log reduction), no viable cells were detected and did not affect the egg quality preserving all the tested characteristics ($p > 0.05$). It was observed that there was a synergistic effect between ultrasound and caffeine against Salmonella during microbial inactivation in eggshells. The cavitation from ultrasound was able to remove and start the disruption of the microbial cells from the eggshell, and the caffeine had antimicrobial effect, leading to cellular death. Further studies are required to scale up and validate this novel approach to ensure energy during cavitation is delivered homogeneously on a bigger scale. This synergy represents an affordable and green option from small to big egg farms to ensure the food safety of the product before it reaches the consumer.

Keywords: Salmonella; eggshell; egg quality; egg processing; natural antimicrobial

4112 Impact of various storage conditions on quality changes in table eggs

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Eggs are an excellent source of nutrients in the human diet. Naturally protected by their shells, they represent a self-contained food product whose quality encompasses all characteristics that consumers consider acceptable or unacceptable. The foundation of high egg quality lies in the health of the laying hens, and the application of appropriate storage conditions. The maximum shelf life of eggs varies depending on national regulations; in the European Union and the Republic of Serbia, the prescribed shelf life for commercial chicken eggs is 28 days when stored at above 5 °C.

The aim of this study was to evaluate the effects of different temperatures (5 °C, 15 °C, and 25 °C) on the microbiological status and on both external and internal quality parameters of medium weight category table eggs obtained from 32-week-old Isa Brown hens. A total of 570 M-class eggs were randomly divided into 19 groups and analyzed on the day of production and after 7, 14, 21, 28, 35, and 42 days of storage. Data were analyzed by ANOVA with Duncan's test using R software (v. 3.2.2); $p < 0.05$ was considered significant.

Microbiological analysis of egg contents (yolk and albumen) revealed that Salmonella spp. and Listeria monocytogenes were not detected, while total microorganism, Enterobacteriaceae, Escherichia coli, coagulase-positive staphylococci, yeasts and molds were all below the quantification limit (<10 CFU/g) during 42 days of storage at 5 °C, 15 °C, and 25 °C.

No changes ($p > 0.05$) were observed in egg weight, shell colour, shell cleanliness, egg shape, shell strength, shell thickness, or shell weight during storage of table eggs at 5 °C, 15 °C, and 25 °C for 42 days. On contrary, significant differences ($p < 0.05$) were observed on internal quality, including yolk height (YH), yolk width (YW), albumen height (AH), and Haugh unit (HU). The greatest changes occurred in eggs stored at 25 °C, where YH decreased from the initial 19.64±0.98 mm to 12.74±0.89 mm, YW increased from 37.97±1.02 mm to 44.93±1.72 mm, AH decreased from 8.95±1.22 mm to 1.74±0.46 mm, and the HU decreased from 94.34±6.70 to 26.03±10.36. Slightly smaller changes were recorded in eggs stored at 15 °C, where, at the end of the storage period, YH measured 17.12±0.71 mm, YW 41.47±1.26 mm, AH 5.16±0.54 mm, and HU 70.92±4.66.

After 28 days, eggs at 25 °C showed altered quality (YH 14.03±0.69 mm, YW 37.63±10.14 mm, AH of 2.46±0.72 mm, HU of 39.51±11.97), while at 15 °C and 5 °C only AH and HU changed slightly (15 °C: AH 6.31±0.64 mm, HU 79.47±4.65; 5 °C: AH 7.09±0.76, HU 84.28±4.49).

Although changes were also observed in eggs stored at 5 °C, they were less pronounced, indicating that eggs stored at 5 °C retain their initial quality for longer period.

Keywords: egg quality; storage temperature; microbiological safety; internal quality degradation; shelf life

4113 Yolk pigmentation in laying hens is affected by season, genotype and age: analysis of a large commercial dataset

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The UK market is well adapted to the needs of brown-feathered laying hens, and feed pigment levels have been developed to suit the brown layer. However, recent changes have led to the introduction of white birds into the UK system, which now make up approximately 15% of the national flock. In order to investigate the impact of hen genotype on yolk pigmentation, a large dataset (n=17876) was compiled from egg yolk pigmentation readings taken by multiple commercial UK egg packers. As birds were fed from different feed mills and over an extended period, formulations varied (though all were based on wheat/barley/suns/soybean with minimal or no maize addition). Pigment supplementation was provided entirely through the premix (supplying supplied 1500 mg/kg saponified paprika extract or 22.5 mg/kg canthaxanthin 10%, and 250 mg/kg lutein rich extract) and dictated by egg brand, rather than genotype. 470 commercial flocks were assessed over 80 weeks using the Roche colour scale. All readings were taken by trained QC staff. For each reading, 5 individual eggs were scored and averaged. At each weekly reading, supplemental data about each flock was collected including hen colour, age and target yolk score. Roche scores were analysed with JMP 19. Differences were considered significant at $P<0.05$; $P<0.1$ was considered a near-significant trend.

There was a clear effect of season on yolk score. There was a seasonal decrease in yolk pigmentation, with the lowest scores recorded from May to September. Decreases in yolk score coincided with spring and summer, and the onset of higher temperatures. Distribution of genotype in the dataset reflected wider commercial realities in the UK (Brown n=14884, White n=2992). Across the dataset, there was a highly significant difference in egg pigmentation between white and brown birds (Brown=11.47, White=10.52, $P<0.0001$), as well as a difference in target achievement (Brown=115.90%, White=114.52%, $P=0.0069$). Across the dataset, yolk score increased with bird age ($P<0.0001$); data suggests that the effect of age differs between white and brown birds, with white birds pigmenting less well with age.

While these differences are minimal, they are consistent and may have implications for the commercial nutrition of laying hens. There is no data to suggest differences in pigmentation deposition ability between white and brown birds, though white birds are known to consume less feed – and thereby less pigment – and produce more egg mass than brown birds. This, combined with the impact of a declining yolk score as white birds ages, may have

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substantial implications for pigment requirements in aging white flocks and require a new, dual-season approach to pigment levels in commercial feeds.
Keywords: Yolk score; Laying hens; Pigmentation

4114 Increasing bioactive properties and maintaining high quality of egg yolk through enzymatic treatment and exposure to ultrasound
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The aim of this research was to develop new products resulting from enzymatic modification of egg yolk through limited proteins proteolysis, exhibiting bioactive properties i.e. antioxidant, antihypertensive and antidiabetic, and maintaining or improving egg yolk's emulsification, cake-forming, and gelling properties. The goal was to achieve high-quality yolk by lowering the pasteurization temperature (TP) by 2°C in combination with ultrasonic treatment (UST) without compromising microbiological standards.

The raw material for small-scale (1000 kg) or industrial-scale (4000 kg) studies was liquid egg yolk (caged and free-range eggs) treated with the enzymes endoprotease Protamex® (YE) or transglutaminase (YTGA) and/or phospholipase 2 (YF). The properties of the enzyme-treated yolks were compared with those of a control yolk without enzymatic treatment (YC). The yolks were then sonicated (20 kHz) for 20 minutes or not, pasteurized at 66 or 68°C, and stored refrigerated for 42 days.

The following parameters were analyzed: chemical composition, fatty acid and volatile compounds profile (GC/MS), oxidation -amount of thiobarbituric acid reducing substances (TBARS) and radical ABTS, inhibition of angiotensin-converting enzyme (ACE) and glucosidase inhibition (AD) as an indicator of antidiabetic properties; technological properties: emulsion stability (SE), sponge cake volume (V), gel strength, and total microbial, fungal, and pathogen counts. ANOVA was used to analyze the results.

The SE prepared with YF was, as expected, higher than that of the control and other enzymatically modified egg yolks (94% YF vs. 81-84%). Lowering TP to 66°C and UST increased the SE and V cake. The gel strength for YTGA was higher (12.3 N) than for other egg yolks (10.2 N). TBARS of the yolks after ultrasonic treatment decreased (from 17 to 9 mg/kg), as well as oxidized volatile compounds. The amount of free amino acids increased only by 2-3% due to limited proteolysis of yolk proteins, but the biological activity (ABTS, ACE, AD) increased at least twice. The results of the industrial experiment indicate that enzymatic and UST treatment reduced the microbiological contamination of stored egg yolks by at least 1 log cycle.

Conclusions: Enzymatic modification of the yolk, including limited proteolysis of proteins, and ultrasound improve the product's bioactivity, technological properties, and microbiological standards, even at lower pasteurization temperatures.

This research was co-financed by the National Centre for Research and Development (Poland) under the European Funds program - project number POIR.01.01.01-00-1459/19.
Keywords: yolk; proteolysis; bioactivity; emulsification; ultrasound

4115 Effect of sampling time on helminth egg shedding patterns in naturally infected Nigerian local chickens
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Hypothesis / Objective:
This study evaluated whether sampling time influences helminth egg shedding (expressed as eggs per gram, EPG) in naturally infected local chickens.
Experimental Design:
A longitudinal observational study was conducted to assess variation in helminth egg output across different times within a 24-hour cycle over six days.
Materials and Methods:
The study was conducted over six consecutive days between September 29 and October 4, 2023 at the Poultry Pen, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Forty apparently healthy, naturally infected Nigerian local chickens (aged 7–8 months and mean body weight 703.21±162.96) were enrolled. Birds were maintained under intensive management conditions, characterized by individual confinement in battery cages (one bird per cage), provision of a controlled diet, ad libitum access to water, routine sanitation, and prevention of scavenging. Faecal samples were collected from each bird at six-hour intervals (6:00 am, 12:00 pm, 6:00 pm, and 12:00 am), generating repeated measurements per individual. Helminth egg shedding was quantified as eggs per gram (EPG) using the McMaster technique, with all eggs observed within the counting chambers evaluated and recorded (922 eggs). All experimental procedures were approved by the university animal research ethics commitee(UDUS/IACUC/2023/R06)
Statistical Analysis:
Data were analysed using a zero-inflated negative binomial generalized linear mixed model to accommodate over-dispersed count data with an excess of zero observations. Sampling time was specified as a fixed effect, while individual birds were included as random effects to account for the repeated-measures structure of the data.
Results:
Sampling time significantly affected helminth egg shedding ($p = 0.002$). The highest EPG values occurred at 12:00 pm, while the lowest were recorded at 6:00 am, indicating a distinct diurnal pattern.
Conclusions:
Helminth egg shedding in local chickens varies markedly with sampling time, with midday sampling offering the highest diagnostic sensitivity. Optimal sampling periods should therefore be considered when interpreting faecal egg counts.
Keywords: sampling time; helminth eggs; EPG variation; local chickens; McMaster technique

General Health/Disease/One Health VI

4116 Anti-parasitic activity and dosage response of VERMI (DOSTOFARM) on A. galli, H. gallinarum and H. meleagridis in vitro
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Parasitic infections in poultry are a major concern for long-live birds, including turkeys, laying hens and broiler breeders. These infections can cause high mortality, such as the case with Histomoniasis (blackhead disease), caused by Histomonas meleagridis and maintained in the environment through its intermediate host, Heterakis gallinarum. Ascaridia galli, though not deadly, harbors pathogens of concern such as Salmonella and can reduce efficien-

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cy and production. The objective of this study was to determine whether a plant-derived phytogenic extract (Vermi) inhibits H. meleagridis and reduces the viability of H. gallinarum and Ascaridia galli eggs in vitro. Two independent assays were performed. For the Histomonas culture assay, 100,000 H. meleagridis were inoculated into 9 mL of medium containing 0 (negative control; NC), dimetridazole at 12.5 ppm (positive control; PC), or Vermi at 125, 250, 500, 1,000, 2,000, 4,000, 8,000, and 16,000 ppm, and incubated at 42°C for 48 h. Parasite survival was quantified by hemocytometer counts, and bacterial growth was estimated via OD600. For the nematode assay, ~50 A. galli or H. gallinarum eggs were isolated from gravid female worms and exposed to the same concentration range for 1–24 h in 24-well plates (6 replicates/treatment). Embryonation and hatchability were evaluated microscopically, and eggshell integrity was determined by trypan blue exclusion. Data were analyzed using one-way ANOVA with Dunnett's test ($P \leq 0.05$). Vermi exhibited dose- and time-dependent inhibition of both A. galli and H. gallinarum egg development. Embryonation and hatchability of A. galli eggs declined significantly within 8–24 h at concentrations ≥0.1%, with complete inhibition and eggshell disruption at 0.8–1.6% ($P < 0.0001$). Similarly, H. gallinarum embryonation and hatchability were suppressed after 8 h exposure to 0.1–0.8%, with total loss of viability at 1.6%. Trypan blue uptake increased with concentration and exposure duration, showing progressive compromise of eggshell integrity. For H. meleagridis, Vermi at 8,000 and 16,000 ppm eliminated all viable protozoa similar to the PC, while 1,000–4,000 ppm reduced parasite counts by approximately 95% ($P < 0.0001$). Bacterial levels were unaffected except for elevated OD600 at 2,000–8,000 ppm ($P < 0.0001$). These findings indicate that Vermi exerts broad anti-parasitic activity by inhibiting H. meleagridis and reducing nematode egg viability in vitro. Further research is warranted to characterize lethal concentrations, optimize formulation, and evaluate efficacy in vivo as a non-antibiotic strategy for histomoniasis control.

Keywords: Histomonas meleagridis; Roundworms; Blackhead; Dewormer

4117 Broiler chicken coccidiosis management: The efficacy of a botanical formulation standardized in saponins, validated under commercial production conditions

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Botanicals solutions are gaining interest in managing coccidiosis. However, for considerable part of theme there is a lack in scientific evidence. To build credibility and reassure industry stakeholders, it is essential to conduct studies demonstrating their efficacy and safety in the management of this issue.

Several studies have demonstrated that standardized saponin-rich plants are efficient in reducing coccidial risk and improving resilience of infected broilers. However, most investigation had been conducted in vitro or experimental conditions while really few data from large commercial-scale settings are available. The present study aims to reevaluate the effectiveness of a standardized saponin-rich feed additive (SP, Norponin® XO2, Nor-Feed) under commercial rearing conditions.

A total of 573668 broiler (Ross 308) from 22 buildings located in Centre-Val de Loire regions (France) implemented from 22nd November to 27th December 2024 were included. 9 buildings (TC, n= 265284) had the basal diet supplemented with the conventional anticoccidial program (monensin 40 ppm + nicarbazin 40 ppm); while 13 buildings (SP, n= 308384) received the same basal diet supplemented with the saponin-rich additive at 300 g per ton of complete feed. 5 birds per building in both groups were randomly selected around 20 days of age to score cocci-related intestinal lesion (ILS). Growth performances and coccidiosis occurrence were also recorded. Fisher's test was used to analyze differences in coccidial occurrence, while Student's t-test (parametric) or the Mann–Whitney test (non-parametric) was applied to compare overall ILS and performance parameters between groups.

No difference was observed in coccoidal occurrence($P>0.999$) and global ILS ($P=0.43$) between groups. The average slaughter age was 33 days in TC Day and 34 days in SP buildings. Consequently, SP birds were slightly heavier (1,800 g) than TC birds (1,757 g), although not significantly ($P = 0.317$). Feed conversion ratio was comparable between 2 groups (1.54 vs. 1.56 in TC and SP group respectively, $P=0.186$). Mortality was recorded at 3.3% (TC) and 3.7% (SP) with no significant difference.

These results evidenced that SP supplementation was as efficient as monensin-nicarbazin combination. Indeed, coccidiosis occurrence and growth performance were comparable between the two treatments. These findings confirm previous results. They evidence the fact that a scientifically formulated, standardized botanical solution can be a valuable additional tool in managing coccidiosis. Thus, offering agility in building coccidiosis management programs for broiler chicken producers.

Keywords: Coccidiosis; chicken; Eimeria; botanicals; saponins

4118 Comparative Evaluation of Different Anticoccidial program and β-Mannanase Supplementation on Broiler Performance and Gut Health in Thailand

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Coccidiosis imposes major economic losses to the poultry industry through intestinal damage, impaired nutrient absorption, and reduced growth performance. Although coccidiostats are widely used to control Eimeria infections, comparative data on how different programs perform under severe challenge conditions remain limited. This study evaluated two shuttle coccidiostat programs in broilers exposed to a severe coccidiosis vaccine challenge and assessed whether Hemicell (β-Mannanase enzyme) supplementation could further enhance performance. A total of 540 day-old male Ross 308 broilers were assigned to 5 dietary treatments with 6 replicate pens of 18 birds each in a completely randomized design. Treatments included an unchallenged control (UC), a challenged control (CC), two shuttle programs T1: Narasin–Nicarbazin (D1-21) to Salinomycin (D22-35), T2: Monensin–Nicarbazin (D1-21) to Salinomycin (D22-35) and T3: Narasin–Nicarbazin (D1-21) to Salinomycin (D22-35) with β-Mannanase (D1-35). All groups except UC were challenged with 10X commercial live coccidiosis vaccine on days 1 and 7. Weekly BW and FI, daily mortality, final FCR and production index (PI) at day 35 were collected, with intestinal integrity evaluated at day 32 using the Elanco Health Tracking System (HTSi). Statistical analysis was performed using one-way ANOVA with Tukey's test. The CC group showed the lowest BW and FI, along with the poorest FCR and PI at Day 35, confirming successful challenge establishment. T1 and T3 significantly improved BW, FI, and FCR relative to CC ($P<0.05$), whereas T2 improved BW and PI but did not differ from CC in FI or FCR. T1 consistently outperformed T2 in growth and feed efficiency parameters. T3 achieved the highest BW and PI, and the lowest adjusted FCR among challenged groups. Interestingly, T3 achieved a significantly lower FCR than even the UC group ($P<0.05$). At day 35, PI values showed a numerical ranking of T3 (455.4) > T1 (452.6) > T2 (443.4). HTSi results at day 32 indicated superior

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intestinal integrity in T3, with lower E. maxima and thick-intestine lesion scores compared with all other challenged groups. These results confirm successful establishment of the coccidial challenge model, with CC performing worst across all parameters. Both anticoccidial shuttle programs effectively mitigated performance losses, with the Narasin–based program (T1) showed stronger recovery than the Monensin-based program (T2), while β-mannanase supplementation (T3) further improved performance and gut health, producing the most favorable overall response under severe coccidiosis pressure.

Keywords: Anticoccidial, Broiler performance, β-Mannanase, HTSi, Intestinal Integrity
Keywords: Anticoccidial; Broiler performance; β-Mannanase; HTSi; Intestinal Integrity

4119 In ovo LPS exposure decreases γδ T cells and induces the expression of IFN-γ and IL-1β genes in the developing chicken spleen

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The chicken embryo is a suitable model to study the early immune development and the host–pathogen interactions in avian species. However, the functional immune responses that occur during embryogenesis are not well characterized. Hence, this study investigated the impact of bacterial lipopolysaccharide (LPS) on the composition of immune cells in the spleen and the associated molecular responses.

For this purpose, Escherichia coli LPS (O111:B4; 32 µg per egg) was administered to 30 specific pathogen-free layer embryos on the embryonic day (ED) 15 via the allantoic route, while 30 control embryos received PBS. Following additional three days of incubation, the spleens were collected and mononuclear cells were isolated individually from each spleen to identify phenotypic changes of immune cells by flow cytometry. A panel of antibodies was used to identify the following cell subsets: CD45+ leukocytes; CD4+ and CD8+ T cells; γδ T cells; Bu-1+ B cells; and KUL01+ macrophages/monocytes. In addition, RT-qPCR was used to investigate the expression of the cytokine genes IFN-γ, IL-1β, IL-6, and IL-10, as well as the Toll-like receptor (TLR) genes TLR1A, TLR2B, TLR2A, TLR3, TLR4, TLR5, TLR7 and TLR21.

Flow cytometric analysis revealed that LPS exposure significantly reduced the proportion of γδ T cells compared to the control group, while the number of other cell subsets remained similar between the two groups. Furthermore, RT-qPCR analysis showed that IFN-γ and IL-1β were upregulated, while TLR1A, TLR2B, and TLR4 were downregulated in the LPS-treated group compared with the control group.

The reduction in γδ T cells indicates early cellular alterations in the spleen that potentially reflect migration to peripheral tissues. The upregulation of IFN-γ and IL-1β observed demonstrates the activation of pro-inflammatory and innate immune pathways in response to LPS exposure. Notably, the downregulation of TLR1A, TLR2B, and TLR4 indicate a feedback regulation following receptor activation - a mechanism that can limit excessive inflammatory signalling. Overall, the results suggest that the embryonic immune system can mount specific defence mechanisms against LPS. Based on the present work, further studies can be conducted to investigate embryonic immunocompetence, in ovo vaccination, and early host–pathogen interactions.

Keywords: chicken; flow cytometry; in ovo; spleen; immune response

4120 Fatty liver syndrome challenge induces persistent nutrient utilization variability and jejunal transcriptomic shifts in late-laying ISA Brown hens

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Liver health is central to egg yolk formation and lay persistency in laying hens, and disturbances such as fatty liver syndrome (FLS) may have long-lasting consequences beyond the liver. This study aimed to determine whether a prior dietary FLS challenge induces persistent changes in nutrient utilization and intestinal transcriptomics after hens return to a common control diet. Forty-eight ISA Brown hens (73 wk of age) that had been fed either a control diet (CON; commercial-type diet) or an FLS challenge diet from 58 to 73 wk were switched to a commercial control diet until 85 wk. The high-energy FLS challenge diet, characterized by elevated starch and reduced oil and protein, successfully increased liver dry matter by 2.6% and liver fat by 9.3% at 73 wk, validating the model. Two groups (CON, FLS; n = 24 each) were then compared for body weight, liver composition, nutrient digestibility, and jejunal transcriptomics. At 85 wk, liver traits (weight, dry matter, and fat), apparent ileal digestibility (AID) of dry matter, crude protein, and ether extract, apparent metabolizable energy (AME), and coefficients of total tract apparent retention (CTTAR) of dry matter, Ca, and P were measured. Data were analyzed using mixed models (GLIMMIX, SAS 9.4), and variance heterogeneity was tested by Levene's test (GLM, SAS 9.4). RNA-seq was performed, and transcriptomic data were processed with the SAVVY™ platform. During the 12 wk on a common control diet, egg production parameters did not differ between groups ($P > 0.05$), with average lay percentage and egg mass of 95.0% ± 9.94 and 61.1 g ± 6.84, respectively. However, body weight gain was lower in FLS hens compared with CON hens ($P < 0.001$). No differences were observed in liver traits, AID, AME, or CTTAR ($P > 0.05$). FLS hens exhibited greater variance in Ca digestibility ($P = 0.045$) and tended toward more uniform ether extract digestibility ($P = 0.068$). Transcriptomic analysis revealed downregulation of the PPAR-α signaling pathway (P-adj = 0.021), along with immune defense and cellular senescence pathways (P-adj = 0.004), both linked to gut integrity. In contrast, the most strongly upregulated pathways were deubiquitination (P-adj = 0.030), associated with jejunal immune function, and WNT signaling (P-adj = 0.003), associated with gut integrity. In conclusion, although liver morphology normalized after 12 wk, a prior FLS challenge induced subtle instability in Ca utilization and persistent transcriptomic stress signatures in the intestine. These findings highlight the lasting systemic impact of liver health on Ca metabolism and gut resilience in late-laying hens.

Keywords: Liver health; Fatty liver syndrome; Gut-liver axis; Calcium utilization

4121 Genome editing of gliotoxin genes in Aspergillus fumigatus reduces aspergillosis in broilers

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Gliotoxin of Aspergillus fumigatus has been extensively studied for its role in pathogenesis in animals and humans. It triggers pathogenesis by its immunosuppressive and cytotoxic effects. Biosynthetic gene cluster consisting of 13 genes regulates its biosynthesis. We targeted gliZ, gliP and gliA genes of this cluster using CRISPR/Cas9 system in a multigene editing approach and

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checked the pathogenesis in broilers. crRNAs were designed using EuPaGDT and 3 single guide RNAs (sgRNA) were commercially synthesized. Each sgRNA was combined with Cas9 to form ribonucleoprotein complexes which were then used for simultaneously transfecting fungal protoplasts. Thin-layer chromatography showed the absence of gliotoxin on silica plate. DNA sequencing showed various indels in target genes post-transfection: a double missense mutation in gliZ, a synonymous mutation in gliP, and a doule nucleotide substitution in gliA. Mutations is gliZ resulted in substitution of Cysteine to Tryptophan and Serine to Proline at positions 57 and 58, respectively. Mutation in gliP resulted in no change of amino acid at position 90 because both the codons (AGC and TCG) code for Serine and, thus, it was likely not functionally relevant. Mutation in gliA resulted in substitution of Leucine to Histidine at position 114. Regenerated protoplasts were matured to form fungal hyphae and spore production was induced. These spores were inoculated intra-air sac in day-old broiler chicks (n=10) in experimantal group. Birds in positive control group (n=10) were inoculated with the wild-type A. fumigatus spores. The third group of birds (n=10) served as negarive control. During one-week infection trial, birds inoculated with RNP-treated spores showed mild clinical signs, while birds infected with wild-type spores showed morbidity (100%) and mortality (30%). Histopathological analysis of lungs showed necrosis and congestion, and presence of mixed population of inflammatory cells in wild-type infected birds, while no such lesions were seen in birds infected with RNP-treated spores and in control group. These results suggest that the multigene editing approach was able to introduce indels simultaneously in three gliotoxin-related genes, leading to amino acid substitutions that may have affected gliotoxin biosynthesis and export. In vivo experiments indicated that RNP-treated fungal spores showed a reduced ability to induce A. fumigatus pathogenicity in broilers. Collectively, these findings indicate that targeting gliotoxin biosynthesis may represent a potential strategy for the development of antifungal therapies.

Keywords: CRISPR; Aspergillus; Gliotoxin; Pathogenicity; Histopathology

4122 Development of an indigenous strain based efficacious attenuated live ARV vaccine and its potency testing for tenosynovitis and osteoarthritis inducing ARV in poultry.

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This study developed a live attenuated vaccine against circulating Avian Reovirus (ARV) in India. Field strains were isolated from synovial tissues, with ARV confirmed through RNA extraction, PCR targeting L1/S2 segments, and virus isolation in Vero cells showing cytopathic effects. Genetic characterization identified Genotype I as the predominant lineage.

The in vivo design comprised six poultry groups: untreated control, wild-type challenged, commercial vaccine, live vaccine, prime-boost (live+killed), and miRNA-adjuvanted prime-boost. Post-challenge analysis assessed pathology, immunity, and viral load using Kruskal-Wallis/Dunn's tests for lesion scores, ANOVA/Tukey's HSD for antibody/viral data, and log-rank tests for survival.

Vaccine development was achieved after 40 serial passages of DVB04 strain for attenuation and evaluated conventional and innovative miRNA-enhanced vaccination strategies.

The research successfully confirmed the presence of Avian Reovirus (ARV) in clinical samples from birds exhibiting characteristic signs of joint pathogenesis, including tendon swelling and hemorrhages. The virus was then successfully isolated and propagated in Vero cells. This identified Genotype I as the primary ARV lineage circulating in India, making it a suitable candidate for vaccine development. To create a vaccine candidate, the wild-type ARV strain (DVB04) is being attenuated through serial passaging in Vero cells for 40 generations. The study successfully identified differentially expressed microRNAs (miRNAs). 479 known miRNAs were identified, 24 were upregulated and 18 were downregulated. Two miRNAs were selected for functional validation gga-miR-146a-3p (log2FC = 3.375) and gga-miR7474-5p (log2FC = 5.794). The experimental validation of the identified target genes—PAWR, MAP2 and PDCD7 was performed using quantitative reverse transcription PCR (qRT-PCR). Transfection of miRNAs and anti-miRNAs. The increase in ARV gene copy number following anti-miR-3 transfection suggests that this miRNA typically functions to repress viral replication; thus, its inhibition facilitates an increase in viral load Experimental design for the final efficacy trial has been established, comprising six groups to test the live attenuated vaccine candidate, a prime-boost strategy (live followed by killed vaccine). In summary, the project has successfully isolated and genotyped circulating ARV strains and development a live attenuated vaccine candidate and enhancing vaccine efficacy using a novel miRNA-based adjuvant system. The stage is set to be pre-clinical testing of the developed vaccine candidate as per Indian Pharmacopoeia.

Keywords: Avian Orthoreovirus ; Live attenuated vaccine; potency testing ; field isolate

4123 Evaluation of the systemic/mucosal and passive humoral immune response initiated by next-generation inactivated Salmonella vaccine complexes.

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This study evaluated the systemic and mucosal humoral responses to prime and boost vaccination, as well as the IgY passive transfer of two next-generation inactivated Salmonella Enteritidis (SE) vaccine complexes (mannosylated chitosan (MCA)-based or agonistic CD40 aptamer-based (CD40-AP)) in Light-brown Leghorn (LBL) pullets. Twelve-week-old LBL pullets were randomly placed into floor pens. Pens were randomly assigned to four treatments: g1, PBS subcutaneous (s.c).; g2, MCA oral (MCA o.); g3, MCA s.c.; g4, CD40-AP s.c. At 14 weeks of age, pullets were primed with either PBS s.c., MCA o., MCA s.c., or CD40-AP s.c. (n = 6 per group). At 18 weeks of age, booster doses were administered. Blood and cloacal swabs were collected before (0d) and at 3, 5, 7, 10, 14, 21, and 28 days after prime and boost vaccination to quantify the relative levels of SE-specific plasma IgM, IgY, IgA, and mucosal IgA using ELISAs. Eighteen weeks after boost vaccination (34 weeks of age), eggs were collected consecutively for ten days to assess passive immunity by measuring yolk IgY levels using ELISA. A two-way ANOVA was used to determine the effects of treatment, time, and their interaction for blood and cloacal swab data. A one-way ANOVA was used to determine the effects of treatment on yolk sample outcomes. Tukey's HSD multiple means comparison tests were conducted as needed. Significance was set at $P \leq 0.05$. The MCA s.c. vaccine elicited higher plasma IgM levels after prime, and plasma IgM and IgY levels after boost ($P \leq 0.05$), compared to all other groups. Moreover, it triggered higher IgM, IgY, and IgA plasma levels during recall compared to those in the prime response, alongside increased IgA mucosal levels ($P \leq 0.05$). The CD40-AP s.c. vaccine produced higher levels of mucosal IgA compared to all other treatments after boost, and increased plasma

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IgY, IgA, and mucosal IgA levels during recall compared to those found in the prime response ($P \leq 0.05$). The MCA o. vaccine did not trigger significant changes across time in IgM and IgY levels, but it triggered higher mucosal IgA levels during recall compared to the prime response ($P \leq 0.05$). These observations suggest that MCA s.c. vaccine preferentially stimulates a robust systemic humoral response, whereas CD40-AP administration preferentially stimulates a mucosal IgA response. In summary, the humoral immune responses triggered by the MCA o., MCA s.c., and CD40-AP s.c. show the potential of next-generation Salmonella vaccine platforms for targeted activation of humoral immune responses. Future studies should characterize the local and systemic leukocyte dynamics, cytokine responses, and functional protection following Salmonella challenge.

Keywords: Salmonella; Vaccine; Immune response; Aptamer; Mannosylated chitosan

4124 Biosecurity as a potential tool to improve poultry farm production and profitability and reduce antibiotic usage and resistance in Cameroon

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In Cameroon, poultry plays an important role in terms of human nutrition. Due to the lack of strict legislation, antimicrobials are extensively used to treat diseases, the main hindrance of the sector. Among the solutions to minimize the risk of disease, strengthening biosecurity is the key strategy. This study aimed to evaluate the relationship between biosecurity implementation with production performance and antibiotic usage and resistance in poultry farms in Cameroon. A total of 185 poultry farms were visited and data concerning biosecurity, production performance (average daily gain or ADG, mortality rate, feed conversion ratio or FCR and performance index or PI) and antimicrobial usage (AMU) were collected in one batch of broiler farms (45 days). In addition, fecal, drinking water, and biofilm samples were collected in both broiler and layer farms and subsequently analyzed by real-time PCR with PowerUp™ SYBR® Green Master Mix in a LightCycler®480 Roche and 16S rRNA NGS to evaluate the prevalence of antimicrobial resistance genes (ARGs) conferring resistance to carbapenems (blaVIM-2 and blaNDM), (fluoro) quinolones (qnrS, qnrA, and qnrB), polymyxins (mcr1 to mcr5), and macrolides (ermA and ermB). One-way analysis of variance followed by Duncan's comparison test was used to compare more than two means. Student's test was used to compare two means. The overall biosecurity level in poultry farms was moderate and less than half of farms had a good biosecurity score. The ADG was significantly ($P=0.034$) higher in farms with good biosecurity (46.54±5.18 g/day) compared to those with poor biosecurity (43.80±4.16 g/day). Similarly, FCR (1.59±0.61 versus 1.75±0.58), mortality rate (2.47% versus 6.65%) and PI (339.21±105.79 versus 268.22±101.09) were statistically better in farms with good biosecurity. No correlation was noted between biosecurity and AMU, although there was a trend towards reduced use in farms with good biosecurity. All samples tested positive for genes conferring resistance to (fluoro) quinolones, 97.8% to macrolides, 64.4% to polymyxins, and 11.1% to carbapenems. Good practices toward AMU were found to be correlated with a reduction in polymyxin resistance. This study highlights the importance of biosecurity in improving poultry farmers' profitability and reducing AMR and its potential impact on human and environmental health.

Keywords: Biosecurity; Antibiotic; production performance; Broiler; Layer

4125 Coaching improve biosecurity and production and reduce antibiotic usage in broiler farms in Adamawa (Cameroon)

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In Cameroon, the chicken sector plays an important role for human nutrition. Unfortunately, recent studies have shown a poor implementation of biosecurity in broiler farms that lead to an increase of infectious diseases, antimicrobial usage (AMU) and resistance. Behavior change promoted by coaching may increase biosecurity compliance. This study aimed to evaluate the efficacy of coaching to improve biosecurity and production as well as reduce AMU in broiler farms in the Adamawa region of Cameroon. A total of 15 farms were randomly selected and coached. Coaching consisted in training farmers on the importance of biosecurity implementation based on the weakness observed in their farms. This was done based on a guideline developed by the authors. Biosecurity compliance using Biocheck.UGent™ tool, AMU and production performance (average daily gain or ADG, mortality rate, feed conversion ratio or FCR and performance index or PI) were assessed before and after the coaching. The pre and post assessment were performed on two different batches of broiler in the same farm. A batch was followed for 45 days. The results showed an overall improvement on biosecurity level in broiler farms after the coaching. In addition, the coaching helped to reduce AMU and specially the use of highest priority critically important antimicrobials. Data analysis is still ongoing to finalize this study but the preliminary finding highlights the efficacy of coaching in improving biosecurity compliance and antimicrobial stewardship in broiler farms in the study area in Cameroon.

Keywords: Antimicrobial usage; Biosecurity; Coaching; Poultry

4126 Coccidiosis control in hot climates

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Heat stress is recognised by the poultry industry as one of the main hurdles for broiler performance in many areas in the world, this is because feed must be restricted to minimise heat stress and related mortality. There is evidence that some anticoccidials can increase metabolic rate and therefore aggravate the heat stress situation further.

A commercial broiler farm at high altitude, humidity, and temperature with 16 open sided houses was used for this study. 5 different coccidiosis control strategies were used: Narasin+ nicarbazin (Control, T01), diclazuril + lasalocid (T02), diclazuril(T03), lasalocid(T04) and a coccidia vaccine.

Treatment 01 was the control (standard coccidiosis control for all broilers in the company). Replicas in each treatment: T01 (control)= 4 replicas, T02= 4, T03= 3, T04=3 and T05= 2. Each house with approximately 10.000 broilers.

Whole blood was taken from the brachial vein from 5 males marked at day old in each house, the same individuals sampled each time at week 3, 4, 5 and 6 from all the houses, at week 3 only houses 1, 2, 3 and 4 sampled.

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Environmental conditions were measured every 2 minutes in 6 houses using artificial intelligence (AI) equipment (brightness, fine dust, CO2, NH3, temperature, humidity and air speed).
17 parameters were analysed in whole blood by a gas analyser machine.
Statistical analysis: For the statistical tests, Alpha risk is 5%. SAS software was used to run statistical tests, for quantitative traits analysis, fixed effects were tested using variance analysis, the mathematical models used was Xij (measured trait) = m(average)+ ai (fixed effect of ith treatment) + bj(fixed effect of jth age) + Eij(residual).
pH, glucose, lactate, pCO2 and pO2 results were statistically analysed for each treatment, with pH and pCO2 finally selected.
Their results showed statistically significant differences between treatments, with the vaccinated group showing lower pH and higher pCO2:
T04 (7.42) > T02 (7.41) ; T01 (7.40) > T03 (7.40) > T05 (7.36) (pkw = 0.004 **) for pH
T05 (50.5) > T03 (48.9) ; T01 (48.6) ; T02 (48.1) > T04 (47.1) (pkw = 0.095) for

Environmental conditions: temperature between 34oC and 25oC, humidity from 55 to 85%. Data collected from 6 houses showed wide variation, more equipment needed to evaluate all houses.
Conclusions: Vaccination to control coccidiosis can help to maintain pH closes to neutral and pCO2 lower which indicates that it can potentially be an alternative to anticoccidials in hot and high-altitude conditions, helping to keep broiler metabolisms lower, controlling heat stress and cardiovascular problems, resulting in better zootechnical results. More trials required.
Keywords: Coccidiosis control; Vaccines; Anticoccidials; Hot climates; Feed restriction

4127 Improving Hatchery Hygiene through Validated Biosecurity and Disinfection Protocols: A Field Study from Serbia

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Biosecurity measures are widely recognized as one of the most important factors of infectious disease prevention in poultry production. This study evaluated the effectiveness of routine biosecurity and disinfection procedures in three commercial hatcheries in Serbia in reducing microbial contamination and limiting the persistence of Salmonella spp. on critical surfaces involved in the production of day-old-chickens. The hatcheries included in this study each had an annual production capacity of 5 to 7 million chickens, representing nearly a third of the national broiler production in Serbia. Sampling was performed in multiple production zones before and after routine cleaning and disinfection procedures. Swab samples were collected from floors, walls, tables, and equipment in direct contact with eggs, chickens, and hatchery waste. Microbiological analyses included determination of total aerobic bacterial counts (CFU/cm2) and detection of Salmonella spp. using standard bacteriological methods. Statistical analysis was conducted using paired t-tests to compare mean total bacterial counts before and after disinfection in each hatchery, and a Chi-square ($\chi 2$) test to assess differences in the frequency of Salmonella-positive samples. A substantial and statistically significant reduction (97-98%; $p<0.01$) in total aerobic bacterial counts was observed in all hatcheries following sanitation, indicating the overall effectiveness of routine hygiene measures in lowering environmental bacterial load. The result showed that Salmonella spp. was detected in all hatcheries before sanitation and remained present in a limited number of samples after disinfection, with no statistically significant difference in detection frequency ($\chi 2=3.26$; $p=0.071$). These findings suggest that while routine biosecurity and disinfection protocols are effective in reducing overall microbial contamination, they may be insufficient to consistently eliminate Salmonella spp., particularly in critical areas such as hatching rooms, chicken handling areas, and waste zones. Although based on a limited number of facilities, this study provides field-based evidence that standard hygiene programs substantially reduce bacterial loads but may leave residual risk for Salmonella persistence in hatchery. The results highlight the need for improved hygiene monitoring, validation of disinfection efficacy, and the potential integration of additional control technologies (e.g., hydrogen peroxide, UV, ozone) to enhance pathogen control in commercial hatchery environments. Although this is already well described, continuous staff training and regular hygiene control programs are recommended for maintaining high standards of hatchery biosecurity.
Keywords: Biosecurity; hatchery hygiene; Salmonella spp.; disinfection efficacy; microbial safety

Meat Processing Technology II

4137 Developments in primary poultry meat processing

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Poultry meat production continues to grow around the world and so is processing speed at dedicated modern meat planes. The industry has seen an impressive increase in automation resulting in a fivefold increase in processing speed (3,000 to 15,000 birds per hour; bph) within the past half a century. This reflects a contribution of technological innovation, industrial reorganization, and sophisticated approaches to process control. Together, those developments have shaped the efficiency, scale, and economics of global poultry meat production. A century ago, poultry processing was constrained by manual operation, limited mechanization, and relatively simple processing systems. The first major shift came with the introduction of semi-automated / automated de-feathering equipment, followed by evisceration and chilling systems. All helped to reduce manual labor requirements and assisted in standardizing product handling. Mechanical innovations, such as rotary pickers, automated vent cutters and transfer systems enabled continues flow operation that significantly raised capacity. By the late 20th century advances in line synchronization process, ergonomics and shackle design allowed planes to increase speed to 8000 bpm. At the same time regulatory agencies started shifting from visual inspection to microbiological and process control verification, which were required to allow increase in line speed. The most significant gains in the past 20 years have come from automation and digitalization (part of the Industry-4.0 revolution). Robotic manipulators, high speed vision systems, and sensor driven quality control devices have allowed raising the speed to 15,000 bpm seen today. Examples of some recent cutting-edge technologies include the introduction of x-ray imaging to adjust each individual portioning, which provide higher yields, and less defects while deboning > 3,000 breast fillets per hour. Another example is

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the recent introduction of an AI system to monitor and adjust vent cutting, while helping reduce contamination and improve efficiency. Overall, using data driven sensors and monitoring platforms that can track parameters (e.g., carcass weight, equipment performance, contamination risk) in real time, enables the industry to run efficiently at peak capacity. As the industry continues to evolve, further gains are likely to come from deeper automation and AI driven developments

4138 Development of a nanobody-horseradish peroxidase fusion-based competitive ELISA to rapidly and sensitively detect Enrofloxacin residues in animal-derived foods

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As a third-generation fluoroquinolone antibiotic, enrofloxacin (ENR) misuse could fuel the development of fluoroquinolone-resistant pathogens that can be transmitted between animals and humans. When ENR residues are present in the food chain, this can lead to a range of adverse outcomes and human diseases. Therefore, it was thus an urgent need to establish sensitive, rapid, and high-throughput approaches to detecting ENR in animal-derived foods in order to ensure food safety and to protect against antibiotic resistance. In this study, a nanobody-horseradish peroxidase fusion specific for ENR was generated to enable a sensitive and rapid competitive ELISA suitable for detecting enrofloxacin in samples of eggs and chickens. An enrofloxacin hapten generated via the glutaraldehyde method was initially used to immunize an adult Bactrian camel as a means of constructing a phage library. Enrofloxacin specific nanobodies were then selected through three rounds of biopanning, and HRP-fused versions of these nanobodies were then expressed. Lastly, these nanobodies were used to develop a sensitive cELISA for enrofloxacin detection in eggs and chickens (obtained from breast and leg, number of repeated samples=3), these samples was combined with ENR and homogenized to obtained the extraction solution, and the operation process of ic-ELISA experiments following by standard solution dilution. The resultant assay were exhibiting an IC 50 of 37.41 ng/mL and a linear detection range (IC20-IC80) of 10.89 to 244.34 ng/mL. The limit of detection for this cELISA was 6.48 ng/mL, with 4.66 % cross-reactivity with ciprofloxacin, and recovery rates that ranged from 84.99 % to 107.72 % together with an RSD below 10.70 %. This novel cELISA platform can thus enable the sensitive, specific, straightforward, rapid, and cost-effective immunoanalytical detection of residual ENR levels within food products.

Keywords: Nanobody; competitive ELISA; Enrofloxacin; Residue determination

4139 Prevalence, serogrouping and antibiotic susceptibility of Listeria monocytogenes from raw, frozen and processed chicken meat in Bangladesh

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Objective: This study aimed to determine the prevalence, serogroup distribution, and antimicrobial susceptibility patterns of Listeria monocytogenes isolated from raw, frozen, and processed chicken meat in Bangladesh, based on the hypothesis that locally marketed poultry meat carries virulent and antimicrobial-resistant strains posing health risks.

Description of the problem: A cross-sectional investigation was conducted to detect L. monocytogenes in different poultry meat types and to characterize isolates according to virulence genes and antimicrobial resistance, addressing the growing concern of multidrug-resistant L. monocytogenes contamination in the poultry supply chain.

Materials and Methods: A total of 122 samples—raw (n = 87), frozen (n = 23), and processed chicken meat (n = 12)—were collected from broiler farms and retail markets in Mymensingh. Samples were examined using cultural and biochemical methods, followed by PCR confirmation targeting prs and hlyA genes. Strain-specific PCR was performed to detect virulence genes D1 and MAMA-A. Antimicrobial susceptibility was assessed using the disk diffusion method against β-lactams, macrolides, fluoroquinolones, glycopeptides, and aminoglycosides.

Statistical analysis: Data analysis included descriptive statistics, one-way ANOVA for group comparisons, and correlation analyses to evaluate associations between virulence genes and resistance profiles.

Results: The overall prevalence of L. monocytogenes was 13.93% (17/122). Raw meat showed the highest contamination (14.71%), followed by frozen (13.04%) and processed meat (8.33%). Virulence gene detection varied across sample types: prs (66.67–100%), hlyA (61.54–100%), D1 (38.46–66.67%), and MAMA-A (15.38–33.33%). High resistance rates were observed to penicillin G (90.91%), oxacillin (81.82%), and ampicillin (72.73%), while erythromycin, vancomycin, and gentamicin remained effective. Correlation analysis indicated strong associations between β-lactam resistance and virulence genes, such as prs with penicillin G ($r \approx +0.50$) and hlyA with oxacillin ($r \approx +0.55$).

Conclusions: These findings reveal the presence of multidrug-resistant and virulent L. monocytogenes in poultry meat in Bangladesh, underscoring the need for improved hygiene, prudent antibiotic use, and regular monitoring to safeguard public health.

Five Keywords

Listeria monocytogenes; poultry meat; prevalence; antimicrobial resistance; serogrouping

Keywords: Listeria monocytogenes; Poultry meat; Prevalence; Antimicrobial resistance; Serogrouping

4140 Cannabidiol and nanoselenium as a mediators of meat properties in C. Perfringens-Challenged Chickens

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Recently, growing evidence indicates a close relationship between the functional state of the gut, the factors determining it, and the processes that condition the physicochemical properties of poultry meat. Cannabidiol (CBD) and Se-nanoparticles (SeNPs) have shown promising results in supporting health status in chickens but their mechanisms of action and optimal inclusion levels is unknown. Therefore, we hypothesized that dietary CBD and SeNPs may modulate mechanisms influencing breast muscle characteristics under various conditions. A total of 432 one-day-old male Ross 308 broilers were reared for 35 days. Chickens were housed in cages and divided into 6 experimental groups. The negative control (CON) received a basal diet without supplementation or challenge, while the positive control (CON+) was fed the same basal diet but challenged with Clostridium perfringens. The CBD+ group was challenged with C. perfringens and supplemented with 15 g/kg Cannabis sativa extract, while the SeNPs+ group was similarly challenged but received 0.3 mg/kg SeNPs in the diet. Chicks from CBD + SeNPs and CBD + SeNPs + C. perfringens were fed a CON supplemented with both additives, with the latter group also challenged C. perfringens. On day 35 of the experiment, caecal digesta samples were collected for bacterial analysis, and breast muscle was sampled for meat quality, fatty acid profile and sarcoplasmic protein evaluation. Data were analyzed using one-way ANOVA. Supplementation with CBD and/or SeNPs did not negatively impact breast meat quality or the desaturation, thrombogenic and atherogenicity indices. Dietary supplementation with CBD and

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CBD + SeNPs significantly increased polyunsaturated fatty acid (PUFA) and omega-3 PUFA concentrations of C. perfringens-challenged chickens. Principal component analysis revealed that the PUFA n-6/n-3 ratios in muscle lipids were most significantly associated with groups receiving SeNPs alone or in combination with CBD. The levels of pyruvate kinase/phosphoglucose isomerase in meat exudate from the CBD + SeNPs group were significantly different from those in the CON, CON+ and CBD+ groups, showing the lowest level of this enzyme ($P<0.005$). Feeding cannabis extract resulted in the meat transfer of CBD, while accumulation of THC remained below the maximum allowable level (10 ppm) in the food for humans. These findings provide new understanding of how CBD and SeNPs influence the nutrition-challenge-meat quality relationship, highlighting the need for additional research to fully elucidate their effects. Funded by the Minister of Science under the Regional Initiative of Excellence Program.
Keywords: cannabidiol; gut health; c. perfringens; meat properties; cannabinoid transfer

4141 Breast meat quality as affected by major european farming systems varying in intensification level: Insights from the H2020 INTAQT project

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Chicken meat consumption continues to increase across Europe, supporting the predominance of fast-growing production systems. However, rising societal concerns about animal welfare and product quality are promoting the development of alternative, less intensive, and more welfare-friendly farming approaches. Within this context, the H2020 INTAQT project aims to assess how different farming practices affect chicken meat quality across nutritional, sensory, health, and technological dimensions. Breast and thigh meat from 90 farms representing 18 production systems in France, Belgium, Italy, Poland, and Switzerland were evaluated. Systems were classified into six categories based on growth rate, slaughter age and weight, and outdoor access: Indoor_Fast_Heavy, Indoor_Fast, Indoor_Fast_Med, Outdoor_Fast_Heavy, Outdoor_Medium, and Outdoor_Slow. Breast meat proximate composition, sensory attributes, and technological traits were assessed. Data were analyzed using one-way ANOVA to evaluate the effect of farming system. Breast meat from fast-growing broilers used for Indoor_Fast_Heavy, Indoor_Fast, Indoor_Fast_Med and Outdoor_Fast_Heavy farming systems showed the highest lipid content, regardless of whether they were raised in intensive or extensive systems. Conversely, fillets from intensive systems (Indoor_Fast_Heavy, Indoor_Fast) had the lowest protein content and the highest water-to-protein ratios. These systems also produced meat with higher pH values compared with those using slower-growing strains. Notably, when high growth rate birds were raised more extensively and longer (Outdoor_Fast_Heavy), their pH values matched those of low-productivity, highly extensive systems. Despite their higher pH, meats from indoor system with fast growing broilers exhibited greater drip and cooking losses. In contrast, breast meat from ECC-type indoor systems (Indoor_Fast_Med), using intermediate-growing strains slaughtered at heavier weights than Indoor_Fast, showed the highest processing yields after curing and cooking and the lowest shear-force values. This superior tenderness appears related to a normal pH (~5.8) combined with reduced cooking losses, and was confirmed by sensory evaluation. Ongoing Random Forest analyses aim to clarify the contribution of individual farming practices and to provide recommendations that help producers tailor meat quality to specific market demands.
Keywords: Meat quality; Farming; Intesification level; chicken

4142 Evaluation of Meat Quality Parameters in Fast-Growing Ross 308 and Slow-Growing Hubbard JA57 Broilers

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This study was conducted to determine the differences in meat quality parameters between fast- and slow-growing broilers. The Ross-308 genotype was used as the fast-growing genotype, and Hubbard JA57 as the slow-growing genotype. Both genotypes were reared until they reached the accepted market weight of 2.5 kg. Chickens were slaughtered by cervical dislocation, and meat quality analysis was conducted 24 hours after slaughter. Subsequently, colour and shear force values were evaluated in 12 samples, including six breast and six thigh muscles from each genotype. Colour and shear force measurements of thigh muscles were conducted, and no significant differences were observed in lightness (L), redness (a), yellowness (b), Hue, or Chroma between genotypes ($p > 0.05$), although Chroma value tended to be higher in Hubbard JA57 ($p = 0.085$). Significant differences were detected in shear force indices: Fi3 (Hubbard: 32.18 and Ross: 18.73, $p = 0.004$), Si3 (Hubbard: 23.46 and Ross: 14.22, $p = 0.003$), and Ei1 (Hubbard: 313.51 and Ross: 119.97, $p < 0.001$), indicating higher muscle firmness in the slow-growing genotype. Colour and shear force parameters of breast muscles were compared between fast-growing Ross 308 and slow-growing Hubbard JA57 broilers ($n = 6$ per genotype). A statistical comparison was made using the independent sample t-test in the SPSS statistical program. Significant differences were observed in lightness (L: Hubbard 79.17 and Ross 88.56, $p = 0.045$), yellowness (b: Hubbard 2.81 and Ross 6.80, $p = 0.032$), hue angle (Hue: Hubbard -20.60 and Ross -44.16, $p = 0.048$), and Chroma (Chrome: Hubbard 8.01 and Ross 10.00, $p= 0.009$). No significant differences were detected for redness (a) or shear force indices (Fi3, Si3, Ei1) between genotypes ($p > 0.05$). Overall, slow-growing Hubbard JA57 broilers exhibited higher thigh muscle shear force compared to fast-growing Ross 308, while significant differences in breast meat colour parameters were also observed between genotypes. These findings indicate that growth rate influences specific meat quality traits, which may have implications for consumer preferences and broiler breeding strategies.
Keywords: genotype; growth rate; shear force; broiler; meat quality

4143 NIX COLOR SENSOR AS POTENTIAL TOOL FOR EVALUATING COLOUR IN BROILER BREAST MEAT

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A study was carried out to evaluate the efficacy of an innovative and portable sensor (Nix Spectro Color Sensor) for the instrumental assessment of chicken breast meat color. Three individual replicates of the experiment were conducted using a total of 90 boneless, skinless, 24 h post-mortem fillets from 6-week-old female Ross 308 broilers (2.8 kg live weight). Samples were selected in the deboning area of a commercial processing plant based on their lightness (L*) values, which were measured with a standard

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tristimulus colorimeter (Minolta CR-400) and used to classify them as dark, normal, or pale fillets. The L* thresholds used to discriminate the three color groups were established from measurements obtained in a preliminary trial: dark (L* < 54), normal (55 < L* < 58), and pale (L* > 59). Upon arrival at the laboratory, fillets (30/group) were analyzed for color using both the Minolta CR-400 colorimeter and the Nix Spectro Color Sensor, as well as for pHu, drip loss, and cooking loss. Data were analyzed using one-way ANOVA to evaluate the effect of color group. In addition, Pearson's correlation coefficients were calculated. Overall, when comparing the colorimetric data obtained with the tristimulus Chroma Meter and the Color Sensor, differences were found with the values measured with the last being lower than that observed with the Minolta. These differences may be attributed to the characteristics of the light-emitting sources integrated in the devices, which can lead to different scattering of the incident light due to its interaction with the meat surface and/or structure. Nevertheless, significant differences among color groups were detected for all color parameters when the Color Sensor was used. Also, as expected, paler breast meat was associated with lower ($P < 0.001$) pHu and poorer ($P < 0.001$) water-holding capacity, while dark breast meat showed an opposite behavior. When correlations were considered, a high degree of significance was observed particularly between the L* values measured with the two color-measuring instruments ($r = +0.90$; $P < 0.001$), whereas associations were weaker for redness (a*; $r = +0.61$; $P < 0.001$) and yellowness (b*; $r = +0.52$; $P < 0.001$). Notably, the correlations of lightness with pHu, drip loss, and cooking loss showed similar coefficients regardless of the instrument used. In conclusion, these results indicate that the Nix Spectro Color Sensor can represent a viable alternative to the conventional chroma meters for both research and industrial applications.

Keywords: Chicken breast meat; color; Minolta CR-400 colorimeter; Nix Pro2 Color Sensor; quality traits

4144 Impact of Ochratoxin A on Muscle Metabolites and Meat Quality in Broiler Chickens

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Ochratoxin A (OTA) is a mycotoxin produced by Aspergillus and Penicillium species and is known as common contaminant of poultry feeds that adversely affects health, growth and product quality parameters in chicken. OTA induced oxidative stress and metabolic disruption may alter muscle biochemistry leading to reduced meat quality. Muscle metabolites such as carnosine, anserine, creatine and creatinine are key indicators of muscle energy metabolism and antioxidant potential. Present study was planned to evaluate the impact of OTA exposure on muscle metabolite concentrations and meat quality in broiler chickens using high performance liquid chromatography (HPLC). Day-old broilers were randomly assigned to four dietary groups containing OTA 0, 1.6, 3.2, and 6.4 mg/kg of feed for 10 days to induce acute ochratoxicosis in day old broilers. The experiment duration was 42 days with feed and water offered ad libitum. Six birds per group were sacrificed at days 10 and 42 and pectoralis major muscles were analyzed for quantification of anserine, carnosine, creatine and creatinine. After extraction and deproteinization, quantification of these metabolites was carried out using an Atlantis® HILIC silica column (3 µm, 4.6×150 mm) with a HPLC-UV detection system. Results indicated that carnosine levels were significantly reduced in the 6.4 mg/kg OTA group compared with control at day 10, while differences among other groups were non-significant. Anserine concentrations were significantly lowered in 3.2 and 6.4 mg/kg OTA groups at day 10 and by day 42 where a dose-dependent decline was observed across OTA-fed groups. Creatine content decreased significantly at day 10 in 3.2 and 6.4 mg/kg groups whereas at day 42 reduction was observed only in highest OTA fed group. However, creatinine levels did not differ significantly among treatment groups at any stage. In conclusion, OTA exposure induced dose-related reductions in carnosine, anserine and creatine concentrations, reflecting impaired energy metabolism and antioxidant capacity in broiler muscles. These alterations suggest that OTA compromises muscle biochemical integrity and overall meat quality. This study appears to be the first to report the direct effect of OTA on muscle histidine-containing dipeptides in broilers, contributing novel insight into the biochemical pathways underlying OTA-induced meat quality deterioration.

Keywords: Ochratoxin A (OTA); Broiler chickens; Muscle metabolites; Meat quality; HPLC analysis

4145 Comparative Analysis of Flavor Profiles in Yanjin Black-Bone and Jinling Partridge Chickens using multi-omics

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Objective:

This study aimed to systematically compare the flavor profiles of Yanjin black-bone chicken (YJ) and Jinling partridge black-bone chicken (JL) by analyzing volatile organic compounds, fatty acid composition, and associated metabolic pathways. The findings provide insights into breed-specific flavor attributes and support genetic improvements for enhanced meat quality.

Experimental Design:

A combined flavoromics and metabolomics approach was employed. Meat samples from 30 female chickens per breed were analyzed using gas chromatography–tandem mass spectrometry (GC-MS/MS) and electronic nose (e-nose) technology to identify volatile aroma-active compounds. Fatty acid profiles and lipid contents (triglycerides, phospholipids, and cholesterol) were characterized. Non-targeted metabolomics and Weighted Gene Co-expression Network Analysis (WGCNA) were applied to identify key metabolic pathways influencing flavor formation. Multivariate statistical methods were used to compare compositional differences.

Materials and Methods:

Meat samples were collected from 30 female chickens of each breed (YJ and JL). Volatile compounds were extracted and analyzed by GC-MS/MS, with 718 compounds identified; among these, compounds with an odor activity value (OAV) greater than 1 were considered aroma-relevant. Fatty acid composition was determined by gas chromatography following lipid extraction. Triglyceride, phospholipid, and cholesterol contents were measured using standardized commercial assay kits.

Statistical Analysis:

All data were processed using SPSS 22.0. Independent samples t-tests were applied to compare compound levels and flavor indices between breeds, with statistical significance set at $p < 0.05$.

Results:

E-nose analysis revealed distinct aroma profiles between YJ and JL. Fatty acid analysis showed significantly higher levels of saturated, monounsaturated, and polyunsaturated fatty acids in YJ, including elevated concentrations of lauric acid (C12:0), myristic acid (C14:0), and oleic acid (C18:1n9c). Key odorants such as (E)-2-hexenal, hexanal, and ethyl acetate, with divergent odor activity values (OAVs), were identified as major contributors to flavor differentiation.

Conclusions:

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Significant flavor differences between YJ and JL chickens were attributed to distinct volatile compound compositions and fatty acid profiles. Critical flavor determinants, including (E)-2-hexenal and ethyl acetate, along with metabolic precursors like 6-hydroxymelatonin and α-linolenic acid, underlie the sensory divergence. These findings establish a molecular basis for flavor differentiation and provide a foundation for breeding programs targeting improved meat flavor quality.

Keywords: Flavor; black-bone chicken; melanin; volatile compounds; fatty acids

4146 Enhancing chicken meat with omega-3 DHA by including algal oil high in DHA in the diets of broiler chickens

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What if the key to better nutrition isn't new or more food, but smarter food? Omega-3 docosahexaenoic acid (DHA) is an important long-chain polyunsaturated fatty acid for human health especially brain, eye, and cardiac health and function. Many people do not consume enough or indeed any of the traditional source of DHA, namely oily fish. An alternative source of obtaining DHA could be in poultry meat; by supplementing broiler chickens with DHA in their diet. This study followed 960 commercial Ross 708 broiler male and female chickens that were placed equally among 40 floor pens, provided ad libitum feed and water, and raised until 34 days of age (DOA). The chicks were raised on a typical commercial starter diet from 0 to 13 DOA, and grower diet from 14 to 22 DOA. From 23 DOA to the end of the trial, five experimental finisher diets were distributed equally among the pens: CON (control, standard Canadian broiler diet, antibiotic and animal by-product free), LC (low crude algal oil from Aurantiochytrium limacinum), HC (high crude algal oil), LA (low OmegaPro Advance, algal oil + sunflower oil), and HA (high OmegaPro Advance). Pen body weights and feed weights were taken at day 0 and at the end of each phase to calculate ADG and FCR. Mortality was recorded and parameters adjusted for accuracy. On day 34, two birds per pen were sacrificed, weighed, and dissected for chicken breast and thigh tissue samples on which omega-3 DHA fatty acid content was analyzed. The data were analyzed in R version 4.3.1. and the FCR was analysed using the Kruskal-Wallis test and Dunns post-hoc comparison. Throughout the finisher stage, for FCR, there was no significant difference amongst treatment groups. In addition there was no statistical difference in ADG amongst treatment groups. The omega-3 DHA concentration in the CON tissues was below the limit of detection (<20 mg DHA/100g meat). However, successful incorporation was found in the tissues of the treatment groups; the lower inclusion groups had approximately 73 mg DHA/100g breast meat and 199 mg DHA/100g thigh meat, and the higher inclusion groups had approximately 93 mg DHA/100g breast meat and 271 mg DHA/100g thigh meat. Based on this trial, it is proven that the DHA-rich algal oil tested included in feed for broiler chickens successfully incorporated omega-3 DHA into the meat products, without compromising the production parameters of the broiler chickens. Omega-3 DHA enriched chicken meat is an alternative source of this important fatty acid for consumers.

Keywords: docosahexaenoic acid; microalgae; Ross 708

Nutrition: Enzymes II

4147 Beyond nutrient release: emerging roles and future opportunities for feed enzymes in poultry nutrition

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The sustained growth in the application of exogenous enzymes in poultry feed is driven by their ability to enhance nutrient utilization and mitigate the environmental impacts of intensive poultry production. Enzymes targeting viscosity-inducing factors in cereal grains, such as wheat and barley, have both helped reduce the negative effects of these cereal grains, especially in young birds, and enabled their inclusion at higher levels in diets. In addition, the application of phytase has enabled a substantial reduction in dietary use of inorganic phosphorus, with the potential to further reduce or completely eliminate its use in diets. In the quest to further exploit the benefits of enzyme use, the additional benefits of using enzymes in combination were explored through further characterization of enzyme combination relationships, including interactions (negative or positive) and additivity. More recent developments in feed enzyme application are informed by the several theoretical and practical frameworks including: 1) beneficial effect of enzymes can not be completely accounted for by response to the deficient nutrient that the enzyme is intended to release, and 2) there is potential for positive (or perhaps negative) effects of "downstream" substrates resulting from the action of the enzyme on the target substrate. Despite or as a result of decades of research and development, new generations of enzymes are still being discovered with the potential to yield further economic and environmental benefits for poultry production. In the future, the use of enzymes to support gut health (already an important research area) will remain an active area of investigation, with potential benefits for the industry. It will be important to understand the biological mechanisms linking enzyme activity to microbiome and host physiology. The question remains whether and how enzymes could be designed/developed to target specific microbiota pathways or metabolic responses. In the context of feed formulation, the use of enzymes to optimize the utilization of, for example, reduced-protein diets or alternative feedstuffs, or to further reduce nutrient excretion to the environment, will continue to be relevant areas of investigation and frontiers for enzyme technology and development.

Keywords: Feed enzymes, gut health, host, microbiome, enzyme technology

4148 Performance, egg quality, phosphorus digestibility, phytate disappearance and bone mineralization responses of laying hens to a novel bacterial 6-phytase in nutrient-reduced diets without supplemental inorganic phosphorus

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This study investigated the effects of graded levels of a novel biosynthetic bacterial 6-phytase (Rovabio® PhyPlus, Adisseo, France) on the laying performance, eggshell and egg quality, phosphorus (P) digestibility, phytate disappearance, and bone mineralization in hens fed corn–soybean meal diets reduced in energy and digestible amino acids, without supplemental inorganic P. From 39 to 59 weeks of age, 1,152 Hy-Line Brown hens were randomly assigned to six dietary treatments with 16 replicate cages of 12 hens each. Treatments included: a positive control (PC) diet with adequate energy and nutrients; a negative control (NC) diet reduced in metabolizable energy (−30 kcal/kg), digestible amino acids (−2%), and without inorganic P; and the NC diet supplemented with phytase at 250, 500, 1,000, or 1,500 FTU/kg. Egg production, average

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daily feed intake (ADFI), egg weight, egg mass, feed conversion ratio (FCR), and eggshell and internal egg quality variables were evaluated every 4 weeks. At 59 weeks of age, prececal digestibility (pcd) of P, phytate disappearance, and tibia mineralization were determined. Data were analyzed using a randomized complete block design with cage as the experimental unit. Significant least square means were compared by the Tukey-Kramer test, and phytase dose effects were assessed using orthogonal polynomials, with significance declared at $P < 0.05$. Nutrient reduction in NC significantly ($P < 0.05$) decreased ADFI, laying rate, egg weight, egg mass, pcd of P, and tibia ash, and increased FCR compared to PC. Phytase supplementation improved (linear and quadratic, $P < 0.05$) ADFI, laying rate, egg mass, and FCR, with a linear effect on egg weight ($P = 0.04$). Dietary treatments had limited effects on eggshell and egg quality variables, except at weeks 16 and 20 of the experimental period, where phytase exhibited a linear response ($P < 0.05$) for eggshell strength, eggshell thickness, albumen height, and yolk color. Both linear and quadratic responses ($P < 0.05$) were observed for pcd of P, phytate disappearance, and tibia ash content. These findings demonstrate that dietary supplementation of a novel bacterial 6-phytase enhances laying performance, P digestibility, phytate disappearance, and bone mineralization in nutrient-reduced diets without inorganic P in a dose-dependent manner.

Keywords: bacterial 6-phytase; performance; digestibility; laying hens; bone mineralization

4149 Efficacy of a new biosynthetic 6-phytase on growth efficiency in broilers fed low crude protein, energy and inorganic P-free diets

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An experiment was carried out to investigate the influence of different commercial phytases on growth performance in broilers fed inorganic-phosphorus (P)-free diets, and reduced in crude protein and metabolizable energy, from 1 to 35 days of age. A randomized complete block design (2,904 Ross 308 mixed-sex birds; 121 pens, 11 pens per treatment) tested 11 treatments: 1) a positive control diet (PC, T1), containing 2950, 3050, 3150 kcal AME/kg and 22.0, 20.5 and 18.0% crude protein (CP) during 1 to 10, 11 to 21, and 22 to 35 d of age, respectively; 2) a negative control diet reduced in AME and CP by 50, 75 and 75 kcal/kg and 1.5, 2.0 and 2.0% units in phases 1, 2 and 3, respectively, compared to PC diet (NC1, T2)); 3) as NC 1 but additionally reduced in Ca and without inorganic P incorporation (NC2, T3); 4) as NC2 but additionally reduced in some amino acids including Thr, Arg, Val, Ile and Gly+Ser (NC3, T4); and NC3 supplemented with phytase A (T5), B (T6), C (T7), D (T8), E (T9) each at 500 FTU/kg diet. The phytases A and B were also added to NC3 at 1000 and 6000 FTU/kg diet, respectively. Body weight (BW) and feed intake were measured at 21 and 35 days of age, and BW gain, feed conversion ratio (FCR) and European performance efficiency factor (EPEF) were calculated. At 21 days of age, compared with birds fed the PC diet, the reduction in CP and AME (NC1) did not affect ($P > 0.05$) feed intake, BW gain, FCR and EPEF, while the additional reductions in Ca, P and some amino acids (NC2 and NC3 diets) reduced dramatically ($P < 0.001$) feed intake, BW gain and EPEF, and increased FCR compared to birds fed the PC and NC1 diets. Due to these dramatic consequences and for the welfare of birds, the treatments NC2 and NC3 were removed at 21 days of age from the experimental design. For the entire experimental period, broilers fed the NC1 diet had similar final BW (2414 vs 2435 g; $P = 0.99$), BW gain (2369 vs 2389 g; $P = 0.99$), feed intake (3717 vs 3779 g; $P = 0.93$), FCR (1.569 vs 1,582; $P = 0.99$), and EPEF (422 vs 422; $P = 0.99$) as birds fed the PC diet. Among the evaluated phytases at 500 FTU/kg diet, phytase A showed advantages ($P < 0.05$) in restoring feed intake and BW gain. The addition of phytases A and B at 1000 and 6000 FTU/kg, respectively, to NC3 diets fully restored ($P > 0.28$) feed intake (3662 and 3662 g), BW gain (2341 and 2349 g) and EPEF (418 and 427), and had similar performances as birds fed the PC and NC1 diet. The results of the present study showed that phytase A (a novel biosynthetic bacterial phytase) could be an effective alternative to improve growth efficiency and support sustainable poultry production by reducing crude protein and energy levels and fully removing inorganic P from diets.

Keywords: broilers; low protein; inorganic P-free; performance; phytase

4150 Effects of a high dose of phytase on growth performance and bone mineralization of broiler chickens

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A study was conducted to evaluate the effects of a high phytase dose on the growth performance and bone characteristics of broiler chickens up to 40 days of age. A total of 1,000 one-day-old male Cobb 500 chicks were allocated to 40 floor pens in a commercial poultry farm. Birds were assigned to one of four dietary treatments, with 10 replicate pens of 25 birds each, in a completely randomized design. The experimental diets consisted of: a positive control (PC; diet formulated to meet commercial levels of metabolizable energy and nutrients, containing 1,500 phytase units [FYT]/kg); PC with 3,000 FYT/kg (PC3,000); a negative control (NC; reduced by 0.16% calcium and 0.08% available phosphorus compared with the PC); and NC with 3,000 FYT/kg (NC3,000). All diets were formulated with a standard phytase nutrient matrix equivalent to 0.19% calcium and available phosphorus. Broilers were fed corn–soybean meal–based pelleted diets in five feeding phases (pre-starter, starter, grower 1, grower 2, and finisher). Feed intake, body weight gain (BWG), and feed conversion ratio (FCR) were measured weekly from 1 to 40 days. On day 40, tibia samples were collected from two birds per pen for tibia breaking strength and ash analysis. Data were subjected to one-way ANOVA and treatment means were compared by the Tukey test ($P \leq 0.05$). Contrasts were applied to assess the effects of phytase compared to each control diet. Feed intake and BWG did not differ among dietary treatments; however, the final body weight increased by 2.7% in the NC3,000 group compared with the NC. Broilers fed the PC3,000 diet showed the best FCR, whereas the NC had the poorest FCR ($P < 0.05$). Phytase improved tibia strength and ash content, with higher values observed in broilers fed the PC3,000 diet compared with the PC ($P \leq 0.05$). In conclusion, these results demonstrate that a high dose of phytase (3,000 FYT/kg) can effectively enhance growth performance and bone mineralization in broilers reared under commercial conditions.

Keywords: broiler; calcium; feed efficiency ; nutrient matrix; tibia ash

4151 Limestone solubility, calcium level, and phytase supplementation: Impacts on performance, bone mineralization, and mineral utilization in late-phase laying hens

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The aim of this study was to evaluate the effects of limestone solubility, calcium (Ca) levels, and phytase (Ronozyme HiPhorius) supplementation on egg production, bone mineralization, and apparent ileal digestibility of P in laying hens aged 58 to 82 weeks. Ninety-six Hy-Line Brown hens were assigned to eight treatments in a 2 × 2 × 2 factorial design: two limestone types (slow vs. fast solubility), two Ca levels (3.4% vs. 4.0%), and two HiPhorius levels (0 or 600 FYT/kg of feed). Egg production, egg quality, mineral digestibility, tibia mineralization, and blood biochemistry were assessed. Three-way ANOVA was used to analyze the data.

The results showed that HiPhorius supplementation at 600 FYT/kg of feed significantly increased egg production by 5.7% over the 24-week period (87.9% vs 92.9%). Fast-soluble limestone reduced (P = 0.05) eggshell strength at week 12 of trial, though this effect diminished with the progression of the study. Fast-soluble limestone and high Ca level (4.0%) also significantly decreased tibia ash, Ca, and P content. Apparent ileal digestibility of phosphorus was significantly higher in hens fed slow-soluble limestone (66.7% vs 55.9%), 3.4% Ca (64.6% vs 58.1%) or HiPhorius at 600 FYT/kg of feed (69.7% vs 53.1%). Fast-soluble limestone led to significantly higher plasma Ca, blood bicarbonate, and carbon dioxide levels under high Ca diets.

In conclusion, this study indicates that phytase supplementation supports egg production, mineral utilization and physiological balance in aging hens, while fast-soluble limestone may compromise bone and eggshell quality and increase metabolic stress. Lowering Ca levels from 4.0% to 3.4% appear more favorable for skeletal health. These findings emphasize the importance of considering all three factors—limestone solubility, Ca level, and enzyme supplementation—together when formulating diets for aging laying hens.

Keywords: calcium; laying hens; limestone solubility; phosphorus; phytase

4152 Total vs. Digestible Calcium Formulations and Limestone Source Effects on Broiler Performance and Nutrient Digestibility in Response to Phytase

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The aim of this study was to evaluate the effects of limestone source and calcium (Ca) formulation system on broiler growth performance, bone mineralization, and nutrient digestibility through two trials. In the 37-day performance trial, 512 broilers were assigned to four dietary treatments in a 2×2 factorial design: two limestone sources (Chalk vs. Marble) and two Ca formulation systems (total vs. digestible Ca), with all diets supplemented with phytase (Ronozyme HiPhorius at 1000 FYT/kg). In the 23-day digestibility trial, 160 birds were allocated to four treatments (Chalk vs. Marble, with or without phytase), with eight replicates of five birds each. Data was analyzed using two-way ANOVA.

In the performance trial, no interaction between limestone source and Ca formulation was observed. Marble limestone significantly reduced feed intake and improved feed conversion ratio during the grower phase (P < 0.05), and increased tibia ash, Ca, and P content at day 37 of age (P < 0.05). No significant differences were found between Ca formulation systems, likely due to discrepancies between predicted and measured Ca digestibility values—Chalk (57.8% vs. 46.7%) and Marble (33.4% vs. 52.4%).

In the digestibility trial, phytase significantly increased apparent ileal digestibility (AID) of phosphorus (P < 0.01), and enhanced inositol hexakisphosphate degradation (P < 0.05). A significant limestone × phytase interaction was observed for AID of Ca: phytase improved Ca digestibility with Chalk limestone (P < 0.05), but not with Marble, likely due to Marble's slower solubility and reduced Ca-phytate precipitation.

These findings highlight the need for accurate prediction of Ca digestibility and adjustment of phytase matrix values based on limestone characteristics to optimize broiler nutrition.

Keywords: apparent ileal digestibility; bone mineralization; digestible calcium; limestone solubility; phytase

4153 Efficacy of a 6-phytase on performance parameters and bone mineralization in broiler starters

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The current study evaluated the effects of graded levels of bacterial 6-phytase (Rovabio PhyPlus, Adisseo, France) on the growth performance and bone mineralization in broiler starters. A total of 1,440-day-old male Ross 308 chicks were randomly allocated to six dietary treatments using 12 replicates per treatment and 20 birds per pen from 1 to 21 days of age. Treatments included: a nutrient adequate-positive control (PC) diet with calcium (Ca) level of 0.70% and available phosphorus (avP) of 0.45%, a negative control (NC) diet with Ca level of 0.70% and avP of 0.18%, and the NC diet supplemented with phytase at 500, 1,000, 1,500, and 2,000 phytase units (FTU)/kg. The NC diets were obtained by monocalcium phosphate reduction. Body weight (BW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were determined from 1 to 21 days of age. The right tibias from one bird per replicate (n = 12 tibia samples per treatment) were collected on day 21. All data were analyzed using GLM procedures of SAS software (2012). The pen was used as an experimental unit for the parameters reported. Significant least square means were compared by the Tukey-Kramer test, with significance declared at P < 0.05. Birds fed the NC diet had lower (P < 0.001) final BW (-47%), WG (-49%), feed intake (-48%), and FCR (+2.9 points) compared to the birds fed the PC diet. The birds fed the NC diets supplemented with phytase reached similar BW and WG compared to the birds fed a nutrient-adequate PC diet. The FCR of birds fed NC supplemented with 1,000, 1,500 and 2,000 FTU/kg phytase was lower (P < 0.05) compared to groups that fed the NC or PC diets. Moreover, reducing the percentage of avP in the NC diet resulted in lower (P < 0.001) tibia weight, dry matter and ash contents compared to birds fed the PC diet. However, 500, 1,000, 1,500 and 2,000 FTU/kg of phytase supplementation improved (P < 0.001) the tibia weight by 35%, 47%, 52% and 48% and ash content by 28%, 35%, 33% and 33%, respectively, compared to the NC group. There were no significant differences (P > 0.05) between PC and diets supplemented with phytase doses for bone mineralization parameters. These results revealed that bacterial 6-phytase can improve the growth performance and bone mineralization of broilers fed reduced P diets during the starter phase. The magnitude of the response depends on the phytase dose.

Keywords: bacterial 6-phytase; broilers; performance; bone mineralization; phytase dose

4154 Enzymatic Detoxification of Ochratoxin A in Broilers – Efficacy of an OTA Amidohydrolase

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Ochratoxin A (OTA), a nephrotoxic mycotoxin produced by Aspergillus and Penicillium species, is a significant concern for feed and food safety due to its widespread presence in agricultural crops and processed foods, prolonged half-life, and its potential for bioaccumulation via dietary exposure1. The European Food Safety Author-

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ity (EFSA) has recently established a reference threshold of 0.03 mg OTA/kg feed for adverse health effects in growing chickens and hens2.

This study investigated the effectiveness of an OTA amidohydrolase (OAH; OCHRAzyme®) as a feed additive to detoxify OTA by enzymatically converting it into the non-toxic metabolites ochratoxin α (OTα) and phenylalanine within the digestive tract of broiler chickens. In a 36-day feeding trial, 192 one-day-old broilers were assigned to four dietary groups with eight pens per group: (i) negative control, (ii) 1 mg/kg OTA, (iii) 1 mg/kg OTA plus 15 U/kg OAH, and (iv) 1 mg/kg OTA plus 75 U/kg OAH. Growth performance was monitored, and plasma and kidney samples were collected at the end of the trial to assess OTA and OTα concentrations as biomarkers of exposure. Hypothesis of this study was, that OTA levels in plasma and kidney were significantly reduced in the enzyme groups (iii and iv) compared to the toxin group (ii). Statistical analysis was done with ANOVA and non-parametric comparisons.

OAH supplementation resulted in significant reductions in OTA levels ($p < 0.0001$) and corresponding increases in OTα ($p < 0.0001$) in both plasma and kidney samples. Moreover, the detrimental impact of OTA on final body weight and feed intake was effectively mitigated by both OAH dosages ($p < 0.01$). These findings highlight the promise of OAH as a preventive feed additive strategy to counteract OTA-induced health and performance challenges in poultry production.

[1] Malir, F.; Ostry, V.; Pfohl-Leszkowicz, A.; Malir, J.; Toman, J., Ochratoxin A: 50 Years of Research. Toxins, 2016, 8(7), 191. doi: 10.3390/toxins8070191
[2] EFSA Journal 2023, 21(11), 8375
Keywords: Mycotoxins; Ochratoxin A; Biomarker

Nutrition: Feed Additives VI

4155 The Role of Feed Additives in Shaping the Future of Poultry Production in the Antibiotic-Free Era

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Modern poultry production faces the challenge of increasing productivity while improving animal health, reducing the use of antibiotic growth promoter (AGP), and minimizing environmental impact. In this context, feed additives have become essential tools for supporting gut health, enhancing nutrient utilization, and improving overall production efficiency. A wide range of functional additives have demonstrated the ability to modulate intestinal microbiota, improve intestinal integrity, and support immune and metabolic functions in poultry. These strategies may contribute to improvements in growth performance, intestinal homeostasis, egg production, eggshell quality, and carcass yield. However, no single product appears capable of fully replicating the benefits historically associated with AGP, and therefore a combination of nutritional and management strategies will likely be required. Consequently, sustainability, environmental impact, and the potential risk of antimicrobial resistance should be carefully considered when developing and regulating alternatives to AGP in animal production

4156 Evaluating Bioavailable Silicium (PoulSil®) Supplementation for Enhanced Egg Integrity and Production Performance in Laying hens: Insights from Controlled and Commercial Trials

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Optimizing layer nutrition to improve egg quality and maintain productivity remains a key challenge in modern egg production. Among innovative feed strategies, bioavailable silicium (PoulSil®) has shown potential to influence structural and internal egg integrity. The present work combines results from a controlled research trial and a large-scale commercial validation to assess the effects of PoulSil supplementation on egg performance and quality in laying hens.

In the controlled study, 1,170 hens aged 26–30 weeks were assigned to three dietary treatments (T1: control; T2: control + PoulSil 150 g/ton; T3: control + PoulSil 450 g/ton) over four weeks. Performance indicators and egg quality traits (egg weight, shell strength, vitelline membrane strength, Haugh Units) were recorded and analyzed ($\alpha=0.05$). PoulSil supplementation significantly increased egg weight (T2: 58.5 g; T3: 58.4 g; T1: 57.9 g; $P=0.004$) and vitelline membrane rigidity (T3: 9.30 mN/mm vs T1; $P=0.034$), suggesting improved internal egg integrity. While overall zootechnical performance remained the same.

To validate these findings under field conditions, a commercial trial was carried out on 5,174 layers aged 52 weeks, divided into two flocks (Control: receiving a standard layer diet vs. PoulSil 150 g/ton). PoulSil was supplemented for five weeks (53–57 weeks). Production parameters, including laying rate, egg weight, feed intake, feed conversion ratio, and the incidence of cracked and shell-less eggs, were monitored. PoulSil supplementation led to a significant reduction in cracked and shell-less eggs (control: 1.19% vs. PoulSil: 0.59%, $P<0.001$), indicating a clear improvement in eggshell and membrane integrity. No significant differences were observed in feed intake ($P=0.510$) or laying rate ($P=0.466$), confirming that the improved egg integrity was achieved without compromising productivity or feed efficiency.

Collectively, these results demonstrate that dietary inclusion of PoulSil can enhance internal egg resilience and reduce shell defects in both experimental and commercial conditions. The consistency between controlled and field data supports the use of bioavailable silicium as a practical nutritional strategy to strengthen egg integrity and minimize egg losses in commercial layer operations.

Keywords: bioavailable silicium; egg integrity; shell quality; layer performance

4157 A nanovesicle-rich apple extract provided through drinking water improves feed efficiency and intestinal homeostasis in broiler chickens

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Plant-derived nanovesicles/exosomes are a group of molecules that is receiving growing attention because of their peculiar properties, such as the capacity of bypassing biological barriers and carrying small RNAs, messenger RNAs, cytosolic proteins, and vitamin precursors. The aim of this study was to evaluate the effects of the drinking water administration of a nanovesicle-rich apple extract on the growth performance and intestinal inflammation in broiler chickens. A total of 450 day-old male chicks were divided into three experimental groups (6 replicate pens of 25 birds per group): CON group, receiving no water supplementation, and EX2 and EX5 in which the drinking water was supplemented with 2 and 5% apple extract. All groups were fed the same 3-phase basal diet and birds were weighed at the end of each feeding phase (14, 28 and 41 days). At slaughtering (41 days), intestinal samples were collected

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for evaluating the gene expression of pro-inflammatory cytokines. Data were analyzed by means of one-way ANOVA considering the water supplementation of the apple extract as independent variable. The water supplementation of apple extract did not alter the growth performance in the starter and grower phases. In the finisher phase, EX2 and EX5 groups showed lower daily feed intake (171.4 and 174.8 vs. 184.3 g/bird/day, respectively for EX2, EX5 and CON group; P<0.001) and improved feed conversion ratio (1.548 and 1.561 vs. 1.659, respectively; P<0.001). Considering the overall trial period (0-41 days), EX2 and EX5 groups achieved similar final body weight (3,126 and 3,180 vs. 3,133 kg/bird, respectively for EX2, EX5 and CON group; P=0.59) and a tendency for reduced feed intake if compared to CON (4.417 and 4.483 vs. 4.570 kg/bird, respectively; P=0.09), resulting in a significantly lower feed conversion ratio (1.436 and 1.430 vs. 1.486, respectively for EX2, EX5 and CON group; P<0.001). Mucosal transcriptomics revealed that apple exosomes induced a coordinated repression of pro-inflammatory cytokines, chemokines, and upstream innate immune sensors - including IL1β, IL6R, IL17A, CXCL8, TNFα, CCL20, TLR4, and MYD88 - indicating broad attenuation of NF-κB–driven inflammatory programs. Collectively, the data obtained in this study supports a model in which apple-derived nanovesicles/exosomes operate as multifunctional stabilizers of intestinal homeostasis, simultaneously dampening inflammatory signaling, enhancing regulatory immune tone, and reinforcing barrier architecture, which in turn could be responsible for the observed improvements of feed efficiency.

Keywords: broiler chicken; water; apple extract; productive performance; inflammation

4158 EVALUATION OF BLACK WATTLE CONDENSED TANNIN SUPPLEMENTATION ON THE REPRODUCTION OF BROWN LAYER BREEDERS

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The search for natural alternatives to antimicrobial growth promoters in poultry production has intensified due to restrictions on antibiotics and the demand for safer, more sustainable food systems. In this context, condensed tannins—especially those extracted from black wattle (Acacia mearnsii)—stand out for their antioxidant, anti-inflammatory, and antimicrobial properties. Although widely studied in broilers and swine, their effects on the reproduction of laying breeders remain limited. Considering the importance of fertility, egg quality, embryonic viability, and offspring performance, condensed tannin supplementation emerges as a promising alternative for breeder nutrition. This study evaluated the effect of different levels of black wattle condensed tannin on the reproduction of brown laying hen breeders. Forty Red Rhode Island males housed individually and 216 females kept in groups of six per cage were used. The trial was conducted from 40 to 56 weeks of age, divided into four 28-day periods. Birds received corn–soybean diets according to the treatments: T1 – control; T2 – 50 g/ton zinc bacitracin; T3 – 500 g/ton condensed tannin; T4 – 1000 g/ton condensed tannin. Feed and water were offered ad libitum. Semen was collected weekly to assess volume, motility, vigor, and morphology. In females, feed intake, body weight, egg production, fertility, hatchability, embryonic mortality, and pecked eggs were evaluated. Body weight and feed intake were not affected by treatments (P>0.05). Infertility rate was highest in T2 (10.58%) and increased over time in T1. In contrast, T3 and T4 maintained low and stable infertility rates (1.26% and 0.75%), indicating a protective effect. Early embryonic mortality was also reduced in the tannin groups (P<0.0001), and the incidence of pecked eggs was lower between days 56–84 in T3 and T4 (P=0.0100). Hatchability was higher in T4 during the 1–28, 56–84, and 84–112 day periods (P<0.05), and total hatch rate was superior in T4 during the first three periods, especially between days 1–28 (70.15% vs. 53.51% in control; P=0.0245). Regarding semen quality, roosters supplemented with tannins (T3 and T4) showed greater semen volume and fewer abnormal sperm cells (P<0.0001), whereas T2 had the poorest results. Sperm motility and vigor did not differ among treatments (P>0.05). It is concluded that black wattle condensed tannin, particularly at 1000 g/ton, improves fertility, hatchability, and semen quality, representing an effective alternative to zinc bacitracin.

Keywords: Fertility; Hatching; Phytogenic; Feed additive; Antioxidant

4159 Effect of Aqueous Bitter Leaf (Vernonia amygdalina) Extract on Growth Response, Gut Histomorphology, and Serum Biochemistry of Cobb500 Broiler Chickens

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Objective:

To evaluate the effects of aqueous bitter leaf extract (ABLE) on growth response, feed efficiency, gut histomorphology, and serum biochemistry of Cobb500 broiler chickens.

Experimental Design:

One hundred and twenty day-old Cobb500 chicks were randomly assigned to four groups (A–D) of 30 birds, with three replicates of 10 birds. Group A served as control; Groups B, C, and D received ABLE at 2.5, 5.0, and 10.0 g/L in drinking water for six weeks.

Materials and Methods:

Birds were reared in battery cages with ad libitum feed and water. Feed phases included super starter (0–2 wk), starter (2–4 wk), and finisher (4–6 wk). Birds were vaccinated against Gumboro (weeks 1, 3) and Newcastle disease (weeks 2, 4). Growth parameters were measured weekly. Blood samples were collected at days 21 and 42 for liver, kidney, and lipid profile analyses. Duodenal and jejunal segments were collected, fixed, processed, and stained for villus height (VH) and crypt depth (CD) measurements.Ethical approval was obtained (UDUS/IACUC/2023/R04)

Statistical Analysis:

Growth data were analyzed using a 2-way repeated measures mixed model; carcass, serum, and histology data by one-way ANOVA with Tukey's test. Significance was set at $p < 0.05$.

Results:

At day 42, birds receiving 2.5 g/L ABLE had significantly higher body weight (2369 g), weight gain, and feed intake with improved FCR compared to control and 10 g/L groups ($p < 0.05$). Liver, kidney, and lipid profile parameters showed no significant differences ($p > 0.05$). Duodenal and jejunal VH, CD, and VH:CD ratios were not significantly different, though 5 g/L ABLE tended to increase VH and VH:CD ratio.

Conclusions:

ABLE at 2.5 g/L enhanced growth response and feed efficiency without adverse effects on organ function or gut histomorphology. Higher doses (10 g/L) reduced growth, suggesting dose-dependent effects. ABLE is a safe prebiotic supplement for broilers.

Keywords:

Bitter leaf, Vernonia amygdalina, broiler chickens, growth response, gut histomorphology

Keywords: Bitter leaf; Vernonia amygdalina; Broiler chickens; Growth

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response; Gut histomorphology

4160 Feeding effects of probiotic, synbiotic and organic acids as alternatives to antibiotics in broiler production

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The aim of the experiment was to investigate the feeding effects of dietary supplementations of prebiotic, synbiotic and organic acid as alternative to antibiotic on growth performance and carcass characteristics of broiler chickens. A total of 240 day-old Arbor Acres broiler chickens of either sex were randomly assigned to five dietary treatments each consisting of four replications and each replicate having 12 birds for 5 weeks of rearing period. The dietary treatments were (T1) control group with basal diet, (T2) Basal diet supplemented with antibiotic (at 250 gm/100 kg of starter as well as finisher ration), (T3) Basal diet supplemented with probiotic (at 10 gm/100 kg of starter as well as finisher ration), (T4) Basal diet supplemented with synbiotic (at 25 gm/100 kg of starter ration as well as finisher ration), and (T5) Basal diet supplemented with organic acid (at 150 gm/100 kg of starter as well as finisher ration). The birds were provided with ad-libitum feed and drinking water during the entire experimental period. The results revealed that the highest body weight observed in a probiotic group (1777.75± 6.4 g) , which was significantly ($p<0.05$) higher than that of control group (1700.75±7.44 g). Antibiotic, synbiotic and organic acid groups were showed comparatively (1671.10±13.19, 1697.65±6.18 and 1703.70 ± 5.61 g, respectively) lower body weight than probiotic group. A total feed intake did not show any significant ($p>0.05$) differences among experimental groups. There were no significant ($p>0.05$) differences in feed conversion ratio of broiler chickens in antibiotic, prebiotic, synbiotic and organic acid groups as compared with control group. The study further exhibited that non-significant ($p>0.05$) differences in the carcass traits with respect to dressing percentage, carcass percentage, heart weight, liver weight and gizzard weight, wing percentage, breast percentage, back percentage, thigh percentage, and drumstick percentage in Arbor Acres broilers among treatment gropus. The growth performance and percentage of carcass yield did not show any significant difference by the dietary inclusion of antibiotic, probiotic, synbiotic, and organic acid compared with un- supplemented control in a commercial broiler chicken. Therefore, it may be concluded that probiotic, synbiotic, and organic acid can be used as alternatives to antibiotic for broiler production.

Keywords: Probiotic, ; Synbiotic, ; Antibiotic,; Organic acids; Growth Performance

4161 Effects of aqueous Moringa oleifera leaves on growth performance and carcass characteristics of broilers (Cobb 500)

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The use of antibiotics as growth promoters in animal husbandry has been banned due to its concerns about the accumulation of their residues in animal tissues and the bacterial resistance they confer. In view of this situation, Between February and April 2024, a study was conducted at the Ferme AvicoleT3F of Makong with the aim of evaluating the effects of aqueous Moringa oleifera leaves on growth performance and carcass characteristics of broilers.. For that, a total of 150 day-old Cobb 500 strain chicks with respective average weights of 37 ± 0.09 g and 43 ± 0.03 g were used and allocated to 20 experimental units in a completely randomized design with 4 replicates of 7 to 8 chicks each for seven weeks. At the starter as well as the finisher phase, 5 experimental diets were formulated from a negative control ration (T0-) by adding, 0,25 g(T1), 0,50 g(T2), 0,75 g(T3) and 1g(T4) of aqueous Moringa oleifera extract. The main results revealed that at the starter as well as the finisher phase the supplementation of aqueous Moringa oleifera leaves at different rate did not significantly affect ($p>0,05$) the growth performance of broilers. On the other hand, the incorporation of aqueous Moringa oleifera leaves at a rate of 0,25 g/ kg significantly affect the carcass yield 1 and 2 of the animal. Furthermore, whatever the incorporation rate in comparison with the control ration, the inclusion did not have a significant effect on the parameters of the immune system. The cost of food consumption and the cost of production per kilogram of chicken turn to increase with the increase rate of inclusion regardless of the different stage of production. In conclusion, the results of this study revealed that the inclusion of aqueous Moringa oleifera leaves at a rate of 0, 25 g/kg made it possible to obtain the best carcass yields. Aqueous extract of Moringa oleifera leaves in the food has no beneficial effect on growth performance but makes it possible to produce meat with less bacterial residue for the benefit of health conscious consumers.

Keywords: Moringa oleifera, ; Aqueous extract; Growth performance; Phytobiotics; Broilers chicken

4162 Metabolism of β-carotene in Laying Hens and Its Effects on Follicular Atresia

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The quantity and quality of follicles in the ovary of laying hens are important factors affecting the laying performance. Previous studies have shown that feeding β-carotene has a certain protective effect on mammalian ovarian health. In this study, the deposition and metabolism law of β-carotene in the body of laying hens was analyzed. The results showed that the amount of β-carotene in egg yolk reached a plateau after two weeks of supplementation. It was found that with the increase of β-carotene in the diet, the deposition of β-carotene in yolk was gradually increased, but the amount of lutein and zeaxanthin were significantly decreased. The different forms of vitamin A in yolk and the content of β-carotene in serum, liver and ovary of laying hens were significantly increased respectively. When diet supplemented with 60 mg/kg of β-carotene, the content of retinol and retinyl propionate in serum, liver and ovary were significantly increased. But the β-carotene supplemented 120 mg/kg, the content of retinol and retinyl palmitate in serum of laying hens were significantly decreased compared with β-carotene 60 mg/kg group, indicating that long-term high dose of β-carotene intake of hens could lead to negative feedback regulation of β-carotene conversion to vitamin A. When study further explored the effect and possible mechanism of β-carotene on follicular atresia of laying hens at late laying period. β-carotene may restrain follicle atresia of laying hens at late laying period by up-regulate anti-apoptotic gene BCL-2, down-regulated pro-apoptotic gene Caspase-3 and Caspase-9. At the same time, β-carotene could be deposited in laying hens and enhance the antioxidant capacity of laying hens, and could also regulate the oxidation of polyunsaturated fatty acids and stimulate the secretion of steroid hormones. The

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above physiological processes work together to alleviate follicular atresia of laying hens at the late laying period.
Keywords: β-carotene; vitamin A; laying hens; follicle; supercritical fluid chromatography

4163 Moriga oleifera leaves as an organic feed additive in broiler diets

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Moringa oleifera a miracle plant which is a member of the Moringaceae family distributed in the tropical and subtropical areas around the world. The leaves of M. oleifera are highly nutritious, being a good source of protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals, antioxidant, flavonoids and various phenolics compounds. Based on their nutritional and biochemical properties moringa leaves was used in animal or poultry diets for producing value added food. The goal of modern poultry production approaches is to maximize profits at the minimum reasonable cost of production, about 60–70% of this production cost. M. oleifera leaf (MOL) meal has been shown to improve growth performance, and potentially even improve the quality of value-added meat when added to poultry diet. Therefore, the objectives of the study was to evaluate the feeding effect of M. oleifera leaf meal on the growth performance, oxidative stability and fatty acid profiles of broiler meat. A total of 240 old mixed sex Cobb 500 broiler chicks were split into 4 dietary treatments for four weeks, with four replications having 15 chicks in each replication i.e. 60 birds in each treatment group. Four dietary treatment groups were produced from the basal feed as follows: control (T1), MOL 0.5% (T2), MOL 1% (T3), and MOL 1.5% (T4). The birds were randomly distributed in each pen, and the data were analyzed using the SPSS IBM statistical package 27. The analytical measurements were done in triplicates, and the results were presented as the average of 3 analyses ± the standard deviation (SD). The final body weight (BW) gain of broiler was significantly ($p<0.05$) higher in the T3 and T4 groups, and the feed conversion ratio improved in the T3 group (1.68). The lowest ($p \leq 0.05$) thiobarbituric acid-reactive substances values (TBARS) of breast and thigh were obtained in T3 groups compared to other group after the second week of preservation. Regarding fatty acid profile of breast and thigh meat, the omega-3 fatty acid levels, such as those of linolenic and docosahexaenoic acid (DHA), were increased significantly ($p<0.05$) in the additive's groups. Feeding MOL at 1 and 1.5% increased omega-3 fatty-acid levels by 1.35 and 1.46%, respectively for T3 and T4 groups. Overall, the results indicate that the addition of 1 to 1.5% MOL to broiler feed could be an effective way to improve meat fatty-acid profile and reduce cholesterol in meat.
Keywords: Broiler diets; Moringa oleifera; Thiobarbuturic acid; docosahexaenoic acid; linolenic acid

Nutrition: Vitamins & Minerals II

4164 Broiler responses to the interaction between dietary total calcium and digestible phosphorus levels during the grower phase

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Current nutritional models require refinement based on digestible phosphorus (dP) to more accurately predict broiler requirements and optimize performance. Given the close interaction between calcium (Ca) and P, Ca levels must also be carefully considered. The objective of this study was to determine the interactive effects of dietary total Ca (tCa) and dP levels on broiler growth performance, tibia mineralization, blood mineral concentration, footpad dermatitis incidence, and litter characteristics from 10 to 28 days of age. A total of 3,200 one-day-old male Ross 308 broilers were fed a common diet (0.70% tCa and 0.40% dP) from 0 to 10 days of age. From 10 to 28 days, nine experimental diets were offered following a 3 × 3 factorial scheme with three levels of tCa (0.51, 0.68, or 0.85%) and three levels of dP (0.23, 0.31, 0.39%) as the main factors. Each treatment was randomly assigned to an experimental unit of 40 chicks, with nine replicates per treatment, except for the diet containing 0.68% tCa and 0.31% dP, which had eight replicates. Data were analyzed using a randomized complete block design in which treatments were combined according to the factorial scheme aforementioned. Treatment means were compared using Tukey's test, with differences considered significant at $P < 0.05$ and trends at $P<0.10$. From 10 to 28 d of age, a significant tCa × dP interaction was observed ($P < 0.001$) for feed intake (FI) and BW gain, and a tendency ($P = 0.09$) for feed conversion ratio (FCR). Increasing tCa in combination with low dP reduced FI and consequently growth, with the greatest negative effect occurring at 0.85% tCa and 0.23% dP. Birds receiving an unbalanced diet relative to CVB requirements in tCa (0.85%) and dP (0.23%) showed a reduction of 11.6% in BW gain, and 9.2% in FI compared to the control diet (0.68% tCa and 0.31% dP). Tibia ash was also influenced by the tCa × dP interaction ($P = 0.002$). Low dP reduced ash content, particularly when tCa was high, whereas higher dP improved bone mineralization under elevated tCa levels. Bone breaking strength increased with tCa level ($P < 0.001$). Litter moisture at 27 d was unaffected by high levels of tCa ($P = 0.11$) and tended to decrease as dP increased ($P = 0.09$). A significant interaction between tCa and dP was detected for serum P at 28 d ($P < 0.001$); birds receiving higher tCa showed elevated serum P, accentuated when dietary dP was low. No significant differences were found for footpad dermatitis incidence. In conclusion, the interaction between dietary tCa and dP strongly influenced broiler responses. Excessive tCa combined with low dP impaired growth performance and bone mineralization and tended to worsen litter quality from 10 and 28 d of age.
Keywords: Calcium; Broiler performance; Digestible phosphorus; Bone mineralization; Grower phase

4165 Jefo Gut Harmony enhances growth performance, antioxidant status, and intestinal integrity in broiler chickens

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Jefo Matrix Technology (JMT), a triglyceride-based encapsulation system, is the core technology behind the Jefo Gut Harmony (GH) concept for microencapsulating vitamin and trace mineral (VTM) premixes. The concept ensures micronutrient stability during feed processing and storage while enabling a progressive release along the intestinal tract for optimal absorption and bioavailability. The study aimed to evaluate the effects of GH on growth performance, antioxidant status, and intestinal integrity in broiler chickens. A total of 1,200 as-hatched Ross 308 broilers were randomly allocated to three dietary treatments for 33 days: a basal diet containing a standard commercial vitamin and trace mineral premix (control), and two diets where the free premix was replaced by GH at 25% or 50% of the control premix equivalence. Diets were corn–soybean meal based. Data were analyzed using one-way ANOVA with

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significance declared at $P \leq 0.05$. Five birds per treatment were randomly selected on day 33 for blood biochemistry and gene expression analyses. Final body weight was significantly higher ($P < 0.0001$) in birds fed 50% GH than in those fed 25% GH or the control, corresponding to distinct superscript groupings (a, b, and c, respectively). The 50% and 25% GH groups showed 11.8% and 8.5% increases over the control body weight of 2051 g, respectively. Feed conversion ratio was significantly ($P=0.012$) improved by 6 (1.40) and 7 (1.39) points for the 25% and 50% GH groups, respectively, compared to the control FCR of 1.46. The joined improvement in body weights and FCR allowed for significant ($P<0.0001$) improvements to the European Production Efficiency Factors of both GH treatments compared to the control, where 25% and 50% GH treatments showed an improvement of 13% and 16%, respectively, compared to the control which had an EPEF of 393. Expression of hepatic and intestinal antioxidant (SOD, GPX), immune (IL-10), nutrient-sensing (mTOR, IGF-2), and epithelial integrity (FABP2) genes increased significantly ($P < 0.0001$) with GH inclusion, where the 50% group showed the highest expression across all markers, with 25% displaying intermediate yet distinct values compared to the control. Blood biochemistry reflected the same trend, where the total antioxidant capacity rose in both GH groups, while malondialdehyde, ALT, AST, creatinine, uric acid, triglycerides, and VLDL declined, and HDL increased, all in a dose-dependent manner ($P < 0.05$). Overall, both Gut Harmony inclusions improved oxidative status, lipid metabolism, and gut health compared with the control, with the 50% level providing the greatest response and 25% yielding intermediate but significant benefits under commercial-like conditions.

Keywords: Gut Harmony; Broiler; Nutrition; Vitamins and Trace Minerals; Premix

4166 Influence of nano form of dicalcium phosphate at reduced dietary levels on mineral balance and bone mineralization in broilers

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PPhosphorus (P) represents the third most costly nutrient component in poultry diets, necessitating improved efficiency of its utilization from inorganic sources such as di-calcium phosphate (DCP) to achieve sustainable poultry nutrition. Nanotechnology offers a promising strategy to enhance P utilization efficiency. In this context, an experiment was carried out to evaluate the effects of substituting conventional dietary DCP with nano-sized DCP at reduced inclusion levels on mineral balance and bone mineralization in broiler chickens. A control diet containing rock-derived conventional DCP was formulated to meet the P requirement, while five experimental diets incorporated nano-DCP at 90, 80, 70, 60, and 50% of the conventional DCP inclusion level. Calcite powder was added to satisfy the remaining calcium (Ca) requirement, and a constant Ca-to-available P ratio was maintained across all diets. Each dietary treatment was allotted to six replicates of ten unsexed Cobb broiler chicks and fed for a period of 35 days. A metabolism trial was conducted during the terminal three days using two birds per replicate, following the total collection method in metabolic cages. Feed, excreta, and tibia samples were analyzed for Ca and P concentrations using inductively coupled plasma–optical emission spectroscopy (ICP-OES), and the data were subjected to statistical analysis using SPSS version 16 with Tukey's post-hoc test. The findings indicated a significant reduction ($P < 0.01$) in Ca and P excretion at lower nano-DCP inclusion levels (50 and 25%) compared with the conventional DCP group. However, Ca and P retention expressed as mg/day, percentage of intake, and mg/kg body weight did not differ significantly ($P > 0.05$) among treatments. On day 35, two birds per replicate were sacrificed for tibia morphometric and mineralization assessments. Tibia parameters including length, width, and weight showed no significant differences ($P > 0.05$) among dietary treatments, and tibia breaking strength, total ash content, as well as Ca and P contents was comparable across all groups. It was concluded that conventional DCP in broiler diets can be effectively replaced with nano-DCP at 50% of the standard inclusion level, resulting in improved Ca and P utilization without adversely affecting bone mineralization. Further research is required to establish optimal inclusion levels of nano-DCP relative to conventional DCP and to elucidate the mechanisms governing its effects on mineral bioavailability, skeletal integrity, and P excretion, thereby confirming its potential contribution to environmentally responsible poultry production.

Keywords: Broilers; Bone mineralization; Mineral balance; Nano di-calcium phosphate; Phosphorus

4167 Effect of nano form of dicalcium phosphate at lower dietary levels on growth response, gut health and immunity in broilers

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Phosphorus (P) is a major mineral essential for energy metabolism and skeletal development in birds. The supplementation of exclusive P sources such as di-calcium phosphate (DCP) is a common practice in poultry diets and strategies to improve the P availability in such sources is required for sustainable poultry production. In this context, a study was undertaken to assess the effect of replacing conventional DCP with nano from of DCP at lower dietary inclusion levels on growth performance, gut health and immunity in broilers. A total of 360 day-old broiler chicks (straight run, Cobb strain) were allotted into six dietary treatments and each treatment consisted of 6 replicates of 10 chicks each. The control diet contained rock DCP to meet P requirement while five test diets contained nano DCP at 90, 80, 70, 60 and 50 % of rock DCP inclusion level. The breeder's nutrient specification with a constant Ca to available P ratio was followed to prepare diets for pre-starter (1-10 days), starter (11-21 days) and finisher (22-35 days) phases. The results indicated that there was no significant difference ($P>0.05$) in body weight gain, feed intake, feed conversion ratio and survivability of broilers among different groups during different phases and cumulatively. The histomorphometry study (12 birds per treatment, 35 d age) indicated that the nano DCP at lower levels did not affect the villi length and crypt depth of duodenum, jejunum and ileum. The serum antibody titres against Newcastle disease and infectious bursal disease virus also remained comparable among different treatments. It was concluded that nano DCP at 50 % inclusion level of conventional DCP in diets does not compromise with the growth repose, gut health and immunity in broilers. However, more comparative studies are required to quantify the bioavailability of nano-DCP relative to conventional DCP and other inorganic or organic P sources including its interaction with nutrient absorption and gut microbial populations to confirm its possible role in sustainable and eco-friendly poultry production.

Keywords: Broilers; Growth; Gut health; Nano di-calcium phosphate; Phosphorus

4168 Copper Nanoparticles: boosting broiler growth and immunity while ensuring low toxicity in a dose-dependent manner

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Copper (Cu) is essential for maximum growth in broiler chickens,

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and its nanoparticles (Cu-NP) incorporated into feed improve growth, immunity, and gut microbiota. However, the excess usage of Cu in feed causes economic losses and human health hazards after consumption. This study aims to determine overall production performance, gut health, immunological parameters, and toxicity level by determining residual concentrations of Cu in different organs, excreta, and histopathological examination in broiler. Hence, it was assumed that Cu-NP could be used for boosting growth and immunity along with ensuring low toxicity in broiler. 160 broiler chicks were randomly divided into five treatment groups: T0, T1, T2, T3, and T4, with four replications for each group. The T0 group received basal diet; the T1, T2, and T3 groups received 5, 10, and 15 mg/L Cu-NP (<100 nm) powder, respectively, while the T4 group received 10 mg/L coarse Cu powder.Cu-NP were added to the drinking water prior to supply, and complete consumption of the water was ensured to confirm the intake of Cu-NP. Similar iso-nitrogenous and iso-caloric starter (1 to 14 days) and grower (15 to 28 days) diet were used having 3000Kcal/kg, 22% CP and 3100Kcal/kg and 21% CP respectively. Weekly live weight, weekly feed consumption, FCR, mortality and accessory organs like liver, bursa, thymus weight were recorded. Sample from liver, ileum part of intestine and caeca swab was collected at 4th week of age to evalute fibrosis, villi health, count Salmonella sp. and E coli sp. Data were subjected to analysis of variance (ANOVA) using SPSS Statistics version 26, and means were compared using Dancan Multiple Range Test. Results showed a significant improvement in production parameters ($P<0.05$), including body weight gain, feed conversion ratio, dressing percentage, and meat yield in the T3 group.The highest final weight of the bird in the T3 group was 1775 g, whereas the commercial recommendation was 1600 g. Moreover, Cu-NP significantly ($P<0.05$) improved the liver and bursa of fabricius weight and the villi health (villi length and width). Importantly, Cu-NP residual concentration was significantly ($P<0.05$) lower, while coarse Cu was found in excreta. The E. coli and Salmonella spp. count was significantly ($P<0.05$) lower in T3 compared to others. The results of the study demonstrated the beneficial effects of supplementing Cu-NP in a dose-dependent manner, and 15 mg Cu-NP/L drinking water showed the most promising outcome in growth performance, gut microbiota, with minimal toxicity. So, using Cu-NP supplementations with specific doses can be a wonderful source of growth-promoting agents, gut health, and immunity stimulators in a sustained approach.
Keywords: Nano copper; Gut health; Immunity; Broiler; Toxicity

4169 Effect of in ovo feeding and supplementation of inorganic and nano zinc on the colour, texture and chemical composition of breast and thigh muscle of commercial broilers

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Comprehensive studies on supplementation vis-à-vis in ovo feeding of zinc in commercial broilers are scanty. Thus, a study was conducted to assess the effect of in ovo vis-à-vis supplementation of inorganic and nano zinc on the performance of commercial broilers. Fertile eggs of commercial broiler breeder birds were set for incubation. On 18th day of incubation, eight hundred and sixty fertile eggs were divided into four groups: un-injected control (UC), sham-injected group (injected with 0.1 ml of distilled water) (SC), in ovo injected with ZnSO4 (75 µg/egg in 0.1 ml distilled water) (INIZ) and in ovo injected with nano Zn (50 µg/egg in 0.1 ml distilled water) (INNZ). After hatching, one hundred and eight day-old chicks from aforesaid each group of incubated eggs were further divided into three subgroups, each with three replicates and 12 birds in each replicate. One subgroup received a basal diet (BIS, 2007; broiler starter diet, 22% CP and 3100 ME till 3 weeks and thereafter broiler finisher diet, 20% CP and 3200 ME till 6 weeks), while the other two received a basal diet with ZnSO4 @ 110 ppm/L and basal diet with nano Zn @ 0.4 ppm/L administered in drinking water. There was no significant difference in the colour and texture of breast and thigh muscle among the different treatment groups after in ovo feeding or supplementation with two forms of zinc. The chemical composition of breast and thigh muscles of birds at 6 weeks of age showed no significant differences in percent crude protein, and ether extract after in ovo feeding and/or supplementation with two forms of zinc. However, total ash (%) in breast and thigh muscle was significantly lower ($P<0.001$) in birds of INNZ group compared to UC and INIZ. Interaction of in ovo feeding and supplementation of nano zinc revealed significantly lower ($P<0.001$) total ash % in breast and thigh muscle compared to un-injected basal diet group. Percent MUFA and omega-6 fatty acid was significantly higher ($P<0.05$) and percent SFA was significantly lower ($P<0.05$) in breast muscle of INIZ birds compared to UC and INNZ group birds. In ovo feeding along with dietary supplementation of inorganic zinc resulted in significantly lower percent SFA where as in ovo feeding along with dietary supplementation of nano zinc resulted in significantly higher percent omega-3 fatty acids in breast muscle. Supplementation of inorganic zinc resulted in significantly lower ($P=0.019$) percent SFA and significantly higher ($P<0.001$) percent MUFA in thigh meat of broilers whereas nano Zn supplementation resulted in numerically higher percent PUFA and omega-6 fatty acids and significantly higher omega-3 fatty acids in thigh meat of broilers.
Keywords: Zinc; Nano zinc; Chemical Composition; broilers; Meat texture

Physiology II

4170 Dynamic regulation of calcium and phosphorus homeostasis in commercial layers: impacts of age on the daily lay cycle

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Commercial layers are kept in extended production through 100 weeks-of-age, exacerbating economic and welfare concerns related to reduced eggshell quality and skeletal integrity in older hens. These changes are due, in part, to alterations in mineral homeostasis that make uptake and utilization of Ca and P less efficient as hens age. Recent studies have revealed physiological adaptations that support the onset of egg production and investigated how these systems change over the daily lay cycle and throughout an extended production period. At the onset of lay, increased responsiveness to hormones regulating Ca and P homeostasis is evident by higher levels of receptors and binding proteins in the intestine, kidney, and shell gland. The shell gland was identified as a novel target of parathyroid hormone (PTH), calcitonin (CALC), 1,25(OH)2D3, and fibroblast growth factor (FGF) 23 based on changes in expression of PTH1R, CALCR, VDR, and FGFR1 – 4 that occurred between 18 and 31 weeks-of-age and over the daily lay cycle. Age-related declines in the expression of several of these receptors were observed in hens over 80 weeks, further suggesting that these hormones play a critical role in the shell gland's ability to efficiently use Ca and P for eggshell mineralization. Candidate plasma membrane and intracellular mineral transporters that support the hen's dynamic capacity for eggshell mineralization

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were identified, with changes related to hen age and the daily lay cycle observed in the intestine, kidney, and shell gland. Higher circulating levels of active 1,25(OH)2D3 and lower circulating levels of inactive 24,25(OH)2D3 are associated with the onset of lay, and as hens age, changes observed throughout the daily lay cycle are disrupted such that higher levels of 24,25(OH)2D3 and lower levels of 1,25(OH)2D3 are present at times of peak eggshell mineralization in hens over 80 weeks when compared to younger hens. Daily patterns in circulating ionized Ca (iCa) and total P (tP) reflect periods of mineral utilization for eggshell formation, medullary bone repletion, and transitions between the two. Reductions in plasma iCa are apparent once the egg reaches the shell gland, with a sharp decline during peak eggshell mineralization. Conversely, tP is highest at this time, as levels rise when medullary bone is broken down to support calcification. Similar to what was observed for vitamin D3 metabolites, disruptions to the daily circulating patterns of iCa and tP in older hens were apparent and are likely driven by altered expression of mineral transporters in the kidney and shell gland. Altogether, these physiological changes highlight that older hens have a reduced ability to efficiently transition between mineral utilization for shell deposition or bone repletion, suggesting that strategies aimed at enhancing this transition could help alleviate declines in eggshell and skeletal quality that occur towards the end of an extended production period.

4171 Organ-specific antioxidant and heat shock proteins response to extreme heat stress in broilers

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As global temperatures rise, the adverse effects of extreme heat stress (EHS) are intensifying, with temperatures above 35°C proving fatal to broilers. The liver and gut are particularly sensitive to thermal stress. Since high temperatures induce oxidative stress, this study evaluated the molecular responses of the liver and gut under acute and prolonged EHS conditions. At 28 days of age, 252 broilers were randomly assigned to Control (CON) and EHS groups, each comprising 18 cages (7 birds per cage). From days 29–35, the EHS room temperature was gradually increased by 3.5°C per hour to 35°C, maintained for 3 hours, and then reduced to 21°C over 1 hour (total 8-hour exposure). The CON birds were maintained at 21°C throughout the trial. Hepatic and jejunal tissues were collected from six randomly selected birds per treatment at 0, 4, and 8 hours of EHS exposure on days 1 and 7 to analyse mRNA expression of heat shock proteins (HSPs) and antioxidant genes. On day 1, jejunal gene expression was more responsive under EHS, showing significant upregulation ($p < 0.05$) of HSP70, HSP90, NADPH oxidase 1 (NOX1), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase 1 (GPX1) compared to CON. HSP90, NOX1, and GPX1 exhibited significant hour effects ($p < 0.05$), with the highest expression observed at 8 hours. HSP90 and GPX1 displayed significant interaction effects (EHS × hour; $p < 0.05$) in the jejunum. Hepatic gene expression showed hour effects, and HSP70, HSP90, NOX1, and NOX4 were downregulated after 8 hours ($p < 0.05$), while GPX1 was elevated ($p < 0.05$). By day 7, only jejunal HSP90 remained significantly upregulated in the EHS group ($p < 0.05$) compared to CON, whereas time-dependent expression changes were more evident. NOX1, NOX4, NRF2, SOD, and CAT showed higher expression at 4 and 8 hours ($p < 0.05$). Interaction effects were seen in HSP70 and HSP90, peaking in the EHS group after 4 hours of exposure ($p < 0.05$). Hepatic SOD, CAT, and GPX1 were upregulated in EHS birds ($p < 0.05$). Notably, hepatic HSP90 showed an interaction effect and was most strongly expressed in the EHS group after 4 hours of exposure ($p < 0.05$). Overall, the jejunum exhibited a stronger and earlier mRNA transcription response to acute EHS compared to the liver, highlighting its sensitivity to environmental fluctuations. At day 7, the marked upregulation of hepatic and jejunal HSP90, particularly at 4 hours of EHS, underscores its central role in cellular defence against thermal stress. Collectively, these results suggest that oxidative responses in the jejunum under EHS may parallel those of the liver.

Keywords: extreme heat stress; broilers; gene expression; heat shock protein; jejunum

4172 Home is where the hen is: How housing transitions affect reproductive and sress-related gene expression in the brain of laying hens

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Laying hen housing systems vary based on bird age, production stage, and producer preference. Currently, no system optimally balances hen welfare, productivity, and environmental impact. While numerous studies have examined how housing affects egg characteristics, space use, and labor, limited research has explored how rearing pullets in one housing type and transferring them to another affects brain mRNA expression of hormones related to stress and puberty. Our objective was to evaluate how housing transitions affect reproductive and stress-related gene expression in the brain of laying hens. At 17 weeks of age (WOA), 400 pullets reared in simple floor rooms were randomly assigned to remain in floor housing or to multi-tier aviary rooms, where they stayed until 85 WOA. Brains were collected from six birds at 17 WOA (before transfer) and from six birds per housing treatment at 25, 55, and 85 WOA. The diencephalon (DI) was microdissected for qRT-PCR analysis of genes involved in puberty, egg laying, and stress regulation. Data were analyzed using a generalized linear mixed model (GLIMMIX procedure, SAS v9.4) with a lognormal distribution to examine the effects of age, housing type, and their interaction. Pairwise comparisons were performed using Tukey's. $P \leq 0.05$ was considered significant. Relative opsin-2 (OPN2) expression was significantly higher in floor birds compared to aviary birds ($p=0.0364$) and was higher at 55 WOA compared to 25 WOA ($p=0.0201$). OPN2, a red light sensitive DBP, may reflect differences in lighting or developmental conditions. Relative corticotropic releasing hormone (CRH) expression was not affected by age or treatment. However, arginine vasotocin receptor (AVT-R1) expression was significantly lower in aviary birds compared to floor birds ($p=0.0425$) and was higher at 55 WOA compared to 25 WOA, suggesting a down regulation of expression due to prolonged stress to housing change from pullet to hen. Overall, these results suggest that maintaining consistency between pullet and layer housing may improve welfare outcomes.

Keywords: layers; reproduction; stress; housing; deep brain photoreceptors

4173 Heart Rate as a Welfare Indicator in Commercial Broilers During Transport: A Novel Non-Invasive Monitoring Approach – A Proof of Concept

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Transport represents a critical yet often underestimated phase in broiler production, during which birds experience multiple

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stressors that can compromise welfare and product quality. Continuous heart rate (HR) monitoring provides an objective physiological measure of stress, yet non-invasive systems suitable for commercial settings are limited. This study presents a proof of concept for a novel, non-invasive monitoring system developed in collaboration with novu.track GmbH to record HR in freely moving chickens during commercial transport. The lightweight (< 90 g) backpack device uses Ag/AgCl gel electrodes adapted from human medical technology, enabling continuous electrocardiogram (ECG) recording (250–500 Hz) without restraint or sedation. Microcontroller-based firmware (ESP32) allows real-time ECG acquisition and onboard data logging, with HR derived via a peak-detection algorithm. Validation against standard veterinary ECG equipment under controlled conditions in broilers and adult laying hens is ongoing. Field testing involved 24 broilers across six transport events, with synchronized video and environmental data collection. A preliminary analysis of HR-data and Temperature (Spearman-Correlation) showed a weak but statistically significant negative correlation between temperature and HR for one individual (Spearman's $\rho = -0.26$, $p = 0.026$), indicating a monotonic decrease of the measured parameter with increasing temperature. In contrast, no significant correlations were detected between temperature and HR-data of the other three individuals ($\rho = 0.19$, $p = 0.14$),($\rho = -0.03$, $p = 0.81$) and ($\rho = 0.21$, $p = 0.07$).
Full statistical analysis including data of the remaining twenty individuals will follow validation. These findings demonstrate the feasibility of continuous, non-invasive HR monitoring for real-time welfare assessment in broilers, offering new opportunities to identify and mitigate stressors during commercial transport.
Keywords: broiler welfare; transport stress; ECG monitoring; heart rate; poultry behavior

4174 SGLT1 is one of the key molecules for sensing sweet taste and inducing rewarding effect in chickens

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Taste perception determines what is edible and what is not. The aim of this study was to elucidate the mechanism underlying sweet taste sensing in chickens. In mammals, the T1R2/T1R3 heterodimer functions as the primary sweet taste receptor; however, birds lack a functional T1R2/T1R3 receptor because the t1r2 gene is pseudogenic. In a previous study, we suggested that chickens may sense sweet taste via sodium–glucose cotransporter 1 (SGLT1). In the present study, we generated chicken SGLT1 (cSGLT1)-expressing cells by transfecting a cSGLT1/pcDNA3.1(+) plasmid into human embryonic kidney 293T (HEK293T) cells. Using whole-cell patch-clamp recordings (n = 3–7 individual cells per treatment, seven treatments in total), we confirmed that cSGLT1-expressing cells were significantly activated by NaCl in the presence of glucose compared with empty vector-transfected cells ($P < 0.05$, unpaired t-test). This activation was abolished when NaCl was replaced with choline chloride ($P < 0.05$, unpaired t-test). cSGLT1-expressing cells were also activated by NaCl with galactose. In addition, significant activation was observed with NaCl and the non-metabolizable sugar methyl-α-D-glucopyranoside (MDG) under sodium-dependent conditions ($P < 0.05$, unpaired t-test). These experiments were one-time replicate. These results indicate that cSGLT1 is activated by sodium-dependent sugar transport. However, it remains unclear whether sweet taste perception induces rewarding effects in chickens. Identifying taste stimuli that elicit reward responses would provide important insights for improving animal welfare in the poultry industry. Therefore, we employed a conditioned place preference (CPP) test to assess rewarding effects in chicks. Three-day-old Rhode Island Red chicks were tested with various sweet solutions (glucose, galactose, fructose, sucrose, MDG, maltose, lactose) and water as a control (n = 5–9 birds per treatment, eight treatments in total). After conditioning, the time spent in the compartment paired with sugars was significantly increased for glucose, galactose, sucrose, MDG, maltose, and lactose compared with pre-conditioning values ($P < 0.05$, paired t-test). In contrast, fructose and water did not induce significant place preference. These experiments were one-time replicate. These results demonstrate that chickens receive rewarding effects from specific sugars, but not from fructose. Sucrose, maltose, and lactose are likely hydrolyzed into monosaccharides, some of which indirectly activate cSGLT1. Fructose, in contrast, is presumed not to be a substrate for cSGLT1. Collectively, these findings suggest that sugars transported by SGLT1, with or without caloric value, induce rewarding effects in chickens.
Keywords: taste; rewarding effect; sweet; SGLT1

4175 Adipokines as Key Energy Sensors: Integrating Metabolism and Reproduction in Poultry

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Reproduction in avian species is a complex physiological process regulated by the interaction between metabolic homeostasis and hormonal axes. While adipose tissue was long considered a mere energy reservoir, it is now recognized as a dynamic endocrine organ secreting adipokines such adiponectin, visfatin, and chemerin. This review highlights the divergent roles of these molecules between mammals and birds, noting that several mammalian adipokines remain unmapped in the avian genome. Research demonstrates that adipokines and their receptors are locally synthesized within the avian reproductive tract and embryonic annexes, showing significant fluctuations during egg formation and embryo development. Specifically, chemerin is highly abundant in egg albumen and amniotic fluid, where it exerts antibacterial properties and promotes angiogenesis. In ovo neutralization of the chemerin system significantly increases embryo mortality, suggesting its role as a vital survival factor. Conversely, in males, chemerin appears to negatively regulate fertility by inhibiting testosterone production and reducing sperm motility and concentration in roosters. Comparative studies between broiler and layer breeds reveal distinct expression patterns, yet confirm that the chemerin system is a common driver of embryonic growth. Ultimately, these adipokines serve as essential metabolic signals and could be potential biomarkers for improving egg fertility and quality of offsprings.

4176 Effect of Crossbreeding on Physiological Adaptability and Growth Regulation of the Pure and Reciprocal Crosses Between the Sasso and Wassachie Chickens

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The study evaluated hematological and hormonal responses (growth hormone [GH]) and insulin growth factor 1 [IGF1]) in purebred and reciprocal crosses of Sasso (S) and Wassache (W) chickens. Crosses from the parent stocks produced four genotypes (SS, WW, SW, and WS), each replicated three times. Eggs collected were hatched and reared for 12 weeks. Blood samples (six birds per genotype) collected at 12 weeks were analyzed for hematology using an automatic hematology analyzer. And samples collected on days 18, 19, and 21 of incubation and at weeks 2, 4, 6, 10,

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and 12 post-hatch were assayed for GH and IGF-1 using specific double-coated antibody kits. Data analysis using one-way ANOVA showed no genotype effect (P>0.05) on mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or platelet counts. However, the WS genotype exhibited higher (P<0.05) white blood cell counts, hemoglobin concentration, and hematocrit values, indicating enhanced immune response and oxygen-carrying capacity. The WW genotype recorded higher white blood cell counts than SS, suggesting better adaptability to environmental stress. Genotype effects (P<0.05) were observed for GH at day 19 of incubation and at weeks 2, 10, and 12, and for IGF-1 at day 18 and weeks 2, 4, and 12, reflecting episodic hormonal secretion. Overall, the WS genotype showed superior hematological performance and adaptability, highlighting the potential of crossbreeding for developing resilient dual-purpose chickens for tropical environments.

Keywords: Adaptability; Crossbreeding; Growth-hormone; Hematology ; IGF1-hormone

4177 Estimating genetic parameters for multivariate beak shape traits in a White Leghorn pure line population

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Selection of blunter beak phenotypes may help mitigate severe feather pecking damage to the benefit of hen welfare. However, previous work in pure line hens suggests that certain beak shape traits (e.g., beak tip curvature) may negatively correlate to egg production. The reason for this relationship is unclear. This study examined the influence of maxillary beak shape and size traits on egg production and quality traits and performed a genome-wide association study (GWAS) to identify candidate genomic regions and genes associated with maxillary beak shape and size. The maxillary beak shapes of 2,973 White Leghorn pure line hens and 203 roosters were analysed using geometric morphometrics. Beak overhang length (BOL; difference between maxillary and mandibular beaks) was also measured. Only female phenotype data was used to estimate genetic parameters. Heritability and genetic correlations were estimated using bivariate models in ASReml and GWAS was performed using univariate and multivariate linear mixed models implemented by GEMMA. Birds were genotyped using a 56K SNP array. After quality control, there were 38, 133 SNPS available for further analysis. Most beak shape variation was attributable to length, curvature, and depth. Surprisingly, sexual dimorphism was only apparent for beak size with males having larger beaks. Heritability estimates for hen beak shape and size traits ranged from 0.17-0.40. Maxillary beak curvature negatively correlated to egg weight (EW). Maxillary beak size was positively correlated with BOL, EW, BW, and feed intake. BOL positively correlated to BW and feed intake. The GWAS revealed significant SNPs on chromosomes 4 and 27 for BW and maxillary beak size. Candidate genes found within these regions have been associated with body size and growth traits in many domestic animal species (e.g., LCORL, IGF2BP1). Two SNPs approached significance on chromosomes 2 and 11 for beak overhang length. No significant SNPs were observed for any other beak shape traits. Overall, the results suggest that beak shape is a complex trait with many contributing genes. Genomic selection of less damaging beak phenotypes appears to be feasible without negatively impacting egg production or quality. However, the effect of beak shape traits on production in pure lines is inconsistent highlighting the need to investigate beak shape traits in multiple different genetic lines.

Keywords: severe feather pecking; geometric morphometrics; welfare; genetic selection; laying hen

4178 Regulation of mitochondrial content and function: lessons from poultry and other farm animals

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The mitochondrion has been implicated in numerous production traits including muscle mass and feed conversion efficiency. In general, farm animals have a skeletal musculature possessing a low mitochondrial content for their size. Broiler chickens artificially selected on feed conversion efficiency are canonical examples. Here, the pectoralis muscle possesses a mitochondrial content of just 2% based on planimetric analysis of electron micrographs. Contrast this with the 35% documented for the same tissue in highly athletic hummingbirds. The diminished content in chickens can still support the ATP demand of growth but with little spare capacity in place. It is as though the animal has been 'designed' for efficient production by an engineer intent on reducing unnecessary overheads. As part of a strategy to investigate relationships between individual variation in tissue mitochondrial content (using mtDNA copy number as a proxy) and performance phenotypes, high throughput duplex Taqman qRT-PCR assays have been developed. Clear coordination across sheep digestive tissues and chicken muscles have been discovered. In chickens, the low content individuals are both more muscular and fatter than their conspecifics. Finally, a mitochondrially anchored co-expression network built from RNAseq data from 91 bovine tissues has been reconstructed using the PCIT algorithm. This network retains only highly conserved pairwise associations and represents >50% of the mRNA encoding the mammalian mitoproteome. A number of biologically coherent clusters are detected including one bearing the mRNA and tRNA products of the mtDNA genome. Moreover, de novo predictions can be made for unannotated transcripts based on their presence in a particular cluster. This mitochondrial network can help metabolically interpret 'omics data from any tissue or species.

Keywords: mitochondrial content; comparative biology

4179 Role of vitamin D metabolites on avian T-cell proliferation

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Mammalian immune cells express 1α-hydroxylase (CYP27B1) to convert 25-hydroxyvitamin D3 (25(OH)D3) to its active form 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) as well as the vitamin D receptor (VDR), thus linking vitamin D metabolism and immunity. Unlike its role in the Ca metabolism, the actions of 1,25(OH)2D3 in the immune system are mainly autocrine. While several studies showed a modulatory effect of 1,25(OH)2D3 on the mammalian innate and adaptive immune system, its role in the avian immune system has only recently gained interest. In a previous study we could show that upon activation of B-cells, there was no change in CYP27B1 and VDR expression. On the other hand, activated T-cells did not alter CPY27B1 expression during the observation period of 24 h (different to mammals) while VDR expression increased within 6 hours (similar to mammals). As a next step, we tested whether the presence of 25(OH)D3 or 1,25(OH)2D3 has an impact on the proliferation of activated avian T-cells. The spleen of five 12-week-old pullets (White leghorn Line M11) fed commercial standard diets was collected and the lymphocytes were isolated by density gradient centrifugation. Cells from one bird were used for dose finding and method calibration. For dose finding 25(OH)D3 and 1,25(OH)2D3 were added to the cultures at final concentrations of 1, 10 and 100 nM . Non-treat-

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ed and non-stimulated cells served as controls. No effect on cell proliferation in response to T-cell receptor (TCR)-crosslinking was observed with all three concentrations of 25(OH)D3. In contrast, 1,25(OH)2D3 reduced proliferation at 10 and 100 nM but not at 1 nM. Based on these results, the 10 nM concentration was selected for subsequent experiments. These were done by using the cells of the other four birds which were activated and incubated as in the pilot trial with either control medium, medium containing 10 nmol 25(OH)D3 or 10 nmol 1,25(OH)2D3. Each assay was done in triplicates. After 48 hours, proliferation was measured using a commercial BrdU assay. Treatment comparisons were done by GLM. There was no difference between control and 25(OH)D3 treatment on T-cell proliferation ($p = 0.260$) while 1,25(OH)2D3 significantly reduced the proliferative capacity by 71,6 % on average compared to the control treatment ($p < 0.001$) and by about 72.2 % compared 25(OH)D3 ($p < 0.001$). This trial showed that, similar to the observations in mammalian T-cells, 1,25(OH)2D3 reduced the proliferation of T-cells. The analysis of the results is ongoing and will also give insight into the gene expression of interleukins and chemokines This will give more in depth information on how 1,25(OH)2D3 interacts with different T-cell types.
Keywords: Immune system; immune cells; poultry; 25(OH)D3; 1,25(OH)2D3

POSTER SESSION

Poster Session – Sponsored by Cargill

5041P Estimating cortical and medullary tibial bone density in live hens using 2D x-ray validated by post mortem CT

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Background: Accurate measurement of bone quality traits are important if improvements are to be made to advance laying hen welfare.

We believe that medullary bone is critical in maintaining both bone and egg quality during prolonged egg laying cycles.

Aim: To make progress we adapted the pioneering 2D x-ray method we previously developed to measure tibial density in live hens to enable estimates of both cortical and medullary bone density. This was validated using CT of the dissected tibia.

Materials and methods: White leghorn hens, 24 at 13 weeks of age and 23 at 24 weeks of age were subject to x-ray as previously described (Wilson, et al., 2023). After post-mortem a sample was prepared from the tibia mid-shaft for microCT analyses. The average for cortical bone, medullary bone and the marrow from five CT slices were measured using ImageJ for comparison with 2D derived estimates.

Estimation of cortical and medullary bone density from 2D live hen x-ray considered the tibia as a cylinder when a density slice was produced (Wilson, et al., 2023) The peaks represent the extent of cortical bone and the mid area represents an estimate of medullary bone with under/overlying cortical bone which is corrected for.

Statistical analysis: Between age comparisons were made using ANOVA whilst correlation was calculated using "Correlate" in Genstat.

Results: All the total, medullary and cortical bone density measurements by 2D x-ray and CT scans were significantly different and higher in the 24-week laying hen than the 13-week non-laying hen ($P<0.001$). Total density, cortical and medullary density by CT scan and 2D x-ray analysis are well correlated (0.82, 0.80, 0.71 respectively).

Conclusions: The density of bone increased after puberty both using CT data and the estimate from live hen 2D x-ray. Crucially total, cortical and medullary bone density estimates showed high correlation between the novel 2D method on live hens and the gold standard post mortem CT method.

The use of 2D x-ray measurement of bone density has advantages over CT in that it is relatively inexpensive with high throughput and is, most importantly, performed on the living hen thus suggesting it could be a useful tool to incorporate in breeding programs to improve bone quality and associated welfare of the laying hen.

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WILSON, P.W., DUNN, I.C. & MCCORMACK, H.A. (2023) Development of an in vivo radiographic method with potential for use in improving bone quality and the welfare of laying hens through genetic selection. British Poultry Science, 64: 1-10.

Keywords: bone; quality; eggs; welfare; x-ray

5104P Investigation of genetic diversity in native chicken of the Lao People's Democratic Republic (LAO PDR) and Thailand

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The Lao People's Democratic Republic (Lao PDR) exhibits high genetic diversity in its native chickens, which is essential for long-term food security. This study investigated the genetic diversity related to fitness, maternal ability, and performance traits in Lao native chickens compared with a Thai native breed. Blood samples were collected from Ou (n = 32), Black Bone (n = 10), Horn Chou (n = 9), Yolk (n = 121), and Chae (n = 41) chickens from Phongsaly, Luang Prabang, and Savannakhet province, Lao PDR, and Leung Hang Khow (LHK; n = 50) chicken from Suranaree university of technology, Thailand. Genomic DNA was extracted and sequenced using GBS. A total of 647,463 SNPs were identified, and after filtering, 1,484 common SNPs remained. PCA revealed clear group separation, with PC1 and PC2 explaining 37% and 29% of the variation, respectively. LHK formed a distinct cluster, while the Lao breeds showed substantial overlap, indicating similar genetic patterns among Lao populations. Annotation of the 1,484 SNPs identified 56 genes, including those related to adaptive ability (3 genes), maternal behavior (11 genes), performance traits (34 genes), and disease resistance (8 genes). GO analysis identified 17 enriched terms, and KEGG analysis revealed one significant pathway: neuroactive ligand–receptor interaction. LHK chickens exhibited unique SNP variants in five performance-related genes (INPP4B, PGM1, PGM2, ANKRD1, and TGIF2). Genetic differentiation analysis identified four significant genes (LHCGR, HHIP, COL6A2, and IP6K2; $P < 0.05$) associated with maternal behavior and performance. Heterozygosity was lower in LHK compared with Lao native chickens. All Lao breeds showed genetic diversity and conformed to Hardy–Weinberg equilibrium. These results demonstrate that Lao native chickens possess higher genetic diversity than LHK, supporting their value for future genetic improve-

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ment and conservation programs.
Keywords: genetic diversity; genotype by sequencing; Loa native chicken; Thai native chicken

5042P Effect of bedding material on some parameters of leg quality in broilers

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Footpad dermatitis (FPD) and lameness are important factors that lead to reduced production and welfare of broilers. According to available data, FPD occurs in 30-60% of birds in commercial flocks, while mild to moderate lameness occurs in 10-30% of birds. Bedding is one of the a key factors in intensive broiler production as it directly affects the welfare and production performance of broilers. The goal of this study was to determine the effect of different bedding materials on body weight, gait score and occurence of footpad dermatitis in broilers.

A total of 170 one-day old Ross 308 broilers were allocated to 2 treatments with 5 replicates, with 17 chickens per replicate, and raised in floor pens (17/m2) for 42 days. Two bedding materials were used: wheat straw (WS) and peat mixed with wood shavings (PW) which contained 10% of shavings in the total weight of the litter. Birds of both groups were fed by commercial feed: starter (1-10 days), grower 1 (11-21 days) grower 2 (22-31 days) and finisher (32-42 days). The incidence and severity of FPD were measured at 3, 4, 5 and 6 weeks of age by the method described by Eichner (2007): no lesions (0), lesions cover less than 25% of the footpad (1), lesions covering between 25% and 50% of the footpad (2) and lesions covering more than 50% of the footpad (3). The gait score was evaluated at 42 days of age on 20 chickens per treatment according to the method described by Kestin et al. (1992) on a scale from 0 (normal walk) to 5 (bird unable to walk). Body weight was analyzed by ANOVA followed by Fisher LSD post hoc test. FPD and gait score were analyzed using the Mann-Whitney test in the software package STATISTICA 14.0 (Tibco software inc.).

No effect of bedding material was found on final body weight (2,737g (WS) vs. 2,814 (PW)). Bedding material significantly affected the occurrence and severity of FPD ($P<0.01$) starting from 3rd week of age. At 6 weeks of age the mean score of FPD was 2.05 in wheat straw group and 0.46 in peat group ($P<0.01$). However, no significant effect of bedding material was found on gait score. The average gait score of chickens in wheat straw group was 1.65 and for peat group 1.30 ($P>0.05$). Based on the results it can be concluded that broilers raised on wheat straw were found to have a significantly higher incidence and severity of FPD compared to peat with wood shavings. There was no influence of bedding material on body weight and gait score of the birds.
Keywords: broilers; bedding; footpad dermatitis; gait score

5043P Effect of stocking density on gait score and occurrence of footpad dermatitis in broilers

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The main type of bedding used in Serbia and many other European countries is wheat straw. However, numerous studies have shown that much better results in terms of footpad dermatitis (FPD) incidence can be achieved when peat is used as bedding. The question remains whether similarly good results can be achieved at higher stocking densities as well. This study was conducted to evaluate the impact of stocking density on the occurrence of FPD and gait score in broilers raised on peat bedding. Ross 308 broilers were randomly allocated to two treatments with five replicates. Broilers were kept in floor pens with peat bedding. The treatments consisted of two stocking densities: 33 kg/m2 (LD group) or 39 kg/m2 (HD group). The trial lasted for 42 days. Birds of both groups were fed by the commercial feed: starter (1-10 days), grower 1 (11-21 days) grower 2 (22-31 days) and finisher (32-42 days). Housing conditions were controlled automatically and adjusted according to ROSS 308 recommendations. The incidence of FPD was evaluated at the age of 3, 4, 5 and 6 weeks by the method described by Eichner (2007): no lesions (0), lesions cover less than 25% of the footpad (1), lesions in wide areas, covering between 25% and 50% of the footpad (2), more than 50% lesion on the footpads (3). The gait score was evaluated at 6 weeks of age according to the method described by Kestin et al. (1992) on a scale from 0 (normal walk) to 5 (bird unable to walk). Results of final body weights were analyzed by ANOVA followed by Fisher LSD post hoc test. FPD and gait score were analyzed using the Mann-Whitney test (STATISTICA 14.0, Tibco software inc.).

No significant influence of stocking density was established on body weight (2814g (LD) vs. 2756 g (HD)). FPD score slightly increased from 3rd to 6th week in both groups, but there was no effect of stocking density on average FPD score ($P>0.05$) at any week of age. The average FPD score in 6th week was lower in group with low density (0.27) compared to HD group (0.40) ($P>0.05$). No significant effect of stocking density was found on gait score. The average gait score of chickens in LD group was 1.40 and for HD group 1.75 ($P>0.05$). Since the influence of stocking density on body weight, FPD and gait score was not significant, it can be concluded that birds can be raised on peat at higher stocking density without detrimental effect on body weight and leg quality.
Keywords: body weight; footpad dermatitis; gait score; peat

5044P Effects of dietary calcium interaction with L-tryptophan and 25-hydroxycholecalciferol substitution for vitamin D3 on egg production, eggshell quality, bone health, and calcium homeostasis in Dekalb White laying hens

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Calcium (Ca) metabolism plays a vital role in sustaining egg production and skeletal health in laying hens. Given the potential of both L-tryptophan L-Trp and 25-hydroxycholecalciferol (25HyD) to influence Ca metabolism through distinct but complementary pathways, their combined effects with dietary Ca warrant investigation. We hypothesized the dietary supplementation of L-Trp and 25HyD, in combination with varying levels of Ca, would enhance Ca utilization via activation of CaSR and thereby improve performance, egg quality, and bone health in laying hens. This study aimed to investigate the effects of dietary calcium interaction with L-tryptophan (Trp) and 25-hydroxycholecalciferol (25HyD) substitution for vitamin D3 in laying hens. In Experiment 1, seventy-two Dekalb White hens (26 weeks old) were assigned to a 3×3 factorial arrangement (n=8) with three levels of Ca (3.5%, 4.2%, and 4.9%) and three levels of Trp (0.18%, 0.22%, and 0.26%) for 4 weeks. In Experiment 2, forty-eight hens were allocated to a 3×2 factorial arrangement (n=8) with the same Ca levels and with or without 25HyD substitution for an equivalent vitamin D3. Both experiments shared three control treatments containing 0.22% Trp. Data from both experiments were analyzed as a completely randomized design using the MIXED procedure of SAS. In Experiment 1, increasing dietary Trp from 0.22% to 0.26% significantly increased average daily feed intake (ADFI) and body weight (BW), while the greatest egg mass (EM) was observed at a Trp level of 0.18%. Serum total Ca concentration, relative eggshell weight (ESW),

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eggshell thickness (EST), and eggshell breaking strength (EBS) increased linearly with dietary Trp levels ($P < 0.05$). Moreover, a Trp × Ca interaction was observed for relative ESW and EST ($P < 0.05$), with the combination of 0.26% Trp and 4.9% Ca producing the highest values. Tibia weight, ash, Ca, and phosphorus (P) contents were lower at 0.18% Trp than at 0.26% Trp ($P < 0.05$). In Experiment 2, 25HyD replacement for vitamin D3 increased serum total Ca level and tibia P content. Relative ESW, EST, EBS, tibia weight, ash, Ca content, and P content were primarily affected by dietary Ca levels. In conclusion, increasing dietary Trp level enhanced serum Ca concentration, eggshell quality, and tibia mineralization, with significant interactive effects between Trp and Ca on eggshell formation. In contrast, replacing vitamin D3 with 25HyD had a limited impact on improving egg production, eggshell quality, bone health, and Ca homeostasis.
Keywords: L-tryptophan; 25-hydroxycholecalciferol; calcium homeostasis; eggshell quality; bone health

5045P Impact of environmental enrichment on broiler chicken bone strength
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Introduction: Intensive genetic selection in broiler chickens, while optimizing productive performance, imposes significant pressure on the locomotor system, leading to bone fragilities. Objetctive: This study aimed to quantify the impact of dietary additives and environmental enrichment on femur and tibia bone mineral density (BMD), seeking effective strategies to mitigate these skeletal fragilities. Material and Methods: Conducted at the experimental poultry house of the Federal University of Grande Dourados, 1,440 male Ross TM4® chicks (1 to 42 days old) were distributed into 48 experimental units (30 birds/unit). The experimental design was a completely randomized factorial (3x2), generating six treatment combinations (eight replicates each): T1 – negative control with enrichment; T2 – negative control without enrichment; T3 – antibiotic with enrichment; T4 – antibiotic without enrichment; T5 – probiotic with enrichment; and T6 – probiotic without enrichment. Environmental enrichment aimed to enhance bird welfare, stimulating natural behaviors and movement. This enrichment included a permanent plastic ramp and temporary items (mirrors, metallic wig, hay, chains, colorful balls) rotated every three days. At 42 days, one bird per unit was euthanized for femur and tibia collection. Bones were radiographed with a reference aluminum step wedge (0.85 to 9.45 mm) for BMD analysis. Femurs were divided into Q1- Proximal Epiphysis, Q2- Diaphysis, Q3- Distal Epiphysis. Tibias were segmented into Q1- Proximal Extremity, Q2- Diaphysis, Q3- Distal Extremity. Data were evaluated for statistical assumptions and analyzed by ANOVA using the PROC MIXED procedure (SAS). The statistical models included additive type, environmental enrichment, and their interaction as fixed effects, and animal nested within treatment as a random effect. When appropriate, interactions and main effects were compared using Tukey's test. Results: Statistical analysis revealed no significant BMD differences between antibiotic or probiotic treatments in femurs or tibias. However, environmental enrichment showed a positive and statistically significant effect on BMD. In femurs, enrichment increased BMD in the Diaphysis (Q2, avg. 6.11) and Distal Epiphysis (Q3, avg. 6.22). In tibias, enrichment notably increased BMD in the Proximal Extremity (Q1, avg. 6.21) and Diaphysis (Q2, avg. 5.29). Conclusion: This study demonstrates environmental enrichment positively contributed to improved bone gain in broiler chickens, offering a promising solution for skeletal fragilities. These findings suggest environmental management strategies are more decisive for bone health than the dietary additives studied in this context.
Keywords: Bone Mineral Density; Bone Diaphysis; Bone Health; Femur; Tibia

5046P EVALUATION OF SPONDYLOLISTHESIS OCCURRENCE BY COMPUTED TOMOGRAPHY IN BROILER CHICKENS REARED IN ENRICHED ENVIRONMENTS
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The intensive genetic improvement in broiler chickens, aimed at accelerated growth and high carcass yield, increases susceptibility to locomotor disorders such as spondylolisthesis, a vertebral column deformity leading to spinal cord compression, pain, and hind limb paralysis. To mitigate these problems, the industry uses additives like antibiotics (ATB) to treat and prevent bacterial diseases, and seeks alternatives such as probiotics (PROB), which modulate microbiota, stimulate the immune system, and represent a safer, natural option, as well as environmental enrichment (EE) that stimulates physical development. The primary objective of this study was to use Computed Tomography as a diagnostic tool to analyze and quantify the impact of different additives (antibiotic and probiotic), combined or not with environmental enrichment, on the incidence and severity of spondylolisthesis in broiler chickens. A total of 1,440 male Ross TM4® chicks were used, distributed in a completely randomized design, in a 3x2 factorial arrangement, totaling six treatments with eight replicates: T1 - Negative control with environmental enrichment; T2 - Negative control without enrichment; T3 - Antibiotic with enrichment; T4 - Antibiotic without enrichment; T5 - Probiotic with enrichment; T6 - Probiotic without enrichment. The experiment lasted from 1 to 42 days at the Federal University of Grande Dourados. Environmental enrichment consisted of a combination of permanent elements (plastic ramp) and rotated temporary items (mirrors, alfalfa hay, etc.). 42 days, one bird per pen was euthanized and subjected to Computed Tomography to obtain and conduct detailed analysis of the vertebral column images. Statistical analysis of the data, processed via SAS software, revealed no significant difference between treatments. The statistical analysis did not reveal significant differences in spondylolisthesis occurrence or severity among treatments. It is concluded that the use of antibiotics, probiotics, and environmental enrichment, under the conditions of this study, did not yield significant differences regarding spondylolisthesis. However, Computed Tomography proved to be a highly effective and non-invasive tool for the precise and detailed diagnosis of vertebral malformations in broiler chickens, providing a valuable technique for future research on the etiology and prevention of skeletal disorders.
Keywords: Probiotic; Antibiotic; Vertebral Column; Locomotor Health; Spondylolisthesis

5047P Influence of structural differences in chicken wing bones on the occurrence of damage during processing
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Bone damage in poultry processing leads to avoidable food loss. The elbow joint of the wing is frequently affected, where de-

tachment of the cartilage from the distal humerus head causes the bone to protrude through the skin after defeathering, known as "pop-out". The aim of the present study is to investigate the structural root cause of this damage. For this means, 160 wings were obtained from a commercial processing plant representing 2 management systems: The conventional group consisted of Ross 308 broilers with a slaughter age of 41 days, while Hubbard 787 broilers represented the slow growing group with a slaughter age of 56 days. After cleaning of the wing bones with a scalpel, they were further bleached in 30% hydrogen peroxide for 30 minutes to remove any remaining soft tissue. For each group, 60 damaged and 20 intact wings were obtained. Samples showing a damage different from the described 'pop-out' were excluded from further analysis, resulting in 56 and 52 samples for 'dislocated' in the slow growing and conventional group, respectively and 8 samples per group in the 'non-dislocated'. The size of the distal humerus head was markedly different between the management systems, with an average of 16.6 and 16.31 mm for 'non-dislocated' and 'dislocated' in the slow growing group versus 15.66 for 'dislocated' and 15.41 for 'non-dislocated' in the conventional group. A marked depression could be observed at the growth plate of the distal humerus, seemingly more pronounced in the bones from the dislocated groups and conventional system. A small section of approximately 0.5 – 1 cm was cut from this specific area. Samples were analysed under a microscope with a Canon PowerShot G15 camera attached to a Zeiss Stemi 508 microscope. The thickness of the humerus head next to the depression and at the deepest point of the depression was measured as 'highest point' Lh and 'lowest point' Ll using Image J software and the depression expressed in percentage according to the formula (Lh-Li)/Lhx100. A One-way ANOVA showed a significant difference (p-value <0.05) between the four groups, Student's t-test revealed that the depression was significantly more pronounced in the conventional 'dislocated' than in the conventional 'non-dislocated' and more pronounced than in the slow growing 'dislocated'. No significant difference was found between the 'non-dislocated' bones of the two management systems. Within the slow growing group, the depression appeared deeper in 'dislocated' bones, although this difference was not statistically significant. Results indicate an influence of the bone structure at the distal humerus head on wing bone damage in chicken processing, which should be further investigated.

Keywords: Wing bone damage; Chicken processing; Wing structural differences; Broiler management systems; Animal welfare

5048P Continuing education program for the global Poultry sector

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The rising demand for animal proteins by 2050 along with our limited natural resources call for significant improvements within the global poultry sector. The sustainability of poultry production is linked to the ability of the sector to quickly adopt new management practices and tools (e.g. feed ingredients, disease prevention tools, data analysis). Unfortunately, many challenges affect the adoption at scale of new technologies, including the access to new information and their understanding by poultry professionals in the field. For instance, the adoption rate of an innovative feed ingredient or vaccinal strain is directly related to the level of comfort field practitioners have with using them.

In 2010, at the request of the World Organisation for Animal Health (WOAH), the World Veterinary Education in Animal Production Health (WVEPAH) was established to develop various continuing education programs specifically designed for Poultry professionals in the field. These courses, validated by WOAH and leading to a diploma delivered by the University of Montreal, are offered in 3 languages (English, French and Spanish) through a combination of online lectures and on-site sessions. They provide professionals the opportunity to access at their own pace the most recent information about poultry management, nutrition, disease control and biosecurity measures.

In Module I, participants are provided general information about Poultry production and diseases. In Module II, participants can specialize in the 4 main poultry species raised worldwide: broilers and breeder birds, layers, turkeys and waterfowl. WVEPAH has organized courses on 3 continents (Africa, Latin America and Europe), giving a training and certification opportunity to over 400 professionals from 63 countries. In many cases, such level of specialization was not offer by the academic sector of their respective countries. In addition to Latin America and Africa, WVEPAH is now focusing its efforts on Asia, thus covering the regions expected to register the highest growth in production. WVEPAH is a valuable complement to academic Poultry science programs and extension services to support the growth of the poultry sector and has developed alliances with the World's Poultry Science Association, the Latin America Poultry Association, and the World Veterinary Poultry Association. The contribution of WVEPAH was reinforced in May 2025 with its recognition by WOAH as a Collaborating Centre for Advanced training in livestock health. Through continuing education modules and a global certification, WVEPAH empowers Poultry professionals to contribute to the sustainability of human nutrition with the provision of safe and nutritious eggs and poultry products.

Keywords: Continuing education; Extension services; Certification; Sustainability; Training

5049P The creation of the World Animal Biosecurity Association: individual, local, national and international opportunities

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Objective: Describing the creation and development of an international association dedicated to animal biosecurity, as well as the opportunities that it may offer to individuals, organizations, and regional, national and international entities.

Description of the problem:

Emerging or reemerging infectious diseases in animals have always been a serious health, welfare and economic problem worldwide. This dynamic situation is not new but seems to be exacerbated by climate changes impacting the distribution of diseases and pests.

Biosecurity, which is the application of a set of management, behavioural and physical measures designed to reduce the risk of introduction, establishment and spread of pathogenic agents to, within and from an animal population, is the main tool available to contain and prevent these diseases.

Materials and methods

Veterinary and non-veterinary professionals have met over the past 3 years. A mission statement was written with clear objectives. This served as a basis to recruit individuals, institutions and companies with a vested interest in biosecurity. This process led to the election of a council and of an executive that worked to get the association formally registered.

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Results:
The goal of the World Animal Biosecurity Association (WABA) is to be a recognized reference on animal biosecurity by promoting the creation and transfer of knowledge, to advance the science and practice of animal infectious disease prevention and control, with the aim of enhancing sustainable animal, environmental and public health.
Six main objectives were identified:
Provide an international forum dedicated to animal biosecurity
Provide opportunities for members to share information, practices, and collaborate on projects
Inform international standards on matters related to biosecurity
Be a repository of information regarding biosecurity, research and training program funding
Develop and transfer biosecurity knowledge
Inform stakeholders (growers, public, decision makers)

The focus goes beyond production animals, and include companion animals, working animals and wildlife. Consequently, a large spectrum of professionals are welcomed (animal scientists, veterinarians, technicians, public health professionals, psychologists, social scientists, lawyers, administrators, government officials, chemists, microbiologists, engineers, industry leaders).
All info on WABA can be found at https://wababiosecurity.com/

Conclusions:
The creation of WABA is serving as an international hub connecting professionals with an interest in animal biosecurity, providing its membership with learning material and opportunities to present or share data, knowledge and innovations.
Keywords: International; Biosecurity; Animal Health; Association; Training

5050P Mitigating heat stress in laying hens: Effects of strategic feed restriction on egg quality traits
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Heat stress is a major challenge in laying hen production, negatively affecting egg quality, animal welfare, and economic sustainability. This study evaluated whether feed restriction to avoid peak thermal load could modify the egg quality traits in laying hens exposed to summer heat stress. A total of 3,000 Lohmann Brown laying hens were randomly assigned to two experimental groups (n = 1,500 per group; three replicates per treatment) in an aviary system. The control group (CONT) had ad libitum feed access, whereas the treatment group (TRT) experienced complete feed withdrawal between 1100 h and 1700 h, corresponding to the hottest period of the day; feed access resumed during the rest of the day. Water was provided ad libitum. Data were analyzed using mixed-effects models in PROC GLIMMIX (SAS 9.4), including treatment (CONT vs. TRT), time (beginning vs. end), and their interaction as fixed effects, with egg nested within treatment included as a random effect.
Hens subjected to feed restriction, maintained higher body weight compared to ad libitum-fed hens (2009.7 ± 16.6 g vs. 1952.8 ± 16.6 g; $p < 0.05$), indicating improved tolerance to heat stress. The effect of time significantly reduced albumen height, albumen index, Haugh unit, yolk height, and yolk index ($p < 0.05$), reflecting that the negative impact of heat stress. Feed restriction did not significantly affect internal egg quality traits ($p > 0.05$). The interaction of treatment × time effect on albumen height, albumen index, Haugh unit, yolk index and yolk weight were significant ($p \leq 0.05$).
In conclusion, feed restriction represents a practical, low-cost management strategy that buffers the decline in internal egg quality traits during heat stress. Incorporating feed restriction methods, may enhance the internal egg quality traits of laying hens in aviary systems during hot summer conditions.
Keywords: Egg quality; heat stress; Lohmann brown; feeding time

5051P Effect of Different Levels and Sources of Organic and Inorganic Selenium on Laying Hen Performance, Selenium Distribution in Eggs, and Egg Quality
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This study evaluated the impact of various selenium (Se) sources and inclusion levels—both organic and inorganic—on laying performance, Se deposition in eggs, and egg quality in Shaver White hens. A randomized complete block design was implemented over 8 weeks with 270 hens (67–74 weeks of age) allocated to 10 dietary treatments based on a corn-soybean meal diet.
Treatments included a negative control (NC : 0.0ppm Se), sodium selenite alone (I : 0.30ppm inorganic Se), sodium selenite with organic selenium yeast sources (SIO1, SIO2, SIO3 : 0.15 ppm inorganic Se+0.15ppm organic Se) and selenium yeast alone (SO1, SO2, SO3 : 0.30ppm organic Se). An alternative source of inorganic Se (SI4) was also tested through two diets to replace half (SIL4: 0.30ppm inorganic Se) or all (SIH4: 0.30ppm inorganic Se) of the sodium selenite (I). All the 9 Se supplemented diets contained 0.30 ppm of total selenium (organic and inorganic). Performance metrics (laying rate, feed conversion ratio [FCR], egg mass) and egg quality parameters (Se content, Haugh unit, shell integrity) were assessed. Performance data analysis (feed intake, laying rate, egg weight, egg mass and feed conversion ratio) and eggs selenium content was performed by ANOVA according to the blocks and the diets. The mean separation was carried out using a Tukey test with R.2.15.0.
Results showed that selenium yeast 1, either partially (SOI1) or fully replacing inorganic Se (SO1), maintained or numerically improved laying performance (respectively 89.04% and 89.53%) and FCR (respectively 1.84 and 1.89). However, SO1 at 0.30 ppm yielded the highest egg Se content (0.306 ppm) and demonstrated a strong positive correlation between dietary Se and egg Se content ($R2 = 0.77$), indicating superior bioavailability than inorganic selenium. In contrast, other organic Se sources (SO2, SO3) did not enhance egg Se content, and SO3 negatively affected performance and shell quality. The alternative inorganic Se source (SIH4) offered no advantage over sodium selenite and compared to NC, adversely impacted significantly laying performance (-1.12%) , FCR (+20points), and broken eggs rate (+2.61%).
In conclusion, selenium yeast 1 proved to be the most effective Se source in terms of bioavailability and overall performance, while other tested sources showed limited or negative effects.
Keywords: Laying hens; Bioavailability; Egg quality; Selenium; Yeast

5052P Exploring Correlations in Egg and Eggshell Quality Parameters: A Meta-Analysis of 12 Trials Covering 3,448 Eggs
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Egg quality is a multifactorial trait that plays a crucial role in both consumer satisfaction and the economic value of egg production. It involves multiple traits related to both internal components and shell characteristics. While many indicators exist, few datasets combine large-scale, multi-trial data. This study analyzes a comprehensive European database to explore correlations among 21

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egg quality parameters, aiming to better understand their interrelationships and relevance for production standards.
This study compiles 3,448 individual egg data points from 12 European trials conducted between 2013 and 2024, including both commercial farms (33%) and research facilities (66%) with hens between 17 and 77 weeks of age. A total of 21 variables related to egg and eggshell quality were analyzed using Pearson correlation coefficients, with pairwise deletion of missing values (SPSS Statistics 29.0, IBM).
Expected correlations were confirmed between egg weight and its components: eggshell weight (r = 0.66), albumen weight (r = 0.85), and yolk weight (r = 0.67). Interestingly, in this dataset, eggshell weight does not appear to be correlated with eggshell thickness, which is somewhat unexpected. However, positive correlations were observed between eggshell thickness and eggshell breaking strength measurements, including force flat (r = 0.59), workload flat (r = 0.51), force sharp (r = 0.53), and workload sharp (r = 0.43). Specific gravity (SG), a key indicator of eggshell integrity, showed positive correlations with eggshell weight (r = 0.56), thickness (r = 0.87), and strength metrics (force flat: r = 0.57, workload flat: r = 0.36, force sharp: r = 0.55, workload sharp: r = 0.37). These results support SG's relevance as a non-destructive proxy for shell quality. No clear correlation was found between Haugh Units (HU) and shell thickness or weight.
Overall, this analysis highlights key relationships among egg quality traits and reinforces the importance of specific gravity and shell strength in evaluating egg quality across diverse production systems.
Keywords: Egg quality; multi-analysis; Eggshell quality; Egg analysis

5053P Effects of Dietary Calcium Pidolate Supplementation on Performance and Eggshell Quality in Late-Lay Hens
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Poor eggshell quality in late-phase laying hens causes significant economic losses. Standard calcium is often ineffective due to poor absorption in aging hens. Calcium pidolate (Calpid; Norel Animal Nutrition, Spain), an organic calcium salt, is proposed to have higher bioavailability, supported by broiler research on bone strength. Given shared calcium pathways, this study investigated if Calpid could improve eggshell quality in high-performing, late-lay hens.
A total of 360 Hy-Line W36 hens (63 weeks) were allocated to three treatments for 28 days (3 replicates/40 hens). Treatments were: (T1) a corn-soybean meal basal diet (Control); (T2) Control + 250 g/ton Calpid; and (T3) Control + 450 g/ton Calpid. The mash basal diet met Hy-Line requirements (17% CP, 2800 kcal/kg M.E, 4.10% Ca, 0.38% Av.P). Calpid was added on top and directly mixed into the respective treatment diets. Data on hen-day production, egg weight, feed intake, and broken eggs were collected daily. Data were analyzed via a linear mixed model (ANOVA, Tukey's HSD; JMP Pro 17) with significance at $P<0.05$.
The primary finding of the study was a highly significant and specific improvement in eggshell quality ($p=0.005$). Supplementation with Calcium Pidolate at 250 g/ton and 450 g/ton significantly reduced ($p<0.01$) the mean percentage of broken eggs to 1.59% and 1.47%, respectively, compared to 2.46% in the control group. All key production parameters were statistically similar ($p>0.05$) across the control, 250 g/ton, and 450 g/ton groups, respectively. There were no significant differences in Hen-Day Egg Production (86.63%, 86.83%, 87.50%; $p=0.61$), average egg weight (63.09 g, 62.80 g, 62.70 g; $p=0.19$), or daily egg mass (54.65 g, 54.53 g, 54.88 g; $p=0.82$). Furthermore, efficiency metrics remained unchanged, with average daily feed intake (107.14 g, 106.26 g, 107.26 g; $p=0.45$) and Feed Conversion Ratio (1.960, 1.949, 1.955; $p=0.85$) prove to be statistically identical.
In conclusion, while production and feed efficiency were already maximized by the high-specification basal diet, Calcium Pidolate supplementation demonstrated a highly significant and specific economic benefit. It had no effect on production metrics but significantly reduced the percentage of broken eggs by up to 42% (from 2.54% to 1.47%). This study successfully highlights Calpid effect in late-lay hens: enhancing eggshell integrity and improving the percentage of sellable eggs in a high-performing flock. These promising results for a broader research program. Further studies are underway to set the base and validate these findings under different commercial conditions and laying phases.
Keywords: Late-lay hens; Calcium Pidolate; Eggshell Quality; Broken Eggs

5054P "Relationship between eggshell characteristics, variation and measurement of eggshell thickness in laying hens"
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Hypothesis – Objectives: Maintaining good shell quality is economically and productionally important, impacting food security. Our hypothesis was that shell thickness is related to other characteristics and varies in different measurement zones. The objective of this study was to determine the relationship between shell characteristics, variation, and measurement zones of shell thickness in laying hen eggs.
Experimental design: Sixty samples of freshly laid eggs from laying hens were randomly collected. Ten shell thickness measurement zones were established for each egg, from the blunt end to the tip of the egg.
Materials and methods: This study was conducted in the Nutrition Research and Development Laboratory – FMVZ – UNICA. Sixty eggs from 71-week-old HISEX Brown hens were randomly selected. An egg analyzer (Digital Egg Tester-6500) and a Mitutoyo micrometer were used. Shell thickness, shell break resistance, egg weight, and shell weight were evaluated.
Statistical analysis: The SAS v 9.4 correlation procedure was used to examine the relationship between shell characteristics. A GLM ANOVA in SAS was used to examine differences between the 10 measurement zones. A paired-samples Student's T-test was used to compare eggshell thickness measurement zones. Differences were considered statistically significant when $P < 0.05$
Results: A positive correlation was found between shell thickness and breaking strength ($r = 0.424$; $P < 0.0195$), a positive correlation between shell thickness and shell weight ($r = 0.777$; $P < 0.0001$), and shell strength also showed a positive correlation with shell weight ($r = 0.529$; $P < 0.0026$). The average shell thickness of the 10 measured zones ranged from 0.4060 to 0.4240 mm, but this difference was not significant according to the ANOVA ($P = 0.3961$). The blunt zone showed the greatest variation (CV = 8.36), and the equatorial side showed the least variation (CV = 6.48). Based on a paired-samples Student's t-test analysis, the average thickness of the three traditional measurement zones (0.418 ± 0.0298 mm) was found to be significantly different from the average of the ten zones (0.414 ± 0.027 mm). The thickness of the blunt zone (0.424 ± 0.035 mm) was greater and significantly different from that of the sharp zone (0.416 ± 0.033 mm). The thickness of the sharp zone (0.416 ± 0.033 mm) was similar ($P = 0.2420$) to

the average of the ten zones (0.414 ± 0.027 mm).
Conclusions: there is a significant positive relationship between shell thickness and breaking strength. The thickness of the sharp zone is less than that of the blunt zone. The thickness of the sharp zone could be a suitable measurement option in certain situations where measurement of a single zone is required.
Keywords: shell thickness; shell strength; shell weight; egg

5055P Quality of eggs coated with açaí-based coatings formulated with different plasticizers after 21 days of storage

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Açaí residue has emerged as a promising and sustainable raw material for the development of egg coatings due to its functional properties and availability as an agro-industrial by-product. The objective of this study was to evaluate quality parameters of eggs coated with açaí-based edible coatings formulated with different plasticizers after 21 days of storage at room temperature. A completely randomized design was adopted, using forty-four one-day-old eggs obtained from hens of the same age and maintained under similar environmental, management, and feeding conditions. Eggs were randomly assigned to four treatments, with uncoated eggs serving as the control. Edible coatings were prepared using açaí residue flour as the film-forming matrix, into which different plasticizers were incorporated, namely glycerol, propylene glycol, or sorbitol. Eggs were stored at room temperature (24 °C) for 21 days, and egg quality parameters including weight loss, Haugh unit, and albumen pH were evaluated. Data were analyzed by one-way ANOVA using Minitab 18 (Minitab Inc., State College, PA), and differences among means were assessed using Tukey's test at $P < 0.05$. Egg quality parameters were significantly affected by storage time ($P < 0.001$). Uncoated eggs exhibited the highest weight loss (5.18%), whereas eggs coated with açaí-based films containing glycerol (2.66%), propylene glycol (2.45%), or sorbitol (3.23%) showed reduced weight loss compared with the control ($P < 0.001$). After storage, uncoated eggs also presented the lowest Haugh unit (50.25) and the highest albumen pH (9.50) among all treatments ($P < 0.001$). No significant differences were observed among the different plasticizers for the evaluated parameters. In conclusion, açaí-based coatings were associated with improved egg quality attributes after 21 days of storage compared with uncoated eggs. Because açaí residue flour constituted the structural matrix of the coatings, it was not possible to dissociate the individual effects of the matrix from those of the plasticizers within the experimental design adopted. These results indicate the potential of açaí residue as a sustainable material for egg coating applications, while further studies are needed to optimize formulations and evaluate longer storage periods.
Keywords: eggshell; glycerol ; propylene glycol; sorbitol; storage

5056P Effects of bedding material enriched with sodium bisulfate on the concrete floors of European poultry houses

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A critical biosafety control point in European poultry houses is the concrete floor. Placement of day-old chicks requires a thoroughly cleaned and disinfected floor with fresh litter. Litter treatments like sodium bisulfate (SBS) inhibit microbial activity, reduce the risk e.g. of ammonia formation and support animal health. However, in addition to its low pH value, SBS has elevated sulfate and sodium content, which can lead to driving sulfates that can negatively affect concrete surfaces (cracking, deterioration). This study investigated whether litter material treated with SBS would have damaging effects on the concrete barn floor over time compared with litter without SBS. Concrete samples (7.5 x 15 x 7 cm) with Water/Concrete (W/C) ratios of 0.45, 0.5 and 0.6 were exposed to the following three different treatments over eight 40-day cycles, mimicking commercial production conditions in the EU: (a) Control treatment consisting of a mixture of 50% broiler manure and 50% water, (b) treatment (a) with 0.25 kg/m2 SBS (recommended amount), and (c) treatment (a) with 0.50 kg/m2 SBS. The manure used originated from a commercial German broiler farm (65.7% DM), usage of SBS (Grillo Chemicals GmbH, Germany) based on results of Toppel et al. (2019) with a doubling of the amount in (c). After four cycles, samples were analyzed by Kiwa GmbH (Germany) via transmitted light microscopy (TLM) (Jenaval polarizing microscope with linear (II-Pole) and crossed (x-Pole) polarizers, resolution: 500:1 to 1000:1, Carl Zeiss AG), and scanning electron microscopy (SEM) (ZEISS Gemini SEM500 NanoVP Low Vacuum SEM, Carl Zeiss AG). Weekly pH monitoring by Chemad GmbH (Germany) showed initial averages of raw material of pH 2 (SBS) and pH 7 (untreated manure), reaching 8.05 (a), 7.80 (b), and 7.40 (c) after 40 days. TLM and SEM results after four and eight cycles indicated no negative effects from the reduced pH. Findings showed no increased surface concentration of sulfur or sodium and pore filling remained low (both 0.1–0.2%). No gypsum or other driving sulfates could be detected. However, SBS treatments promoted favorable carbonation from 13.9% (c, 0.6 W/C) to 18.0% (b, 0.5 W/C), leading to surface compaction. The W/C ratio demonstrated a greater influence on concrete attrition than the SBS addition. The study concludes that SBS, despite an increased sulfate and sodium content and reduced pH value, has no detrimental effect on concrete and thus may be safely used in poultry houses with concrete floors. This finding is important for European poultry production regarding biosecurity and animal health and may also be beneficial when international husbandry conditions change.
Keywords: Poultry house concrete floor; Sodium bisulfate (SBS); Litter pH; Bedding material; Europe

5057P Effects of light intensity on behavior and seminal performance of White Roman geese

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This study investigated the effects of lighting intensity on behavior and seminal performance among white Roman geese kept in an environmentally controlled house. Two hundred and eighty-eight White Roman geese aged 10 months were randomly distributed among 12 pens, with each pen containing 8 ganders and 16 females on the floor, based on a completely random design (CRD). At the beginning of the study, each treatment group was comprised of 5 ganders and 16 geese. in each pen, besides 3 ganders were individually housed in separate cages.; these groups included: geese under 40 lux (Control), geese under 170 lux, geese under 300 lux, and geese under 430 lux. The birds were maintained under a lighting photoperiod of 7L:17D for 6 weeks. Thereafter, the photoperiod was adjusted to 9L:15D in a controlled-environment house. The pen of the finishing house was 6.5 × 2.8 m (18.2 m2). The tempera-

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ture in the environmentally controlled goose house was maintained between 25 and 35 °C. The results showed that geese under 300 lux in drinking behaviors, motility and alive spermatozoon had higher than among groups. Geese under170 lux in Ingestive behaviors had higher percentage than among groups. Geese under 40 lux in crouching behaviors had higher percentage than among groups. Geese under 40 lux in semen volume had higher than those of 170 and 300 lx groups. Therefore, geese under 300 lux results in had higher motility and alive spermatozoon of semen of White Roman geese in an environmentally controlled house.

Keywords: Lighting intensity ; behavior; semen; White Roman geese; Environmentally controlled house

5058P Evaluation of a high-temperature vertical fermentation tank for the aerobic treatment of Poultry manure

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Manure is the second highest source of greenhouse gas emissions after feeds, accounting for 20% and 6.4% of the total emissions of the egg and poultry meat sectors, respectively. Under pressure from governments, retailers, and consumers, farmers must identify ways to reduce the environmental footprint of Poultry production. Untreated manure may also be a vehicle for the contamination of soils and crops with pathogens, potentially leading to foodborne illness outbreaks and with antibiotic residues, leading to antimicrobial resistance. It is essential for farmers to adopt new mitigation strategies to reduce the environmental burden created by chicken manure.

A high-temperature aerobic vertical fermentation tank was designed, and a study evaluated its performance compared to that of the classic manure piling method used by poultry farmers. Samples of manure were collected at various locations during a 7-day period to monitor temperature, moisture level and bacterial counts. The static chamber technique was used for measuring greenhouse gases.

Within 48 hours, the manure rose above 55°C. Moisture levels decreased from a range of 50-70% to 30% by the end of the process. While the manure piling method with its uncontrolled temperature and cold pockets results in low to uneven pathogen inactivation rates, the vertical fermentation achieved an even and suitable temperature (> 55°C) for at least 3 days, contributing to the reduction in the manure of total coliforms (>4 log), Escherichia coli and Salmonella spp. (> 5 log). Ascaris eggs were also reduced by 99%. The duration and intensity of this thermal treatment allow the denaturation of antibiotic residues, reducing their release in the environment and the risk of AMR. Finally, this even thermal treatment also destroys fly larvae deposited in manure, reducing drastically fly population.

Through its well-controlled aerobic conditions, the vertical tank reduced emissions of methane and carbon dioxide by 90% and 50%, respectively, meaning a lower global warming potential. Hydrogen sulfide and ammonia emissions were decreased by at least 90%, contributing to a reduction in unpleasant smell and acidifying emissions. Through this process, the compost contained more nitrogen, phosphorus and potassium for crop fertilization.

This vertical treatment process, allowing a strict control of the aerobic fermentation, reduces the environmental burden of chicken manure and produce a safer, greener and richer fertilizer to be used on crops. Completed within 7 days, this optimized natural compost allows farmers to generate an additional source of regular income, thus providing more incentive to adopt such mitigation strategy.

Keywords: Manure; Circular economy; Greenhouse gas emissions; Environmental footprint; One Health

5059P Greenhouse Gas (GHG) Emissions in Egg Production Systems: A Comprehensive Global and Regional Review For New Zealand.

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This review presents a comprehensive analysis of greenhouse gas (GHG) emissions associated with commercial egg production systems worldwide, with an emphasis on regional variations and implications for New Zealand. A systematic literature review identified 300 relevant publications, of which 40 high-quality studies were selected based. Reported GHG emission intensities ranged from 1.2 to 5.6 kg CO2-eq per kg of eggs, with most studies identifying feed production as the primary emission hotspot, followed by manure management and on-farm energy use.

Globally, cage and colony systems tend to demonstrate lower GHG emissions per kilogram of eggs due to higher feed efficiency, while free-range and organic systems often exhibit higher emissions, largely influenced by regional factors such as feed sourcing, energy mix, and climatic conditions. Comparative findings across continents highlight the trade-offs between environmental performance and alternative housing systems.

Key mitigation strategies include improving feed conversion ratios, optimizing feed composition, adopting advanced manure management, and incorporating renewable energy sources. However, the review also identifies substantial knowledge gaps, particularly in understudied regions such as New Zealand, and in integrating climate targets with animal welfare and sustainability frameworks. Addressing these gaps is essential for designing region-specific, low-emission egg production strategies aligned with both environmental and ethical objectives.

Key words: Green house gas emissions, Egg production systems, Sustainability, Housing systems, Animal welfare, New Zealand.

Keywords: Green house gas emissions; Egg production systems; Sustainability; Animal welfare; housing systems

5060P Performance, environmental footprint, and economic competitiveness: substituting imported soybean meal with european protein sources in turkey diets

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Introduction

Feed formulation is a critical lever for reducing the environmental impact of poultry production1. This study evaluated the zootechnical performance, environmental footprint, and economic competitiveness of male turkeys when substituting imported soybean meal with alternative European protein sources.

Materials and Methods

The trial was conducted at the Euronutrition experimental station (France) using 288 Aviagen Premium male turkeys raised from 1 to 119 days. Birds were randomly allocated to 24 floor pens (experimental unit), resulting in 4 dietary treatments with 6 replicates of 12 birds each. The feeding program consisted of 6 phases.

All groups received iso-nutritional diets adjusted to commercial specifications, with Metabolizable Energy (ME) ranging from 2750 kcal/kg (0-21d) to 3200 kcal/kg (91-119d) and Digestible Lysine decreasing from 1.55% to 1.01%. Treatments differed solely by the protein raw material profile: Group 1 (Control) used imported soybean meal (Brazil); Group 2 used Hipro sunflower meal (46% CP); Group 3 used an extruded mix of faba bean and French GM-free soy (33% CP); and Group 4 combined dehulled faba bean (31% CP) with Hipro sunflower meal.

Statistical analysis was performed using R software (version 1.2.1335). Growth performance, carcass yields, and environmental criteria were analyzed using one-way ANOVA followed by a

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Tukey post-hoc test with significance set at $p < 0.05$. Mortality rates were compared using a Chi-square test10. Environmental footprints were calculated using Life Cycle Assessment (LCA) based on internal data (GHG, NRE) and the ECOALIM database.
Results
At 119 days, Group 3 (Extruded mix) exhibited significantly lower body weight and feed intake (<p=0.001) compared to other groups. Group 4 (Dehulled faba bean) showed no significant difference in performance compared to the Control. No significant differences were observed for FCR or mortality. Environmentally, substituting imported soybean meal significantly reduced Greenhouse Gas (GHG) emissions by 39% (G2), 46% (G3), and 41% (G4). However, Group 3 significantly increased the phytosanitary treatment index (+293%) and land use (+16%) (<p=0.000). Economically, Group 4 offered the best compromise, minimizing the production cost gap compared to the control.
Conclusion
Complete substitution of imported soybean meal is viable in turkey production without compromising performance, particularly using dehulled faba bean strategies. While GHG emissions are drastically reduced, statistical analysis confirms that trade-offs with other indicators like land use must be monitored to optimize global sustainability.
Keywords: Environnement; Faba bean; Sunpro; Turkey Production; Alternative to soya

5061P Growth Curve Responses of Two ECC–Approved Broiler Chicken Genotypes to Physical Activity, Temperature, and Nutritional challenges

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Current poultry production is dominated by intensive rearing systems, characterised by the use of fast–growing genotypes selected for high performance and muscle hypertrophy. However, growing public concern for animal welfare is shifting European market demand towards alternative production systems: i.e. EU legislation recommended the use of slower–growing genotypes for organic and free–range systems and more recently also for the conventional one (ECC–approved genotype). A key challenge remains choosing a suitable genotype that balances expected welfare improvements with productive performance and contributes to a production chain that is more resilient to stressors (i.e. dietary, environmental, thermal, immunological, etc.). The research aimed to analyse the effects of kinetic activity (C: spontaneous activity; MOVE: 30 min/every day starting from 15 d of age), temperature variations (CT: control temperature, 22.8±2.8 °C; HS: heat stress, 27.2±2.8 °C), and dietary regimens [CON: conventional basal diet for fast–growing broilers; LOW diet: diet with reduced crude protein–to–metabolizable energy ratio (-10% protein)] on the growth curve dynamics of two ECC–approved meat–type chicken strains. At this regards, three trials were performed for testing the response of medium–growing (MG: Ranger Gold) and slow–growing (SG: Kabir) genotypes to physical activity (T1), thermotolerance (T2) and a low–protein diet (T3). The Gompertz model and Generalized Linear Models were employed to analyze the growth curve of the animals. The curve parameters included: A, adult body weight; B, the proportion of asymptotic weight to be gained after birth; k, the instantaneous relative growth rate; MGR, maximum growth rate; T50, 50% of adult body weight. The effects analysed were the genotype, the challenge (heat stress, physical activity and diet) and their interaction.
In each trial, genotype had a significant effect on all the evaluated parameters, with MG outperforming SG across all Gompertz model estimates. However, considering the interaction, in T1, MG genotype exhibited higher values ($P<0.01$) only for B and MGR. In T2, the increase of temperature decreased A ($p < 0.001$) and B ($p < 0.001$) only in MG. Significant effect of temperature or its interaction with genotype were also recorded for T50 and MGR where SG showed a better resilience. In T3, the administration of the LOW diet reduced the MGR ($p = 0.009$), particularly in MG birds, while SG birds maintained similar values of those of fed the CON diet. In conclusion, new sustainable rearing strategies should be developed that accept the expected performance reduction of SG strains but seek to maximise overall system resilience and value.
Keywords: Poultry; ECC approved genotypes; growing rate; Gompertz curve ; Resilience

5062P The response of laying hens in cage free housing to two sequential exposures of Campylobacter hepaticus and high ambient temperature

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The study objective was to assess the response of barn housed ISA Brown hens to two exposures of Campylobacter hepaticus with/out exposure to high ambient temperature. When hens were 20 weeks of age (woa) they were allocated to initial groups: 1. Control: no C. hepaticus, 2. C. hepaticus gavage: oral exposure to C. hepaticus via gavage (109 cfu) and, 3. Contact: 10-weeks ongoing contact with hens gavaged with C. hepaticus (109 cfu). The vibroid C. hepaticus used was passaged 6 times (i.e. high passage, likely low virulence). From 26-30 woa half of the hens in groups 1, 2 and 3 were held at high ambient temperature i.e. 330C for 6 hrs/day, and 210C for 18 hrs/day (i.e. Heat treatment), and the other half remained at constant 210C (Cool treatment). When 30 woa, 20 hens/treatment were sacrificed, liver lesions scored (range 0-4) and C. hepaticus detected in bile via PCR. At 30 woa all remaining hens (30 hens/treatment) received C. hepaticus (109 cfu), passaged only two times (i.e. low passage, comparatively higher virulence), orally via gavage, half receiving coccoid, the other half vibroid form. Concurrently the temperature treatments were swapped i.e. hens from Cool temperature received Heat treatment, and vice versa. One week later. i.e. 31 woa, all hens were sacrificed, and liver lesions scored. At 30 and 31 woa total C. hepaticus antibody titres in serum were assayed by ELISA. Liver scores were analysed via factorial ANOVA and antibody titres via repeated measures ANOVA.
At 30 woa bile was C. hepaticus positive in 82% C. hepaticus Gavage and 33% Contact hens, with 0% positive in Control hens. At 30 woa liver scores in all C. hepaticus exposed and both temperature treatments were similar ($P>0.05$). At 31 woa Oral exposure to low passage C. hepaticus generated higher liver scores in Control compared to Gavage and Contact hens (average score 1.5, 0.6 and 0.4 respectively, $P<0.01$), with no difference between the latter two. Vibroid low passage C. hepaticus generated higher liver scores in previous Control hens than coccoid form (average score 2.2 and 0.9 respectively, $P<0.01$). Temperature had no effect. At 30 and 31 woa C. hepaticus antibody titres were higher in hens initially exposed via Gavage and Contact than Control ($P<0.01$). Ambient temperature and hen age (30 v 31 woa) did not alter antibody titres.

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Hens unexposed to high passage C. hepaticus (Control) had higher liver scores and lower antibody titres following oral exposure to the low passage C. hepaticus compared to hens previously exposed to high passage C. hepaticus either by Gavage or Contact. Further, compared to the coccoid form, vibroid C. hepaticus induced higher liver scores and may be a candidate agent for a C. hepaticus vaccine.
Keywords: C. hepaticus; heat; liver score; in-vitro passage; vibroid

5063P Control of field Infectious Bronchitis pressure by using a hatchery vaccination program with Genotype strains GI-13 (1/96) and GI-1 (B48) in broilers
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Infectious Bronchitis (IB) is a highly contagious viral disease affecting the respiratory, urogenital, and intestinal systems of broilers, laying hens and breeders as well. It is well established that Infectious Bronchitis Coronavirus (IBV) is one of the pathogens that causes major economic losses for the poultry industry worldwide. The aim of this study was to evaluate the effectiveness in terms of protection, conferred by a combined vaccination program with genotype strains belonging to Massachusetts and 793B groups, applied in the hatchery by spray at Day-Old (DO) chicks, through the study of production performance parameters. Four hundred and seven (407) flocks of heavy broilers (Ross 308) were studied between January 2020 and May 2025. Birds were simultaneously vaccinated at DO in the hatchery with a Massachusetts genotype strain GI-1 (Cevac Mass L®, strain B48) and a group 793/B genotype strain GI-13 (Cevac IBird®, strain 1/96) by spray in accordance with the recommendations of the products' SPC. Ten (10) blood samples were randomly collected from each flock at slaughter age, and all sera were serologically analyzed using the ELISA method (BioChek IBV kit). All broiler flocks were classified into two groups according to the results of serological analysis: one group with titers falling into the expected range following a hatchery vaccination (average titer around 3000) and a second group with 20% titers above 4500 which suggest a field viral passage. All technical and economical results of the two groups were statistically compared by using Student's T-test. The statistical analysis revealed that more than one third of the groups studied were subjected to field IB virus pressure (challenged group), whereas the serological profile of the rest of the groups was in accordance with titers expectations (non-challenged group). Performance parameters such as feed conversion ratio, average daily gain, performance index, mortality and condemnation were analyzed, and the results revealed no significant differences between these two groups ($p > 0.05$). These serological findings showed that IBV was actively circulating in some areas where the broiler flocks were grown and a comprehensive vaccination program combining strains belonging to Mass and 793B groups, when administered at the hatchery, can effectively protect against IB viral infection improving therefore the technical and economical results. In conclusion, this study shows that the vaccination of chickens at DO with the combination of Cevac Mass L® and Cevac IBird® provides stability of technical performance regardless the field pressure of Infectious Bronchitis that may occur in the area.
Keywords: Infectious Bronchitis; broilers; Mass L; IBird

5064P An innovative liquid solution to prevent coccidiosis and enhance performances in broiler chickens
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Coccidiosis remains a major challenge in the poultry industry, significantly impacting the health and productivity of broiler chickens worldwide. This parasitic disease, caused by species of Eimeria, leads to considerable economic losses due to decreased growth rates, increased mortality, and higher costs associated with pharmacological treatment. Developing nutritional solutions for broiler chickens is a promising approach to improve their overall health and resilience against diseases like coccidiosis. The objective of this study was to evaluate the preventive effect of an innovative liquid nutritional solution on performance and coccidiosis-related parameters.
A total of 204 male Ross308 broiler chickens were assigned to two treatment groups in a commercial farm with a history of coccidiosis (endogenous challenge): the control group (CTRL) that received commercial feed without coccidiostats, and the treated group (TRT) that received the same commercial feed and an innovative nutritionnal solution based on plant extracts in the water from day 1. Birds were housed in pens (6 per treatment) containing 17 animals each and were individually tagged. Animal weights and feed consumption were recorded on days 10, 22, and 29. Oocyst counts and lesion scores associated with coccidiosis were assessed on days 22 and 29. Data were analyzed using analysis of variance, with statistical significance defined as $p < 0.05$. When appropriate, starting body weight was used as a covariable in the model.
The TRT group exhibited higher body weight gains compared to the CTRL group for the D3-D10 period (22.05 g/day vs 20.73 g/day; $p = 0.023$), for the D10-D22 period (32.52 g/day vs 31.53 g/day; $p = 0.0.349$), and for the D22-D29 period (55.15 g/day vs 51.7 g/day; $p = 0.051$). Feed consumption did not differ significantly between groups for all periods. On day 22, a significant reduction in lesions caused by E.acervulina was observed in the TRT group ($p = 0.008$), indicating targeted efficacy in the early stages of infection. Furthermore, on day 22, the TRT group showed significantly lower mean oocyst excretion (44,333 oocysts/g) compared to the CTRL group (125,000 oocysts/g; $p = 0.037$), suggesting a positive effect of the treatment on coccidiosis management during the acute phase. On day 29, although the mean oocyst count remained lower in the TRT group (8,833 oocysts/g vs 31,000 oocysts/g), the difference was not statistically significant ($p = 0.126$), possibly due to an overall reduction in excretion at this stage.
In conclusion, this study highlights the promising potential of nutrition-based strategies in supporting animals facing coccidiosis challenge, providing a pathway to healthier and more productive poultry production.
Keywords: broiler; coccidiosis; nutrition; sustainability

5065P fosA3 Plasmid-Mediated Co-resistance in ESBL-Producing E. coli Isolated from the Poultry Industry and Human Urinary Tract Infections
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Objective: To investigate the co-resistance to fosfomycin and third-generation cephalosporins in extended-spectrum β-lactamase (ESBL)-producing Escherichia coli from poultry, retail chicken, and human urinary tract infections (UTIs), and to evaluate the

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plasmid-mediated transfer of fosA3 and blaCTX-M genes.
Experimental Design: A comparative cross-sectional study analyzed 609 ESBL-producing E. coli isolates from broiler farms (n=122), chicken carcasses (n=258), and community-acquired UTIs (n=229).
Materials and Methods: Fosfomycin susceptibility was determined by agar microdilution following CLSI (2024). PCR was used to detect blaCTX-M allelic groups and fosA3. Conjugation assays employed E. coli J53 as recipient strain, and transconjugants were typed by PCR-based replicon typing (PBRT). Clonality was assessed by two-locus MLST (fimH, fumC) and analyzed via Minimum Spanning Tree (PHYLOViZ). Statistical analyses (Chi-square, Fisher's exact test) were performed with JASP v0.19.2.
Results: Fosfomycin non-susceptibility was 50.8% in animal, 49.2% in food, and 23.1% in human isolates. fosA3 was detected in 80.5% of resistant strains, frequently co-occurring with blaCTX-M. Conjugation assays confirmed simultaneous transfer of both genes with an efficiency of 54.2%. Predominant plasmid incompatibility groups were IncI1 (30.8%) and IncN (29.8%), with occasional co-occurrence of IncI1–IncFIC and IncI1–IncN plasmids. Phylogenetic analysis revealed shared clones among animal, food, and human isolates, suggesting cross-transmission along the food chain.
Conclusions: The co-selection of fosA3 and blaCTX-M through transferable IncI1 and IncN plasmids highlights poultry production as a potential reservoir for multidrug-resistant E. coli. Continuous use of fosfomycin in poultry may indirectly promote resistance to third-generation cephalosporins. These findings underscore the necessity of integrated One Health surveillance to control antimicrobial resistance dissemination at the animal–human–environment interface.
Keywords: Escherichia coli; fosA3; blaCTX-M; fosfomycin resistance; One Health

5066P High-Resolution Detection of Salmonella Serovar Diversity in Broilers from Ecuador Using CRISPR-SeroSeq
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Objective: To characterize the diversity of Salmonella enterica serovars in commercial broiler flocks from Ecuador using CRISPR-SeroSeq, a high-resolution molecular serotyping tool capable of detecting multiple serovars within a single sample.
Experimental Design: Bootsock samples were collected from broiler houses of a single integrated poultry company between May 2023 and March 2024 and tested for Salmonella according to ISO 6579. From positive samples, DNA was extracted and a subset was analyzed by CRISPR-SeroSeq to determine serovar composition.
Materials and Methods: Seventy-six environmental samples were processed; Salmonella was confirmed by PCR targeting invA. DNA from 32 positive samples was amplified for CRISPR loci and sequenced on an Illumina NextSeq platform (150 cycles, single-end). Sequence reads were aligned via BLAST to a database of CRISPR spacers from 150 Salmonella serovars. Relative frequencies of each serovar were calculated as the proportion of total CRISPR spacer reads.
Results: Overall Salmonella prevalence was 77.6% (59/76). Eighteen out of nineteen farms were positive. CRISPR-SeroSeq identified single-serovar populations in 81.3% of samples and mixed serovars in 18.8%. Salmonella Infantis (S. Infantis) was the predominant serovar, detected in nearly all samples, including mixed populations. Importantly, Slmonella Enteritidis and Salmonella Typhimurium (serovars of major public health concern) were detected at relative abundances below 0.05, levels unlikely to be recovered by culture-based methods. Several farms showed both mono- and multi-serovar profiles, indicating complex contamination dynamics within the same company.
Conclusions: This study provides the first application of CRISPR-SeroSeq in Ecuador, revealing hidden Salmonella serovar diversity in broiler flocks. The predominance of S. Infantis and detection of low-abundance pathogenic serovars underscore the need for high-resolution surveillance tools. Incorporating CRISPR-SeroSeq into poultry monitoring programs could substantially improve food safety and biosecurity through more accurate detection and targeted control of Salmonella in the production chain.
Keywords: Salmonella enterica; broilers; CRISPR-SeroSeq; Ecuador; serovar diversity

5067P A formula of standardized essential oils to support parasite control methods in layers
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Infestations of external parasites, notably lice and mites, remain a persistent issue in laying farms. Their presence can have an economic impact through production losses, treatment costs, and workers' discomfort. Their management can be challenging, as their life cycle varies between species. To address this issue, the use of essential oils with a non-specific mode of action is proposed as an alternative to chemical treatment to protect laying hens from several external parasites. This study aims to assess the efficacy of Nor-Mite, a standardized essential oil blend of lemongrass and clove essential oils (SEOB) against lice and red poultry mite.

Two trials in commercial conditions were conducted:

*The first trial was conducted at a 22,000-caged layer farm with a high lice infestation. Hens have been supplemented with 750 g/T of SEOB in their feed for 7 weeks, 12 days after a Spinosad treatment, to control lice population. Fifteen lice traps were put in three random rows, and the number of lice in the trap was assessed at Week 0, Week 6, and Week 10, which is 3 weeks after the end of SEOB supplementation.

*For the second trial, 10,000-caged laying hens were divided into two groups. 5000 hens were kept as a control, and the other 5000 received a supplementation of SEOB at 100mL/day through drinking water for 2 weeks. The red mite population was assessed five times using traps, at W0 (before treatment), W1, W2 (during treatment), W3 and W4 (after treatment). The proportion of engorged mites was also recorded in each trap, as the mode of action of the SEOB is reported to prevent red mites from sucking hens' blood. Statistical analysis were performed with a T-test, using R Studio.

Trial 1: a significant (p-value <0.05) reduction of the number of lice per trap was observed during and after the supplementation. The farmer also reports that lice stopped crawling on his body after supplementation.
Trial 2: No differences in the number of red mites per trap or the proportion of engorged mites were found between the groups before treatment. After the treatment started, the supplemented group showed a significant reduction of the number of mites per trap and a lower proportion of engorged mites in traps compared to the control group (p-value < 0.05).

Overall, the study demonstrates the potential of standardized essen-

tial oil formula, in both powder and liquid forms, as a natural tool for managing external parasites in laying hens.
Keywords: Laying; Poultry red mite; Poultry Lice; Essential Oil; Parasite population management

5068P Comparative Evaluation of Peat Moss and Wood Shavings as Bedding in Commercial Broiler Production
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Effective litter management is critical to ensuring animal health and welfare in broiler production. Peat moss mixed with wood shavings (Naturesorb) is proposed as an alternative to traditional bedding materials, such as wood shavings. It is marketed for its high absorbency, antibacterial properties and potential to reduce ammonia and pathogen loads. This study aims to compare the two substrates in paired barns under commercial conditions. We hypothesized that peat moss would decrease bacterial loads, improve zootechnical performance and environmental parameters such as moisture and ammonia levels.
For this paired observational study, we recruited a total of 12 farms with two similar barns that received either Naturesorb or wood shavings as bedding. Flocks were visited once at 3 weeks of age, birds examined, and samples collected. qPCR assays were performed for Enterococcus cecorum (commensal and cpsO pathogenic), Clostridium perfringens (cpa, netB), Escherichia coli, Campylobacter jejuni, and Salmonella spp. from four sample types (darkling beetles, ventilation, droppings, and litter). In addition, litter moisture, litter pH, and air parameters (moisture, temperature, ammonia (NH3) and carbon dioxide (CO2) were measured at the time of visit. Footpad burn scores, bird cleanliness, and presence of breast lesions (burns and ulcers) were assessed during the visit and total mortality and slaughter data later obtained. Wilcoxon signed-rank tests were used to compare mean values between substrates per sample type (significance threshold: $p<0.05$). Substrate type significantly affected microbial loads in ventilation samples for several pathogens, with peat moss generally associated with lower pathogen detection. For most other parameters and sample types, including temperature, humidity, NH3 and CO2 levels, no differences were observed between the two bedding substrates. Total mortality rates were similar for broilers raised on peat moss and wood shavings, indicating that peat moss litter performed comparably to traditional bedding materials. Additionally, there were fewer footpad lesions in birds raised on peat moss $p<0.001$.This paired observational field study demonstrates that peat moss was as effective as traditional wood shavings in commercial broiler production settings. Overall, these results suggest that peat moss can maintain health, welfare, and performance outcomes equivalent to wood shavings while providing a viable option for broiler operations. Moreover, certain parameters, such as footpad health and some microbiological indicators, showed a tendency to improve with peat moss use, suggesting its potential additional benefits under specific conditions.
Keywords: Broiler chikens; Peat moss bedding; Wood shavings; Zootechnical performance; Bacterial load

5069P Elucidation of Vasculature-Associated Lymphoid Tissue as a Key Player in Avian Respiratory Immunity
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As fast-growing broilers are raised in intensive farms to meet the high demand for meat, they are exposed to a variety of pathogens, including respiratory pathogens. Importantly, immune cells originating from lymphoid tissue control these respiratory pathogens. Importantly, we discovered a unique lymphoid tissue in the kidney named vasculature-associated lymphoid tissue (VALT) which plays a vital role in the aggravation of renal lesions. In this study, we demonstrated the presence of this VALT in the lungs of healthy chickens and its role in lung immunity. Lung samples were collected from 10 chickens at 1, 15, and 28 days of age. They were examined by routine histopathological and immunofluorescence techniques. Pearson's chi-square test was performed to analyze significant differences between positive and negative samples at different ages. VALT appeared as scattered immune cells around blood vessels in the lungs of day 1 chicks and became diffusely organized by day 15 but it took on the shape of typical lymphoid tissue in 30 days ($P=0.659$). It indicates that VALT is a secondary lymphoid tissue in the lungs of chicken. VALT at 28 days of age consisted of B cells, T cells, and macrophages. It also contained blood vessels, lymphatic vessels and high endothelial venules. VALT had a well-organized connective tissue network which supported immune cells to organize within it. In addition, VALT contained macrophages and MHCII+ molecules, indicating antigen presentation occurred in it. Therefore, VALT will offer a new avenue for therapeutic investigations and vaccine development for respiratory diseases.
Keywords: Lymphoid tissue; Lungs; Chicken; Immunity

5070P Serological Monitoring and Field IBDV and NDV Pressure Assessment Following In Ovo Vaccination with rHVT-ND-IBD Vector Vaccine in Broilers in France
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In France, IBD vaccination strategies have traditionally relied on live attenuated vaccines administered orally via drinking water. However, this method presents several limitations, including interference with maternally derived antibodies, variability in antibody levels, and the risk of vaccine inactivation. That's why an increasing number of farmers are switching for hatchery vaccination. The objectives of this study were to evaluate serological responses and field viral pressure in broilers vaccinated in ovo with a rHVT-ND-IBD vector vaccine in a large-scale French broiler production. A field study was conducted from November 2024 to June 2025 in a single production organization involving 23.5 million broilers, including 9 million vaccinated in ovo. From 403 in ovo vaccinated flocks, 60 were blood-sampled at slaughter between 33 and 47 days of age. All birds were vaccinated in five hatcheries using the same rHVT-ND-IBD vector vaccine (Innovax®-ND-IBD, MSD Animal Health). Two broiler breeds were included: ROSS 308 and a hybrid one. For each flock, 20 birds were sampled for serology and 4 bursas of Fabricius were collected for IBD PCR and VP1/VP2 sequencing (Sanger method, Islam classification). Sera were analyzed using ID Screen® IBD VP2, Newcastle Disease Indirect (F protein specific), and Newcastle Disease Nucleoprotein Indirect ELISA kits (used as DIVA for ND). Statistical analyses were performed using R software, including non-parametric ANOVA and t-tests to assess the effects of hatchery, breeds, age, and viral challenge on serological parameters. Mean VP2 titer was 7358 ± 3953 with 83% ± 17% positivity. F protein titer averaged 3799 ± 2203 with 65% ± 19% positivity. NDV nucleoprotein titer was 115 ± 125 with 2.25% ± 4.3% positivity; all flocks had negative mean NP titers. Among 36 flocks with no wild IBD PCR results

(negative or Faragher 52/70), VP2 titer was 6014 ± 3622 and positivity 77% ± 16%. In 20 flocks with wild IBD virus (A3 or A3B1), VP2 titer was 10,187 ± 3,336 and positivity 94% ± 11% ($p = 0.006$). No significant hatchery or breed effects were observed. Age significantly influenced F protein titers ($p = 0.001$) and positivity ($p = 0.001$), but not VP2 titers ($p = 0.166$). In conclusion, in ovo vaccination with rHVT-ND-IBD vaccine provides consistent serological responses in broilers. ELISA kits used in this experiment were effective for monitoring vaccine response and detecting field viral pressure. None of the flocks with wild IBD virus PCR detection showed clinical or subclinical signs. Further analysis of zootechnical performance will be conducted based on vaccination type and viral challenge status.

Keywords: IBD; ND; in ovo vaccination; rHVT-ND-IBD vector vaccine; broiler

5071P Correlation between classical and whole-genome-based typing methods of Pasteurella multocida poultry isolates

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The objective of this study was to evaluate the added value of whole genome sequencing (WGS) for supporting strain selection in autogenous vaccine development for poultry Pasteurella multocida infections by comparing genomic clustering with classical typing methods. Poultry-origin P. multocida isolates collected from multiple European countries were characterized using capsular typing and multilocus sequence typing (MLST), followed by WGS-based single nucleotide polymorphism (SNP) analysis to assess genomic relatedness. Hierarchical clustering of SNP distances was applied to identify genetically related groups and to evaluate concordance with classical typing results. WGS-based clustering showed a high level of agreement with capsular and MLST profiles, confirming that isolates sharing identical classical types clustered consistently at the genomic level, while also revealing finer genetic differentiation within certain lineages. These findings demonstrate that WGS complements established typing methods by providing higher-resolution discrimination without contradicting classical classification schemes. The combined use of classical typing and WGS-based analysis offers a robust, practical framework for informed strain selection in autogenous vaccine development and enhances decision-making in the control of Pasteurella multocida infections in poultry production systems.

Keywords: Pasteurella multocida; whole genome sequencing; SNP analysis; strain typing; development of targeted vaccines

5072P Shiny Floors, Dirty Data: The Reality of Cleaning and Disinfection in Broiler Farms – A review

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Effective cleaning and disinfection (C&D) of broiler houses are crucial for reducing pathogen persistence, preventing disease outbreaks, and minimizing antimicrobial use. However, despite the central role of hygiene in biosecurity, on-farm success rates vary widely, and evidence-based standards are lacking. This systematic review evaluated 77 scientific papers addressing C&D procedures and disinfectant efficacy in broiler production worldwide. Aldehyde- and quaternary ammonium compound (QAC)-based disinfectants were the most frequently investigated (57% and 48% of papers, respectively), while only 45% of studies reported key procedural parameters such as detergent use, water temperature, waiting time, or application technique. This lack of methodological transparency impedes reproducibility and limits practical recommendations.

Quantitative assessment identified peracetic acid- and aldehyde-based disinfectants as most effective (mean efficacy > 90%), whereas potassium-peroxymonosulfate products frequently underperformed (< 80%). The presence of organic material impacted the disinfectant efficiency of several components, confirming that thorough cleaning and detergent use are prerequisites for success of disinfection. Pathogen-specific variations were evident: phenol-based products were less effective against Salmonella, while sodium hydroxide was insufficient against Campylobacter. Emerging technologies—such as the integration of nanoparticles, electrolyzed water, or bacteriophage-based disinfection—show promising results in vitro, yet robust field validation remains scarce.

Analysis of data revealed that research output has increased since 2020, paralleling the global focus on antimicrobial resistance. Nevertheless, the average "information density" across studies was below 50%, demonstrating that most papers still omit critical procedural details. This knowledge gap obstructs the development of harmonized hygiene protocols and undermines confidence in disinfection outcomes.

Overall, the review emphasizes that the success of C&D in broiler houses depends not only on the choice of compound but also on execution quality, documentation, and verification. Regular susceptibility testing of farm-specific isolates, transparent reporting of parameters, and implementation of validated, standardized protocols are urgently needed. Improving C&D effectiveness will enhance animal health, reduce antibiotic reliance, and contribute to safer and more sustainable poultry production systems.

Keywords: biosecurity; hygiene ; management; pathogens; cleaning

5073P Aflatoxicosis in Broilers: Clinical, Pathological, and Diagnostic Findings from Outbreaks in the northern Serbia

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Aflatoxicosis is a toxicosis in broiler chickens caused by ingestion of feed contaminated with aflatoxins, the secondary metabolites produced by molds of the Aspergillus genus, mainly A. flavus and A. parasiticus. The most potent and toxic form of aflatoxins is aflatoxin B1, which primarily affects the liver, leading to reduced growth, poor feed efficiency, immunosuppression, and increased susceptibility to secondary infections in broilers. Clinical signs include lethargy, loss of appetite, stunted growth, and diarrhea. Chronic aflatoxicosis may occur without overt clinical symptoms but negatively affects long-term production performance. In severe cases, hemorrhages, ascites, abnormally discolored organs (especially liver and kidneys), and elevated mortality are observed. The aim of this study is to present cases of broiler aflatoxicosis. Between December 2024 and June 2025, increased mortality was observed in broilers from fourth week of age, accompanied by decreased daily weight gain and poor feed conversion, on 13 farms of different capacities located in northern Serbia. From each farm, complete feed samples were tested for the presence of aflatoxin B1, using the ELISA method, while pathomorphological and histopathological examinations of the dead broilers were performed. The results showed that all feed samples contained elevated levels of aflatoxin B1, ranging from 5.24 to 33.63 µg/kg, which is significantly higher than the legal limit in Serbia for complete broiler feed (5 µg/kg). Pathomorphological findings revealed liver

enlargement, yellow to greenish discoloration, and hemorrhages, as well as renal hypertrophy. Histologically, vacuolar degeneration of hepatocytes, necrosis, and bile duct proliferation were observed, lesions typical of chronic aflatoxicosis. Based on clinical, laboratory, and histopathological findings, aflatoxicosis was confirmed as the cause of the observed health and productive issues.
In conclusion, the results emphasize the importance of continuous feed quality monitoring in intensive poultry production, as well as the implementation of other preventive measures to reduce the risk of aflatoxicosis in broilers, including the addition of mycotoxin adsorbents to the feed.
Keywords: aflatoxin B1; broiler chickens; liver pathology; mycotoxin adsorbents; feed safety

5074P Helminth pressure in organic and free-range poultry: insights from a national observatory (2019–2024)
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Expansion of free-range poultry systems encourages natural behaviours but increases birds' exposure to helminths. In European organic farming, parasite control is challenging due to restrictions on conventional anthelmintics and the downgrading of eggs after treatment. This study aimed to characterize helminth infestations in free-range and organic poultry farms in France and to identify management factors associated with parasite load.
An observational study was conducted in France from 2019 to 2023 on 106 free-range and organic laying hen farms and 65 broiler farms. In 2024, continuous monitoring in 3 organic laying hen farms assessed parasite dynamics through targeted sampling (arrival on farm, before and after deworming). Parasites were identified using the Anses–ITAVI sieving method. Coproscopy was performed in 9 farms to compare diagnostic methods. A chi-square test assessed the relationship between helminth presence and treatment strategy. An exploratory multiple correspondence analysis (MCA) was used on 48 laying hen farms to explore multivariate associations between parasite burden and co-occurring management factors (poultry age, number of bird by flock, treatment strategy, and treatment-to-sampling interval) beyond pairwise comparisons.
Parasite prevalence was higher in laying hens (93%) than in broilers (46%). Ascaridia were detected in 74% of laying hen farms and 38% of broiler farms, and Heterakis in 88% and 28%, respectively. Cestodes and Capillaria were only detected in laying hens (43% and 9%, respectively). Use of classic (C) anthelmintics was associated with a non-significant trend toward lower contamination rates in laying hen farms (91%) compared with alternative (A: 97%) or no treatment (NT: 94%) strategies ($p = 0.07$). A similar pattern was observed in broilers (C: 34%, A: 56%, NT 58%). The sieving method outperformed coproscopy, (+83% detection worm identification). MCA indicated that high parasite burdens (nemat./bird > 10) clustered primarily with older flocks (>70 weeks) and longer treatment-to-sampling intervals (>6 weeks) rather than treatment type alone. Continuous monitoring provided illustrative evidence of a short-term reduction in detectable nematode burden following classical treatment, followed by rapid parasite reappearance when treatment-free intervals exceeded six weeks. One plausible hypothesis for this reappearance is the environmental persistence of parasite eggs not targeted by anthelmintics.
Overall, these results suggest that treatment timing, flock age, and environmental management are important factors associated with parasite burden in free-range and organic poultry systems and, therefore, should be considered when designing parasite control strategies.
Keywords: Helminths; Free-range; Broiler; Laying hen; Anthelminthic

5075P Genomic surveillance of zoonotic-potential Salmonella Newport in Brazilian chicken meat with global relevance
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Objective: To analyze the phenotypic and genomic profile of a Salmonella Newport isolate from chicken meat in Brazil, with emphasis on antimicrobial resistance and its zoonotical potential for global dissemination.
Methodology: A Salmonella enterica serovar Newport isolate (Sal 2608-18) from chicken meat (Gallus gallus) in Brazil was characterized by phenotypic antimicrobial susceptibility testing (AST) and whole-genome sequencing - WGS (Illumina MiSeq). Disk diffusion was performed for cephalosporins, fluoroquinolones, carbapenems, and sulfonamides, while Minimum Inhibitory Concentration - MICs were determined for colistin and ciprofloxacin. Genomic analyses included assembly, molecular typing, detection of resistance and virulence genes, pathogenicity islands, plasmids, and SNP-based phylogenomics, with comparison to 57 international human genomes from EnteroBase.
Results: The isolate was assigned to ST45 and showed resistance to fluoroquinolones and second- and third-generation cephalosporins. Most pathogenicity islands were conserved, differing from the human-associated genomes mainly by the absence of SPI-4;2 and SPI-12. Resistome analysis identified plasmids carrying resistance determinants, including the AmpC β-lactamase CMY-2 and the plasmid-mediated quinolone resistance gene qnrB19; the latter, together with multiple efflux and regulatory determinants, was consistent with reduced susceptibility to ciprofloxacin and nalidixic acid in the absence of classical QRDR mutations. Phylogenomic analysis placed the isolate within a predominantly human-associated subclade, closely related to genomes from France, the United States, and the United Kingdom, supporting the international circulation of a conserved genomic backbone, possibly linked to the food chain.
Conclusion: The antimicrobial resistance profile of the Brazilian isolate, characterized by the concomitant presence of blaCMY-2 and qnrB19, adds a functional dimension to its phylogenetic placement. The absence of these determinants in related genomes suggests recent acquisition via mobile genetic elements. High genomic similarity to human-associated strains from multiple countries, combined with Brazil's role as the world's largest poultry meat exporter and the conservation of pathogenicity islands shared with clinical isolates, highlights the potential for zoonotic transmission and international dissemination of resistant clones.
Keywords: Salmonella Newport; Antimicrobioal resistence; Genomic surveillance; Poultry production; Brazil

5076P Serology as a tool to assess different vaccination protocols against Newcastle disease by using different Elisa kits in Polish broiler flocks between 2017-2025
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Newcastle disease (ND) is a major viral disease of poultry presenting continuous threat to commercial poultry production. In addition to good biosecurity practices, preventive vaccination, using live and / or vector vaccines, of flocks are the common strategies. Monitoring and surveillance are also vital to identifying outbreaks early and implementing rapid response actions in affect-

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ed areas. In Poland, biosecurity and vaccination schedules for ND kept the country free of ND up to 2023 when the first major ND outbreak was manifasted. The aim of this study was to measure the efficacy of various vaccination programs with different Elisa kits as depicted by AMT (Arithmetic Mean Titers), CV (%) and positivity (%) before and after the introduction of the disease in Poland. 23 blood samples per flock, from 17 to 56 days of age, from 2493 flocks were analyzed for ND serology between 2017 to 2025. The analysis was split in two periods, 2017-2023 before ND first outbreak and 2023-2025 after the outbreak. Broiler flocks were regularly monitored for ND serology since 2017, and all data were stored in PHP, Ceva Animal Health database. Elisa kits based on different technologies (Biochek and Biochek NDV-F, Idexx, IDVet NDV-F indirect and IDVet indirect conventional vaccines) were used for titers evaluation and all data were statistically analysed by using Student's T-test. Before the first ND outbreak, flocks were vaccinated with mainly one live vaccine in the hatchery and occasionally in the field and some farms with r-HVT-F vaccine in the hatchery as well. The results showed significant icrease in titers and positivity and decrease in CV after r-HVT-F vaccination in the hatchery, whereas absense of ND field stains was confirmed with the low titers after the use of conventional vaccines Elisa kits. Nevertheless, cases of significantly high titers of ND after one single shot of live vaccine in the hatchery were detected, but not invstigated further, indicating either live vaccine cisculation or field strains. In 2023-2025, the vaccination was intensified with the use of r-HVT-F and live vaccine in the hatchery and various additional live vaccine applications in the field mainly using Lasota strain. The results revealed significantly high levels of protection when r-HVT-F vaccines were used. Flocks vaccinated with lives vaccines in the hatchery and in the field performed as expected in terms of serology significantly higher than the vaccination with just one live vaccine but still exhibited low positivity (27%) indicating poor vaccination methods and increasing the risk of outbreak. In conclusion, monitoring is a simple tool to access the vaccination programs efficacy and helping to decide the right strategy.

Keywords: Newcastle Disease; serology; broilers; Elisa

5077P Divergence between Salmonella serovars from serogroup D regarding the survival rate during infection of chicken-derived HD-11 macrophages.

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Salmonella enterica subsp. enterica serovar Enteritidis (Salmonella Enteritidis - SE) has great importance to public health and poultry production, while Salmonella Gallinarum biovars Gallinarum (SG) and Pullorum (SP) are a burden to the poultry industry. These bacteria are genetically related since they belong to the serogroup D, whose members whose all bacteria from this group that have the O9 antigen as a key component of lipopolysaccharide (LPS) in the bacterial cell wall. However, they cause completely different diseases in poultry, with the macrophages playing an essential role in both the infection course and severity. Therefore, our study aimed to evaluate the capture and survival of SE, SG and SP within chicken-derived HD-11 macrophage-like cells at 20 hours post-infection (hpi).

Bacterial strains Salmonella Enteritidis str. P125109, Salmonella Gallinarum biovars Gallinarum str. 287/91 and Pullorum str. 449/87 were used to evaluate the uptake and survival at 20-hpi throughout chicken-derived HD-11 macrophage-like infection, in vitro. The experiment was conducted with three technical and biological replicates with a multiplicity of infection (MOI) 1:10. The bacterial counts of uptake, survival, and inoculum were logarithmically transformed, previously to comparison of strains' results. One-way ANOVA was used for uptake, while Two-way ANOVA evaluated the fold-net and CFU/mL of bacterial counts, followed by Tukey's multiple comparisons test. Statistical difference was considered with a P-value lower than 0.05 ($P < 0.05$). All statistical analyses were performed using Graphpad Prism 10.3.0 for Mac OS.

Although no difference was observed in uptake between SG and SE, SE showed greater uptake than SP strain, demonstrating that the three serovars are recognized differently by HD-11 cells; it's important to highlight that no difference between inocula was found. Moreover, SE showed a greater intra-HD-11 cells' survival than SG and SP strains. Interestingly, the fold-net replication (20h vs Uptake) had no difference between those serovars studied, suggesting that these serovars exhibit similar behaviors after HD-11 cells' uptake, despite the strong difference in survival. The survival rate of SE can be related to bacterial metabolism, which is less fastidious than that of SG and SP. On the other hand, SP had the worst survival, although this bacterium can persist in host macrophages for up to 70 days.

Our results suggest that the chicken-derived HD-11 macrophage infection model can be a good in vitro alternative to study serogroup D Salmonella strains, and, ultimately, they indicate that bacterial metabolism can be a rule for high bacterial rate inside the HD-11 cells and their persistence.

Keywords: O9 antigen; Salmonella Enteritidis; Salmonella Gallinarum; Salmonella Pullorum; serogroup D

5078P In vitro evaluation of bacteriophages as biofilm-disrupting agents against Salmonella Infantis in poultry facilities – Preliminary results

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Introduction: Salmonella continues to represent a major food safety hazard worldwide, with significant repercussions for human health, and the overall economy of the food supply chain. The persistence of Salmonella in food-processing environments, particularly through biofilm formation on equipment and surfaces, poses an additional challenge by increasing bacterial resistance to cleaning, disinfection, and antimicrobial treatments. Therefore, research efforts are needed to identify and validate alternative control strategies to conventional chemical and antimicrobial agents.

Among emerging alternatives, bacteriophages, viruses that specifically infect and lyse bacterial hosts, have gained growing attention as sustainable, natural, and highly specific tools for bacterial control. They hold potential both as therapeutic agents and as environmentally friendly disinfectants for food-related environments.

Objective: In a previous study, newly isolated phages were shown to be effective against Salmonella Infantis planktonic cells. However, their potential to act against biofilm-forming strains remained unexplored. The present work aims to provide a preliminary assessment of these bacteriophages as surface-disinfecting agents in poultry facilities in Spain.

Materials and methods: To this end, field Salmonella strains were characterized in vitro to determine their biofilm-forming capacity at 24, 48, and 72 h at 37 °C, quantified through crystal violet staining. The reference strain CECT 4594 served as a positive control. After biofilm establishment (24 h and 48 h), the biofilms were exposed to 109 PFU/well of the tested bacteriophages for 4 h, after which biofilm biomass was re-quantified. Statistical analyses were performed using two-way ANOVA.

Results: they confirmed that all field strains were able to form

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stable biofilms within 24-48 h. Moreover, treatment with specific bacteriophages led to a measurable and statistically significant reduction in biofilm biomass after short incubation periods. These findings demonstrate that the tested phages exhibit biofilm-disrupting activity against S. Infantis field strains to different degrees. Conclusions: beyond demonstrating proof of concept, these results highlight the potential of bacteriophages as an effective biocontrol strategy to mitigate Salmonella persistence in poultry production environments. Furthermore, incorporating phage-based interventions into hygiene management programs could help decrease dependence on chemical disinfectants and antibiotics, supporting both food safety and sustainable antimicrobial stewardship. Continued research is warranted to optimize phage-host matching, and integrate this approach into comprehensive Salmonella control frameworks.

Keywords: Salmonella; biofilms; bacteriophages; disinfectants; Infantis

5079P Hidden Cost of Avian Diseases in Tropical Extensive Poultry Production Systems : An Estimation Based on Partial Budgeting in Sub-Saharan Africa

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Family poultry production is an essential source of income, food and nutrition security, and women's socio-economic empowerment in tropical rural areas of sub-Saharan Africa (SSA). Despite this essential role, these tropical extensive poultry production systems (TEPPSs) are characterized by high poultry mortality, mainly due to infectious diseases such as Newcastle disease and parasitic infections. This disease burden reduces productivity, weakens household incomes, and undermines poultry welfare as a result of avoidable losses and damages. The objective of this study was to estimate the economic magnitude of these losses in order to guide priority animal health interventions.

A survey was conducted on 137 poultry-keeping households in the communes of Houéyogbé and Bopa, southern Benin. Data collected through semi-structured interviews, observations, and questionnaires were used to characterize health practices and mortality levels. Economic assessment was based on the partial budgeting method, comparing the observed situation (50.98% average mortality up to market age, five months) with a reference scenario (20%) inspired by the zootechnical and health profile of poultry flocks where a network of trained community-based animal health workers provides basic prophylaxis (vaccination, antiparasitic treatment, and awareness raising).

Diseases were identified by 84.7% of farmers as the main cause of mortality in chickens. The average prophylaxis cost was FCFA 4,862 (≈ USD 7.95) per chicken per year. Avoidable economic losses and damages reached FCFA 13,761.72 per hen per year, corresponding to 9.3 unproduced birds. This disease burden has a direct negative impact on household food and nutrition security, socio-economic stability, and live chickens' welfare. Avian diseases constitute a major health and socio-economic constraint in TEPPSs in SSA. Effective deployment of community-based animal health workers could significantly reduce mortality, increase productivity, improve poultry welfare, and support women's socio-economic empowerment and rural livelihoods.

Keywords: Animal health workers; Family poultry; Newcastle disease; Rural Africa; Welfare

5080P Cerebellar Malacia in 7-Day-Old Broilers: Evidence for Avian Encephalomyelitis

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Neurotropic viral infections in young chicks cause rapid neurologic disease and high mortality in unvaccinated flocks. Documented clinicopathologic and histopathologic features of an acute neurologic outbreak in 7-day-old broiler chicks and evaluated the relative likelihood of Avian Encephalomyelitis virus (AEV) versus Newcastle disease virus (NDV). Representative freshly dead chicks from a broiler flock (n=100) presenting clinical signs of progressive ataxia, circling, and 15 deaths over seven days were necropsied. Postmortem examination revealed minimal gross changes, limited to pulmonary congestion, mild enteritis, retained yolk sac, and abdominal exudate. The brain (cerebrum, cerebellum, and brainstem), proventriculus, and sciatic nerve were fixed, paraffin-embedded, and stained with hematoxylin and eosin for light microscopy. Histology showed severe, diffuse non-suppurative leptomeningoencephalitis with dense perivascular mononuclear cuffing, endothelial swelling, extensive gliosis, neuronal degeneration, and neuronophagia. Notably, the cerebellum exhibited multifocal to coalescing granular layer necrosis with liquefactive encephalomalacia; marked ependymal hyperplasia and subependymal lymphocytic infiltration were also present. There was no convincing transmural fibrinoid necrosis of vessels or widespread visceral necrosis/hemorrhage. In birds this young, the lesion distribution (prominent cerebellar malacia and granular cell loss), epidemiologic history (early onset, possible vertical transmission), and lack of systemic hemorrhagic or necrotizing visceral lesions favour avian encephalomyelitis virus as the most likely cause; NDV remains an important differential. This case emphasizes the critical role of histopathology in diagnosing central nervous system infections in poultry and highlights the importance of early vaccination and biosecurity in preventing severe neurologic diseases in broiler flocks.

Keywords: Avian encephalitis; broiler chickens; neurotropic viruses; histopathology; Newcastle disease virus

5081P Effects of feeding barley, oats and extracted sunflower meal containing diets with broiler chickens affect the tight junction and mucine mRNA expressions in the jejunum and caeca

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Dietary fibers have several impacts on gut morphology, integrity, viscosity, microbiota and digestion. The aim of this trial was to get more understanding, how the feeding of barley, oats, and sunflower meal (SFM) affects the gene expression of tight proteins and mucins in the jejunum and caeca at different age categories. In total, 288 male Ross 308 one-day-old chickens were randomly assorted into 3 dietary group using four replicate pens of 24 birds. Besides a corn-wheat-soybean based control diet (C), two fiber treatments were applied. The fiber 1 (F1) diets contained 15% barley on the expense of corn, while the fiber 2 diets (F2) contained barley, oats and SFM. The insoluble dietary fiber content of diets increased from 12.54% to 16.07%; 11.85% to 14.12% and 11.11% to 16.4% in the starter (day 0-10); grower; finisher, grower (day 11-24) and finisher (day 25-40) phases respectively. The increments in the precipitable soluble dietary fiber fractions were 2.08% -3.11%; 2.03% – 3.02% and 2.08% – 3.19% in the starter, grower and finisher phases. On day 21 jejunum and caecum samples, on day 46 of life caecum samples were collected for gene expression analysis of selected tight junction (CLDN1, ZO-1, CLDN10, OCLDN) and mucin (MUC2) proteins. The RNA extraction, and subsequent purity and integrity examinations were carried out according to the manufacturer's instructions (RNeasy Mini kits). The mRNA gene

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expression results were evaluated by one-way ANOVA, at each time points and sampling place the fiber treatment results were compared with the control. In the jejunum, on day 21, both F1 and F2 treatments significantly downregulated the mRNA expression of OCLDN, while F1 diets also decreased MUC2 gene expression. On the other hand, feeding the F2 diets significantly upregulated CLDN1. In the caecum, the different ages of birds affected the expressions in a different way. In the young, 21-day old chickens almost all tight junction protein genes were downregulated in the F1and F2 groups. The only exception was OCLDN in the F1 treatment group. No significant MUC2 response was found. On the other hand, at day 46, the barley supplemented F1 diets upregulated all the investigated genes, but the difference was only in the case of OCLDN significant. Interestingly, treatment F2 did not modify the mRNA expression. The result showed that the different levels of soluble and insoluble fiber content of diet have different effects on the gene tight junction expressions in the jejunum and in the caeca. The age of chickens is also an important factor that determines the response. Among the measured parameters occludin seems to be the most sensitive tight junction protein.

Keywords: dietary fiber; broiler chicken; tight junction protein; mucine; mRNA

5082P Efficacy of a live bivalent Salmonella Enteritidis and S. Typhimurium vaccine against Fowl Typhoid

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Fowl typhoid (FT), caused by Salmonella Gallinarum (SG), is a poultry disease distributed worldwide that persists in many regions and in the last decade has re-emerged in some regions where it was considered to be eradicated. Besides biosecurity, vaccines are frequently used to reduce mortality, clinical signs and vertical or horizontal transmission. The aim of this study was to assess the protection against FT conferred by a live bivalent attenuated vaccine that includes S. Enteritidis (SE) and S. Typhimurium (ST) in its formulation. Two experimental groups of brown layers were included: 1) 60 birds receiving 3 doses of the vaccine (AviPro™ SALMONELLA DUO) administered by gavage into the crop on day 1, week 6 and week 16 of life, and 2) a control group of 60 unvaccinated birds. Three independent challenges were done on weeks 26, 30 and 32 of life in which hens (n=20) from both experimental groups were infected via the oral route with a virulent strain of SG – INTA91 – 106CFU/hen. Mortality was recorded for 15 days and all hens that died from FT or survived were necropsied, and isolation of the pathogen from liver samples was attempted. Mortality and isolation of SG rates were compared by the Fisher exact test with a significance level of 0.05. In all challenge experiments, hens started to die from day 5 onwards in the unvaccinated groups. After the challenge in week 26, 90% of the hens from the unvaccinated group died from FT whereas none of the hens from the vaccinated groups died. All dead hens from control groups were found to be positive for SG in their livers but the microorganism could not be isolated from any of the surviving hens from control or vaccinated groups. Similar results were found when the challenge was done in week 30, where 90% of the unvaccinated and none of the vaccinated birds died from FT during the observation period. In this trial, SG was isolated from 1 of the 2 surviving hens from the control group and from none of the vaccinated hens. Finally, when the challenge was performed in week 32, 50% of the unvaccinated and none of the vaccinated hens died from FT, and SG was not isolated from any of the livers from the surviving birds from both groups. In all challenges, differences between mortality were significant (for challenges at weeks 26 and 30, $P<0.00001$; and for challenge on week 32, $P=0.001$). Vaccination with the bivalent live SE & ST vaccine was able to reduce mortality due to FT significantly and could be considered a useful tool to control the disease.

Keywords: Salmonella; Fowl Typhoid; Vaccines; Poultry

5083P Efficacy of the vaccination with bivalent Salmonella Enteritidis and S. Typhimurium live and inactivated vaccines against Fowl Typhoid

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Fowl typhoid (FT) caused by Salmonella ser. Gallinarum (SG) is a disease distributed worldwide that persists in Latin American, Africa, and Asia as well as in some European countries. Besides biosecurity, vaccines are frequently used to reduce mortality, clinical signs and vertical or horizontal transmission. There are several types of immunogens available in the market, such as inactivated and live-attenuated vaccines, whose efficacy to control different serotypes of Salmonella may differ. The aim of this study was to compare the protection against the infection with SG conferred by live or inactivated vaccines that include S. Enteritidis (SE) and S. Typhimurium (ST) in their formulation. Three experimental groups of 20 brown layers each were considered: 1) birds receiving 3 doses of a vaccine formulated with live attenuated strains of SE and ST that was administered by gavage into the crop on day 1 and weeks 6 and 16 of life, 2) birds receiving 2 doses of an inactivated vaccine (bacterin) that included SE and ST and was administered by the intramuscular route on weeks 12 and 16 of life, and 3) unvaccinated birds. Thereafter, in week 26 hens from all groups were infected by the oral route with a virulent strain of SG. Mortality was recorded for 15 days post infection (pi) and all hens that died from FT or survived were necropsied and samples of livers were analyzed for isolation of the pathogen. Comparison the mortality and isolation of SG rates were compared by the Fisher exact test with a significance level of 0.05. Hens started to die from day 5 pi onwards. At the end of the trial, 90% of the hens from the unvaccinated group, 65% of the hens from the group that received the inactivated vaccine and none (0%) of the hens immunized with the live bivalent vaccine died form FT. SG was isolated from the livers of all hens dead from FT and from none of the surviving hens, except from 1 bird from the group that received the inactivated vaccine. No significant differences in the mortality rates were found between the group of unvaccinated hens and the birds that were immunized with the inactivated vaccine ($P= 0.1274$). In contrast, vaccination with the bivalent live vaccine significantly reduced the mortality due to FT ($P< 0.0001$). Vaccination with the bivalent live SE & ST vaccine was able to reduce the mortality due to FT in a very efficient way, and could be considered as a useful tool to control the disease.

Keywords: Salmonella; Fowl Typhoid; Vaccines

5084P The impact of using macrophage-like HD-11 cells in the uptake and intracellular survival of Salmonella serogroup D

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Background:

Despite differences in host range and infection biology, Salmonella serogroup D comprises are closely related genomically, including Salmonella Enteritidis (SE), commonly associated with

poultry-related foodborne outbreaks, and Salmonella Gallinarum biovars Gallinarum (SG) and Pullorum (SP), which are responsible for some of the most important diseases in poultry. Salmonella invades macrophages for replication, making these cells crucial for coordinating immune responses and determining infection progression. Therefore, this study aimed to compare the uptake and intracellular survival of SE, SG and SP between immortalized macrophage-like HD-11 cell lines and chicken primary macrophages (CPM).

Methods:
Bacterial strains SE (P125109), SG (287/91) and SP (449/87) were used to assess bacterial uptake (30 min) and survival (20 hours post-infection) in vitro in chicken primary macrophages and HD-11 cells. All assays were carried out with three technical and three biological replicates at a multiplicity of infection (MOI) of 10:1. All statistical analyses were performed using GraphPad Prism 10.0.0 for Mac OS. A Two-way analysis with Sidak's multiple comparison test was used to analyze statistical significance in uptake assays. Survival data were analyzed by the Kruskal-Wallis multiple comparison test, and SE was selected as the control group due to its higher intramacrophage survival. A P-value ≤ 0.05 was considered statistically significant.

Results and Discussion:
HD-11 cells exhibited significant differences in bacteria uptake among SG vs SE (P=0.0003) and SP vs SE (P<0.0001), whereas chicken primary macrophages showed lower uptake rates with no differences between strains. The biovar SP displayed the lowest uptake levels in primary macrophages, demonstrating that SP are differently recognized between cells.
SP showed a lower intra-macrophage survival in chicken primary macrophage compared to SE used as a control (P<0.0001), following SG (P=0.0007), suggesting that these biovars present different behaviors after macrophage uptake in that cell model. This divergence could be related to the genetic apparatus within the SPI-2 type III Secretion System, which is required for intracellular persistence, contributing to the reduced survival of SP.

Conclusion:
Our findings suggest that the intracellular environment shapes the behavior of the biovars. This underscores the importance of using primary cells to better reflect physiological Salmonella-macrophage interactions. Moreover, the reduced uptake and survival of SP in CPM may reflect an alternative infection strategy, potentially involving limited recognition or reducing the intracellular adaptation.
Keywords: Cell immune responses ; Poultry; Salmonella Enteritidis; Salmonella Gallinarum; Salmonella Pullorum

5085P Interference of antimicrobials with the re-isolation of a bivalent Salmonella live vaccine

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Live-attenuated vaccines are used to reduce tissue colonization and prevent vertical or horizontal transmission of Salmonella strains in commercial poultry. They can be administered from the first day of life onwards in drinking water, offering an early protection to young chicks. At the same time, antimicrobials are commonly added to feed or drinking water during the first days of life of the birds, and it may be necessary to observe a withdrawal period to avoid any potential negative impact of the antimicrobial on the viability of the vaccine strains, thus reducing their capacity to colonize the gut. The aim of this study was to assess the minimum withdrawal period of antimicrobials commonly used in poultry to avoid interference with a bivalent S. Enteritidis (SE) and S. Typhimurium (ST) live vaccine (AviProTM SALMONELLA DUO, Elanco Animal Health).
Two antimicrobials were tested: tylosin (Tylan Soluble®, Elanco) and enrofloxacin (Baytril®, Elanco). Different groups of birds received the antimicrobials from day 1 to 5 of life in the drinking water at concentrations recommended by the manufacturer. Non-treated control groups of birds were included in the trial. Thereafter, birds (n=20) from each group were vaccinated by oral gavage 2, 3 or 5 days after the administration of the antimicrobials.
Two days after vaccination, birds were euthanized. Enumeration of the vaccine strains from cecal contents was done by plating log10 dilutions onto brilliant green agar (BGA) plates plus rifampicin and streptomycin (to detect SE vaccine strain) and onto BGA plates plus rifampicin and nalidixic acid (to detect ST vaccine strain).
In the case of the SE vaccine strain, no significant differences were found in the levels of the vaccine strain in cecal contents when birds were immunized 2 (P= 0.5100) or 3 days (P=0.6887) after the administration of tylosin when compared to the control group. In contrast, vaccine levels were significantly reduced when birds were vaccinated 2 (P<0.0001) or 3 (0.0181) days after administration of enrofloxacin. However, 5 days after the administration of enrofloxacin, vaccine levels were similar to the ones found in the control group (P=0.6045). In the case of ST, no significant differences were found in the levels of the vaccine strains in the birds treated with either tylosin or enrofloxacin in the groups of birds vaccinated after 2, 3 or 5 days after the administration of the antimicrobials (P>0.05).
The results show that it is possible to administer the vaccine 2 days after administration of tylosin, but that a minimum withdrawal period of 5 days is necessary after the administration of enrofloxacin, in order to reach acceptable colonization rates of the vaccine strains in the gut.
Keywords: Salmonella; live vaccine; antibiotics; tylosin; enrofloxacin

5086P In vitro trial to assess the effect of dewormers or a parasiticide on the viability of a live bivalent Salmonella vaccine applied via drinking water

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Live Salmonella vaccines are routinely used in laying hens and breeding birds in most European countries and across the globe. The bivalent vaccine AviProTM SALMONELLA DUO, administered via the drinking water line, provides protection against S. Enteritidis and S. Typhimurium, the two most important Salmonella serovars with regards to Public Health.
Dewormers, such as fenbendazole (Panacur® Aquasol and Gallifen) or flubendazole (Flimabend® and Fluboral), and an anti-mite treatment (Exzolt®) are applied via drinking water as well, raising the question if there might be any negative impact of these substances on the viability of the vaccine strains.
In order to investigate if both the vaccine and the anthelmintic or parasiticide product can be administered on the same day via drinking water, an in vitro trial was conducted where one of the products and the vaccine were added to clean drinking water at a concentration representing the situation in the field. To assess the impact of residues of the products in the drinking water lines, additional trials were run using 50%, 10% and 1% of the recommended dose of either the wormer or Exzolt®.

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Water samples containing the vaccine plus either the dewormer or the parasiticide were incubated at 15°C and 30°C, both with and without the addition of a water stabilizer (AviBlueTM), and the two Salmonella vaccine strains were enumerated after an incubation period of 0 hrs, 2 hrs and 4 hrs, using a ten-fold dilution series. All dewormer products tested had a detrimental effect on the vaccine strains in a dose-, time- and temperature-dependent manner. The ST vaccine strain was generally more impacted than the SE strain, and all products led to a greater reduction in vaccine strain counts at the higher temperature. Fenbendazole impacted the vaccine strains more than flubendazole. Even at a concentration of 1/00 of the recommended dosage, the dewormers still had a negative effect on the vaccine strains. The use of AviBlueTM was able to mitigate the effect to some degree, but differences were seen depending on the product and temperature, and it cannot be guaranteed that a sufficient vaccine dose will reach the birds even if AviBlueTM is used. Exzolt® had a minor effect on vaccine viability at 15°C, but reduced vaccine counts significantly at 30°C. Customers are therefore advised to administer neither dewormers nor Exzolt® on the same day as AviProTM live vaccines, even if a water stabilizer is added. For the dewormers, even low concentrations will have a negative impact on the vaccine strains, and it is therefore important to make sure that no residues of such substances are present in the drinking water line when administering the vaccine.

Keywords: Salmonella; live vaccine; flubendazole; fenbendazole; anti-mite

5087P Salmonella and Campylobacter cross-contamination between animal sectors (poultry, cattle, pigs): testing a systemic approach to biosecurity support

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Salmonella and Campylobacter's ability to persist in the environment and their asymptomatic carriage in various species complicate efforts to control them in the field, especially on farms with diverse livestock activities (e.g., poultry, cattle and swine). This setup, common in France, increases the risk of cross-contamination through the movement of vehicles, people, and equipment. Despite the challenge, it has received little attention. This project adresses it by using a spatial and dynamic approach of Salmonella and Campylobacter's epidemiology, to identify effective control measures.

Six poultry farms with diverse livestock activities and a history of Salmonella contamination were selected to implement this approach, taking into account all production units and the flows between them. In November 2023, a first farm was recruited with rabbit, broiler, duck, cattle units and 350 ha of crops, following the detection of Salmonella Typhimurium affecting the rabbit flock. Potential contamination routes were identified through flow mapping, supported by longitudinal monitoring of the farm environmental contamination. Three sampling campaigns were conducted in different seasons, gathering in total 149 samples (wipes, boot swabs, fecal samples) in all production units, on farming equipment, and along traffic routes in the professional area. The samples were analyzed for the detection of Salmonella and Campylobacter, following the recommendations of standards NF U 47-100 and NF EN ISO 10272-1, respectively. Finally, a participatory approach involving the farmer and the veterinarian was used to identify sanitary barriers, workflows, and risk-management strategies to limit cross-contamination.

In April 2024, 17/39 samples tested positive for C. jejuni in cattle and duck units, and 1/39 tested positive for S. Arizonae in the rabbit manure pit. In November 2024, 32/55 samples tested positive for C. jejuni and 2/55 for C. coli in all units. Three out of 55 were tested positive for S. Typhimurium, detected on equipment shared with other farms and on pasture, and two out of 55 were positive for S. Veneziana in a cattle stall. The participatory workshop took place in December 2024. At-risk flows and areas were mapped out on the farm. In order to mitigate the risks, the farmer proposed moving the carcass freezer and shared equipment storage, and modifying the cattle manure disposal routes. In May 2025, no Salmonella was detected and 20/55 samples tested positive for C. jejuni.

This approach revealed significant Campylobacter and Salmonella contamination in animals' living areas and shared equipment, while effectively illustrating contamination routes and motivating farmers to implement targeted biosecurity changes.

Keywords: Salmonella; Campylobacter; Cross-contamination; Biosecurity; Participative approach

5088P Assessment of Salmonella Enteritidis and Salmonella Typhimurium challenge in commercial 113-weeks of age layers vaccinated with a Salmonella Enteritidis live vaccine.

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Salmonella Enteritidis (SE) and Salmonella Typhimurium (ST) are the main Salmonella serovars causing for foodborne infections in humans, with poultry products as an important source of transmission. Vaccination is a key tool for broiler breeder and laying hen producers and is an essential part of Salmonella control programs. In modern poultry production, laying hens are kept in long production cycles, extending beyond of peak immunity. Consequently, the risk of SE and ST contamination persists for many weeks after the rearing phase, when vaccination is usually applied. This raises the question of how long vaccine-induced protection in older hens and whether hey can control Salmonella infection at advanced ages. The aim of this study was to assess the protection provided by a live Salmonella Enteritidis vaccine (Cevac®Salmovac) against SE and ST in 113-week-old vaccinated hens after 3 drinking water applications during rearing at 2, 7 and 11 weeks of age. Forty hens were assigned to three groups: a negative control (T1; n=14), SE-challenged group (T2; n=13), and ST-challenged group (T3; n=13). Birds in T2 and T3 were orally infected with about1.9×108 CFU of their respective strains. Salmonella colonization was evaluated 48 hours post-challenge using cloacal swabs, cecal content, liver and spleen samples. Differences in positive birds were analyzed with a binomial model, and bacterial counts (log10 CFU) by regression analysis, with $p < 0.05$ (IBM SPSS v29). All birds tested negative upon arrival, and the control group remained negative throughout the trial. Cloacal shedding was minimal in T2 (7.6%) and moderate in T3 (38.4%), with statistically significant differences observed between groups ($p < 0.05$). Cecal colonization occurred in 92% of infected hens, yet bacterial loads were low despite the high infective dose, averaging 0.31 log10 CFU in T2 and 0.82 log10 CFU in T3 with no statistically significant differences between groups ($p > 0.05$). No Salmonella was detected in liver or spleen samples, suggesting absence of systemic dissemination. These findings highlight that long-term

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immunity conferred by vaccination during rearing with Cevac® Salmovac can persist in very old hens without revaccination requested during laying period. Despite being challenged at 113 weeks of age with a high dose (~108 CFU), Salmonella colonization was limited and bacterial loads remained low. However, since this study did not include an unvaccinated challenged group, further studies are needed to directly compare vaccinated and non-vaccinated hens at advanced ages in order to fully quantify the specific contribution of vaccination to the observed protection.
Keywords: Salmonella Enteritidis; Salmonella Typhimurium; Vaccination; Colonization; Laying Hens

5089P Translating Field Conditions to Laboratory: Establishment of an in vivo Model of Colibacillosis in Laying Hens

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Avian colibacillosis, caused by avian pathogenic Escherichia coli (APEC), poses a major challenge for the egg industry, contributing to economic losses through increased mortality, impaired hen health, and reduced egg production during peak production at 27–28 weeks of age. Serogroups O1, O2, and O78 predominate in clinical cases, with virulence of strains mediated through expression of adhesins, siderophore-mediated iron uptake, and protective surface structures. The absence of consensus regarding inoculation conditions to reliably reproduce extra-intestinal APEC infections in laying hens at peak production remains a limitation for establishing a reproducible experimental model. Yet, such a model is essential for evaluating efficacy of antimicrobial and alternative strategies to manage the disease.

The present study aimed to define inoculation parameters capable of reproducing clinical and pathological features of field colibacillosis in Canada. Based on results from pilot studies comparing different inoculation routes, 60 28-week-old white Lohmann hens were divided in three control groups (n=8) and three infected groups (n=12). Hens were exposed to a light stress (100 lux) for 0, 6 or 12 hours prior to inoculation. Challenged groups received an intraperitoneal (IP) dose of 1x109 CFU of a rifampicin-resistant APEC O78:H9 strain in 0.5 mL of 0.9% NaCl, while control groups for each of the stress conditions were given 0.5mL of 0.9% NaCl IP. Clinical signs, mortality, lesion scores and bacterial loads in the ileum and cecum (generic E. coli and the challenge strain) were measured. Mortality reached 16.6% in the non-stressed infected group within 96 hours post-infection (PI), whereas exposure to 6 and 12 hours of light stress elevated mortality to 33.3% and 50.0%, respectively. T-tests analysis showed significant differences in total lesion scores between challenged and control groups at 24, 48, and 72 hours PI. Two-way ANOVA identified differences among infected groups only at 48 hours PI. No significant differences were detected in generic E. coli loads, suggesting IP inoculation did not trigger intestinal dysbiosis.

Overall, the results of the present study suggest that an IP challenge with APEC O78:H9 in the exponential growth phase is sufficient to induce characteristic lesions of extraintestinal infections in hens at peak production. While colibacillosis often arises as a secondary infection under predisposing stress conditions, the incorporation of a 6-hour light stress appears to better mimic field conditions, while limiting mortality in hens. This unique model therefore represents a valuable tool for evaluating of prevention and treatment strategies for colibacillosis in laying hens.
Keywords: Avian pathogenic Escherichia coli (APEC); Colibacillosis; Laying Hens; In vivo; Model

5090P In Ovo Supplementation of Lactobacillus acidophilus Provides Comparable Protection to Weekly Post-Hatch Dosing Against Salmonella Typhimurium in Broiler Chickens

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The consumption of poultry products, including meat and eggs, has been associated with increased incidence of foodborne illnesses in humans, particularly infections caused by Salmonella. In the US, over 19,000 people are hospitalized annually due to Salmonella infections, resulting in approximately 380 deaths. Recognizing chickens as a major reservoir of these pathogens, implementing pre-harvest mitigation strategies is crucial to reducing foodborne illnesses and supporting the production of safe, sustainable, and high-quality poultry products. While probiotics given to hatched chicks have demonstrated potential against Salmonella, their efficacy when administered in ovo remains largely unexplored. Therefore, this study investigated the efficacy of in ovo supplementation of Lactobacillus acidophilus in chick embryos to enhance resistance to Salmonella colonization post-hatch. A total of 110 chicks were divided into five groups (n = 22 each): 1) in ovo, 2) post-hatch (weekly oral gavage), 3) in ovo + post-hatch, 4) positive control (infected, non-treated), and 5) negative control (non-infected, non-treated). Birds were challenged with S. Typhimurium on days 2 and 37, and necropsies were conducted on days 10 and 42 to collect cecal contents for Salmonella enumeration and 16S rRNA-based microbiome analysis. Tissues, including the Bursa of Fabricius and cecal tonsils, were collected for analysis of immune system gene expression. Salmonella colony counts were analyzed using one-way ANOVA followed by Tukey's post hoc test. The gene expression and microbiome analyses are ongoing. Results showed that on day 10, Salmonella counts were reduced by 0.45, 0.55, and 0.7 log10 in the in ovo, post-hatch, and in ovo + post-hatch groups, respectively. Salmonella was detected in 1 of 8 birds in all treatment groups, compared to 6 of 8 in the positive control group. By day 42, Salmonella was detected in only one bird per treatment group, likely due to antibody-mediated clearance from the early infection. In conclusion, both in ovo and post-hatch administration of L. acidophilus provided protection against Salmonella colonization. Notably, a single in ovo dose produced effects comparable to weekly post-hatch oral administration, suggesting that in ovo inoculation is a practical approach for controlling Salmonella in broiler chickens. Further optimization of dosage may be necessary to achieve complete elimination of the pathogen.
Keywords: Chicken; Broiler; Probiotic; Salmonella; Lactobacillus

5091P Molecular characterization of avian reovirus cases of tenosynovitis in commercial broilers in Belgium and the Philippines

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Avian reoviruses (ARVs) are frequently isolated from both healthy and diseased poultry and are associated in several disease clinical

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signs, including tenosynovitis/lameness (green hocks), runting-stunting syndrome (RSS), malabsorption syndrome (MAS), and respiratory and enteric disorders. ARV also imposes significant losses in carcass quality and value during slaughter due to condemnations and downgrading. One of the ARV control and prevention depends on the vaccination of breeder flocks using live attenuated and inactivated vaccines, providing passive immunity to their offspring.

This study presents field cases of ARV-associated tenosynovitis and lameness in broilers from Belgium and the Philippines. In Belgium, ten birds aged 26–35 days—five symptomatic (showing lameness and poor growth) and five asymptomatic—were collected from three farms for diagnostic investigation. In the Philippines, ten ARV cases were reported and diagnosed between 2023 and 2025 in some commercial broiler farms with signs of tenosynovitis and pododermatitis.

In both regions, necropsy revealed no significant internal organ lesions, but affected birds consistently shown peritendinous thickening and inflammation within tendon sheaths which leads to typical lesion in the hock called "green hocks". Tissue samples (caecal tonsils, jejunum, duodenum, spleen, liver, and tendons) were processed and analyzed by qPCR and virus isolation method using chicken embryo liver cell lines. ARV isolates were genotyped through S1 gene sequencing, and additional bacteriological and molecular assays were performed to exclude other causes of lameness.

Among the Belgian samples, nine were positive by virus isolation and were subjected to gene sequencing. This revealed that Clusters 5 were most frequently present in tendons of clinically affected birds. Cluster 4 appeared to be the most predominant cluster found in both sick and healthy birds followed by Cluster 2. In the Philippines, molecular characterization of ARV-positive samples identified Cluster 2 as the predominant serotype, detected in seven out of nine positive cases. Only one Cluster 5 and Cluster 4, and one still Unknown were identified from the samples tested.

These findings highlight the circulation of multiple ARV clusters within and across regions, with Cluster 5 primarily linked to tenosynovitis in Belgium and Cluster 2 associated with "green hock" and pododermatitis lesions in Philippine broilers. The concurrent detection of diverse ARV clusters underscores the dynamic nature of ARV evolution and the need for ongoing molecular surveillance and virulence studies to guide effective vaccination and control strategies globally.

Keywords: Avian Reovirus (ARV); tenosynovitis; green hocks; malabsorption

5092P BACTERIOPHAGES: A THERAPEUTIC APPROACH TO CONTROL SALMONELLA IN POULTRY PRODUCTION

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Intensified poultry production has supported global demand for affordable, high-quality protein but has also contributed to foodborne illnesses, notably salmonellosis. Traditional Salmonella control methods face limitations, creating a need for innovative approaches. Bacteriophages (phages), viruses that selectively target bacteria, offer a promising biocontrol strategy to eliminate pathogens like Salmonella. The PhagoVet consortium aims to register bacteriophages as a zootechnical additive authorized by the European Food Safety Authority. Efficacy trials were conducted on 324 male broilers divided into three groups: T1 (negative control), T2 (positive control, challenged with Salmonella Typhimurium), and T3 (phage-treated, challenged with Salmonella Typhimurium and treated with PhagoVet via drinking water). Results revealed significant reductions in Salmonella Typhimurium levels in the phage-treated group (T3) during the rearing cycle. Additionally, PhagoVet nearly eliminated Salmonella contamination in the farm environment by the cycle's end, demonstrating the potential of phage therapy as a preventative and biocontrol strategy. This study underscores the efficacy and safety of bacteriophages to control Salmonella infections in poultry production. The authorization of bacteriophages as a zootechnical additive could transform on-farm Salmonella control, enhancing poultry product safety and reducing foodborne illness risks.

Keywords: bacteriophages; Salmonella; biocontrol; poultry production; PhagoVet

5093P Effect of Ethnoveterinary Medicine to Control Ranikhet Disease in Small-Scale Native Chicken Farming

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Native chicken farming is emerging as an important venture for people in rural areas, as it demands little to no resources. In small-scale poultry operations, farmers typically handle the care of sick birds themselves using various ethnoveterinary medicines (EVMs). The current study was undertaken to evaluate the effect of ethnoveterinary medicines in controlling Ranikhet disease by incorporating ethnomedicinal plants into the feed. An entrepreneur bought 800 day-old Aseel chicken from the Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, South India and reared them under a deep litter management system. At eight weeks of age, the chicken showed dullness, depression, greenish-white watery droppings, anorexia with 15% morbidity and 16% mortality. It is an outbreak at the farmers' field and hence, the experimental design was not planned. The vaccination schedule was followed as per the recommendation of the veterinarian. On necropsy, pinpoint haemorrhages were observed in the proventriculus and the caecal tonsil. Clinical samples, such as proventriculus and intestinal tissues were collected and subjected to histopathological examination and molecular analysis, respectively, at the Central University Laboratory, TANUVAS, Chennai, South India. Histopathological examination revealed severe congestion, haemorrhage and epithelial hypertrophy. Upon molecular analysis of intestine and cloacal swabs, it was positive for Ranikhet disease virus during the clinical course of the disease. Qualitative phytochemical analysis of medicinal plants was conducted at the Ethno Veterinary Herbal Research Centre for Poultry, Namakkal, TANUVAS. Medicinal plants contain active compounds such as allicin found in Allium sativum, cuminaldehyde found in Cuminum cyminum, terpenoids found in Ocimum sanctum, flavonoids found in Phyllanthus amarus, alkaloids found in Piper nigrum and azadirachtin found in Azadirachta indica. The affected birds were treated with TANUVAS recommended ethnoveterinary herbal formulation for Ranikhet disease. For each 10 birds, cumin seeds - 10 g, pepper - 5 g, turmeric powder - 5 g, stone breaker leaves - 50 g, onion - 5 and garlic - 5 were ground and made into a mixture. This mixture was incorporated into the mash feed and administered over a period of 10 days. At the end of the treatment, the antibody level against the Ranikhet disease virus was determined. The observed Ranikhet disease HI titre was satisfactory (above 128) and around 500 birds recovered. The findings of the study indicated that the ethnoveterinary herbal formulation recommended by TANUVAS is effective in preventing Ranikhet

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disease and has demonstrated success in lowering its incidence.
Keywords: Ranikhet disease; Native chicken; Ethnoveterinary medicine; Photochemical analysis; HI titre

5094P Whole blood biochemical and gas parameters using two diagnostic systems according to laying hens fed enriched- and none-enriched linseed diets.
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By enriching designer eggs with nutrients like omega-3s which improve blood lipid profiles and antioxydants reducing inflammation, objective is to mitigate effects of egg consumption while boosting beneficial components that support overall health, including potentially liver health.

In a first experiment, hundred laying hens were fed to produce designer eggs (high linseed content). Eight and six hens were then blood sampled in each group, respectively: linseed enriched and control groups. In a second experiment, a number of seven and eighty-nine hens were blood sampled, respectively: linseed omega-3 and non-omega-3 fed hens. Measurements of blood chemistries and electrolytes, hematology and blood gases were obtained with the I-Stat CG-8+ cartridge (Abbott) (EPOC) and the VetScan© Avian Diagnostic Profile reagent rotor (Zoetis) (VETSCAN). In the first method, pH, partial pressure of carbon dioxide (pCO2, mm Hg), partial pressure of oxygen dioxide (pO2), base excess (BE) BEecf, HCO3, total concentration of carbon dioxide (TCO2), saturation of hemoglobin (sO2%), sodium (Na, mmol/L), potassium (K+, mmol/L), ionized calcium (iCa, mmol/L), Glucose (Glu, mg/dL), hematocrit (Hct % packed cell volume [PCV]) and hemoglobin (Hb, g/dL) were analysed. Second method established values of aspartate aminotransferase (AST), bile-acid (BA), creatine kinase (CK), uric acid (UA), total calcium (Ca++), phosphorous (PHOS), total protein (TP), albumin (ALB), globulin (GLOB), hemolyse (HEM), lipemia (LIP) and icteria (ICT). For statistical analysis, comparisons of means between diet treatments were assessed by least significant differences using Statistix 10,0 for a one-way ANOVA. Statements of significance were based on $P \leq 0,05$.

In the first experiment, data were significant between treatments for AST ($P \leq 0,01$) and iCa mmol/L ($P \leq 0.05$). A blood profile database was then datamined comparing layers on linseed diets and non linseed diets. Similar effect on AST was observed ($P \leq 0,01$). In addition, glu mg/dL, total protein g/L, albumin g/L, Na mmol/L and Hct% were all significant between treatments ($P \leq 0,05$). The significantly lower AST in the omega 3 group with no significant difference in CK indicates that the difference comes from liver metabolism. Low AST levels in layer type birds has been associated with antinutritional factors in linseed that are antagonists with vitamin B6. These results indicate that antinutritional factors could be involved in liver health and possibly anemia in linseed fed omega-3 egg producing layers. Further research should be undertaken to evaluate these effect and possible association with reduced production, amino acid metabolism, gut integrity and blood loss from gut lesions.
Keywords: Designer eggs; Omega-3; Flaxseed; liver health; metabolic profile

5095P Genomic Monitoring and Typing of Infectious Bronchitis Virus (IBV) Strains in Poultry Populations Across Europe, Asia, and Latin America
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Infectious Bronchitis Virus (IBV) is a highly contagious avian coronavirus causing respiratory disease, reduced egg production, and significant economic losses in poultry worldwide. Due to high mutation and recombination rates, numerous IBV variants continue to emerge, often providing limited cross-protection. Continuous surveillance and molecular characterization are therefore essential to understand IBV epidemiology and optimize vaccination strategies.

This study investigated the distribution and genetic diversity of IBV strains across Europe (Belgium), Asia (Vietnam and the Philippines), and Latin America (Brazil) using molecular diagnostic methods.

A total of 14,068 samples were collected from commercial poultry farms between 2022 and 2024. Samples originated from broiler and layer flocks showing respiratory signs, production losses, or included in routine monitoring programs. Inclusion criteria consisted of farm submissions to regional diagnostic laboratories (MercoLab, Brazil; Vetworks/Poulpharm laboratories in Belgium, Vietnam, and the Philippines) during periods of suspected IBV circulation.

Samples primarily included tracheal, cloacal, and organ swabs or tissues and were processed using standardized laboratory protocols. All samples were analyzed by real-time quantitative PCR (qPCR) for IBV detection, followed by lineage-specific typing assays for strain identification.

In total, 8,978 samples were analyzed from Brazil, 4,860 from Belgium, and 229 from Vietnam and the Philippines. Multiple IBV lineages were identified, including Massachusetts (GI-1), Brazilian (BR), GI-23, GI-19 (QX), GI-13 (4/91, 793B), GI-12 (D274), IBV 181, and several emerging variants.

Data analysis included calculation of annual detection rates and relative lineage prevalence by country. Temporal trends were assessed descriptively by comparing yearly and monthly distributions of detected strains; no inferential statistical analyses were performed due to the surveillance-based study design.

IBV detection in Brazil increased from 43% in 2022 to 85% in 2024, with GI-23 surpassing Massachusetts and BR strains. In Belgium, GI-13 (4/91, 793B) and GI-19 (QX) predominated, with GI-19 cases increasing from 47 in 2022 to 110 in 2023. Massachusetts strains remained stable, while IBV 181 increased in 2024. In Vietnam and the Philippines, QX-like and GI-23 strains co-circulated, mirroring patterns observed in Brazil.

Overall, this study demonstrates clear regional differences in IBV strain prevalence and highlights the importance of sustained molecular surveillance and region-specific vaccination strategies to support effective IBV control.

Keywords: Infectious Bronchitis Virus, Molecular surveillance, IBV genotypes, Poultry health
Keywords: IBV; GI-19, GI-23, QX-like, GI-13; Massachusetts; phylogenetic analysis; vaccine adaptation

5096P Longitudinal monitoring of reovirus reveals high losses in vaccinated broiler parent flocks
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Reovirus infection of broiler parent flocks can induce serious losses because of tenosynovitis and stunting of young animals. As health of legs is of high priority in parent flocks, even mild changes could be complicated with secondary bacterial infections, what could result in significant selection and exclusion of animals from production. We monitored longitudinally flocks on the broiler parent farm during last 6 years, 13 flocks in total, during rearing period. Detailed description and number of excluded birds was marked and analyzed as weekly losses. Also, from some flocks, carcasses were sent for necropsy and diagnostics, including molecular detection of reovirus using qPCR reaction. All flocks were vaccinated with commercial vaccines, one live at 7 weeks of age, and one inactivated at 18 weeks of age, except in last two flocks in which, because of poor live vaccine supply, only one time inactivated or two time inactivated vaccine was used. Flocks are negative on M gallisepticum and vaccinated against M. synoviae. Monitoring reveals start of the clinical manifestation already at 8 weeks of age, and the latest start at 15 weeks of age. Peaks and dominant losses were present between 12 and 17 weeks of age. Average losses because of leg problems in monitored flocks were between 0,08% and 3,61%. Birds sent before peak clinics for necropsy and later for qPCR detection of reovirus were 12,5% to 42,8% positive in tendon samples. Results show high losses in some flocks despite vaccination what imply probable presence of local specific, non-homologus strains compared to commercial vaccine. Also, highest losses of 3,61% and 3,5% were in flock vaccinated with one live and one inactivated or only one inactivate vaccine, respectively. The lowest losses of 0,08% were present in a flock vaccinated with two inactivated vaccines. This could imply that high levels of humoral immunity could provide better protection. It is known that quality of chickens results in higher or lower problems with reoviral tenosynovitis, so control should be placed on parents as possible source for vertical transmission to broiler flocks. Special focus should be on improvement of general health and immunity during rearing, which is hard and riskiest period in broiler parent life, with emphasis on the biosecurity measures in reduction of mentioned problems.
Keywords: reoviral tenosynovitis; broiler parent flock; longitudinal monitoring; qPCR

5097P Study of chicken immune responses to long noncoding RNAs (lncRNAs) against highly pathogenic avian influenza H5N1 infection

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Introduction/objective: Avian influenza viruses (AIV) pose a significant threat to global poultry production, necessitating effective control strategies to mitigate economic losses and ensure animal welfare. Long non-coding RNAs (lncRNAs) have emerged as crucial regulators of immune responses, yet their roles in AIV-infected chickens remain poorly understood. In this study, we aimed to identify novel lncRNAs associated with host responses to AIV H5N1 infection and examine DE transcripts, including lncRNAs, in chicken lung tissue. Specifically, we analyzed DE lncRNAs and their targets in both highly pathogenic AIV (HPAIV)-resistant and -susceptible indigenous Vietnamese Ri chickens.
Materials and Methods:
The HPAIV-H5N1-resistant and -susceptible Ri chickens (5 chickens/group) were infected with HPAIV-H5N1 and lung tissue samples were collected 1- and 3-days post-infection (dpi). After RNA sequencing and transcriptome analysis, we identified novel lncRNAs and analyzed differentially expressed (DE) transcripts at 1- and 3-dpi in chicken lung tissue. The connections between lncRNAs and mRNAs were visualized using Cytoscape, and predicted cis- and trans-acting lncRNA and their target genes. Validation for DE lncRNAs and mRNAs were performed using RT-qPCR.
Results: Our results revealed a higher number of DE lncRNAs and mRNAs at 1 dpi and 3 dpi, respectively, compared to control, with resistant chickens exhibiting a notably stronger immune response than susceptible chickens at 3 dpi. Functional analysis implicated these lncRNAs in immune-related pathways crucial for host responses to H5N1 viral infection. Furthermore, we identified lncRNA–mRNA interactions associated with antiviral responses and immune function. Notably, several genes involved in antiviral resistance and immune responses showed higher expression in resistant chickens, confirming their stronger antiviral response.
Conclusions: Overall, our study provides insights into the role of lncRNAs in the host's response to HPAIV H5N1 infection in chickens and highlights potential candidates for further investigation into host–pathogen interactions. These findings could drive the development of novel control strategies for AIVs, significantly enhancing poultry health and biosecurity.
Keywords: long non-coding RNA; chicken; lung; avian influenza virus; H5N1

5098P A Multi-Omics Analysis Reveals Genes Potentially Involved in Resistance to High-Temperature Environments in Broilers

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Temperature is a major stressor in poultry production. When ambient temperature exceeds the thermal comfort zone (>30 °C), chickens are prone to heat stress, leading to performance loss, immunosuppression, increased mortality, and oxidative damage. We integrated whole-genome, multi-tissue transcriptomics (hypothalamus, pituitary, heart, liver), and serum metabolomics to investigate host genetic mechanisms of heat stress resistance. Breeds from hot regions—Wenchang (WC), Fayoumi (FA), Wuding (WD), and Dandarawi (DA)—and from cooler regions—Dagu (DG) and Beijing-You (BY)—were analyzed. At 40 °C , 283 WC chickens were exposed for 2 and 4 h, with mortality of 54% and 82%. Birds were classified as resistant or susceptible based on survival and moribund status, and heat-exposure time was recorded for moribund individuals. Phylogeny and TreeMix showed clustering consistent with thermal origin (BY–DG, WC–WD, FA–DA). Contrasting low vs high-temperature lineages identified 3,782 shared positively selected genes (PSGs). A GWAS using time to death as the phenotype annotated 50 genes within ±100 kb of significant loci; 11 overlapped PSGs. Fine-mapping of Chr3 (91.535–91.635 Mb) pinpointed two causal variants: Chr3_91585075 (PIP = 0.95; AA favorable) and Chr3_91588802 (PIP = 0.64; GG favorable). Genes within ±100 kb included FBXO25, ERICH1, TDRP; FBXO25 and ERICH1 were both PSGs and GWAS hits. In 4 tissues, FBXO25 expression was negatively correlated with time to death ($P < 0.05$). Serum metabolomics under an OPLS-DA framework (VIP > 1.0, $P < 0.05$, $|FC| \geq 1.2$) identified key heat-resistance metabolites—10,20-dihydroxyeicosanoic acid, 3-methoxypropylamine, hydantoin-5-propionic acid, N-acetyl-D-phenylalanine, N-stearoyl proline, oxoglutaric acid, sphingosine, and stearic acid—which were lower in resistant birds. RNA-seq comparisons of resistant vs susceptible birds yielded 55 common DEGs across tissues ($|FC| \geq 1.5$, FDR < 0.05). WGCNA integrating key metabolites and time to death identified 19 cross-tissue hub genes; intersecting these with the common DEGs produced 18 key DEGs. Correlation analysis linked FBXO25 with JUN, FOS, and HSPH1 and so on. Protein interaction experiments demonstrated that FBXO25 can bind to and

inhibit JUN/FOS (AP-1). Further functional assays showed that under heat stress si-JUN suppressed HSPA2, HSPH1, DNAJB1 and si-FBXO25 enhanced HSPA4, DNAJA4 in HD11 cells($P<0.05$). KEGG enrichment implicated apoptosis, MAPK, ErbB, and FoxO pathways. In sum, as a component of the E3 ubiquitin-ligase complex, FBXO25 was expressed at lower levels in the heat-resistant group and influenced adaptation to high-temperature environments by regulating AP-1 and downstream stress-response pathways.
Keywords: Heat stress; Selective-sweep; GWAS; RNA-seq; Metabolomics

5099P Molecular Mechanisms Underlying Eggshell Quality in Indigenous Chinese Chicken Breeds

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Hypothesis or objective

Eggshell quality—including thickness, strength, and color—differs between the indigenous Yaoshan chicken (YS) and its high-producing crossbreed (GYR). We hypothesized that these differences are regulated by uterine gene–metabolite networks controlling mineral deposition and pigment synthesis. This study aimed to identify key genes, metabolites, and pathways underlying eggshell traits using integrated transcriptomic and metabolomic analyses.

Experimental design and methods

A total of 1,000 hens (500 YS, 500 GYR) were reared under standard conditions. At 260 days, uterine tissues from six hens per breed were collected during eggshell calcification. Thirty eggs were measured for thickness, strength, and color. RNA-seq and LC-MS/MS were used to profile transcriptomic and metabolomic changes. Differentially expressed genes (DEGs; $|\log2(\text{fold change})| > 1$, $p < 0.05$) and metabolites (DMs; $|\log2(\text{fold change})| > 1$, VIP > 1) were identified. GO and KEGG analyses, along with integrated transcriptome–metabolome analysis, were performed to identify key gene–metabolite pairs and associated pathways.

Statement of statistical analysis

Eggshell traits were tested for normality and variance homogeneity. Differences between GYR and YS were evaluated using t-tests or Mann–Whitney U tests, with $p < 0.05$ considered significant. Data are presented as "mean ± SEM". Spearman's rank correlation was used to assess relationships between variables.

Results

GYR hens produced green-shelled eggs with significantly greater thickness and strength compared with the light-brown eggs of YS hens. RNA-seq identified 877 DEGs (196 upregulated, 681 downregulated in GYR), including SLCO1B3, PTGR1, LGR6, MELTF, CRISP2, OVSTL, enriched in calcium signaling, arachidonic acid metabolism, neuroactive ligand–receptor interaction, and immune pathways. Metabolomics detected 1,619 metabolites, with 79 significantly altered, including prostaglandin-related compounds and biliverdin. Integrated analyses highlighted key gene–metabolite networks, notably PTGDS–prostaglandin E2 and PTGS1–prostaglandin E2, as regulators of eggshell traits.

Conclusions

YS and GYR uterine analyses revealed key genes, metabolites, and pathways regulating eggshell quality. Notably, PTGDS–prostaglandin E2 and PTGS1–prostaglandin E2 were highlighted as major gene–metabolite pairs, offering insights into molecular mechanisms and potential targets for improving eggshell traits in poultry.
Keywords: eggshell quality; uterus; chicken; transcriptome; metabolome

5100P Genetic diversity analysis of indigenous Siruvidai chicken of Tamil Nadu, South India,

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A study was conducted to analyse the genetic diversity of indigenous Siruvidai chicken and assess their genetic distance from established Indian (Aseel, Kadaknath) and exotic breeds (Rhode Island Red, White Leghorn). A total of 240 blood samples were collected from Rhode Island Red (30 samples), Aseel (30 samples), Kadaknath (30 samples), Peruvidai (30 samples) and Siruvidai (4 regions and 30 samples from each region) ecotypes. DNA was extracted using the phenol-chloroform method. The 397 bp region of the chicken is widely used in genetic studies to analyse the maternal origins, diversity and phylogenetic relationships of domestic chickens (Gallus gallus domesticus).D-loop fragment is used to construct phylogenetic trees. The hypervariable region I (HV1) of the mtDNA D-loop was amplified using primers L16750 and CR1b. PCR reactions were performed with optimized buffer systems and cycling conditions. Amplicons were visualized using agarose gel electrophoresis and purified using the QIAquick PCR Purification Kit. Direct sequencing of the HV1 region was performed using internal primers (CR-forward and CR-reverse) and the BigDye Terminator v3.1 kit on an ABI 3730XL sequencer. Chromas was used for sequence viewing and editing. Sequences were aligned using ClustalX 1.83, and polymorphic sites were identified using MacClade 4.0. MEGA 3.0 was employed for phylogenetic tree construction and evolutionary analysis. D-loop sequences were compared to reference GenBank sequences. The aligned sequences (397 bp) of theD-loop HV1 region showed significant variation among the populations. Polymorphic sites distinguished the Siruvidai ecotypes and Peruvidai from other breeds. Based on the 397 bp HV1 region, 14 haplotypes were identified across the studied populations. Siruvidai ecotypes exhibited high haplotypic diversity, while Peruvidai had 2 unique haplotypes. However, Aseel and Kadaknath had more shared haplotypes. Exotic breeds (RIR and WLH) had 1 single dominant haplotype each. A detailed matrix was prepared and it was found that all Siruvidai ecotypes are more closely related to Indian breeds (Aseel, Kadaknath, Peruvidai) than to exotic breeds (WLH, RIR). The Red Jungle Fowl the ancestral node was genetically closest to Aseel and Siruvidai. The study revealed substantial genetic diversity among Siruvidai ecotypes and their clear differentiation from other indigenous and exotic breeds. The phylogenetic and distance analyses establishes Siruvidai chicken as a genetically distinct breed.
Keywords: Indian indigenous chicken; Genetic diversity; phylogenetic tree; Genetic distance; Exotic breeds

5101P A chicken genome-wide CRISPR screening identifies ABHD12 as a key host factor promoting Salmonella infection via the NOD1/NF-κB pathway

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Objective:

This study aimed to identify key host factors regulating Salmonella typhimurium infection in chicken macrophages (HD11) using a genome-wide CRISPR/Cas9 screen and to elucidate the molecular mechanism of the candidate gene ABHD12.

Experimental Design:

We employed a loss-of-function genetic screen in the HD11 chicken macrophage line using a genome-wide CRISPR/Cas9 sgRNA library. Screening under S. typhimurium infection pressure,

followed by high-throughput sequencing and bioinformatics, identified host genes critical for Salmonella infection. The role of ABHD12 was further investigated.
Materials and Methods:
A lentiviral sgRNA library (93,961 sgRNAs targeting 16,821 genes) was used to generate a genome-wide knockout pool in HD11 cells. Cells were infected with mCherry-S. typhimurium SL1344, and uninfected (mCherry-negative) cells were isolated by FACS. Enriched sgRNAs and candidate genes were identified using MaGeCK. ABHD12's interaction with NOD1 and its impact on NF-κB signaling were validated by Co-IP, WB, and a NF-κB reporter assay.
Statistical Analysis:
sgRNA enrichment was analyzed with MaGeCK (FDR<0.005, |LFC|>1). Bacterial loads and cytokine levels were compared by unpaired t-test; survival data were analyzed by Log-rank test. Data are mean±SD.
Results:
We constructed a chicken genome-wide CRISPR library and, through three rounds of screening in HD11 cells, identified 215 candidate host factors, including ABHD12. These genes are implicated in phagocytosis, calcium homeostasis, vesicle transport, and innate immunity. Mechanistically, ABHD12 directly interacts with NOD1, promotes its degradation, and negatively regulates the NOD1/NF-κB pathway to facilitate infection. Consistently, ABHD12 knockout mice exhibited prolonged survival, reduced hepatic bacterial load, and upregulated pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) upon infection.
Conclusions:
We identify ABHD12 as a critical negative regulator of host defense against S. typhimurium. It promotes NOD1 degradation to suppress NF-κB signaling, thereby compromising immunity. This finding provides a novel target for breeding Salmonella-resistant poultry.
Keywords: Chicken; Salmonella typhimurium; CRISPR; genome-wide screen; ABHD12

5103P Comparative Methylation Analysis of Different Poultry Genotypes Reveal Distinct Epigenomic Signatures
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Epigenetics explores heritable changes in gene expression without altering the DNA sequence. Key mechanisms include DNA methylation at cytosine (5-methylcytosine, 5mC) and its oxidized form, 5-hydroxymethylcytosine (5hmC). These markers regulate developmental plasticity, environmental adaptation, and speciation. Understanding epigenetic variation among poultry breeds can reveal how environmental factors shape adaptation. Commercial Layer and Broiler chickens, bred for egg and meat production respectively, provide an ideal model to study breed-specific methylation patterns. This study compares genome-wide 5mC and 5hmC profiles in male Layers and Broilers to identify differentially methylated regions (DMRs) and assess their potential role in production traits. We hypothesize that distinct methylation landscapes reflect divergent selective breeding histories. Blood samples (1 ml) were collected from 15 males per breed. Genomic DNA was extracted and pooled (100 ng per bird) to minimize individual variation and reduce sequencing costs. Pooled samples were sequenced using Oxford Nanopore Technologies PromethION (R9/R10.4.1 flow cell). Single-base methylation status was determined directly from ONT reads. Quality control and mapping were performed against the chicken genome (GCA_000002315.5). Global methylation statistics were computed using Methylartist v1.3.1. Gene features were annotated from GTF files. DMRs were identified using Modkit's "dmr pair" tool and mapped to genomic regions with ChIPseeker. DMR scores were calculated using a hidden Markov model and Bayesian framework; significant DMRs met criteria: corrected P-value < 0.05, score > 20, and fold-change > 2. Broilers exhibited 52.42% 5mC and 4.58% 5hmC, while Layers showed 53.70% and 4.17%, respectively. Methylation was most abundant in introns and 3′ UTRs. A total of 9,610 significant DMRs were detected: 61% intronic, 30% exonic, and 9% promoter regions. Twenty-three genes were hypomethylated in Layer exons, and 28 in Broilers.The LINGO1, EFNB1, RUNX1 genes were hypomethylated in layers and are involved in neural and vacular development, feather morphogenesis and oocytes maturation, respectively supporting the high rate of egg production and physiological stability in layers. Hypomethylation of PCDHA and CUX1 genes in broilers are involved with cell proliferation, rapid growth and neuroendocrinal control that drives feed intake behavior and. This first genome-wide comparison in Layers and Broilers reveals extensive breed-specific epigenetic variation. Integrating epigenomic signatures with traditional breeding strategies could enhance genetic potential and production efficiency in poultry.
Keywords: Epigenomics; 5mC; 5hmC; Broilers; Layers

5105P A chicken genome structural variation atlas
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The chicken (Gallus gallus) is one of the domestic animal species with the richest genetic and phenotypic diversity. Throughout human civilization, chickens have been raised for thousands of years, and poultry production has long played an important role in global agriculture. However, to further unlock the genetic potential of chickens, it is essential to establish a comprehensive, accurate, and queryable map of genomic variation, enabling the systematic identification of functional variants underlying economically important traits. Genomic variation forms the foundation of phenotypic interpretation, particularly structural variants (SVs).
In this study, we integrated chromosome-level assemblies and HiFi sequencing data (Source: NCBI public database) into a pangenome framework to construct a comprehensive SV catalog. Using this catalog, we genotyped SVs across 2,018 chickens representing 76 breeds worldwide, thereby generating a high-resolution SV database for the chicken.
Firstly,pan-genome characterization added 57.63 Mb of novel sequence relative to GRCg7b, 58.86 % of which is transposable elements (mainly long terminal repeat and long interspersed nuclear element). After cross-validation, a total of 61,246 SVs repository were identified. Approximately 50% of SVs were distributed within intronic regions, followed by upstream gene regions. Subsequently, we compared SVs, SNPs and INDELs across 2,018 individuals (wild, native, and commercial) for select effect, genetic diversity, and concordance in population structure inference. Our results indicate that SVs matched SNPs and INDELs in accurately resolving genetic structure, yet exhibited distinct selective effects,

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~50% weak linkage disequilibrium and higher genetic diversity. Thus, SVs play a distinct role from SNPs during chicken domestication.
This study confirms that SVs provide an independent "SV dimension" complementary to point mutations, substantially improving the resolution for deciphering domestication history and breeding selection.
Keywords: Graph-based pan-genome; Structural variant; Multiple-type variant; HIFi reads

5106P Effects of Quillaja Extracts on Growth Performance and Immune Response in Broilers During Disease Challenges

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Coccidiosis caused by Eimeria spp. is one of the most important diseases in poultry. Antimicrobial resistance and antibiotic residues have become an issue in recent years, which has increased the interest in phytogenics to improve the immune system and reduce the impact of diseases. Quillaja saponaria (QS) is a tree rich in saponins, a metabolite proven to improve animal health.

QS extracts were supplemented in Ross 308 broiler chickens challenged with Eimeria spp. and Dextran Sulfate Sodium (DSS), used as a model of intestinal inflammation (Dal Pont GC et al. 2021), to evaluate its effects on growth performance, intestinal integrity, immune modulation, and oxidative balance. One in vivo study was conducted in Dankook University, using 360 broilers (9 replicates) in a 42-day trial, distributed into 4 treatments, challenged control with vaccine + DSS (T1), negative control without vaccine and DSS (T2) and diets supplemented with QS at two levels (30 g/T and 60 g/T; T3 and T4). On day 14, coccidiosis vaccine LIVACOX® Q (Biopharm) was administered via oral gavage, at 10x higher than standard dose to all broiler chickens (except T2), to create a stressful environment, while DSS challenge was administered between days 22 and 28 to exacerbate intestinal stress. On day 42, Immune-related lymphocyte populations were analyzed by flow cytometry using CD4 and CD8 surface markers. Oxidative parameters were measured from the serum with ELISA kits. All data were processed by Duncan's multiple range test using the General Linear Model procedure of SAS (2013) to test the significance between the means ($p<0.05$).

In overall growth performance, T4 achieved results comparable to the unchallenged group ($p>0.05$), outperforming the positive control T1 ($p<0.05$) in BWG and FCR, indicating the positive effects of natural supplementation. Regarding intestinal lesions, groups receiving QS displayed significantly reduced lesion scores in duodenum, jejunum and ileum when compared to T1, even equaling the untreated control, suggesting a protective effect against different Eimeria species damage. Flow cytometry analysis showed that T4 increased the CD4/CD8 ratio, surpassing T1 and reflecting improved immune function. Oocyst counts in feces were also significantly lower in T4 and compared with T1. In blood antioxidant profile, T2, T4, and presented higher levels of SOD, GPx, and CAT, together with reduced levels of MDA, compared to T1 ($p<0.05$).

In conclusion, supplementing QS, at 60 g/T, improved growth performance, intestinal health, immune related parameters, and oxidative balance in broiler chickens under Eimeria spp challenge, highlighting its potential as a natural, residue-free alternative for coccidiosis management.

Keywords: Saponins; Gut health; Immunity; Antioxidant

5107P Bark contributes bioactive properties to lignocellulose feed supplements

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Objective
Lignocellulose products (LC) are used in livestock feeding to support gut health and performance. Previous work suggested that bark inclusion positively modulates the intestinal microbiome. We hypothesized that eubiotic LC (stem wood + bark) provides bioactive compounds that improve performance and microbiome compared to 1st-generation LC (stem wood only).
Experimental design
The hypothesis was investigated under moderate heat stress in broilers and in a mode-of-action study using a species-appropriate stressor in Drosophila melanogaster, as flies do not respond uniformly to heat challenge. Broiler trial 1 compared a basal diet with different LC products or bark alone. In the 2nd trial, bark was included at different dosages.
Materials and methods
Male Cobb500 were reared till d35 with all groups exposed to 28 °C from d15. In Trial 1, diets were supplemented with either 8 g/kg of 1st -generation LC, or eubiotic LC (OptiCell®, Agromed Austria GmbH), or 2.5 g/kg of bark (90 birds/treatment; 6 repetitions). In Trial 2, diets were supplemented with bark at 2 or 4 g/kg from d0–d21, followed by a 14-day withdrawal, or continuously at 2 g/kg until d35 (80 birds/treatment; 8 repetitions). Zootechnical parameters were recorded. At d35, 6 caecal digesta samples/treatment were individually analyzed by qPCR to selectively quantify bacterial groups of known functional relevance to gut health. Drosophila were exposed to oxidative stress induced by iron sulphate and treated with bark-derived preparations. Reactive oxygen species (ROS) were measured after 72 h in Drosophila homogenate using H2DCFDA probe that produces fluorescent DCF products upon reaction with ROS. Survival was evaluated after 7 days of oxidative stress and parallel treatment.
Statistical analysis
Data were tested for normality of the distribution and analyzed using one-way ANOVA with Dunnett's post-hoc test.
Results
Eubiotic LC improved weight gain compared to the control (+5 %; $p<0.001$) and to 1st-generation LC (+2.4 %; $p<0.001$). Bark supplementation improved performance (EPEF 510 vs.550/566/579; $p<0.001$) and resulted in higher caecal Lactobacillus spp. abundance (8.77 vs. 9.24 log/g;$p=0.045$). In Drosophila, bark-derived preparations reduced ROS levels ($p=0.01932$) and increased survival by 28 %($p=0.00089$) under oxidative stress.
Conclusions
Bark inclusion in LC products exerted antioxidative effects in a Drosophila model, which may contribute to the observed performance enhancement in broilers. In addition, beneficial caecal microbiota was promoted. These positive effects support the role of bark as a source of bioactive compounds. Bark-containing LC products may represent a nutritional strategy to improve performance under stress conditions.
Keywords: broiler performance; lignocellulose; drosophila melanogaster; microbiota; oxidative stress

5108P The effect of a natural liquid smoke condensate on growth performance and Salmonella Typhimurium colonization in experimentally challenged broilers

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Poultry is recognized as a primary vector for foodborne Salmonella infections. Developing interventions to reduce Salmonella in poultry production systems is critical for enhancing food safety and ensuring regulatory compliance across the supply chain. Smoke has been used historically to preserve foods and to impart desirable color, flavor, aroma, and textures. Liquid smoke extracts produced from the condensation, purification, and fractionation of pyrolyzed wood vapors have been demonstrated to have potent antimicrobial activities. We have demonstrated the ability of liquid smoke condensates to mitigate effects of necrotic enteritis in broilers previously. In this study, we evaluated the effect of liquid smoke condensate administration on growth performance and Salmonella colonization in experimentally challenged broiler chickens. Day-old male Cobb 500 broiler chicks (n=1,248) were allocated randomly to 4 treatment groups with 12 replicate pens of 26 birds arranged as a randomized complete block design. Broilers were fed untreated (NC) feed or feed treated with liquid smoke condensate at either 200 g/ton (LO) or 400 g/ton (HI) or a direct-fed Bacillus subtilis culture (DFM, 3.5 × 108 CFU/kg). Broilers were experimentally challenged with Salmonella Typhimurium (~109 CFU/bird) by oral gavage at 7 d post-hatch, cecal Salmonella colonization was evaluated at 14 d and 21 d post-hatch, and growth performance was evaluated over the 21-d study. Growth performance and Salmonella counts were analyzed using ANOVA, whereas Salmonella prevalence was analyzed using a $\chi 2$ test. A significant treatment effect was not observed on BWG ($P>0.05$) but was observed on FCR ($P<0.001$). Administration of liquid smoke at both the LO and HI doses improved FCR by ~2 pts over 0 to 21 d when compared to the NC and DFM-treated broilers. No effect of mortality was observed ($P>0.05$). A significant treatment effect was not observed on Salmonella colonization at 14-d post-hatch ($P>0.05$) but was observed at 21 d post hatch ($P=0.016$). A reduction of 0.8 log10 CFU/g cecal contents, an 84% reduction, was observed when broilers were treated with liquid smoke at the HI dose when compared to the remaining treatments. Although the effect was not significant ($P =0.145$), a 12% reduction in Salmonella positive broilers was observed in the HI dose smoke treatment when compared to the NC. Overall, we have demonstrated the ability of a natural liquid smoke condensate to improve growth performance and reduce cecal colonization of Salmonella in experimentally challenged broilers. The results of this study suggest the potential of liquid smoke condensates as natural interventions to improve preharvest food safety in poultry production.

Keywords: liquid smoke; Salmonella; Antimicrobial; Food safety; Broiler

5109P Effect of reduced-protein diets on performance, intestinal lesion score and histomorphometry of broilers challenged with Eimeria spp.

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Excess protein intake may be associated with a low ability to cope with pathogens. The aim of this experiment was to evaluate the effect of reducing dietary crude protein (CP) levels on performance, intestinal macroscopic lesions, and histomorphometry parameters in broilers challenged with Eimeria spp. from a live coccidial vaccine containing E. acervulina, E. maxima and E. tenella. A total of 480 one-day-old male chicks (Cobb-500) were allocated in floor pens in a completely randomized block design with a 2x2 factorial arrangement (2 nutritional levels x 2 coccidial challenge levels), with 8 replicates per treatment. The 3 phases diets were: starter (0-13 d), grower (14-27 d) and finisher (28-41 d). Control diets (CTRL) were formulated according to industry levels, and Low Crude Protein diets (LCP) were formulated with a 7.5% reduction in CP and main digestible amino acids (Lys, Met+Cys, Thr). At 21 days of age half pens of each diet were challenged (COCC), being orally gavaged with a commercial coccidial vaccine (10x vaccination dose). One bird per pen was randomly selected and euthanized 7 days post-challenge. Intestinal samples were taken for: (1) macroscopic lesions scoring according to the Johnson–Reid method (LS; grade 1-4); (2) the amount of parasitic forms (schizonts and oocysts) by direct microscopic examination of serial mucosal scrapping from the duodenum, jejunum and ceca (MO); and (3) histomorphometric analysis of duodenal flexure fixed with 10% formalin for villus height (VH) and crypt depth (CD). Fecal samples were collected on day 28 for Eimeria enumeration (EN). Birds and feed were weighed weekly and mortality was recorded daily. Parametric data were analyzed as a 2-way ANOVA, and non-parametric data with the Kruskal-Wallis Test. No significant interactions between treatments were detected for the evaluated parameters ($P>.05$). Significant differences ($P\leq.05$) in body weight were observed from day 14 to 42, being CTRL birds heavier than LCP ones. Cumulative feed conversion ratio (FCR) from 0 to 21, 35, and 42 days and cumulative feed intake (FI) for 0-21 d also was better for CTRL broilers ($P<.05$). After the challenge, there were no differences between diets for FCR and FI ($P>.05$), except for greater FI in the LCP group at 28-35 d ($P<.05$). For LS, MO, VH, CD and EN there were no significant differences between diets ($P>.05$). However, COCC bids showed lower VH and higher EN compared to unchallenged ones ($P<.05$), with a tendency to have a lower cecal LS ($P<0.1$). In conclusion, the reduction in CP and key AA was enough to affect performance without challenge, but had no effect afterward. The LCP did not show better intestinal results after Eimeria challenge.

Keywords: coccidiosis; Eimeria; broilers; crude protein intake; intestinal health

5110P Use of Elanco Health tracking System (HTSi®) to evaluate status of digestive parasitism in standard commercial turkeys in France

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In standard commercial turkey production, digestive disorders represent a major cause of performance loss and the need for treatments. Among the possible causes are digestive parasites, but recent prevalence data in this species remains limited. The objective of this study was to document the frequency of the main endoparasites identified during parasitological monitoring in France using the Elanco Health Tracking System. HTSi® is a necropsy-based tool that goes beyond simply lesion scoring; it's a broiler and turkey health platform for data capture, processing and analysis. The standardized HTSi® turkey protocol includes the macroscopic assessment of 32 parameters, among which coccidiosis lesion scoring (LS) for the two major pathogenic species Eimeria meleagrimitis (ML) and Eimeria adenoeides (AD), detection of protozoa by intestinal and cecal scrapings, and screening for ascarids. All collected data were analysed using SAS/STAT® Software, version 18.1.0 - GLM procedure (means compared using t Test).

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Between February 2023 and July 2025, a total of 1,894 conventional turkeys aged between 1 to 9 weeks were necropsied, with a sample of 4 birds per flock. Necropsies were conducted at the request of farmers, with a tendency to examine flocks showing digestive disorders. Coccidial lesions were confirmed only in cases where oocysts were observed microscopically.
Over the study period, the prevalence of birds with lesions attributable to Eimeria meleagrimitis reached 40% (mean LS 0.63), while that associated with Eimeria adenoeides was 14% (mean LS 0.17). Lesions were more frequent in winter ($p < 0,005$) and between 3 and 5 weeks of age ($p < ,0001$ for ML), although some were detected up to 49 days (ML) and 60 days (AD). Non-coccidial protozoa (intestinal or cecal flagellates) were identified in 2% of cases, mostly in summer ($p < ,0001$). Ascarids remained very rare (<0.5%) with no seasonal pattern ($p = 0,1920$).
The frequency observed in this survey underlines the importance of including coccidiosis in the differential diagnosis of digestive disorders in standard turkey production, particularly between 21 and 41 days of age. This epidemiological data provides objective elements to guide prevention plans: optimizing anticoccidial feeding programs in farms and recommending regular monitoring through parasitological checks.
Keywords: Coccidiosis; Turkey; Monitoring; Gut health

5111P Coccidiosis situation and trends in French broiler production from 2018 to 2024
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Coccidiosis remains a major disease in broilers, compromising gut integrity, feed efficiency and growth, and impacting the sector's sustainability. Subclinical forms, far more common than clinical outbreaks, cause significant but often invisible performance losses, with an estimated cost that could reach €0.187 per bird. Monitoring flock health is essential to assess the epidemiological situation and guide control strategies.

The Elanco Health Tracking System (HTSi) is a standardized monitoring tool based on post-mortem evaluation of five birds per flock, assessing 38 health criteria including coccidial lesion scoring (LS) by Reid & Johnson method. Data presented include 16,734 broiler flocks aged 19–30 days, collected across France between 2018 and 2024. Standard birds slaughtered up to 42 days (10,340 flocks) and heavy broilers slaughtered after 42 days (6,394 flocks) were separated; Label, ECC and certified productions were excluded.

Eimeria acervulina remained the most prevalent species in 2024, with a mean LS of 0.98 in heavy flocks (+15% vs 2020, highest level since 2018) and 0.84 in standard (+17%). The main risk peak occurred at 21 days, followed by a second rise around 25 days. Unlike previous years, mean LS was higher in summer than in autumn–winter. The proportion of birds with LS ≥2, potentially affecting growth and feed conversion, reached 29.5% in heavy and 25.5% in standard production.
Eimeria maxima showed an LS of 0.35 in heavy flocks (+46% vs 2020) and 0.08 in standard (stable). Lesion occurrence was observed in 21.8% of heavy and 5.4% of standard birds, with a first risk peak around 20 days and a second around 26 days.
Eimeria tenella remained infrequent (LS 0.12 in heavy, 0.05 in standard), detected mainly at 27 days but sometimes earlier.
Overall, 25–30% of broilers fail to reach growth potential due to coccidiosis. The rise in mean LS for E. maxima in heavy production highlights the need for regular monitoring to detect subclinical dynamics and adjust control strategies. The epidemiological situation differs greatly between production types, influenced by rearing duration and technical factors such as litter management and concrete floor hygiene (for pododermatitis control) in heavy production, which heighten infection risk.

In conclusion, routine monitoring such as HTSi is essential to understand the evolving epidemiological picture and adapt control programs. In heavy broilers, the extended cycle and higher exposure justify a reinforced and longer anticoccidial program, combined with good hygiene, nutrition and flock management. Such an integrated approach strengthens prevention, supports bird performance, and contributes to the long-term sustainability of poultry production.
Keywords: Coccidiosis; Broiler; Monitoring; Gut health

5112P Impact of gut sampling methods on the reliability of analysis results of microbiome of broilers chickens
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Understanding and analyzing the gut microbiome in commercial broiler production is pivotal for optimizing both the productivity and health of flocks. By delving into the complex microbial ecosystem of the intestine, producers can gain critical insights into nutrient absorption, disease resilience and overall bird well-being. Caeca are considered the gold standard and most informative digestive compartment for broiler microbiome profiling as they have the highest microbial load and diversity (main fermentation chamber), its microbiome is linked to key functions (short chain fatty acids production, nutrient fermentation and gut health) and they have a better signal-to-noise (with observation of clearer differences between treatments) compared to small intestine. On the other side, cloacal digesta samples usually offer more transient and less representative results (mix of gut content with urinary contamination). This study evaluated the efficacy of different microbiome sampling methods targeting two different compartments of the digestive tract (caeca and cloaca) across two distinct poultry farms: a commercial operation and a contract research organization. These farms differed in diet, sampling age (33 vs. 36 days), and management practices, which were expected to cause significant variation in the gut microbiome. A total of 15 healthy birds were randomly selected per farm for the sampling, with each bird sampled 3 times through the different methods of interest. 45 microbiome samples were collected per farm (15 cloacal swabs, 15 cecal swabs, and 15 frozen ceca) to assess alpha diversity, microbial composition, and suitability for downstream applications such as machine learning and product development. DNA from all samples was analyzed using 16S rRNA (variable region V1-V8) amplicon sequencing via Oxford Nanopore long-read sequencing. Sequences were mapped to Silva database v.138.1 and analyzed using Qiime2 and R.
Results demonstrated that cecal swabs stored at ambient temperature are comparable to frozen cecal samples in terms of microbial diversity and taxonomic resolution. This suggests cecal swabbing is a practical and reliable alternative for microbiome profiling.
In contrast, cloacal swabs showed significantly ($p < 0.05$) lower alpha-diversity, inconsistent microbial composition resulting in a tendency for lower beta-diversity uniformity ($p = 0.06$), and insufficient DNA yield for deep sequencing. These findings align with prior research and industry observations, confirming that cloacal sampling method is inadequate for robust microbiome-based health assessments. Cecal swabs, on the other hand, offer a promising approach for poultry microbiome studies due to their reliability and ease of use.
Keywords: Microbiome; Sampling; Caeca; Analysis; Broilers

5113P Enterococcus cecorum challenge models in chickens

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Enterococcous-cecorum-infection represents one of the most important bacterial diseases in broiler production worldwide. It causes an increase of mortality, antibiotic use and condemnation at the processing plants. Compared to other bacterial pathogens of poultry, the knowledge about the disease is poor. However, more knowledge about the disease is urgently needed, for example to find intervention methods against it or to understand certain aspects of the pathogenesis. For this, animal models are needed. In this talk I will show different types of Enterococcus cecorum challenge models, advantages and disadvantages of these models, sampling methods and open questions.

Short duration trials, which are completed after one to two weeks are only suited for the examination of limited parameters of the disease. The main disadvantage of these models is that no skeletal lesions can develop during the trial. For the evaluation of this parameter, the trial duration should be at least six weeks in length. The required animal number is dependent on the used challenge strain, parameter of interest and if chickens are selected for necropsy during the trial or just at the end of the trial. The most important challenge route is oral gavage and the challenge dose is mostly between 106 to 108 cfu/animal. Important samples from living chickens are blood samples and cloacal swabs. Important samples from animals during necropsy additionally comprise heart, liver, spleen, intestine, femoral head and free thoracic vertebra samples.

Limited information is available concerning the influence of the age of the chickens at challenge, transmission dynamics and the Enterococcus cecorum strain as well as the genetic origin of the birds.
Keywords: Enterococcus cecorum; Challenge models

5114P Genetic characterization of Salmonella Minnesota isolates from poultry origin
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Salmonella spp. is a global One Health issue, and poultry products are the main sources of these pathogens. Although the serotypes Enteritidis and Typhimurium are most frequently associated with infections in animals and humans, other serotypes, such as Salmonella Minnesota (SM), have been isolated from poultry and poultry products in Brazil, raising concerns among health authorities. Among SM isolates, sequence type 548 (ST548) has been one of the most common in poultry. This study aimed to analyze the complete genomes of five SM isolates recovered from broilers reared on commercial farms in southeastern Brazil, together with other ST548 sequences of this serotype available in EnteroBase. Briefly, DNA extraction was performed using a commercial kit, and whole-genome sequencing was carried out on the Illumina MiSeq platform. Subsequently, the genomes were assembled using CLC Genomics Workbench with de novo assembly. The resulting assembled sequences were annotated with RAST. The five sequenced genomes and 263 publicly available sequences were analyzed to identify virulence and resistance genes. Bioinformatics tools from the Center for Genomic Epidemiology (CGE), including SPIFinder and ResFinder, were used. In addition, plasmid replicons were detected with PlasmidFinder, and prophage sequences were identified using PHASTER. Phylogenetic analysis was performed with IQ-TREE2. The analyses revealed that 191 strains were multidrug-resistant (MDR), carrying several resistance genes to aminoglycosides (aac(6′)-Iaa, aph(3′)-Ia, and aadA1), quinolones (qnrB19), tetracyclines (tetA), sulfonamides (sul2), and β-lactams (blaCMY-2, blaCTX-M-8, blaCTX-M-55, and blaTEM-126), including strains resistant to fourth-generation cephalosporins. Furthermore, several virulence genes were identified, related to factors such as adhesins (faeC, faeE, and fimB), non-fimbrial adhesins (pegA, staA, fimA, and csgD), iron uptake (mgtB and mig-14), and type III secretion system components (ssaM, sseE, sseJ, and cdtB). Twenty-five plasmid replicons and fifty-five different prophages were identified among the analyzed sequences. Moreover, the phylogenetic tree demonstrated a close relationship between SM isolates from poultry in southeastern Brazil and SM sequences from various sources and countries, including those linked to human outbreaks. Therefore, infections of poultry flocks by SM should not be neglected, as they represent a potential risk to public health.
Keywords: Resistance genes; Virulence genes; Foodborne pathogen; Molecular epidemiology; Whole Genome Sequencing

5115P Resistome Dynamics in Laying Hens: Insights from Alternative Housing Systems Using Metagenomics
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This study aimed to characterize the fecal resistome and microbiome of commercial laying hens housed in enriched colonies and aviaries using next generation metagenomic sequencing. A total of 87 fecal samples were collected at the beginning (19 -24 wks of age) and end (57 -65 wks of age) of the laying cycle to assess the abundance and diversity of antimicrobial resistance genes (ARGs) and bacterial taxa in 12 enriched colonies and 12 aviairies. DNA was extracted and sequenced using Illumina NovaSeq technology, generating over 100 million reads per sample. Bioinformatic analyses were performed using Kraken2 and Bracken for microbial taxonomy while detection and quantification of ARGs was done using ResFinder, AMR++ and MEGARes databases.
Lactobacillus and Psychrobacter were among the most abundant genera, with shifts in dominant taxa observed between housing types and production stages. The relative abundance of Lactobacillus tended to be higher in aviary, particularly at the end of the cycle while Psychrobacter was more abundant in EC. The resistome revealed that enriched colonies exhibited a higher initial load of ARGs, while aviaries showed a marked increase over time. By the end of the cycle, resistome profiles converged between systems, suggesting homogenization of microbial pressures. Resistance genes to tetracyclines and MLS (macrolides, lincosamides, streptogramins) were most prevalent. These gene correspond to antimicrobials used in poultry: chlortetracycline is the only antimicrobial authorized for use during lay in Canada, while tylosin (macrolide), lincomycin (lincosamide), and virginiamycin (streptogramin) are sometimes used during the pullet rearing phase. Environmental persistence, historical exposure, and housing conditions, particularly in complex environments such as aviaries, were identified as contributing factors to ARG dissemination. ARGs associated with disinfectants and heavy metals were also detected.
It is important to note that resistome analysis identifies the genetic potential for resistance but does not confirm phenotypic expres-

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sion. However, compared to conventional AMR surveillance programs, which focus on culturable pathogens, most commonly Escherichia coli, Salmonella and Campylobacter, resistome analysis offers broader and more sensitive detection of ARGs, including those in uncultivable bacteria and non-targeted taxa. This approach provides a more comprehensive understanding of antimicrobial resistance dynamics in poultry production. The findings underscore the importance of integrating resistome profiling into national surveillance frameworks to enhance risk assessment and guide responsible antimicrobial stewardship in the industry.
Keywords: Resistome; Laying hens; Antimicrobial resistance; Alternative housing systems; Metagenomic sequencing

5116P Bacillus spp. as a Litter-Level Biocontrol Strategy Against Enterococcus cecorum, in Broiler Chicken Litter
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Enterococcus cecorum (EC) is an emerging bacterial pathogen in broiler chickens, associated with skeletal infections and significant economic losses. Preventive strategies targeting EC colonization in litter could be an innovative way to reduce the risk of infection. BIO-LITTERWS (BL) is a commercial product (PROBIOTECH International Inc.) composed of three Bacillus species strains designed to be applied to litter before chicks are placed in the barn. We aimed to verify how this product behaves over time when applied to wood shavings and whether it can target other poultry pathogens. Our objectives were: (1) characterize the survival and stability of BL bacteria on wood shavings under simulated barn conditions (including the effects of pH and humidity); (2) assess the biofilm-forming capacity of these Bacillus strains; and (3) determine the antimicrobial activity of BL and its individual strains, on Enterococcus cecorum (EC) in vitro.
For objective 1, BL was inoculated onto sterile wood shavings and monitored for nine days at room temperature. Bacterial counts and pH were measured at multiple time points. For objective 2, we quantified biofilm formation via crystal violet staining and spectrophotometry after six days of incubation in glucose-supplemented TSB. For objective 3, antimicrobial activity against pathogenic EC strains (cpsO-positive) was assessed using an agar well diffusion (spot) test and confirmation assays.
The results indicated that the populations of BL bacteria remained stable (approximately 105 CFU/g) over nine days, with Bacillus #3 demonstrating the greatest persistence. Litter pH measurements revealed consistent acidification, suggesting persistence of the Bacillus spp.'s metabolic activity. All three strains formed biofilms under optimal conditions; Bacillus #1 and #3 produced the densest matrices (OD550: Bacillus #1 = 1.977, Bacillus #3 = 2.173; positive control = 0.417, negative control = 0.004), and Bacillus #3 was motile as well. Inhibitory activity against EC was observed, with Bacillus #3 showing the most pronounced effect (10-12 mm zones).
In conclusion, BIO-LITTERWS sprayed on wood shavings demonstrated stability, biofilm formation, acidification, and in vitro inhibition of Enterococcus cecorum under simulated field conditions. These findings support its potential role in competitive exclusion and modulation of the barn litter microbiota to reduce EC colonization in broiler chickens.
Keywords: Enterococcus cecorum; Bacillus spp; BIO-LITTERWS; Biofilm formation; Antimicrobial activity

5117P Evaluation of two commercially available potentiated ionophores under field conditions in broilers.
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Coccidiosis compromises intestinal integrity (I2) of conventional broilers throughout the production cycle and is a major cause of production losses. The global economic cost of coccidiosis is estimated at €11,8 billion or €0,18 per produced broiler. Impaired I2 due to mild to severe coccidiosis is a concern worldwide and not only results in loss of profitability but also impacts welfare throughout the life span of the birds. Since coccidiosis prevention strategies play a crucial role in maintaining gut health, welfare and profitability in modern poultry farming, small differences in efficacy will result in a significant impact.

The objective of this study is to assess the efficacy of two different potentiated ionophores NN (narasin 50ppm + nicarbazin 50ppm) and MN (monensin 50ppm + nicarbazin 50ppm) in a shuttle program with SA (salinomycin 70ppm) to prevent coccidiosis and to establish how these two potentiated ionophores differ in their impact on bird gut health, welfare and performance.

In total 4 within house comparisons were made on a Belgian poultry farm, including more than 160,000 Ross 308 broilers. Each house was longitudinally divided in two equal compartments (20,500 broilers per compartment) ruling out differences related to climate conditions or general disease pressure. Broilers in compartment A received NN from day 0-28 followed by SA untill slaughter, while broilers in compartment B received MN from day 0-28 followed by SA untill slaughter. During the study, from each compartment 100 broilers were weekly individually weighed (BW), and five broilers randomly selected, euthanized, necropsied, and scored using HTSi at the age of 21, 24, 28 and 35 days. Technical performance parameters such as average daily gain (ADG) and feed conversion ratio (FCR) were calculated for each compartment. All collected data were analysed using SAS/STAT® Software, version 18.1.0 - GLM procedure (means compared using t Test).

At 35 days of age broilers fed MN-SA had a lower I2 score compared to NN-SA (91,09 vs 94,17) mainly due to higher coccidiosis lesions in the MN-SA group. A fourfold increase of lesions due to E. acervulina and a twofold increase of E. maxima and E. tenella lesions were noticed in the MN-SA group compared to NN-SA at 35 days of age. At 28 days of age birds fed MN had a significant lower ADG (-1g/day) and BW (-34g) ($p<0,05$) compared to the NN-group, differences were maintained till slaughter. At slaughter also a higher FCR was noticed for MN-SA.

Based on this study, it can be concluded that NN results in higher welfare due to better coccidiosis control resulting in an increased profitability based on BW, ADG and FCR.
Keywords: coccidiosis; performance; ionophore; gut health; broiler

5118P Effects of Dietary Supplementation with Bacillus subtilis C-3102 on Health, Laying Performance, and Egg Quality of Laying Hens during the Late Production Period
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This study explored the effects of dietary Bacillus subtilis C-3102 (Calsporin®) supplementation on health, production performance, and egg quality in Hy-Line Brown laying hens during the late laying period (≥68 weeks old). 594 healthy 68-week-old hens (uniform weight/feed intake) were housed in 4-row × 8-tier systems and randomized into 2 groups (9 replicates/group, 33 hens/replicate). Controls got a basal diet; treatment got basal

diet + C-3102 (sprayed): 600 mg/kg (Weeks 1-2), then 300 mg/kg. The 12-week trial collected egg number, dirty/cracked eggs, mortality. Egg quality (Weeks 4,8,12); carcass traits, intestinal morphology, gut microbiota (Week12); serum indicators (Weeks 8, 12) were assessed. Compared with controls, C-3102 raised total egg production (+533 eggs, P<0.05), lowered dirty egg rate (−3.49 pp, P<0.05), and maintained higher laying rate. Yolk color improved (Weeks4,8,12, P<0.05); eggshell thickness increased (Week8, P<0.05); eggshell color decreased (Week12, P<0.05). At Week12, treatment had more ovarian follicles (+54.9%, P<0.05) and trend of lower abdominal fat. Serum AMH, E2, FSH were higher (Weeks8,12, P<0.05). PD-Ab was lower (Week8, P<0.05) but higher (Week12, P<0.05). Serum ALP lower; GLU-YA higher (Week12, P<0.05); TG higher (Week8, P<0.05); ALT, AST, ALB altered. At Week12, treatment showed intestinal changes: lower crypt depth (−29.37%, P<0.05), higher VH:CD (+35.59%, P<0.05), thicker muscularis (+63.77%, P<0.05). Jejunum: lower crypt depth (−19.67%, P<0.05), thicker muscularis (+49.11%, P<0.05). Ileum: lower crypt depth (−18.10%, P<0.05), higher VH:CD (+37.71%, P<0.05), thicker muscularis (+39.08%, P<0.05). C-3102 modulated gut microbiota: intestine had more Firmicutes_D/Ligilactobacillus (P<0.05), less Proteobacteria/Bacteroidota/Ralstonia(P<0.05); jejunum had more Firmicutes_D/Firmicutes_A/Ligilactobacillus(P<0.05). In conclusion, dietary B. subtilis C-3102 (Calsporin®) improves late-phase laying performance, enhances specific egg quality traits (especially yolk color), increases ovarian follicle count, modulates serum reproductive hormones and biochemical indicators, promotes intestinal morphological development, and optimizes ileal and jejunal microbiota in laying hens. These findings support its use to maintain health and productivity in aging laying hen flocks.

Keywords: Bacillus subtilis C-3102; laying performance; egg quality; intestinal health

5119P Effects of medium-chain fatty acid–rich oils with varying free fatty acid levels on gut morphology, microbiological counts, and cecal fermentation in broilers

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Medium-chain fatty acids (MCFAs) are a rapidly absorbed energy source with antimicrobial properties that may influence digestive tract function. However, different MCFA-rich oil sources may produce distinct effects. This study specifically evaluated the impact of MCFA-rich black soldier fly oil (BSFO), palm kernel oil (PKO), and palm kernel fatty acid distillate (PKFAD; >60% free fatty acids (FFA)) compared to soybean oil (SO) as a control, on intestinal morphology, microbiological counts, and cecal fermentation in broilers during both the starter (0–9 days) and grower (21–36 days) phases. At day 9 and 36, intestinal samples were collected from the distal jejunum and ileum for the histomorphological evaluation and microbiological counts, while cecal contents were used for short-chain fatty acid analysis. Data were analyzed by one-way ANOVA using diet as the main factor, and differences among means were evaluated using Tukey's test. Regarding the morphology assessment, jejunum was unaffected by dietary treatments throughout the trial. In contrast, the ileum showed changes at both ages, with broilers fed PKFAD presented a reduced villus height at 9 days compared with PKO (P = 0.047), suggesting an early negative effect of high dietary FFA, and broilers fed SO showed a greater villus height than PKO (P = 0.040) at 36 days. In terms of microbiological counts, bacterial counts were similar among treatments across all evaluated taxa at day 9. Differentially, at day 36, PKFAD exhibited the lowest Lactobacillus counts (P = 0.001) in both intestinal segments (P < 0.01). These findings show that microbial differences became more evident with age and that the oil with the highest FFA content reduced the counts of a potentially beneficial bacterial population. Cecal fermentation showed few differences among treatments. Although Total short-chain fatty acids (SCFA) concentrations did not differ at 9 or 36 days, propionate proportions varied with diet and age. Concretely, BSFO and PKO presented higher levels than PKFAD at day 9 (P = 0.006), while SO and BSFO showed the higher percentage of this SCFA at 36 day (P = 0.063). Overall, BSFO and PKO supported gut morphology, Lactobacillus abundance, and cecal fermentation patterns, comparable to SO, whereas PKFAD showed early and persistent alterations, particularly reduced villus height and Lactobacillus counts, consistent with its high FFA content. These findings indicate that MCFA-rich crude oils can be used as alternative oil sources, while very high-FFA oil may compromise certain gut health indicators in broilers.

Keywords: MCFA-rich oils; gut health; intestinal morphology; microbiological counts; cecal fermentation

5120P Evaluation of fertility, hatchability and chick quality of meat type chicken strain under standard incubation condition

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Bangladesh Livestock Research Institute has developed a meat type chicken strain named Suborno. The strain was developed from local male line and exotic female line. This study was conducted to evaluate the fertility, hatchability and chick quality performance of the Suborno strain. After performing the artificial insemination, a total of 750 eggs were collected in four consecutive days for experimental materials and they served as independent observational unit. Eggs were then stored at 16°C prior to incubation. All the eggs were then set as a single batch in a PETERSIME VISION incubator at 99.5°F and 55% relative humidity for a total incubation duration of 506.1 hours. Data were analyzed using descriptive statistics in SPSS and the results were presented as percentages and means. Fertility, hatchability, dead-in-shell rate, chick yield, and chick uniformity were calculated using standard hatchery formulas. The average egg weight was 56.41g while the mean day old chick (DOC) weight was 37.03g. Of the total eggs set, 95.73% were fertile, with an infertility rate of 4.26%. Hatchability based on fertile eggs was 94.50% yielding 671 DOC. Embryonic mortality was low with dead-in-shell accounting for 1.11% of fertile eggs. Chick yield was 66% and chick uniformity was reached in 91.67%. Although the incubation trial was conducted without biological replication, the large sample size allowed reliable estimation of key hatchery performance indicators. The results demonstrate high fertility, excellent hatchability, minimal embryonic loss and uniform chick quality in Suborno strain. These findings indicate that Suborno possesses strong reproductive efficiency and is a promising candidate for commercial meat type chicken production in Bangladesh under controlled incubation system.

Keywords: Shuborno; Strain; Fertility; Hatchability; Chick quality

5121P The influence of sex hormones on aggressive behavior violating peck order in local country chickens bred for different production systems

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Aggressive behavior in the poultry industry has been studied for decades. While aggression in agricultural animals is natural, excessive aggression can negatively affect productivity and animal welfare. Chickens are social animals that establish a hierarchy when raised together. With the completion of this hierarchy, dominance–subordinate relationships stabilize and aggression decreases. Aggressive behavior is modulated by multiple factors, with sex hormones being key physiological regulators. In the poultry industry, chickens are selectively bred for different production purposes, resulting in broilers with high meat yield and layers with high egg production. However, such genetic selection may have unintended consequences on physiological profiles, further influencing behavioral traits. Broilers are more aggressive and have higher testosterone levels in yolk compared to layers. Moreover, even when a dominance–subordinate relationship is established, hormone manipulation may alter this balance: administration of testosterone to subordinates induces aggression toward dominants and may even lead to the reversal of social status. This indicates sex hormones may be involved in regulating aggression that violates established social hierarchy (AVH). Nevertheless, this relationship remains unclear among chicken strains selected for different production purposes. Therefore, this study aimed to compare the relationship between sex hormone levels and AVH among chicken strains with different breeding purposes. Two local breeds, Shek-ki (meat-type, SK) and Nagoya (egg-type, NG), were used for this study, and Pearson's correlation was applied to examine the association between hormone levels and AVH rate within each strain. Results showed that AVH rate was higher in SK than NG in both males and females ($P < 0.05$), with higher testosterone and estradiol levels in males ($P < 0.05$) and females ($P < 0.001$ and $P < 0.01$, respectively).Within strains, positive correlations between sex hormone levels and AVH rate were observed. In NG, AVH was moderately correlated with testosterone in males ($r2 = 0.61$, $P < 0.001$) and estradiol in females ($r2 = 0.66$, $P < 0.01$), whereas in SK, only females showed a low correlation between testosterone and AVH ($r2 = 0.47$, $P < 0.05$).These results indicate that even after the establishment of social hierarchy, sex hormones continue to influence birds to perform higher frequencies of AVH. Within strains, although SK (meat-type) chickens exhibit higher sex hormone levels, the influence of sex hormones on AVH appears less pronounced compared to NG (egg-type). Further investigation is required to clarify whether higher sex hormone levels may trigger other mechanisms modulating aggression in SK.

Keywords: aggressive behavior; sex hormones; social hierarchy

5122P Heterologous Production of Functional Bovine Lactoferrin Using Chicken as a Potential Bioreactor System

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Bovine lactoferrin (bLTF) is a monomeric glycoprotein of the transferrin family with high affinity for iron and a molecular weight of ~80 kDa. It plays a crucial role in iron metabolism, including transport, storage, and chelation, and is actively involved in innate defense mechanisms. bLTF exhibits diverse biological functions such as antimicrobial, antiviral, antioxidant, and immunomodulatory activities, as well as regulation of cell growth and binding or inhibition of bioactive molecules like lipopolysaccharides and glycosaminoglycans. By binding iron, bLTF prevents the formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals, thereby reducing oxidative stress. Owing to its multifunctional and health-promoting properties, bLTF has become a major focus of biomedical research and is widely used in infant formulas, functional foods, pet foods, and food packaging materials. In the present study, an avian bioreactor system was developed which could be used for a large-scale, non-invasive production of recombinant bLTF in chicken eggs. A transgenic construct containing the bLTF gene under the control of the Ovalbumin (OVA) promoter and fused with a His-tag was prepared and introduced into chicken sperm via electroporation. Transfected sperm were used for artificial insemination of hens to achieve in vivo gene transfer. Resulting progeny were screened for the transgene at four weeks of age by PCR using five primer sets spanning the entire cassette. Confirmed transgenic hens were monitored until onset of egg laying, after which eggs were collected for protein isolation. The egg white fractions were purified using Ni-NTA affinity chromatography, yielding a protein band of ~80 kDa on SDS-PAGE, consistent with the molecular weight of bLTF. Western blotting using monoclonal anti-bovine lactoferrin antibodies showed strong immunoreactivity in samples from fourteen transgenic hens, confirming expression of recombinant bLTF. Mass spectrometry analysis of samples from four birds further validated the identity of the protein. This study successfully demonstrates the production of recombinant bovine lactoferrin in chicken eggs, establishing a non-invasive, efficient, and cost-effective avian bioreactor platform for the large-scale generation of functionally active therapeutic proteins. Ongoing work focuses on characterizing the biological and functional properties of the egg-derived recombinant bLTF for its potential applications in human health and biotechnology.

Keywords: Chicken; Egg; Bovine Lactoferrin; Transgenic; Bioreactor

5123P Manipulating pullet growth curves through moderate feed restriction influenced production performance in ISA Brown hens

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The objective was to evaluate how alternative growth curves, created through controlled feed allocation, affect pullet development and subsequent laying performance. During rearing (0–17 wk), 192 ISA Brown pullets per treatment (4 replicates of 48 birds, two adjacent cages per replicate) were monitored for growth performance under four strategies: continuous ad libitum feeding (CON), continuous restriction (RES), ad libitum to 9 wk then restriction (LateRES), and restriction to 7 wk then ad libitum (EarlyRES). Restricted groups received a fixed daily allowance, adjusted weekly to remain 7–12% below ad libitum intake. At 17 wk, 160 hens per treatment (8 replicates of 20), selected from the same rearing groups around the average body weight (BW), were transferred to the layer facility (18–40 wk) and fed ad libitum with a common feed. BW differed significantly among treatments throughout rearing and early adulthood ($P<0.001$). At d20 and d49, CON and LateRES pullets showed greater BW compared with EarlyRES and RES. By d65, EarlyRES surpassed RES but was still below CON. At d84, CON pullets had the highest BW and RES the lowest, with EarlyRES and LateRES showing intermediate values. At d120, CON pullets remained the heaviest (1534 g), EarlyRES was intermediate (1484 g), and LateRES (1404 g) and RES (1391 g) were the lightest. During the entire rearing phase, feed intake (FI) for CON, RES, LateRES and EarlyRES were 5795, 5145, 6082 and 5342 g, respectively. During this period, earlyRES had

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a higher feed conversion ratio (FCR) compared to the rest of the treatments (P<0.001). At 15 wk of life, keel bone length was longer in CON and EarlyRES compared to LateRES and RES (P<0.001). In addition, the abdominal fat pad percentage relative to BW was higher in CON compared to the rest of the treatments (P<0.001). In the laying phase (21–40 wk), EarlyRES hens achieved higher egg weight (P=0.022) compared to LateRES. Hen-day egg production was higher in LateRES compared to the RES group (P=0.039). Higher egg mass was observed in EarlyRES only compared to RES (P=0.001). FCR was lower (P=0.005) in CON and EarlyRES compared to the rest of the treatments. BW gain during 17 to 40 wk differed (P=0.001), with previously restricted pullets (RES, LateRES, EarlyRES) showing higher BW gain than CON. However, the final BW at 40 wk was still higher in CON compared to LateRES. These results demonstrate that pullet growth curve manipulation, achieved through feed restriction, affected production performance and BW up to 40 wk of age, highlighting the importance of the long-term effects of the rearing phase on the laying cycle.
Keywords: Growth curve; Feed restriction; Keel bone; Egg production

5124P Temperature stimulation during hatching phase on performance and stress level of Cobb broiler chickens

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Thermal manipulation during egg incubation has been used to improve the thermotolerance and performance of chickens. This study examined how temperature stimulation (TS) in the hatcher affects hatching, growth performance, stress level and organ development of broiler chickens. A total of 355 Cobb 500 breeding eggs were incubated for a 7 days growing trial and 138 eggs for a 35 days growing trial under standard incubation conditions (37.3°C, 55% relative humidity) until day 17, when the living embryos were randomly assigned to a control group (C: standard incubation conditions) and a TS group (+1°C for a maximum of two hours on days 17-20). After hatching, chicks were sexed and assigned to one of four housing pens according to sex and treatment. On day 1, chick quality (Pasgar©Score) and body weight (BW) were determined. Weekly measurements of growth performance included body weight (BW), feed intake (FI), feed conversion (FC), and BW gain (BWG). BW was measured per chicken, while FI, FC and BWG were measured per pen. On days 7 and 35, 40 bursae, hearts and blood samples (10 per gender) were collected for organ weight and heterophil-to-lymphocyte ratio (HLR) determination, respectively. All statistical analyses were performed using GraphPad Prism version 5. Comparisons between groups were analyzed using an unpaired Student's t-test or Mann–Whitney test. Further comparisons were done using one-way ANOVA followed by post hoc tests. TS did not significantly change hatching results, chick weight and chick quality at hatch. 7 days post-hatching, chickens had a statistically significant higher BW (TS: 144.8 g ± 19.2; C: 137.8 g ± 17.0, p = 0.01) and BWG (TS: 17.1 ± 3.2 g/broiler/d; C: 15.9 ± 2.7 g/broiler/d, p = 0.003) and improved FC (TS: 0.98 ± 0.04 kg/kg, C: 1.03 ± 0.03 kg/kg) and FI (TS: 16.5 ± 0.5 g/broiler/d, C: 16.0 ± 0.2 g/broiler/d) when compared to control chickens. 35 days post-hatching, TS chickens showed numerically higher BW (TS: 2336.6 ± 275.3 g; C: 2285.6 ± 265.2 g) and had a statistically significant higher BWG (TS: 116.4 ± 20.6 g/broiler/d; C: 107.3 ± 16.3 g/broiler/d, p = 0.004) than the C group. The C group showed improved FC (TS: 1.5 kg/kg; C: 1.6 kg/kg) and FI (TS: 174.9 g/broiler/d; C: 167.8 g/broiler/d), with no significant differences compared to the TS group. No statistically significant differences were found for the relative bursa and heart weights and HLR between the groups on days 7 and 35 post-hatching. There was no mortality observed in any of the groups. In conclusion, TS had no negative effect on hatching rate, organ development and stress levels and appears to have the potential to improve, BWG, BW, FC and FI after a 7-days and 35-days growing trial.
Keywords: Temperature Stimulation; Incubation; Broiler; Cobb 500; HLR

5125P Effect of In-ovo Glucose Supplementation on Post-Hatch Performance, Intestinal Morphology and Cdx Gene Expression in Coloured Broilers

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Hypothesis: In-ovo glucose supplementation was hypothesized to improve hatchability, early post-hatch performance, intestinal development, and Cdx gene expression in coloured broilers under varying post-hatch feeding regimes.
Experimental Design: A total of 324 healthy, day-old chicks were wing-banded, weighed, and assigned to nine treatment groups, with three replicates per group and 12 birds per replicate.
Materials and Methods: Fertile eggs were divided into three groups: In-ovo glucose supplementation, sham control (saline-injected), and un-injected control. In-ovo injections of sterile glucose solution were administered on day 18 of incubation. Hatched chicks were further divided into subgroups based on post-hatch feeding regimes: immediate feeding, 24-hour fasting, and 36-hour fasting. Chicks were raised under standard management conditions and fed a basal diet. Growth performance metrics (body weight, weight gain, feed conversion ratio-FCR, and European Production Efficiency Factor-EPEF), intestinal morphology (villus height, villus width, and villus height-to-width ratio), and Cdx gene expression in intestinal tissue were evaluated. An economic analysis was conducted to assess profitability.
Statistical Analysis: Data were analyzed using a Completely Randomized Design (CRD) with one-way ANOVA, and statistical significance was set at $P < 0.05$.
Results: Regardless of fasting duration, chicks from the in-ovo glucose group exhibited significantly higher biweekly body weight, weight gain, improved FCR, and higher EPEF compared to controls. Immediate feeding resulted in significantly better performance across all treatments compared to 24-hour and 36-hour fasting. Villus height, villus width, and villus height-to-width ratio were significantly greater in in-ovo glucose and immediate-feeding groups compared to sham and un-injected fasted controls. Cdx gene expression was significantly upregulated in in-ovo glucose-supplemented chicks compared to both control groups. Net return and benefit-cost ratio were highest in the In-ovo glucose and immediate-feeding groups, while the lowest values were observed in the 36-hour fasted controls.
Conclusions: In-ovo glucose supplementation significantly improved post-hatch growth performance, feed efficiency, intestinal morphology, Cdx gene expression, and economic returns in coloured broilers. Immediate feeding further enhanced these outcomes, whereas prolonged fasting negatively impacted performance and profitability. Therefore, in-ovo glucose supplementation is recommended as a viable strategy to enhance productivity in broiler production systems.
Keywords: In-ovo feeding, glucose supplementation, coloured broilers, Cdx gene, intestinal morphology
Keywords: In-ovo feeding; glucose supplementation; coloured broil-

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ers; Cdx gene; intestinal morphology

5126P In silico approach for the construction of a potent multi-epitope poultry vaccine against Salmonella enterica serotype Heidelberg

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Salmonella serotypes remain a global public health concern, with poultry products being the main source of infection. In Brazil, Salmonella Heidelberg is among the most frequently recovered serotypes from commercial poultry and human foodborne outbreaks. Therefore, it is essential to develop safer and more efficient methods to control this pathogen in poultry flocks. In this context, the present study aimed to assess cutting-edge methodologies for the construction of an immunogenic multiepitope protein that could serve as a potential vaccine against infections caused by this serotype in Gallus gallus domesticus. To obtain the final protein sequence, several analyses were performed. First, multiple complete genome sequences of Salmonella Heidelberg were downloaded from the NCBI database and subjected to quality control tests, including serotype confirmation using the SeqSero2 software and evaluation of genome completeness using BUSCO. Genome annotation was performed with Prokka. Conserved genes related to relevant virulence determinants such as outer membrane proteins, fimbriae, type III secretion systems, and flagella were selected and evaluated for antigenicity using VaxiJen v2.0. T-cell epitopes were predicted using NetCTL v1.2 and IEDB tools to identify CD8+ and CD4+ T-cell epitopes, respectively. Sequences were also submitted to ABCpred for B-cell epitope prediction. All selected epitopes were screened with AllerTOP v2.0 to exclude those with allergenic potential. To link epitopes, AAY residues were used between CD8+ T-cell epitopes, GGPPG between CD4+ T-cell epitopes, and KK between B-cell epitopes. The physicochemical properties of the final protein sequence were analyzed using the ProtParam server. The tertiary structure was predicted with RaptorX and refined using the GalaxyRefine server. Structural validation was performed by analyzing the Ramachandran plot generated by PROCHECK. The protein with the best tertiary structure was selected, and its corresponding nucleotide sequence will be synthesized and cloned into the pET30 expression plasmid for in vitro expression in E. coli. A total of 43 complete Salmonella genomes passed quality control and were used in subsequent analyses. Ultimately, 10 highly immunogenic peptides were selected and linked to construct a chimeric protein composed of 620 amino acids. In previous experiments, we evaluated the protective effects of the selected peptides after injection into 18-day-old chick embryos, and the results indicated a reduction in cecal colonization by Salmonella Heidelberg during the first week post-infection. We expect that the designed multiepitope protein will elicit an even more efficient immune response in chicken infection models.

Keywords: Chimeric protein; fowl paratyphoid; in ovo; Reverse vaccinology; protection

5127P Cost-effective ration formulation for native meat-type chicken farming in Bangladesh

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Hypothesis or Objective: The study hypothesized that cost-effective rations for native Hilly chickens could be formulated using locally available ingredients to reduce dependency on imported corn, without sacrificing economic profitability compared to a commercial diet.

Experimental Design: A completely randomized design (CRD) was utilized. Four-hundred-day-old Hilly chicks were assigned to one of four dietary treatments, each with five replications (25 birds/replication) for a period of 60 days. The treatments were T0 (commercial Sonali feed, control), T1 (formulated corn-soya basal diet where no corn was replaced), T2 (formulated diet with 25% corn replaced by Chita Dhan Rice + 5% Black Soldier Fly Meal (BSFM) as a supplement), and T3 (formulated diet with 50% corn replaced by Chita Dhan Rice + 5% BSFM as a supplement).

Materials and Methods: After a screening process of local feed ingredients (including Chita Dhan Rice, Cassava, Ipil-Ipil, and Moringa) based on nutritional composition, availability, and cost, Chita Dhan Rice and BSFM were selected for ration formulation. Iso-caloric and iso-nitrogenous starter (ME=2900 Kcal/kg, CP=20%) and grower (ME=2950 Kcal/kg, CP=19%) diets were prepared. Growth performance was monitored weekly. Carcass characteristics and meat quality were evaluated at termination. A detailed cost-benefit analysis was performed.

Statement of Statistical Analysis: Data were analyzed using a one-way Analysis of Variance (ANOVA) in R software. Treatment means were separated using Tukey's HSD test, with significance declared at $p < 0.05$.

Results: As expected, the commercial diet (T0) resulted in the highest ($p < 0.001$) final body weight (865.03 g/bird) and average daily gain (13.92 g/bird/day). However, the feed conversion ratio (FCR) was statistically similar ($p = 0.706$) across all groups. Crucially, the formulated T1 diet achieved the highest net return (92.65 vs. 78.79 BDT/kg live bird) and benefit-cost ratio (1.36 vs. 1.29), which were significantly higher ($p < 0.05$) than T0. The corn-replacement diets (T2, T3) also showed higher profitability than the commercial control group.

Conclusion: The study demonstrated that while the commercial Sonali diet (T0) led to superior growth and carcass outcomes, its higher feed cost reduced profitability. The diet without maize replacement (T1) achieved the highest net return and economic efficiency, proving most cost-effective. Partial maize replacement (T2 and T3) maintained satisfactory performance and economic viability. Thus, formulated diets using Chita Dhan Rice and BSFM offer practical, affordable alternatives for sustainable native chicken production in Bangladesh, especially when the price of imported corn and soybean meal rises.

Keywords: Native Chicken; Local Feed Ingredients; Feed Formulation; Economic Profitability

5128P Choosing the Right Tool: Best Available Technologies and a Decision Framework for Monitoring Individual Functional-Area Visits in Poultry

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Understanding how individual chickens use feeders, drinkers, nest boxes, perches and outdoor range is fundamental for assessing welfare, resource competition and behavioural organisation. Available tracking technologies differ in spatial precision, identity continuity, invasiveness and feasibility. This study identifies Best Available Technologies (BAT) for monitoring individual visits and dwell time in functional areas and synthesises them into a decision framework for research. A PRISMA-guided systematic review was conducted. Following eligibility screening, 26 technological systems designed for automated tracking in functional areas were extracted from 156 relevant publications (2015–2025). We scored each system on performance, identity tracking, feasibility, and

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(non)invasiveness on a composite scale of 1 (weakest) to 3 (best). The mean composite score was 2.53±0.18, indicating a mature field, with slightly higher values for wearables than for CV systems (2.59 vs 2.48). Computer vision (CV) approaches accounted for 53.8% of systems, while wearable systems represented 46.2%. RFID was the most common subtype (34.6% of systems), achieving an average composite score of 2.60±0.19. It provided stable visit detection and perfect identity persistence, although behavioural ambiguity remains a limitation. Ultra-wideband (UWB) systems reached a composite score of 2.64, offering fine spatial precision but requiring multi-anchor calibration. Infrared (IR) break-beam systems achieved an overall score of 2.59, excelling in temporal resolution but limited by line-of-sight dependency. Within CV, colour-marker pipelines achieved composite scores of 2.58±0.16 and fiducial markers 2.51±0.32, both accurate under controlled lighting conditions. Markerless CV pipelines (basic detect and track, segmentation-based, specialised and re-identification) clustered around composite scores of roughly 2.41 to 2.49, demonstrating promise but still facing challenges in maintaining identity robustness under occlusion and high-density conditions. RFID is the BAT for routine longitudinal monitoring at defined resources, balancing reliability, continuity and low invasiveness. UWB is recommended for studies requiring spatial mapping or trajectory reconstruction. CV with markers provides a viable non-contact alternative in controlled research environments, while markerless CV is an emerging tool for ethological work requiring fully contactless observation. Convergence toward multimodal systems that combine identity tracking from wearables with behavioural context from CV appears a promising direction for comprehensive, welfare-oriented quantification of individual space use in poultry.

Keywords: Functional areas; Poultry behaviour; Individual tracking; Automated behaviour analysis; Precision livestock farming

5129P Evaluation of Electron Beam disinfection for hatching eggs

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Accelerated-electron disinfection offers a promising non-chemical alternative for hatching eggs disinfection. Given the constant temperature, high humidity, and limited air exchange in an incubator, which create ideal conditions for embryos and bacteria, disinfection is crucial. Traditionally, formaldehyde fumigation has been used for microbial control. However, it presents a health risk to hatchery professionals and may impair embryo viability.

This study was designed to assess the effectiveness of the disinfection with accelerated electrons (E-beam) on the eggshell and its impact on hatchability compared with standard formaldehyde fumigation and non-treated hatching eggs.

Hatching eggs were divided into four treatment groups: 1. Conventional treatment (formaldehyde fumigation), 2. E-beam treatment, 3. Formaldehyde fumigation plus E-beam treatment, and 4. Control treatment (untreated hatching eggs). The E-beam treatment utilized accelerated electrons at a controlled dose of 10 kGy, which was less than the maximum dose. In the combination, the E-beam treatment was used first, followed by fumigation. All eggs were incubated under uniform conditions. After incubation, breakout analyses were performed on unhatched eggs to determine embryo development, and hatchability parameters were recorded.

Data were analyzed using non-parametric tests and GLMMs to compare the effects of each treatment and the influence of the flock. As data collection is still ongoing, final statistical analyses are not yet completed.

The results indicate a significant decrease in bacterial load on the eggshells bombarded with electrons compared to conventional and non-treated eggs ($p \geq 0.001$). Moreover, preliminary results suggest that the E-beam treatment did positively impact hatchability. The mean hatchability rates were higher than those of conventionally treated eggs, and much higher than those of non-treated eggs. Furthermore, the E-beam treatment resulted in fewer embryonic abnormalities and improved chick uniformity.

Disinfection with accelerated electrons presents an effective alternative to formaldehyde. This method reduces bacterial load and enhances hatchability while addressing health risks typically associated with the current method. Further research is recommended to optimize dosage to disinfect the eggs until the membrane.

Keywords: Animal health; Decontamination; Electron Beam; Hatchability; Sustainability

5130P Spatiotemporal Video Encoder Integrated with Metadata Fusion for Action Recognition and Behavior Analysis in Smartphone-captured Data

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Automated behavior monitoring is pivotal for enhancing animal welfare and optimizing production in modern poultry farming. However, the dense, dynamic environments of commercial broiler pens, combined with the challenges of handheld smartphone footage, present significant obstacles for reliable computer vision systems. This study develops and validates an integrated pipeline for spatiotemporal action recognition and behavioral analysis of broiler chickens using smartphone-captured video. Our end-to-end framework addresses key technical challenges by first stabilizing raw footage to correct motion artifacts, then employing YOLOv11-Pose for robust detection and pose estimation in crowded scenes, and ByteTrack for multi-object tracking. The core innovation is a novel metadata fusion strategy, where spatiotemporal features from an X3D video encoder are integrated with pose-derived contextual metadata (such as velocity and proximity to feeders and drinkers) using a multilayer perceptron (MLP). This intermediate fusion approach enables the model to disambiguate visually similar but contextually distinct actions, such as drinking versus feeding. The system demonstrated strong performance on five key maintenance behaviors, achieving an average F1-score of 0.871 and an overall test accuracy of 87.08%. The system was particularly effective at detecting drinking and feeding behaviors, with individual F1-scores of 0.949 (94% accuracy) and 0.928 (91.89% accuracy) respectively. The system successfully processed 174 videos, producing valuable insights in the form of behavioral metrics, spatial heatmaps, and individual trajectories. This demonstrates that an integrated system—which combines stabilization, advanced models, and contextual metadata fusion-can effectively use smartphone footage to create a scalable, objective tool for precision livestock farming and welfare research.

Keywords: Computer vision metadata fusion; Action recognition; Precision poultry farming; Pose estimation; Metadata fusion

5131P Innovative accumulation chambers to measure enteric methane emissions in broilers

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Enteric methane emissions in poultry are lower than those of ruminants, but they are not negligible. Broilers are the most commonly raised meat-type chickens in Taiwan, and their enteric

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methane emissions are still worth investigating. This study aimed to measure the enteric methane emissions of broilers during the warm season (15-25°C) and hot season (>25°C) using an innovated accumulation chamber. Three chambers were used in this study. These were accumulation chambers integrated with an automatic greenhouse gas analyzer and an exhaust system. During measurement, the air inlet and outlet automatically closed, and gas samples were automatically drawn into the analyzer to determine methane concentration; air inlet and outlets reopened automatically during ventilation. Over two decades have passed since enteric methane emissions from broilers were last measured domestically. Breeding advancements, changes in environmental conditions, and evolving rearing practices may have altered emission levels, necessitating updated assessments. Two trials were conducted, one in each season, with 120 broiler chickens per trial. Methane concentration was measured weekly from weeks 1 to 5. Each chamber housed 40 chickens initially, with the number reduced as the chickens grew, down to 10 chickens by 5- 5-week-old, based on space and instrument detection limits. The measurement procedures are as follows (Steps 1–7): 1) Measure the methane concentration of the environment for 1 minute. 2) Measure methane concentration in Chamber 1 for 5 minutes (airflow closed). 3) Open airflow for 7–10 minutes (35–45 m^3/h). 4) Measure methane concentration in Chamber 2 for 5 minutes (airflow closed). 5) Repeat step 3. 6) Measure methane concentration in Chamber 3 for 5 minutes (airflow closed). 7) Repeat step 3. Steps 1-7 are repeated as one measurement cycle throughout the 24-hour experiment. The temperature and humidity inside each chamber are also recorded. Based on the regression curve of body weight and daily methane emissions, the area under the curve was calculated to obtain the enteric methane emissions of broilers over their entire life cycle. The results showed that enteric methane emissions during the warm and hot seasons were 6.77 × 10^ (-6) and 3.33 × 10^ (-6) kg/bird/life cycle, respectively. These values serve as baseline data for enteric methane emissions from broilers in Taiwan.

Keywords: Enteric methane emissions; Greenhouse gas; Broilers; Chicken

5132P Genotype influence on growth and meat quality of Nigerian indigenous chickens: toward sustainable poultry improvement

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Chickens are among the most extensively reared livestock species worldwide, serving as a vital source of animal protein and income, particularly for smallholder farmers. This study evaluated the influence of genotype on growth performance, carcass yield, meat quality, and haematobiochemical parameters in three Nigerian indigenous chicken (NIC) varieties: Normal Feathered (NFC), Frizzle Feathered (FFC), and Naked Neck (NNC), compared with a commercial broiler strain (Arbor Acre, AAC). A total of 140 day-old chicks (35 per genotype) were raised for eight weeks under uniform management conditions in a completely randomized design (CRD). Performance indicators such as body weight, length, and other morphometric traits, including meat quality, were measured using one-way analysis of variance (ANOVA). The AAC birds attained the highest final body weight (2.26 kg) and showed superior carcass characteristics ($p < 0.05$), including slaughter weight (2260 g), carcass yield (1870 g), dressing percentage (82.74%), and visceral proportion (8.0%). Among the indigenous genotypes, the FFC exhibited better feed efficiency (1.81 kg/BW) and desirable meat quality attributes, such as higher water-holding capacity (33.10%) and lower thawing loss (3.48%) ($p <0.05$). The AAC had elevated fat (6.60%) and cholesterol (5.63 mg/dL) levels ($p<0.05$), whereas the indigenous varieties showed higher crude protein content. Haematological and biochemical profiles indicated increased white blood cell counts and globulin concentrations in the NICs, especially FFC, suggesting higher immune competence. Although AAC outperformed the NICs in growth and carcass yield, the indigenous chickens, particularly the FFC, demonstrated favorable meat quality and resilience traits, demonstrating their potential in genetic improvement programmes. These findings highlight the importance of conserving and utilizing indigenous chicken resources to promote sustainable, climate-resilient poultry production systems in Nigeria and similar tropical environments.

Keywords: Genotype; Growth performance; Meat quality; Indigenous chickens; Sustainable poultry production

5133P Effect of Low-protein Diet on Growth Performance, Carcass, and Meat Quality in Dual-purpose Chicken

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Intensive selection has created poultry breeds specialized for eggs or meat, making male layer chicks uneconomical and leading to large-scale culling with ethical concerns. Dual-purpose chickens can provide both eggs and meat, eliminating early culling. Reducing dietary crude protein by 3%, when balanced with essential amino acids, may lower feed costs and nitrogen excretion without harming performance, but research on low-protein diets in dual-purpose chickens remains limited. The aim of this study was to investigate the effects of low-protein diet on growth performance, carcass yield, and meat quality in dual-purpose chicken. A 2 × 3 factorial design was used, testing two CP levels (21% and 18%) with diets formulated to be isoenergetic and balanced for essential amino acids. Three genotypes of cockerels were evaluated, comprising a total of 360 birds (120 birds/genotype) housed in 8 pens and subjected to two CP levels. Taiwan country chicken B strain (B), commercial Lohmann LSL-Ultra Lite (L), and dual-purpose chicken (paternal line: L, maternal line: B). Two-way analysis of variance was performed on the measurement traits using a general linear model. Dual-purpose chickens exhibited intermediate growth rate, carcass weight, and absolute muscle weight compared with the B and L ($P<0.05$). Their meat quality attributes resembled those of the native strains, differing only slightly in color parameters ($P>0.05$). Feeding the low CP diet significantly reduced final live weight across all genotypes, 2006 g vs. 1944 g ($P<0.05$) but did not impair carcass yields or individual cut weights ($P>0.05$). In summary, while lowering dietary CP levels depresses growth, dual-purpose chickens maintain native-like meat quality and display greater tolerance to low protein diet, suggesting their potential to mitigate the culling of male layer chicks. Future research should refine protein level and amino acid requirements in each growth stage for dual-purpose chicken and assess the economic viability within sustainable poultry production systems.

Keywords: dual-purpose chicken; growth performance; low-protein

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diet

5134P Impact of slaughter age and bodyweight on carcass composition and processing yields in heavy male turkeys

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Abstract:
Optimising production costs while maximising carcass utilization is a major challenge in the heavy turkey industry. While breast and thigh meats are the primary value drivers, determining the optimal slaughter age requires a precise understanding of the growth patterns of all meat parts. The aim of this study was to evaluate the effect of slaughter age and bodyweight on processing yields and economic return in male turkeys.

The trial was conducted in Euronutrition, France using 162 Aviagen Premium male turkeys reared under identical conditions. At 106, 113, and 120 days of age, 54 birds per age group were randomly selected, fasted for 12 hours, and slaughtered. Carcasses were hand-deboned to measure the weight of the skin, fat, neck, full breast, wings, and thighs. Statistical analyses were performed using R software. Differences between age groups were assessed with a significance level of $p < 0.05$. Additionally, linear regression models were applied to analyze the relationship between live weight and breast traits.

Results showed a significant increase in overall carcass weight and yield with age ($p<0.05$). However, distinct growth patterns were observed among cut-up parts. Full breast yield increased significantly between 106 days (22.85%) and 113 days (23.39%) but plateaued thereafter, showing no significant difference between 113 and 120 days (23.37%). Conversely, full thigh yield continued to increase significantly from 113 to 120 days ($p<0.05$). Wing yield patterns varied, with drumette weight increasing up to 113 days before declining.
Regression analysis revealed that while bodyweight is the primary predictor of breast weight, age plays a critical role: for a fixed target weight, reaching it at a younger age results in a higher breast weight and yield.
Economically, the feed cost per ton of live weight increased with age (755€ at 106d vs 793€ at 120d) due to increasing FCR. Notably, the feed cost per ton of breast meat rose sharply between 113 and 120 days (+70€/t), driven by the stagnation in breast yield combined with higher maintenance costs.
In conclusion, slaughtering turkeys at 113 days appeared to be the optimal compromise in this study, maximizing breast yield before the onset of diminishing returns observed at 120 days. These data suggest that optimizing the age-to-weight ratio is essential for improving the profitability of heavy turkey production.
Keywords: Turkey, slaughter age, carcass yield, breast meat, economic performance.
Keywords: Turkey Production; carcass yield; breast meat yield; Leg yield; Age

5135P Effect of different levels of branched chain amino acids in low protein diet on performance, carcass characteristics and immune system of broiler

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The present study was conducted with the aim of the effect of different levels of branched-chain amino acids (BCAAs) (L-valine, L-leucine and L-isoleucine) in low protein diets on growth performance, carcass characteristics and blood cells of broiler chickens. In this experiment, during 42 days, 480 one-day-old broiler chickens of Ross 308 strain (equal number of males and females) with an average one-day weight of 42.75±0.47 g were used in a completely randomized design (two-ways ANOVA analysis) with a 2×3 factorial experiment with 6 treatments and 4 replications. The experimental diets included three levels of BCAAs (standard, 10% above the standard and 10 % below the standard) and two levels of crude protein (CP) (standard and 10% below the standard). All diets (treatments) in each period were isocaloric. The lysine level in the diet was between 1-1.3%. The ratios between Val/Leu, Ile/Leu and ratios between BCAAs and Lysine were considered in diet formulation. In the growth stage, the highest weight gain was related to the treatment with 10% less CP and 10% more BCAAs (70.42 g per day). In the finishing stage and also in the total period, the low-protein treatment containing 10% less and 10% more BCAAs did not differ significantly from the other treatments in terms of this trait ($P>=0.05$). The FCR in the initial stage did not show a significant difference between the treatments ($P>=0.05$), but in the growth and finishing stages and in the total period, 10% less CP treatment supplemented with 10% more BCAAs had the best FCR (1.26, 1.90 and 1.56 respectively) ($P<0.05$). The highest carcass percentage was related to the treatment with standard CP and 10% more BCAAs (71.26%) ($P<0.05$). The heterophil percent and heterophil/lymphocyte ratio had significant differences between treatments. The treatment with standard CP and 10% higher BCAAs had more heterophil percent and more heterophil/lymphocyte ratio (28.46% and 0.43 respectively). BCAAs have anabolic effect and immune modulation in broilers. In general, supplementing BCAAs at a rate of 10% higher than the standard in diets with a 10% reduction in CP had a positive effect in improving FCR and growth performance. For improving the immune system of broilers, standard CP with 10% more BCAAs had better effect.
Keywords: crude protein; branched-chan amino acids ; performance; carcass characteristics; immune system

5136P Apparent ileal digestibility of starch and amino acids in low-protein broiler diets with different energy-to-protein ratios

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The present study aimed to investigate the effects of feeding low-protein (LP) diets with different energy-to-protein ratios on the apparent ileal digestibility (AID) of starch and amino acids. A total of 576-day-old Ross 308 broilers were fed a control diet (C) and three low-protein diets (LP1, LP2, LP3) for 41 days (6 pens per treatment; n=24 chickens per pen). Diets were fed in the starter (day 0-10), grower (day 11-24), and finisher (day 25-41) phases. The C diet was formulated according to the requirements of the hybrid (Aviagen, UK). In each feeding phase, the three LP diets contained 1.5% less crude protein (CP) than the C diet, but the SID concentrations of Lys, Met, Met+Cys, Thr, Val, and Ile in the C and LP diets were the same. The LP1 treatment was isocaloric with diet C, while the AMEn contents of LP2 and LP3 diets were reduced by 1.5% and 3% throughout the study, respectively. The dietary ratio of AMEn-to-CP and starch-to-CP was higher in the LP diets compared to the C diet in each phase. The dietary AMEn-to-CP ratio and the crude fat content decreased, while the starch content and starch-to-CP ratio increased from diet LP1 to LP2, and from LP2 to LP3.

In the finisher phase, 12 broilers per treatment were selected for a digestibility trial (day 35-41). The AID of starch and amino acids was determined, and the results were evaluated by one-way analysis of variance (ANOVA). The dietary treatments significant-

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ly (P<0.001) affected the average AID of both starch and amino acids. In the protein- and energy-reduced LP2 (94.8%) and LP3 (95.2%) treatment groups, the AID of starch was significantly higher compared to the isoenergetic C (92.7%) and LP1 (93.2%) groups (P<0.05). The average AID of amino acids was also significantly higher in the LP groups (91.5%, 91.2%, 92.2%) than in the C group (88.2%). In general, the AID of individual amino acids, except for Glu, was significantly (P<0.001) improved when LP diets were fed compared to the feeding of the C diet. For the amino acids Lys, Thr, Leu, Tyr, Cys, Asp, Gly, there were no significant differences among the AID values in the LP treatment groups, so the different dietary AMEn-to-CP and starch-to-CP ratios of LP diets did not affect the results. However, the AID of Ile, His, and Ala in the LP3 group proved to be significantly higher compared to the LP1 and LP2 groups. In conclusion, our results show that the AID of starch in LP diets may be higher than in diets with normal CP content and depends on the dietary AMEn content. Furthermore, the response of the AID of individual amino acids in LP diets to dietary AMEn and starch content can be different.
Keywords: broiler; low-protein diet; dietary energy; amino acids; digestibility

5137P Lemna minor as a protein source for broilers: Amino acid and phosphorus digestibility and dietary integration
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The increasing global demand for poultry meat and concerns regarding soybean cultivation in South America have intensified the search for alternative protein sources for broiler nutrition. Duckweed (Lemna minor, LM) has recently gained attention due to its high growth rate, high protein yield, and simple cultivation. Despite its high potential a key limitation to its broader application is the lack of standardized production and knowledge on nutrient digestibility. Hence, here we examine the nutrient digestibility and integration in broiler diets in two experiments. In experiment 1, the ileal digestibility of crude protein (CP), amino acids (AA), phosphorus (P), and the energy content of indoor-cultivated LM were determined. 32 one-day-old male Ross 308 broiler chicks (16 birds per treatment) were raised with commercial broiler diets. Feces were collected for 5 days, ileal digesta were sampled at the end to calculate digestibility coefficients of CP, AA, P, and energy. In experiment 2, starter, grower, and finisher diets were formulated for 4 treatments with varying LM contents (0/0/0; 2.5/5/7.5; 5/7.5/10; 10/10/10). 36 one-day-old male Ross 308 broiler chicks (9 birds per treatment) were selected for this experiment. Diets were isoenergetic and isonitrogenous, and AA profiles were balanced for digestible AA accordingly to our previous results. All diets complied with german requirements for N- and P-reduced feeding. Fecal and ileal samples were obtained as in experiment 1 to verify the results from experiment 1. All data were analyzed by one-way ANOVA. Results from experiment 1 indicated standardized ileal digestibility (SID) of AA ranging from 48% (Cysteine) to 96% (Tyrosine). Lysine and methionine, as the primary limiting AA in broiler nutrition, exhibit SID values of 87% and 86%, respectively. Approximately 70% of all AA exhibit SID values exceeding 80% Apparent ileal digestibility of P was above 90%. Energy values of LM were determined at 7.05 MJ AME and 6.13 MJ AMEn/kg DM. In the follow-up experiment (2), no significant effects of LM inclusion on nutrient digestibility were observed. Daily weight gain (g/day) did not differ among treatments (66; 67; 68; 70; p=0.29). Feed intake (g/day) (89; 94; 94; 96; p=0.326) and feed conversion ratio (kg/kg) (1.36; 1.38; 1.39; 1.37; p=0.311) were likewise unaffected. This shows that LM cultivated under controlled indoor conditions are a reliable feed source which exhibits high nutrient digestibility. Its inclusion in broiler diets is feasible without impairing performance, provided AA supply is balanced on a digestible basis and biomass quality is standardized. This lays the foundation for further research for higher inclusion rates.
Keywords: indoor vertical farming; duckweed; Lemna minor; protein source; amino acid digestibility

5138P Oral taurine confers thermotolerance and modulates intestinal microflora in chicks
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Amino acids play a vital role in regulating body temperature in chickens. Our previous finding showed that central taurine afforded thermotolerance and induced anorexia in chicks. However, the effects of oral taurine on thermoregulation remain unclear. Therefore, the aim of this study was to evaluate the acute and chronic effects of oral taurine on regulation in body temperature and cecal microflora under thermoneutral and heat stress conditions in chicks. A total of 80 male Julia layer chicks were housed individually from day 2 for acclimatization. In Experiment 1 (acute), 40 chicks (7-day-old) were orally administered water (control, n = 20) or taurine (16 mmol/20 ml/kg, n = 20) at 90-min before exposure either to control thermoneutral temperature (CT; 30 ± 1°C) or high ambient temperature (HT; 35 ± 1°C) in a heat-controlled chamber for 120 min where chicks were equally divided (n = 10 per treatment per temperature condition). Cloacal temperature was recorded at 90- and 30-min pre-administration and at 0-, 30-, and 60-min post-administration of taurine or water. In Experiment 2 (chronic), 40 chicks were orally administered water (control, n = 20) or taurine (16 mmol/20 ml/kg, n = 20) from day 3 to day 10. On day 10, the chicks were exposed to either CT (30 ± 1°C) or HT (40 ± 1°C) for 120 min, where chicks were divided equally (n = 10 per treatment per temperature condition). Cloacal temperature was monitored at 0-, 30-, 60-, 90-, and 120-min post-exposure of CT or HT. Feed intake and body weight were recorded, and cecal content was collected following anesthesia and euthanasia for microflora analysis. Data were subjected to Thompson rejection test to eliminate outliers (P < 0.01). Remaining data were analyzed with 3-way ANOVA for acute and chronic effects on cloacal temperature under HT and 2-way ANOVA for chronic effects on daily body temperatures, body weight, feed intake and cecal microflora. The results showed that acute oral administration of taurine had no significant effect on body temperature. However, chronic oral taurine administration lowered the daily body temperature under CT. In addition, chronic administration of taurine lowered body temperature under both CT and HT. Body weight gains and feed intake were not affected. Microflora analysis showed that Escherichia coli content decreased, while Salmonella showed a strong tendency (P = 0.0509) to decrease under both CT and HT. However, Lactobacillus, and Bifidobacterium were not changed in following taurine administration. These findings indicate that chronic oral taurine administration afforded thermotolerance in chicks and might have reduced pathogenic bacteria under HT without adversely affecting growth performance.
Keywords: Taurine; Thermoregulation; Heat Stress; Chicks; Microflora

5139P Reduced dietary crude protein and essential amino

acids downregulate intestinal T1R1 and T1R3 mRNA expression in broiler chickens

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Intestinal nutrient sensing in poultry is mediated by G protein–coupled receptors, such as T1R1 and T1R3, which recognize amino acids (AA) and peptides, playing a central role in regulating digestive and metabolic responses. Their expression varies along the gut depending on tissue type, developmental stage, and dietary nutrient availability, suggesting that diet composition exerts transcriptional control over nutrient-sensing mechanisms. The objective of this study was to assess the effects of diets reduced in crude protein (CP) and four essential AA on the mRNA expression levels of AA sensors T1R1 and T1R3 in broiler chickens. A 39-day feeding trial was conducted with 64 Ross 308 broilers housed in pairs across 32 floor pens and assigned to four treatments differing in CP and AA inclusion: T1 (control, 23% CP with full Lys, Thr, Met, and Trp), T2 (–30 g/kg CP with complete AA), T3 (–30 g/kg CP with 50% AA reduction), and T4 (–30 g/kg CP without AA). Each treatment had eight replicates. At day 39, six healthy birds per treatment were euthanized by cervical dislocation for intestinal tissue sampling (duodenum, jejunum, ileum). Total RNA was extracted, and gene expression was quantified by qPCR using the ΔΔCT method. Data were analyzed using Kruskal–Wallis tests followed by Dunn's post hoc comparisons in RStudio. Birds fed low-CP or AA-deficient diets (T2, T3, T4) showed a marked reduction in the intestinal expression of both T1R1 and T1R3 mRNA compared with control birds ($p < 0.001$). The coordinated downregulation of both receptors suggests a diet-dependent adaptive mechanism, in which nutrient limitation suppresses AA sensor expression to modulate gut nutrient signaling. These findings highlight the sensitivity of intestinal chemosensory pathways to dietary AA supply and provide insight into molecular adaptations to nutritional stress in poultry.

Keywords: broiler chicken; gene expression; low-protein diet; T1R1; T1R3

5140P Supplementation of enzyme-treated soybean meal during the starter phase improves protein digestibility, disappearance, and performance in broiler chicks

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The digestibility of poultry feed ingredients is typically measured at the ileum, yet digestion and absorption of dietary components occur throughout the intestinal segments. During the process, enterocytes can catabolize starch and protein sources to obtain energy, which can decrease the protein available for tissue synthesis; thus, the synchronization of digestion and absorption of energy and amino acids, avoiding their catabolism, may enhance feed utilization. As in conventional diets, starch is digested faster than protein; increasing the quantity of fast-digestible protein can be considered. In the starter phase, this synchronization may be more crucial, as intestinal structure and function develop, including the secretion of proteolytic enzymes. Enzyme-treated soybean meal (ESBM, Hamlet Protein - Horsens, Denmark & Findlay, Ohio, USA), a specialty feed ingredient with low antinutritional factors and high fast protein, was evaluated during the starter phase (14 days) at two commercial inclusion levels for performance parameters and for protein digestibility in the different intestinal segments. For the trial, 504 male Ross 308 chicks were randomly allocated into 24 cages for a design with three groups (iso-nutritious diets with 0, 5.0, and 7.5% ESBM inclusion), 8 replicates, and 21 birds/replicate. Data was analyzed by ANOVA and least significant differences (LSD). On day 14, 15 birds per cage were euthanized to collect digesta samples from the proximal and distal jejunum and ileum, to determine crude protein digestibility coefficients and crude protein disappearance rates in each section. Results indicate a dose-response effect for ESBM inclusions for protein digestibility and disappearance ($p<0.05$), resulting in 16.6% higher protein disappearance in the proximal jejunum for 5% ESBM, and 34.6% higher for 7.5% ESBM, and 6.5% and 18.8% in the distal ileum, indicating a faster uptake of protein. In agreement, the digestibility coefficients were also higher for ESBM compared to the basal diet. An increasing numerical difference in body weight was observed for ESBM inclusions, without differences in feed intake, resulting in 0.9 and 1.4 points reduced FCR ($p<0.05$) for 5 and 7.5% ESBM, respectively. ESBM in starter feeds can be used to promote broilers' early digestion efficiency and development.

Keywords: digestive dynamics; enzyme-treated soybean meal; protein; early nutrition

5141P Lupin Kernel Meal and Canola Meal as Alternatives to Soybean Meal in Broiler Chickens Fed Reduced Crude Protein Diets

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This study assessed broiler growth performance when soybean meal was replaced with lupin kernel meal and canola meal across two dietary crude protein (CP) levels. A 42-day feeding trial was conducted using 384 Ross 308 male broilers allocated to 48 pens and assigned to 6 experimental diets in a 2×3 factorial design (8 replicate pens per treatment, 8 birds per pen; n=64). Two CP levels were tested: standard CP and reduced CP (minus 1.5%). Three dietary formulas gradually replaced soybean meal (SBM) in control diet: D1 – Control (corn/wheat/SBM), D2 – lupin kernel meal (LKM) + canola meal (CM) + meat and bone meal + crystalline amino acids (AA) replacing 50% of SBM, and D3 – the same alternative ingredients replacing 100% of SBM. Experimental diets were formulated to meet or exceed nutrient recommendations by Aviagen. Birds had ad libitum access to feed and water throughout the trial. Growth performance parameters including final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were recorded. Data were analysed using two-way ANOVA to assess main effects and interactions ($P < 0.05$).

There were no significant main effects of CP level on FBW, ADG, or ADFI. However, reduced CP diets improved FCR compared to standard CP diets (1.49 vs 1.50; $P = 0.02$). The main effects of SBM replacement showed no significant differences in FBW, ADG, or ADFI, though FCR was significantly higher when SBM replaced (1.51 and 1.50 vs 1.48; $P = 0.04$). Notably, significant interactions were observed between CP level and SBM replacement for ADG ($P = 0.03$), ADFI ($P = 0.01$), and FCR ($P = 0.02$). At standard CP, complete SBM replacement resulted in reduced FBW (2789 vs 2908 g), ADG (68 vs 70 g/day), and ADFI (103 vs 106 g/day). In contrast, in reduced CP diets, 100% SBM replacement with alternative ingredients, did not impair growth performance of the birds (FBW 2884 vs 2835 g; ADG 71 vs 70 g/day; FCR 1.50 vs 1.50).

In conclusion, the main findings confirm that alternative protein ingredients including lupin kernel meal and canola meal, combined with meat and bone meal and crystalline AA, can fully replace soybean meal in reduced CP diets without compromising broiler

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growth performance. Full replacement at standard CP levels negatively affected performance, indicating that crystalline AA with their high digestibility and bioavailability are essential for maintaining performance when using alternative protein sources. Based on these findings, the AA digestibility coefficients of lupin kernel meal and canola meal require further evaluation to optimise diet formulation. This approach supports more sustainable and locally sourced feed strategies for the Australian broiler industry.
Keywords: broiler chicken; canola meal; lupin kernel meal; soybean meal; sustainable farming

5142P Effect of glycine equivalent to lysine ratio, and threonine and betaine inclusions in reduced-protein diets during the pre- and starter phases, on growth performance of broiler chickens.

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Reduced-protein levels have been introduced in poultry production to improve the welfare of the animals through the improvements in the litter quality (lower N and moisture content), and to decrease the dependency of Europe from soybean imports. However, when levels of protein are too low, the ratio of available glycine equivalent to lysine (gly:lys) might compromise the growth of the animals, and this was investigated in the present study: protein levels were decreased from the commercial standard wheat-soybean-based diet (Control), by -1.2 and -2.4 percent points in the pre-starter (PSt) and by -1.0 and -2.0 percent points in the starter (St) phases of growth (till 10d and 20d respectively), the lowest protein level was fed as is or with the inclusion of threonine and betaine as possible precursors to gly equivalent values; the gly:lys were 1.23, 1.15 and 1.06. The study had a completely randomized design by blocks, with 12 replicates (floor pens)/group, and 35 day-of-hatch female Ross308 chickens per replicate. Birds were fed commercial feeds for the grower-finisher (GrFi) phase till 35d. Data was analyzed by one-way ANOVA using SAS 9.4 (2012) mixed procedure (diet fixed effect with 3 df, block as random effect with 11 df). BW decreased at 10d (287 g for Control) by -0.7% (NS) and -3.9% (P<0.10) respectively decreasing the gly:lys, while slightly recovered (-2.2%) with the inclusion of the precursors in the 1.06 gly:lys feed. At 20d the differences in BW (889 g for Control) did not reach statistical significance (-1.5%, -3.1% and -2.2% respectively), while at 35d (2309 g for Control) the trend was the same as at 10d (-1.1% NS, -2.1% P<0.10, -1.1% NS). Feed conversion ratio was gradually impaired at each decreasing gly:lys level in the PSt (1.067 for Control, +0.9%, +1.9%, +2.1%, all P<0.05 compared to Control) and the St phase (1.280 for Control, +2.6%, +3.6%, +2.6%, all P<0.05 compared to Control) with NS effect of precursors; the significance was lost for the whole growout (1.421 for Control, +1.0% P<0.10, +0.5% NS, +0.7% NS). Average daily feed intake was not significantly affected either in the PSt or St phases but was significantly lower in the 1.06 gly:lys (without precursors) group in the GrFi phase (-2.5%). According to the results of the present study, the reduction of the crude protein linked to the decrease in gly:lys impaired the performance of the chickens, while the inclusion of precursors partially counteracted the effect; future availability of glycine and serine at commercial prices could help for the reduction of the total protein content of feeds.
Keywords: reduced protein content; glycine equivalent to lysine ratio; amino acid precursors; chickens; performance

5143P Effects of nutritional strategies associated with reduced-protein diets on broiler performance and carcass yield.

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Current challenges in the sector include reducing Europe's dependence on imported soya, lowering litter N and moisture, and decreasing emissions, objectives that can be partly addressed through reduced-protein feeding. However, such diets may impair growth performance. This study evaluated the potential of different feeding strategies to enhance sustainability and effectiveness in broiler chicken farming on performance and carcass yield. A randomized complete block design was used with 6 treatments, each with 8 floor-pen replicates of 41 male Ross308 birds/replicate, in a 4-phase feeding (crumbles in Pre Starter-PSt till 7d, and Starter-St till 21d; pellets in Grower-Gr till 30d, and Finisher-Fi phase till 38d). Commercial corn-wheat-soya based diets were modified according to each strategy (kcal/kg ME and available Lysine-Lys g/kg feed for PSt, St, Gr, Fi phase): T1) "low energy & Lys (except PSt)" 2997 & 1.24 / 3030 & 1.13 / 3050 & 1.04 / 3080 & 0.94; T2) as T1 adding whole wheat grain on top at 5%, 5%, 10% and 10%; T3) "high energy & Lys (except PSt)" 2972 & 1.26 / 3050 & 1.14 / 3075 & 1.06 / 3100 & 1.02; T4) as T3 adding whole wheat grain on top at 5%, 5%, 10% and 10%; T5) "low Lys-high energy" Lys as T1 and energy as T3; T6) "high Lys-low energy" Lys as T3 and energy as T1. Data were analyzed by one-way ANOVA (diet as fixed effect, block as random effect) using SAS 9.4. During the overall growing period, whole wheat grain in T2 resulted in a significant reduction in BW and average daily gain-ADG (−5%; P = 0.01) versus T1 (3039 g and 78.9 g), whereas no differences were observed among other strategies. Feed conversion ratio-FCR improved (−2%; P < 0.001) in the high energy & high Lys (T3) and high Lys & low energy (T6) diets compared with T1 but was impaired (+3%) with whole wheat grain (T2). High Lys (T3, T6) and high energy (T5) increased breast meat percentage (average 21.4%; P < 0.001), whereas T4 showed the lowest value (20.1%). Drumstick and thigh percentages did not differ among strategies. Whole wheat grain (T2 and T4) increased abdominal fat (average 1.81%) compared with the other strategies (1.47%; P < 0.001). Under the conditions of the present study, lower dietary Lys impaired performance and breast yield, and whole wheat grain, negatively affected growth and carcass traits. These results confirm that nutritional strategies intended to enhance welfare or sustainability inherently affect productivity. Further studies should clarify whether potential benefits on welfare, gut function, litter quality, breast myopathies and economic outcomes to compensate for these effects.
Keywords: reduced-protein diets; energy and lysine levels; whole wheat inclusion; broiler performance; carcass yield

5144P Optimizing dietary energy and digestible lysine requirements in broiler chicken: Impacts on performance and cost efficiency

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Energy and amino acids are currently the most expensive and limiting nutrients in broiler chicken diets, thus, these requirements are continuously reviewed. The importance of optimizing the nutrient profile, metabolizable energy (ME) and digestible lysine (dLys) levels, in broiler diets stems from their crucial role in performance, feed cost and environmental sustainability. Hence, more suitable recommendations would achieve both productivity and profitability

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enhancement. Therefore, this study was conducted to assess the effects of applying ME and dLys latest recommendations (Aviagen 2022) in broiler chicken diets across different growth phases on growth performance and profitability. A total of 2,633 one-day-old mixed-sex broiler chickens (Ross 308, 41 ± 0.45 g of body weight [BW]) were randomly distributed into 24 pens (110 chicks/pen, 12 chicks/m2). Trial was designed following 2 x 2 factorial arrangement (n = 6 replicates/treatment), where factors were: nutrient recommendations (2 levels in terms of ME and dLys: Aviagen, 2014 vs. 2022), and pre-slaughter fasting management (2 levels: with or without feed withdrawal for 9 h); the present abstract only reports results of the dietary specifications. A 4-phase feeding program was used: pre-starter (from 0 to 7 d) and starter (from 8 to 21 d), in crumbles, and growing (from 22 to 30 d) and finishing (from 31 to 36 d) in pelleted form. Feeds were mainly composed of corn, soya and wheat. Experimental diets were different in ME and dLys levels according to nutrient specifications and feeding phase. Thus, while the ME was 3,000, 3,030, 3,050 and 3,080 vs. 2,975, 3,050, 3,075 and 3,100 kcal/kg according to Aviagen recommendations 2014 and 2022, respectively; the dLys was 1.24, 1.13, 1.04 and 0.94 vs. 1.26, 1.14, 1.06 and 1.02 %; other requirements followed FEDNA (2018) recommendations and commercial practices. All data were analyzed as a mixed-effects model with repeated measures using MIXED procedure of SAS. Aviagen-2022 group exhibited a greater ($P < 0.01$) final BW and growth, tended to show a lesser ($P = 0.10$) feed intake, and improved ($P < 0.01$) the feed conversion rate in comparison with Aviagen-2014 group. In addition, Aviagen-2022 group resulted in the lowest ($P < 0.05$) feed cost per kg of BW. In conclusion, latest nutrient recommendations (Aviagen, 2022), high levels of ME and dLys, can potentially enhance productivity and profitability in broiler chicken farming.
Keywords: metabolizable energy; digestible lysine; broiler chicken; performance; profitability

5145P MATRIX VALUE ESTIMATION OF A HEAT STABLE E. COLI PHYTASE IN BROILERS

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The objective of this trial was to evaluate the potency of an E. coli derived phytase (OptiPhos® Plus) at two inclusion levels on technical performance and bone formation in broilers. Broilers were randomly assigned to three treatment groups, each with eight pens containing 35 male birds per pen per treatment. Broilers were fed starter (day 0-10), grower (day 11-21) and finisher (day 22-35) diets. Feeds were corn/wheat/soy based and provided as pellet (crumble in starter). Composition was (a) starter: 22 % crude protein, 1.26 % dig. Lys, 2925 kCal (12.23 MJ) AME broiler/kg, 0.90 % Ca and 0.485 % aP; (b) grower: 21.5 % crude protein, 1.14 % dig. Lys, 3000 kCal (12.54 MJ) AME broiler/kg, 0.75 % Ca, 0.38 % aP; and (c) finisher: 20.2 % crude protein, 1.04 % dig. Lys, 3040 kCal (12.71 MJ) AME broiler/kg, 0.55 % Ca, and 0.34 % aP. This feed was reformulated reducing Ca and aP levels by 0.10 % and 0.22 %, respectively in all feeds replacing MCP and limestone, and adding the phytase at levels of 1000 or 2000 FTU/kg (so yielding 3 treatments in total). Phytase analysis according to ISO 30024 revealed average values over the two feeds (corrected for background) of 1305±94 FTU and 2448±122 FTU/kg which indicates values close to the targeted value of 1000 and 2000 FTU/kg, respectively. Growth, feed intake and feed conversion were monitored per feeding phase. At day 21 of age, four birds per pen were euthanised, the tibia bones were collected, pooled per pen and analysed for bone ash. Statistical analysis on the experimental data was performed by ANOVA using GenStat for Windows (24th version).

At a targeted inclusion of 1000 FTU/kg, performance was already brought back to the level of the control (2732 g body weight (BW) and a feed conversion rate (FCR) of 1.379 at 1000 FTU/kg versus 2730 g and 1.389 for the control. However, dosing a targeted value of 2000 FTU/kg resulted in average higher end weights (2781 g) and a lower FCR (1.368) which is indicative of a superdosing effect. Adding 1000 FTU/kg of phytase to the negative control feed brought back bone ash to the positive control (511 g/kg fat-free bone vs 509 g/kg, respectively). Phytase at 2000 FTU/kg increased bone ash to 515 g/kg fat-free bone.

It can be concluded from this trial that at a targeted inclusion level of 1000 FTU/kg, this phytase can compensate for a 0.22 % aP and 0.1 % Ca reduction in the feed, while at a targeted inclusion level of 2000 FTU/kg, a significant improvement in bone ash was noted. This is higher compared to the current claimed effect of 0.176 % and 0.2 % aP for this phytase at 1000 and 2000 FTU/kg, respectively.

Keywords: phytase ; broiler; matrix values; available P; digestible P

5146P Effect of a novel phytase on the phosphorus solubilization of various cereal grains and oilseed meals

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The objective of this trial was to evaluate the potency of an E. coli derived phytase (OptiPhos® Plus) on release of phosphorus (P) from phytate of feed materials, i.e., cereals (corn, wheat, barley) and protein sources (soybean meal, sunflower seed meal and rapeseed meal) in vitro. For this, feed materials were incubated with phytase at 0, 500, 1000 or 2000 FTU/kg. An additional treatment combined 1000 FTU/kg phytase with a novel non-starch polysaccharide (NSPase; Huvezym® neXo) dosed at 1500 EPU/kg, 100 CU/kg and 100 XGU/kg of an endo-1,4-beta-xylanase, endo-1,4-beta-glucanase and xyloglucan-specific endo-beta-1,4-glucanase, respectively. Feed materials were milled at 1.0 mm and analysed for total P.A 0.8 g sample (in triplicate) was incubated in vitro with 20 ml of pepsin-hydrochloric acid (HCl) solution (25 g/l; pH 2.5) at 37 °C and pH 2.5 for 1.5 hours, mixing constantly at 340 rpm. Afterwards, 18 ml acetate buffer (0.1 M and pH 5.5) was added to reach pH 5.5, and 2 ml pancreatin solution (5 g pancreatin solubilized in 50 ml buffer solution) was added. The resulting mixture was incubated for 2.5 h at 37 °C. After 2.5 h, the incubation was stopped, and the tubes were placed into an ice-cold bath. The supernatant and residue were separated by centrifugation (3000 rpm) and the P in solution was determined to calculate the P degradation.

The results indicate that the solubilization of P increased with increasing levels of phytase. In cereals, adding the phytase gave the highest P solubilization in corn (74.6, 80.7 and 88.9 % at 500, 1000 and 2000 FTU/kg, respectively), followed by wheat (62.3, 65.7 and 72.7 % at 500, 1000 and 2000 FTU/kg, respectively) and barley (74.1, 76.9 and 74.6 % at 500, 1000 and 2000 FTU/kg, respectively). For the protein sources, the highest P solubilization was obtained by adding phytase to sunflower seed meal (83.0, 85.0 and 87.9 % at 500, 1000 and 2000 FTU/kg, respectively) followed by rapeseed meal (76.1, 80.7 and 83.6 % for 500, 1000 and 2000 FTU/kg, respectively) and soybean meal (72.3, 79.0 and 81.4 % at 500, 1000 and 2000 FTU/kg, respectively). The addition of the NSPase had a positive effect on a supplemental solubilization of P in corn, barley, wheat, rapeseed meal and soybean meal, increasing it by 6.4, 8.8, 3.6, 4.2 and 4.2 %, respectively at 1000 FTU/kg phytase, while no effect was seen for sunflower seed meal (-0.1 %). This research indicates that phytate breakdown by phytase, as visualized by P solubilization, can be different depending on the cereal and the protein type, and that the addition of an NSPase can

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contribute to a better breakdown of phytate by phytase.
Keywords: phytase ; phytate; in vitro; P digestibility

5147P Effect of exogenous xylanase on feeding value of hybrid rye for broilers

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Hybrid rye is an emerging feed ingredient for broilers, offering high energy value, favourable amino acid composition, and sustainable cultivation benefits. Its inclusion can reduce feed costs and environmental impact. However, relatively high fibre content of rye may have negative impact on broiler growth performance variables. This study evaluated the effects of feeding hybrid rye diets with or without xylanase (XYL) supplementation on broiler performance. Four well characterised cultivar samples of hybrid rye (average AMEn of 9.41 MJ/kg DM, with no significant difference between cultivar samples) were used at 20% inclusion rate to produce four balanced diets (12.68 MJ/kg ME, 194 g/kg CP). Each of the diets was then split on two parts as one part was left as is and the other was supplemented with 1100 FXU/g, 125 BGXU/g, 275 FAU/g of commercial XYL (Ronozyme® Multigrain DSM, Kaiseraugst, Switzerland), resulting in eight dietary treatments in total. In total 288 male Ross 308 day-old broilers were purchased from commercial hatchery, reared in a communal pen until 10 days of age and fed the same proprietary starter diets. At 10 d age, birds were randomly allocated to 48 raised floor pens, six birds in each. The experimental diets were presented in meal form and was fed to six pens following randomisation. Excreta were collected between 24 and 28 days of age, oven-dried and with the diets, analysed for marker, energy, N and neutral detergent fibres (NDF). Growth performance variables, including daily feed intake (FI), weight gain (WG), and feed conversion ratio (FCR), were measured from 10 to 28 days of age. Comparisons among studied variables were performed by two-way ANOVA using a 4 × 2 factorial design (rye cultivar samples × enzyme), as the observational unit was the pen with six birds. Data are expressed as means and their pooled standard errors of means (SEM). In all instances, differences were reported as significant at $P < 0.05$. Dietary rye cultivar sample did not have an impact ($P > 0.05$) on any of the studied variables. Xylanase supplementation improved daily WG ($P = 0.002$) and FCR ($P < 0.001$), without affecting feed intake ($P = 0.318$). Dietary xylanase improved dry matter retention ($P < 0.001$), N retention ($P = 0.003$), NDF retention ($P = 0.034$) and N corrected apparent metabolizable energy. No interactions ($P > 0.05$) between rye cultivar and XYL were observed. Therefore, supplementing rye containing diets with XYL may be a strategy to mitigate the reduction in available energy due to higher dietary fibre content, and to increase nutrient availability in broiler diets.
Keywords: Hybrid Rye; Metabolisable Energy; Xylanase; Broilers; Nutrition

5148P Effect of dietary incorporation of rumen content and an enzymatic complex (Natuzyme 1000) on the zootechnical performances of broilers chicken exposed to high temperature

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The present study was conducted with the objective of evaluating the effect of dietary incorporation of bovine rumen content and an enzymatic complex (Natuzyme1000) on the growth performance, carcasses, biochemical and haematological traits of broilers chicken exposed to high temperature. A total of 180 Cobb 500 broiler chicks (14 days of age and weighting 200.75 ± 19.47 g) were divided into six distinct treatment groups in a 3 x 2 factorial arrangement of the incorporation rate of the ruminal content (0; 5.5; 7.5%) and the diet supplementation with the enzymatic complex (supplemented or non-supplemented). Each group consisted of five replicate pens. At the end of experiment, collected data were submitted to a two-way ANOVA tests to determine the main effect of the two factors and their interaction effect. Irrespective of the enzyme supplementation, the feed intake and the body weight gain significantly increased ($P<0.01$) with the dietary level of rumen content. As compared to broilers fed on the non-supplemented diet, those receiving the diet supplemented with Natuzyme1000, showed higher ($P<0.01$) body weight gain. The enzyme supplementation significantly increased ($P<0.01$) the carcass yield and decreased ($P<0.05$) the relative weight of pancreas and the serum content of potassium. It can be concluded that under heat stress conditions, the diet incorporation of the bovine ruminal content improved the body weight gain by increasing feed intake meanwhile, Natuzyme1000 acted by affecting the carcass composition.
Keywords: Bovine ruminal content ; Enzyme complex; Growth performance; High temperature; Broilers chickens

5149P A bacterial xylanase enhances nutrient digestion and intestinal functionality in broiler chickens leading to improved growth performance

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A bacterial xylanase hydrolyzes arabinoxylans, releasing entrapped nutrients and arabinoxylo-oligosaccharides (AXOS), which serve as substrates for short-chain fatty acid (SCFA) –producing bacteria that enhance intestinal health. This study investigates the effects of bacterial xylanase supplementation in energy-reduced diets on nutrient utilization, gut health, and performance in broilers exposed to an intestinal challenge. A total of 900 one-day-old Cobb 500 chicks were randomly assigned to three treatments as follows: T1: Control (CT); T2: metabolizable energy (ME) reduced diet (ER); and T3: ER + 100 g/t of a Bacillus subtilis xylanase (Jefo&Puratos) at 100 g/t (ER+XYL). Each treatment had 10 replicates of 30 birds/pen. The ME reductions for pre-starter, starter, grower, and finisher diets were 80, 82, 87, and 92 kcal/kg, respectively, and were calculated according to their levels of arabynoxylans. All birds were challenged with Eimeria spp. on d 1 and with Clostridium perfringens on d 12-14. Intestinal permeability was measured on d 17 in one bird/pen using fluorescein-isothiocyanate dextran (FITC-d) as marker. Jejunal MUC-2 gene expression was measured on d 21 from two birds/pen. Cecal SCFA, ileal digestible energy (IDE), dry matter (DM) and crude protein (CP) were measured from 4 birds/pen on d 21 and 42. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated weekly. When data were parametric, they were submitted to ANOVA and Fisher's test; when non-parametric, they were analyzed using Kruskal-Wallis and Dunn's test ($P<0.05$, significance; $0.05\leq P<0.10$, tendency). The FITC-d level in the blood was the lowest ($P<0.05$) for ER+XYL, which indicates enhanced intestinal integrity. The ER+XYL group tended to have the highest ($P<0.10$) MUC-2 gene expression, which indicates enhanced mucosa protection. The ER+XYL group presented higher ($P<0.05$) IDE than ER group on d 21 and 42, not differing from CT. At d 21 and 42, ER+XYL group had the highest ($P<0.05$) DM digestibility. The ER+XYL tended to have higher ($P<0.10$) CP digestibility compared to ER, not differing from CT. The CT and ER+XYL had higher ($P<0.05$) concentrations of acetic, propionic and butyric acids compared to

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ER at d 21. The CT group tended to have higher (P<0.10) propionic acid than ER at d 42, being similar to ER+XYL. For the overall period, ER+XYL had higher (P<0.05) BWG and lower (P<0.05) FCR compared to ER, not differing from CT. In summary, the bacterial xylanase improved nutrient digestibility and intestinal health of broilers fed energy reduced diets and subjected to an intestinal challenge, leading to improved growth performance.
Keywords: B. subtilis xylanase; Gut health; Zootechnical performance; Intestinal integrity; Energy utilization

5150P Multicomponent protease as a nutritional additive to improve feed efficiency and egg production in laying hens reared under challenging climate conditions

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The productivity of laying hens is greatly dependent on their protein and amino acids utilization. However, protein metabolism generates a significant amount of metabolic heat which can reduce laying hens' performance especially when reared under high temperature and humidity conditions. A multicomponent protease efficiently hydrolyzes a wide range of protein raw materials and could help animals cope with challenging climates. This study evaluates the effects of a multicomponent protease on the productive performance of laying hens reared in a high temperature and humidity region. A total of 240 Bovans White laying hens, 22-week-old, were randomly assigned to three treatments as follows: T1: Control (CT); T2: Low-density diet (LD: 6% reduction in crude protein and main digestible amino acids); and T3: LD+Protease (Jefo Nutrition). Each treatment had 20 replicates (4 hens/cage). Animals were fed ad libitum with diets in mash form. The study was conducted in Yucatán, Mexico and the temperature and humidity ranged from 12.6 – 39.7°C and 35.4 – 98.4%, respectively. Feed intake, egg production, egg weight, egg mass and feed conversion (FCR) per egg mass and per dozen eggs were evaluated per week and analyzed per feeding phase and overall period. Data were submitted to ANOVA and means were compared by Fisher's test (P<0.05, significance; 0.05≤P<0.10, tendency). From 22 to 28w, laying hens fed the LD+Protease diet showed a lower feed intake (P < 0.05) compared with the LD group (92.2 vs. 95.2 g/d), but similar to CT. Egg production tended to be higher (P < 0.07) in LD+Protease than in LD (97.6% vs. 96.0%), not differing from CT. The FCR per egg mass was better (P < 0.05) for LD+Protease and CT compared with LD (1.69 and 1.71 vs. 1.75, respectively). The multicomponent protease improved (P < 0.05) FCR per dozen eggs in the low-density diets (1.13 vs. 1.19), not differing from CT. From 29 to 50w, FCR per dozen eggs tended to be better (P < 0.07) for LD+Protease compared with CT (1.20 vs. 1.21), with no difference from LD. For the overall period (22–50 w), egg production tended to be higher (P < 0.07) in LD+Protease than in LD (97.6% vs. 96.4%), with no difference from the CT. Both LD+Protease and CT groups exhibited better (P < 0.05) FCR per egg mass compared with LD (1.70 and 1.70 vs. 1.73, respectively), while FCR per dozen eggs tended to be better (P < 0.06) in LD+Protease compared with CT and LD (1.18 vs. 1.20 and 1.20, respectively). Egg mass and egg weight did not differ among treatments. In conclusion, the multicomponent protease enhanced the performance of hens fed low-density diets in high temperature and humidity conditions, yielding results comparable to or better than those fed a standard diet.
Keywords: Heat stress; Laying rate; Metabolic heat; Productive performance; Protein utilization

5151P Evaluating the interaction between diets formulated with varying phytate content and the evaluation of phytase efficacy on nutrient digestibility in broiler chickens

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This study aimed to evaluate the interaction between dietary phytate (PP) concentration and phytase supplementation on nutrient digestibility in broiler chickens from 15 to 21 days post-hatch. A total of 800 one-day-old male Ross 308 chickens were randomly assigned to eight treatments, with 10 replicates of 10 birds each. The treatments were arranged in a factorial design: two PP levels (0.19% and 0.29%, formulated with animal-based meals or rice bran, respectively) and four phytase levels (0, 1000, 1500, and 2000 FTU/kg). Basal diets on each phytate group (zero level of phytase) were nutritionally reduced in calcium (0.36%) and available phosphorus (0.22%). Birds were raised in metabolic cages and fed ad libitum. From 1 to 14 days, birds received a basal diet meeting nutritional requirements. From 15 to 21 days, they were fed with experimental diets containing 0.5% of TiO2 as an indigestible marker. At 21d, birds were slaughtered, and the ileal digesta was collected by flushing. Samples were analyzed for Ca, P, crude protein, gross energy, and Ti concentration to calculate the apparent ileal digestibility (AID). Data were analyzed using a two-way ANOVA in a factorial arrangement, with polynomial contrasts to assess linear (L) and quadratic (Q) responses. Additionally, a non-linear function (Y = a–b × exp (-c×X)) was used to compare enzyme efficacy. All analyses were performed using SAS On Demand, and significance was considered at the 95% confidence interval. Phytase supplementation improved the AID of Ca and P, with a significant increase in Ca AID (Q, p<0.05). Phytase presented an L and Q significant effect (P<0.05) above P AID, for both PP sources. Protein AID was higher for high-phytate diets and showed a significant response to phytase (P<0.05). No significant interactions between phytate and phytase were found for energy digestibility. Non-linear modeling evidenced a different performance for PP diets: Y (0.19% PP) =75.1-22.98×Exp(-0.0018×FTU); Y (0.29% PP) =68.1-14.54×Exp(-0.0024×FTU). The low-PP diet reached a higher digestibility plateau (75.1%) than the high-PP diet (68.1%). Consequently, to achieve a target of 65% AID of phosphorus, the model predicted a requirement of 585 FTU/kg in the low-PP diet compared to 1,150 FTU/kg in the high-PP diet, yielding an equivalence ratio of 2:1. In conclusion, the supplementation of phytase enhances the digestibility of Ca and P, which was significantly influenced by dietary phytate concentration. Independent of phytate dietary content, P digestibility follows an exponential function of phytase levels, with a high-phytate diet resulting in a higher maximum achievable P digestibility than a low-phytate diet.
Keywords: Non-phytic phosphorus; Substrate; Minearls

5152P "Effect of reducing digestible phosphorus and adding phytase to the diet on the productive response in the finishing phase of broiler chickens"

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Hypothesis – Objectives: Phosphorus is a key nutrient in the productive response. During the finishing stage, it is necessary to maximize the productive response, making it essential to define the appropriate level of digestible Phosphorus (digP) at this stage. Our hypothesis was that reducing digP by adding phytase to the diet could maintain the productive response. The objective of this study was to evaluate the effect of reducing digP and adding phytase to the diet on the productive response of broiler chickens during the

finishing phase.
Experimental design: Two study groups were established: a diet with 0.20% digP without phytase (T-1) and a diet with 0.20% digP with phytase (500 FTU/Kg) (T-2), with 4 replicates for each. The experimental treatments were randomly assigned. One experimental unit consisted of 9 chickens.
Materials and methods: This study was conducted in the Nutrition Research and Development Laboratory – FMVZ – UNICA. From a batch of 250 chickens, 72 male chickens of the Cobb 500 line, 28 days of age, were homogeneously selected. The study lasted from 28 to 42 days of age. The variables evaluated were live weight, feed intake, feed conversion ratio, calorie conversion ratio, and relative tibial ash content.
Statistical analysis: The Student's t-test for independent samples was used. Differences were considered statistically significant when $P<0.05$.
Results: The final average live weight was significantly improved ($P=0.0196$) with the inclusion of phytase, reaching 3589.78 g/chicken compared to 3432.6 g/chicken for the group without phytase. Feed intake was not affected ($P=0.6495$). Feed conversion ratio was significantly improved ($P=0.0118$) with the inclusion of phytase, reaching 1.6656 g/g compared to 1.8035 g/g for the group without phytase. Calorific conversion was better ($P=0.0118$) for the group with phytase (5.2060 Mcal/kg) than for the group without phytase (5.6381 Mcal/kg), and the relative weight of tibia ash was similar in both groups (43.74 and 43.79 g/100g). According to the Cobb 500 genetic line guide, growth performance is 3503 g of weight at 42 days of age and the relative tibia weight is around 43 g/100 g, similar to those obtained in this study with the phytase-supplemented diet.
Conclusions: It is concluded that a level of 0.20% digP with inclusion of phytase in the diet at 500 FTU/Kg improves the productive response, without affecting the tibia ash content in the finishing phase of broiler chickens.
Keywords: Digestible Phosphorus; phytase; productive response; broiler chickens
Keywords: digestible Phosphorus; phytase ; productive response; broiler chickens

5153P Effect of Fiber Degrading Enzyme on the Utilization of Dietary Fiber in Japanese Quails (Coturnix japonica)
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Abstract:
The experiment was carried out to investigate the effect of a fiber-degrading enzyme on dietary fiber utilisation in Japanese quails. Three hundred day-old quail chicks were randomly assigned to one of four treatment groups (A, B, C, and D), each with three replicates and each with 25 quail birds. Four iso-nitrogenous and iso-caloric diets (CP: 21%; ME: 2850 kcal/kg) were developed. Diet "A" contained no exogenous enzyme, whereas diets (B, C, and D) contained 200, 300, and 400 g/ton of feed, respectively, of Rovabio® Advances T-Flex 25 granulated exogenous enzyme. Chicks were fed ad libitum, and data on growth performance were collected. After the trial was completed, three birds from each replicate were slaughtered at random to determine carcass parameters and meat quality. Body weight increased ($P < 0.05$) in birds given Rovabio® enzyme, but feed intake and FCR were not significantly different ($P > 0.05$) across all treatments. The enzyme supplementation did not affect carcass characteristics or water-holding capacity. Meat pH was lower ($P < 0.05$) in birds fed Rovabio® enzyme at 400 g/ton of feed. When compared to the control diet, enzyme supplementation significantly improved fiber digestibility ($P < 0.05$). Protein and fat digestibility, on the other hand, remained unaffected across all treatments ($P > 0.05$). The results show that adding Rovabio® enzyme at a rate of 200 g/ton of feed improves body weight, fiber digestibility, and economic efficiency.
Keywords: Enzyme; Fiber; Digestibility; Non-starch polysaccharides; Meat quality
Keywords: Enzyme; Fiber; Digestability ; Non starch Pollysaccharide (NSP); Meat quality

5154P Strategic use of β-mannanase leads to a significant reduction in dirty egg incidence in commercial laying hens
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β-mannans, indigestible fibers in common poultry feed ingredients, are known to trigger a feed-induced immune response (FIIR). This response leads to intestinal inflammation and poor gut health, often resulting in an increased percentage of dirty eggs. Dirty eggs do not meet the high-quality standards for retail markets, causing significant economic losses for producers due to increased labor costs and price downgrades for soiled eggs. This trial aimed to evaluate if supplementing feed with Hemicell® XT, a β-mannanase enzyme, could mitigate the negative effects of dietary β-mannans and thereby reduce the percentage of dirty eggs from commercial laying hens.

A 14-week field trial was conducted on a commercial multi-age farm with 100,000 laying hens receiving the same basal diet. Production data from weeks 16-18 of the year served as the baseline (pre-treatment) period. The subsequent 11 weeks (weeks 19-29 of the year) served as the treatment period, where the hens' feed was supplemented "on top" with Hemicell® XT at a dose of 130 g/ton. Daily records of total eggs and dirty eggs, classified as 'Grade 1' (small, removable stains) or 'Grade 2' (large stains or fecal matter), were collected. An Analysis of Variance (ANOVA) was used to compare the mean percentage of dirty eggs between periods.

Supplementation with Hemicell® XT led to a statistically significant reduction in total dirty eggs, with the weekly average decreasing by 32% during the treatment period compared to baseline. Furthermore, a comparison of the final four weeks of the trial to the pre-treatment period showed a 42% reduction in total dirty eggs. The incidence of 'Grade 1' and 'Grade 2' eggs was reduced by 47% and 23%, respectively. A visible improvement in fecal quality was also noted. Adding Hemicell® XT to the feed is an effective strategy to significantly reduce the incidence of dirty eggs in commercial laying hens. This improvement is linked to enhanced intestinal integrity, as evidenced by better fecal quality. The reduction in dirty eggs provides clear food safety and economic benefits by lowering labor costs, reducing the number of downgraded eggs, and increasing producer profitability.
Keywords: β-mannanase; Hemicell; β-mannans; Laying hens; Dirty eggs

5155P Efficacy of HiPhorius and MultiGrain in Nutrient-Reduced Broiler Diets: Impacts on Growth, Bone Mineralization, and Litter Quality
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A floor pen trial was conducted to validate the efficacy of phytase (Ronozyme HiPhorius) and carbohydrase (Ronozyme MultiGrain) in broilers. A total of 320 male Ross 308 broilers were allocated to four dietary treatments in a completely randomized design,

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each with eight replicate pens containing ten birds. The treatments included: a positive control (PC) diet formulated to meet breed nutrient requirements; a negative control (NC) diet with reductions of 105 kcal metabolizable energy (ME), 0.8% crude protein (CP), 0.01-0.07% amino acids, 0.17% total calcium (Ca), and 0.21% non-phytate phosphorus (nPP); and the NC diet supplemented either with 1500 FYT/kg HiPhorius or with 1500 FYT/kg HiPhorius plus 50 mg/kg MultiGrain. Growth performance was monitored throughout the 37-day trial, and tibia and litter samples were collected at the end of the trial. One-way ANOVA was used to analyze the data.
The results showed that the NC diet significantly reduced body weight gain, final body weight, and feed conversion efficiency compared to the PC diet ($P < 0.05$). Supplementation with HiPhorius numerically improved these parameters, although feed conversion ratio (FCR) remained significantly higher than in the PC group ($P < 0.05$). The combination of HiPhorius and MultiGrain further enhanced growth performance, approaching levels observed in the PC group. Birds fed the NC diet exhibited significantly lower tibia ash, Ca, and P content ($P < 0.05$). These reductions were effectively corrected by either HiPhorius alone or in combination with MultiGrain, restoring bone mineralization to levels comparable with the PC group. Additionally, diets with reduced CP, AAs, Ca, and P led to significant decreases in litter crude protein, phosphorus, and ammonium concentrations ($P < 0.05$).
In conclusion, phytase (HiPhorius) was effective in enhancing Ca and P availability and improving bone mineralization. However, its contribution to energy release appeared limited in the current study. The inclusion of carbohydrase (MultiGrain) provided additional energy, further supporting growth performance under nutrient-reduced conditions.
Keywords: bone mineralization; broilers; carbohydrase; litter; phytase

5156P Determination of true metabolizable energy (TMEn) in roosters and apparent metabolizable energy (AMEn) in broiler chickens fed diets with varying levels of soybean meal and alpha-galactosidase based enzymes

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The objective was to determine metabolizable energy release in corn-soybean meal (SBM)-based diets for poultry by a commercially produced alpha-galactosidase based multicarbohydrase (Kerry, Ireland). Thus, ad libitum-fed broiler chickens were used to evaluate nitrogen-corrected apparent metabolizable energy (AMEn) in Experiment 1, and a precision-fed rooster assay was used to evaluate nitrogen-corrected true metabolizable energy (TMEn) in Experiment 2. In Experiment 1, broiler chickens were raised in alternative design battery cages and fed a nutritionally complete corn-soybean meal-based diet ad-libitum until d13 when feed was removed overnight. On d14, birds were allocated to 1 of 6 mash corn-SBM-based dietary treatments with 5 birds per pen and 12 replicate pens per treatment in a 2×3 factorial arrangement of treatments, with main effects being SBM level (22 and 35%) and alpha-galactosidase dosage (0, 1, and 2x) within the multicarbohydrase. Experimental diets were offered ad-libitum until d21, excreta were collected from d19 to 21 to calculate AMEn, and performance was measured from d14 to 21. Titanium dioxide was included in all diets as an indigestible marker. In Experiment 2, Single Comb White Leghorn roosters were housed individually and fasted for 26 hours before being precision-fed (crop intubated) 1 of the 6 dietary treatments described previously. Excreta were collected quantitatively for the following 48 h to calculate TMEn. Data were analysed using a 2-way ANOVA with SBM level and enzyme dosage as main effects and their interactions. The significance value for all analyses was $P < 0.05$. In Experiment 1, higher levels of SBM resulted in increased BW gain, feed intake, and gain:feed ($P < 0.05$), while enzyme supplementation improved gain:feed in the high SBM diets ($P < 0.05$). There was an interaction for AMEn ($P < 0.05$), where the 2x enzyme dose increased AMEn with both SBM levels, however, the 1x dose only increased AMEn in the 22% SBM diet. In Experiment 2, the TMEn was greater for the 35% SBM diet vs the 22% SBM diet ($P < 0.05$). No effects or interactions were observed for enzyme dose ($P > 0.05$). The lack of effect of enzyme supplementation in precision-fed roosters in Experiment 2 compared with broiler chickens in Experiment 1 could be due to the transient nature of the precision-fed rooster bioassay, which may limit the effects of exogenous enzymes. The improved gain:feed and AMEn in broiler chickens from alpha-galactosidase supplementation in Experiment 1 was likely due to amelioration of the anti-nutritional properties of oligosaccharides in SBM and subsequent energy release from their degradation, demonstrating that this enzyme can be used to increase energy in SBM for poultry.
Keywords: broiler; rooster; alpha-galactosidase; soybean meal; enzyme

5157P Different enzymatic associations improve nutrient digestibility and bone quality in broilers fed nutritionally reduced diets

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The objective of this study was to evaluate the effect of combining different enzymes (xylanase or xylanase + protease) associated with phytase in broiler diets nutritionally reduced on growth performance, nutrient digestibility, and bone quality. A total of 175 male Ross AP95 broilers with 21 days of age (896.77 ± 19.99 g initial body weight), were assigned to 5 treatments, with 7 replicates and 5 birds per experimental unit. The treatments consisted of a positive control diet meeting all nutritional requirements from 21 to 42 days (PC); a negative control diet 1 (NC1; –90 kcal kg-1, –0.15% Ca, –0.15% P, and reduced amino acids); NC1 with the inclusion of 500g/ton of an enzyme blend composed of phytase and xylanase (NC1 + Phy/Xy); a negative control diet 2 (NC2; –105 kcal kg-1, –0.15% Ca, –0.15% P, and reduced amino acids); and NC2 with the inclusion of 500 g/ton of an enzyme blend composed of phytase, xylanase, and protease (NC2 + Phy/Xy/Pro). The enzymes were a novel bacterial 6-phytase (500 FTU/g), a fungal xylanase (1,000 U/g), and a combination of acidic (fungal) and alkaline (bacterial) proteases (2,500 U/g), provided by Tectron, Brazil. The experimental diets were mash-based, formulated with corn and soybean meal according to the nutritional requirements of the strain for the grower (22–35 d) and finisher (36–42 d) phases. Performance parameters (feed intake, weight gain, and feed conversion ratio) were monitored from 22 to 42 days, and digestibility coefficients of gross energy, crude protein, dry matter, ash, and ether extract; as well as metabolizable energy (ME) were determined at 28 days (excreta collection). Bone quality (dry matter and ash content, breaking strength, and Seedor Index) was assessed at 42 days. Data were subjected to tests for homogeneity and normality, followed by ANOVA, and when significant ($P<0.05$), means were compared using Tukey's test at a 5% probability.
The inclusion of exogenous enzyme blends did not affect growth performance or ME values ($P>0.05$). However, both enzyme combinations increased crude protein digestibility coefficients to levels comparable to the positive control ($P<0.001$); the NC2

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+ Phy/Xy/Pro treatment also improved ash digestibility relative to the other treatments (P<0.001). Diets NC1 and NC2, without enzymes, reduced bone strength (P<0.0001) and the Seedor Index (P<0.05), whereas the addition of phytase in both blends improved these parameters, suggesting enhanced release of Ca and P. In conclusion, the results suggest that phytase, likely in synergy with xylanase, was the primary contributor to the observed responses, while the additional inclusion of protease did not provide a distinct advantage under the conditions of this study.
Keywords: Bone strength; Digestibility; Phytase; Protease; Xylanase

5158P The effect of Echinacea purpurea extracts on performance, and interleukin-2 gene expression in broilers
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The effects of various concentrations of Echinacea purpurea extract were evaluated on performance, antioxidant activity, intestinal morphology, immune system, and interleukin-2 gene expression in broiler chickens. To conduct the experiment, 240 one-day-old male chickens of Ross 308 strain were used in a completely randomized design with 4 treatments, and 5 replicates with 12 chickens in each replicate. The experimental treatments included a control diet void of Echinacea purpurea, and diets supplemented with either 10 mg/kg, 20 mg/kg, or 30 mg/kg Echinacea purpurea extract. Addition of Echinacea purpurea extract at 20 and 30 mg/kg in the diet significantly increased the live weight and weight gain of the entire period and decreased the conversion ratio of the entire period (P<0.01). Antioxidant activity was significantly improved only in the treatment group supplemented with 30 mg/kg compared with the control (P<0.05). The villus height and the ratio of villus height to crypt depth in the treatments with 20 and 30 mg/kg extract in feeds were significantly increased compared with the control (P<0.01). Consumption of 20 and 30 mg/kg Echinacea purpurea extract enhanced total antibody production against SRBC and Newcastle vaccine (NDV) in the entire period (P<0.01). Chickens fed diets supplemented t with 30 mg/kg extract had a enhanced relative IL-2 gene expression compared with other treatments (P<0.05). In general, the results of this experiment showed that the consumption of 30 mg/kg extract in the feed of broiler chickens had a positive effect on growth performance, antioxidant activity, and immune system compared with other negative control diet.
Keywords: Echinacea purpurea extract; broiler; performance; Interleukin-2

5159P Microalgae (Spirulina Platensis) improves breast yield in heat-stressed broilers through modulation of proteostasis network
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Heat Stress (HS) has emerged as a critical factor that significantly impacts poultry industry, as it impairs broiler production performance, reproductive efficiency, immune function and overall health. Dietary supplementation with functional nutrients represents a promising strategy to alleviate HS. Spirulina Platensis is a microalgae with rich nutritional composition and variety of bioactive compounds, including proteins, fatty acids, vitamins and antioxidants. In this study, we examined the effect of algae supplementation on growth performance of broilers under HS condition. Day-old male Cobb 500 chicks were randomly allocated according to body weight (BW)-matched groups to 12 controlled-environment chambers (2 pens/chamber, 25 birds/pen), divided into 6 treatments: Thermoneutral-basal diet (C-TN), HS-basal diet(C-HS), TN-basal diet-pair fed(C-PF), TN-0.5% algae-supplemented diet(A-TN), HS-0.5% algae-supplemented diet(A-HS), TN-0.5% algae-supplemented diet-pair fed (A-PF). On day 29, birds were exposed to two environmental conditions: thermoneutral (25°C) or chronic cyclic HS (36°C for 8h/day) until the end of the trial. Daily records were maintained for feed and water intake and BW weekly. On day 42, birds were processed, and carcass parts were weighed. Breast muscles were collected for further analysis (8/diet/environment). Data was analyzed by Two-Way ANOVA and Tukey's multiple range test using Graph Pad Prism version 8.0. Data are shown as mean ± SEM and $p \leq 0.05$ was considered statistically significant. There were no significant effects of diet on BW prior to HS, however, after 2 weeks of heat exposure, both HS and PF birds showed a significantly lower BW than their TN counterparts ($p < 0.001$). There was no effect of algae supplementation on BW. Cumulative feed intake was reduced by HS and PF group ($p < 0.05$) but not affected by algae supplementation. Cumulative water intake increased with HS ($p < 0.05$) but not affected by algae supplementation. The breast yield was significantly lower in HS group compared to TN and PF groups (21.5± 0.02 vs. 22.3 ± 0.02 vs. 22.4 ± 0.02, $p < 0.01$) but was significantly higher in algae compared to control diet (22.3± 2 vs. 21.8 ± 2, $p < 0.05$). To further delineate the mode of action of algae, proteostasis network was determined by qPCR and immunoblot. There was an upregulation of mTOR cascade and down regulation of ubiquitin-proteasome pathway, indicating that algae enhance protein synthesis and decrease protein degradation in heat-stressed broilers, which results in better breast yield.
Keywords: Heat Stress; Spirulina Platensis; Brest Yield; Protein Synthesis; Protein Degradation

5160P Phytoremediation Potential of Vernonia guineensis Root Powder as a Feed Supplement Against Cypermethrin-Induced Damage on Growth, Reproduction, and Biochemical Parameters in Japanese Quails
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This study aimed to evaluate the phytoremediation potential of Vernonia guineensis root powder (VGRP) in alleviating the toxic effects of cypermethrin in Japanese quails (Coturnix japonica), with a focus on growth performance, reproductive parameters, and biochemical markers. A total of 180 quails (90 males and 90 females) of 28 days old, were randomly assigned to five dietary treatments in a completely randomized design with three replicates per treatment. The five groups received: (1) a basal diet (Control), (2) basal diet containing cypermethrin at 655 mg/kg (Cypermethrin only), and (3–5) the same cypermethrin-contaminated diet supplemented with VGRP at 10, 20, or 30 g/kg (Cypermethrin + VGRP10, VGRP20, and VGRP30, respectively). The trial lasted 8 weeks under standard rearing conditions. Growth parameters, carcass traits, reproductive performance, serum biochemical markers (ALT, AST, urea, creatinine), organ weights (liver, kidney), and histopathological profiles were evaluated. Data were subjected to one-way ANOVA, and treatment means were compared using Duncan's multiple range test (p<0.05). Quails exposed to cypermethrin showed reduced feed intake, poor weight gain, decreased carcass yield, elevated liver and kidney function markers, and structural damage to hepatic and renal tissues. VGRP supplementation improved feed intake, growth performance, and carcass traits in cy-

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permethrin-exposed birds, with the highest level (30 g/kg) showing the most notable improvements. Although the toxic effects were not entirely reversed, VGRP reduced the severity of biochemical and histological damage. Reproductive parameters also improved with VGRP, including egg production, egg mass, sperm motility and count, serum testosterone levels, fertility, and hatchability, compared to the Cypermethrin only group. These results suggest that Vernonia guineensis root powder can partially counteract the toxic effects of cypermethrin and enhance performance and reproductive outcomes in Japanese quails.
Keywords: Cypermethrin; Vernonia guineensis; Japanese quail; Toxicity; Phytoremediation

5161P Potential of Red Grape Pomace and Bacillus subtilis as Natural Feed Additives for Improving Broiler Breeder Performance: A Review

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The global poultry industry faces major challenges related to rising feed costs, increasing restrictions on antibiotic growth promoters, and growing demand for sustainable and natural alternatives that enhance bird health and productivity. Red grape pomace, a by-product of winemaking, has attracted considerable attention as a potential feed ingredient because of its high concentrations of polyphenols, dietary fiber, and other bioactive compounds with antioxidant and antimicrobial properties. Similarly, Bacillus subtilis, a well-documented probiotic, is widely recognized for its ability to improve gut microbiota balance, stimulate enzymatic activity, enhance nutrient utilization, and strengthen immune responses in poultry.

This review evaluates the potential benefits of integrating grape pomace and B. subtilis as complementary feed additives for broiler breeder chickens. Recent studies published between 2010 and 2025 were screened for evidence regarding the nutritional value of grape pomace, its capacity to reduce oxidative stress and lipid peroxidation, and the probiotic effects of B. subtilis on gut health and performance. Findings indicate that grape pomace supplementation contributes to antioxidant protection and improved metabolic status, while B. subtilis enhances digestive efficiency and immune modulation. When combined, these additives may act synergistically to promote breeder performance, improve egg quality, and provide a sustainable alternative to synthetic growth promoters.

In conclusion, the integration of grape pomace and B. subtilis offers a promising, eco-friendly feeding strategy for the poultry industry. Nevertheless, controlled experimental trials in broiler breeders remain essential to confirm synergistic effects, determine optimal inclusion levels, and validate their long-term impact on reproductive performance.
Keywords: broiler breeder; grape pomace; Bacillus subtilis; probiotics; feed additives

5162P Effect of Spirulina platensis supplementation on the yolk fatty acid profile of free-range laying hens

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The inclusion of microalgae such as Spirulina platensis in animal feed has gained increasing attention due to its potential to enhance feed palatability and nutritional value while reducing dependence on the fishing industry as a primary source of omega-3 fatty acids for human consumption. Furthermore, Spirulina is rich in crude protein and vitamins, also providing significant amounts of calcium and phosphorus. Owing to these characteristics, several studies have identified Spirulina as a promising alternative to soybean meal and other conventional ingredients used in poultry diets. Therefore, the objective of the present study was to evaluate the effects of Spirulina supplementation at different inclusion levels in the diet of free-range laying hens on the fatty acid profile of egg yolk. Three experimental groups, each consisting of 25 Lohmann Brown laying hens aged 38 weeks, were used in a 10-week trial. The first group was fed a standard commercial layer diet (Control), while the other two groups were fed diets containing 1% (Spi1) and 3% (Spi3) Spirulina platensis, respectively, replacing equivalent proportions of other ingredients in the basal diet. All diets were formulated to be isoproteic. At the end of the 10-week experimental period, ten eggs were randomly collected from each pen. The yolks were separated, and their fatty acid profiles were determined by gas chromatography. Given the limited number of experimental units, statistical analyses were conducted to provide an exploratory comparison among treatments. The effect of diet was assessed by analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. The results showed no significant differences in the proportion of saturated fatty acids (SFA) (Control 33.7±1.2%; Spi1 32.3±2.3%; Spi3 33.2±0.8%), with palmitic acid (C16:0) and stearic acid (C18:0) being the most abundant. However, the percentage of monounsaturated fatty acids (MUFA) was significantly higher in the Control group (51.2±4.0%) compared with Spi1 (45.2±2.6%), with oleic acid (C18:1 n-9) as the predominant component. In contrast, the proportion of polyunsaturated fatty acids (PUFA) was higher in Spi1 (21.5±4.6%) and Spi3 (16.9±1.7%) than in the Control (14.2±4.4%), mainly due to increased levels of linoleic acid (C18:2 n-6). Finally, no significant differences were observed in the omega-3 to omega-6 fatty acid ratio among treatments. These results demonstrate that the inclusion of Spirulina platensis in the diet of laying hens can modify the fatty acid profile of egg yolk. Nevertheless, further long-term studies are needed to evaluate not only egg quality traits but also consumer sensory acceptance.
Keywords: fatty acid profile; Spirulina platensis; egg quality; free-range system

5163P Supplementation of short-chain fatty acids blend in water reduces water Escherichia coli load and enhances growth and economic outcomes in broilers

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Organic acids are increasingly being explored as alternatives to antibiotic growth promoters in poultry production. Among these, short-chain fatty acids (SCFA) have demonstrated the potential to suppress pathogenic bacteria, improve gut health, and enhance growth performance in broilers. This study evaluated the effects of a synergistic SCFA blend (Selko pH E, Trouw Nutrition, The Netherlands) on water microbial load, growth performance, and economic outcomes in broiler chickens. A total of 646 newly hatched, mixed-sex Ross 308 chicks (48.26 ± 0.40 g) were randomly assigned to two treatments, each with 17 replicates of 19 birds per pen: (1) a basal diet (negative control, NC), or (2) a basal diet supplemented with Selko pH E at 1 L/1000 L of water (SpHE). Birds were fed a three-phase, antibiotic-free corn–soybean diet consisting of starter (day 1–10), grower (day 11–21), and finisher (day 22–45) phases, with ad libitum access to feed and water.

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Growth performance parameters [body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and average daily water intake] were recorded from day 1 to 35. Water samples were collected on days 0, 1, 7, 14, 28, and 35 to quantify Escherichia coli loads. Economic efficiency was assessed at the end of the experiment using key financial indicators derived from feed intake, growth performance, and market prices. Data were analyzed using the MIXED procedure in SAS, and treatment means were compared using Tukey's test ($P < 0.05$). Supplementation with SpHE significantly ($P < 0.05$) improved final BW (+109 g/bird) and ADG (+3 g/bird/day), with a tendency ($P = 0.10$) to improve ADFI (96.7 vs 93.3 g/day), FCR (1.780 vs 1.831), and water intake (159.26 vs. 158.60 mL/day). SpHE supplementation also reduced E. coli counts on day 1 ($P = 0.06$), day 7 ($P = 0.10$), day 14 ($P = 0.07$), day 28 ($P = 0.005$), and day 35 ($P = 0.09$), indicating effective suppression of this pathogen over time. Economically, SpHE supplementation resulted in a slightly higher feed cost per bird ($P = 0.07$) without significantly affecting cost per kilogram of BW gain ($P = 0.35$). SpHE significantly increased revenue per bird ($P = 0.04$) and improved both the income-to-feed cost ratio ($P = 0.04$) and benefit-cost ratio ($P = 0.42$). Overall, these findings indicate that supplementation with SpHE via water enhances growth performance and reduce E. coli colonization in broilers. Despite a modest increase in feed cost, the improved revenue per bird and economic efficiency support SpHE as a viable, non-antibiotic alternative for promoting broiler productivity and health.

Keywords: Gut health, ; organic acids; water additives, ; economic efficiency; Escherichia coli

5164P Phytogenic-based additives enhance intestinal health in broilers challenged with Eimeria or inflammatory challenges

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Coccidiosis continues to be one of the most important digestive pathologies affecting broilers by degrading intestinal integrity. Phytogenics have been proposed as anticoccidial alternatives and to strengthen gut health for broiler production. This study aimed to evaluate the effect of phytogenic-based feed or drinking water additives (PBA) on intestinal health and growth performances in broilers exposed to Eimeria or enteric inflammatory challenges. A 35-day trial was conducted using 1,152 Ross 308 males, divided into 12 replicates of 12 birds/cage and subjected to the 8 experimental treatments: non-infected controls (NI); Eimeria challenge (EI); enteric inflammation challenge (IC); EI+conventional anticoccidials control; EI+three PBA with various proportions of star anise, cinnamon, rosemary, garlic and thyme essential oils, Curcuma longa and Yucca schidigera extracts; and IC+Capsicum oleoresins. The EI was induced on day 14 using 160,000 E. acervulina and 40,000 E. tenella sporulated oocysts from field isolates. The IC was provoked by 0.45 g/day dextran sodium sulfate (DSS) oral administrations on days 19 and 20. In addition to performance measurements, the effects of additives on coccidiosis were evaluated by intestinal lesion scores (ILS), oocyst excretion, and Eimeria spp. qPCR on days 20-21. Intestinal permeability was assessed by serum fluorescein isothiocyanate-dextran (FITC-d) levels on day 28. Data were analyzed by a mixed model including treatment and barn section as fixed and random effects and by Kruskal-Wallis tests. ILS were determined on 24 birds/treatment. PBA reduced fecal oocyst excretion (−0.8 log10 oocysts/g, $P<0.05$) and qPCR data showed a trend to reduce E. tenella (−0.7 log10 copies/g, $P=0.10$) 6 days post inoculation. However, they did not improve ILS nor growth performances ($P>0.05$). The Eimeria (+117%, $P<0.05$) and DSS (+125%, $P<0.05$) challenges increased serum FITC-d levels. PBA (−25%, $P<0.05$) and Capsicum oleoresins (−32%, $P<0.05$) reduced FITC-d levels, compared to their challenge controls. Globally, the PBA revealed beneficial results on intestinal permeability during the recovery process following Eimeria and inflammatory challenges, suggesting they could be useful in broilers to promote intestinal health.

Keywords: Coccidiosis; Intestinal inflammation; Intestinal health; Natural additives; Antibiotic-free production

5165P Thermal conditioning and dietary modulation activate the Nrf2–ARE antioxidant pathway and enhance thermotolerance in broilers

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With the increasing prevalence of high ambient temperatures due to climate change, along with the trend toward progressive reduction antibiotic use, the effects of incubation temperature manipulation and dietary strategies on thermotolerance and antioxidant regulatory mechanisms in broilers require further systematic investigation. This study aimed to investigate the effects of incubation temperature manipulation and dietary interventions on feed efficiency, intestinal barrier integrity, and antioxidant responses through the Nrf2–HO-1 pathway in heat-stressed broiler chickens. A total of 240 fertilized eggs were incubated under three temperature profiles: normal (37.8°C), early-elevated (39.0°C from day 7–10), and late-elevated (39.0°C from day 15–17). After hatching, chicks were randomly allocated to three dietary treatments: basal diet (CON) or probiotic-supplemented diet (PRO; Lactobacillus rhamnosus 1×108 CFU/kg). The 3×2 factorial design comprised six treatment groups (n=4 pens/group). From day 28 to 35, chickens were exposed to cyclic heat stress (35°C for 6 h daily). Feed conversion ratio (FCR) was recorded. On day 35, serum and jejunal samples were collected to assess intestinal permeability, antioxidant enzyme activities, lipid peroxidation, and mRNA expression by qPCR. Nuclear Nrf2 activation was measured by TF-ELISA. Data were analyzed using two-way ANOVA with Tukey's post hoc test ($p<0.05$), and correlations were assessed using Pearson's coefficient. A significant incubation × diet interaction was observed for FCR ($p<0.05$); early-elevated incubation combined with probiotic feeding yielded the lowest FCR ($\Delta=-0.05$). FITC-dextran and D-lactate concentrations were markedly reduced in probiotic-fed birds, indicating improved gut barrier integrity. Nuclear Nrf2 activity increased by 32% and upregulated HO-1 and NQO1 mRNA expression, accompanied by elevated SOD, GPx, and CAT activities, while MDA decreased by 25%. Pearson correlation analysis revealed strong positive relationships between Nrf2 activation and antioxidant indices (HO-1, SOD, GPx; $r = 0.60$ to 0.82, $p < 0.01$), as well as negative correlations with oxidative and permeability markers (MDA, FITC-dextran, FCR; $r = -0.45$ to -0.67, $p < 0.05$). Tight junction genes (ZO-1, OCLN) also showed positive associations with Nrf2 and HO-1 expression. Early thermal conditioning, combined with probiotic feeding, enhances antioxidant defense and intestinal integrity through Nrf2–HO–1–mediated regulation. The observed correlations demonstrate a coherent antioxidant–barrier response that links molecular activation to improved feed efficiency and physiological resilience.

Keywords: broiler; incubation temperature; probiotic; heat stress

5166P Protective and performance-enhancing effects of a wood lignan-based feed additive in vitro and in vivo

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Background

High performance demands, variable feed quality, and heat stress trigger oxidative stress and intestinal inflammation, impairing epithelial integrity and performance. In addition, regulatory and societal pressure to reduce or replace antibiotic growth promoters has increased the demand for effective nutritional alternatives.

Objective

We hypothesized that a wood-based lignan product (agromed®ROI Max, Agromed Austria GmbH) protects intestinal health by reducing inflammatory stress and improving the gut barrier function, thereby enhancing broiler performance.

Experimental design

Effects of the lignan product were assessed in vitro in epithelial and immune cell models and in vivo in a 35-day broiler feeding trial.

Materials and methods

Wound closure was analyzed in IPEC-J2 scratch assays. Anti-inflammatory effects were evaluated via nitric oxide (NO) quantification in RAW264.7 macrophages and cytokine expression in Caco-2 monocultures and Caco-2/THP-1 co-cultures. Intestinal epithelial transport of lignans across Caco-2 monolayers was determined and apparent permeability coefficients (Papp) were calculated.

In vivo, the product was compared at 0.1 g/kg with an unsupplemented control in a 35-day feeding trial in broilers. Performance parameters, jejunal cytokine expression, and apparent ileal digestibility (AID) were measured.

Statistical analysis

Data was analyzed by one-way ANOVA with Tukey's post hoc test for multiple-group comparisons or by Student's t-test for two-group comparisons.

Results

In vitro, the lignan product enhanced IPEC-J2 cell migration ($p<0.0001$) and reduced LPS-induced NO production in RAW264.7 cells ($p=0.0009$). In Caco-2 cells, IL-1β expression was decreased ($p<0.0001$) and IL-10 increased ($p<0.0001$), while in Caco-2/THP-1 co-cultures IL-1β tended to decrease ($p=0.0466$) without effects on TNFα, IL-6, or IL-8 ($p>0.05$). Lignans crossed Caco-2 monolayers with molecule-specific Papp values.

In vivo, broilers supplemented with the lignan product showed greater body weight gain (+5.6%), improved feed-to-gain ratio (-6.7%), and higher European Poultry Efficiency Factor (+16%) compared with the control group ($p<0.001$). Lignan supplementation downregulated jejunal IL-8 expression (-81%; $p=0.004$), while TNFα and IL-10 were unaffected ($p>0.05$). Apparent ileal digestibility of crude protein (+2.8%), crude ash (+3.8%) and calcium (+5.1%) was also increased ($p<0.005$).

Conclusions

The lignan product supported epithelial protection and anti-inflammatory responses in vitro and improved performance, markers of intestinal inflammation and nutrient digestibility in vivo, highlighting the potential of this wood-based feed additive.

Keywords: Lignans; broiler performance; intestinal barrier health; apparent ileal digestibility; cytokine expression

5167P Compatibility of a commercial four strain Bacillus-based probiotic with three different ionophores – monensin, salinomycin and narasin.

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In-feed use of ionophores is one of the most common tools to control coccidiosis in poultry production. Ionophores affect the membrane permeability of sporozoites and merozoites to different ions, thus interfere with their metabolism and osmolarity. This effect is not specific to Eimeria but could affect Gram-positive microorganisms as well. The current study aims to assess the compatibility of a four-strain Bacillus spp.-based probiotic containing 4x109 CFU/g total viable spores of B. subtilis NRRL B65574, B. licheniformis ATCC PTA122188, B. amyloliquefaciens NITE BP01844 and B. coagulans ATCC 31284: MicroLife® Prime (MLP) in combination with three different ionophores – salinomycin, narasin and monensin when used at their typical in-feed dose. A total of 320 male day-old Ross308 broilers were allocated to eight treatments with eight replicates each and five birds/replicate in a completely randomized block design. Birds were raised in floor pens provided with ad libitum access to feed supplemented with the probiotic and/or the three different ionophores as follows: T1 - unsupplemented control (CON); T2 – salinomycin at 60 ppm (SAL); T3 – narasin at 90 ppm (NAR); T4 – monensin at 110 ppm (MON); T5 – MLP at 5x105 CFU/g (MLP); T6 – MLP at 5x105 CFU/g and salinomycin at 60 ppm (MLP+SAL); T7 – MLP at 5x105 CFU/g and narasin at 90 ppm (MLP+NAR); and T8– MLP at 5x105 CFU/g and monensin at 110 ppm (MLP+MON). On day 28, fecal samples from each pen were homogenized, diluted with PBS (1:4 w:v), and heat-treated at 75°C for 10 minutes to induce Bacillus sporulation. Ten-fold dilutions were plated on TSA agar and incubated at 37°C for 24 hours to quantify Bacillus spp. spores. Bacillus counts were log-transformed for statistical analysis. Data was analyzed using Standard Least Squares model in JMP software. Means separations were performed using Tukey test. Treatments were considered significantly different at $P < 0.05$. Total viable spores count (Log10/g) were greater ($P < 0.05$) in broilers fed diets supplemented with MicroLife Prime than in other groups (CON 1.21b; SAL 1.96b; NAR 2.70b; MON 2.69b; MLP 4.26a; MLP+SAL 4.39a; MLP+NAR 4.27a; MLP+MON 4.39a); means without common letters in the superscript differ with statistical significance ($P < 0.05$). There was no difference between MLP alone and its combinations with ionophores, indicating that adding ionophores to feed containing MLP did not influence spore counts in excreta. In conclusion the results of the trial indicate that salinomycin, narasin and monensin when used at their registered doses do not exhibit inhibitory effect over the Bacillus spp. provided with MLP, thus indicating the compatibility of MicroLife® Prime with the three ionophores.

Keywords: Bacillus; ionophore; narasin; salinomycin; monensin

5168P Impact of a β-Mannanase Enzyme in Reduced-Energy Broiler Diets on Performance and Economic Returns under Commercial Field Conditions

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Optimizing feed efficiency and nutrient absorption remains important to improve broiler growth performance and welfare. β-Mannans, anti-nutritive polysaccharides, found in common vegetable feed ingredients, can negatively impact broiler performance by triggering a Feed-Induced Immune Response (FIIR), even at low concentrations (as little as 0.05% in vitro). This retrospective cohort study evaluated the efficacy of a β-mannanase enzyme (Hemicell HT™; Elanco AH), administered at the standard recommended dosage of 330 g/tonne, in mitigating FIIR and maintaining broiler performance despite a 50 kcal/kg ME reduction. Two years of historical performance data (2020-2021, without Hemicell HT™ inclusion; 1,777,090 birds) were compared to four rounds of production (2022-2023; 413,390 birds) using feed formulated with a 50 kcal/kg ME reduction and a β-mannanase enzyme inclusion. The basal diets were primarily composed of corn, soybean meal, and wheat. Compared to the Control group, birds in the Enzyme-treated group (supplemented with the exogenous β-mannanase enzyme) showed significant improvements in average body weight (2.61 kg

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vs 2.54 kg; P = 0.0026), feed conversion ratio (1.53 vs. 1.59; P < 0.0001), and feed profit per chick per day (€ 0.0400 vs. € 0.0382; P= 0.0005). No significant differences were observed for feed intake and mortality (P > 0.05). In addition to performance enhancement, a €0.002 increase in gross feed profit per chick per day, the energy reduction resulted in a feed cost saving of € 7 per tonne of feed, considering the cost of the enzyme in this calculation. These findings suggest that a β-mannanase enzyme is a valuable tool for broiler production efficiency and profitability.
Keywords: β-Mannanase; Broilers; Improved Performance; ME reduction; Economic benefit

5169P Supplementing laying hens with probiotic Clostridium butyricum improves zootechnical performance and egg quality

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Objective

Although probiotics are well-established in the poultry industry, their application during lay is limited. This is most noticeable in the European Union: of the 30+ probiotics registered for use in poultry, only about five are registered for use during lay. To evaluate the potential of a novel Clostridium butyricum probiotic, laying hens were supplemented during lay and their production parameters recorded.

Materials and methods

After a pre-experimental phase of six weeks, a completely randomized design was applied to 192 Lohmann Brown laying hens. This allowed for two treatment groups with 16 replicates each (six hens/unit). At 25 weeks of age, hens received either a standard basal control diet or one supplemented with probiotic C. butyricum (1.25 x 108 CFU/kg feed; 250 g Top Gut®/ton, Huvepharma®). Diets were wheat/corn-based and presented in mash form. The trial lasted for 84 days, with feed and water available ad libitum. Zootechnical performance was recorded or calculated. Egg quality was determined 12 weeks after the start of the probiotic supplementation by assessing all eggs laid in a 24-hour period. General health, medication requirement and mortality were also recorded.

Statistics

Using SPSS, dependent variables were analysed by applying a General Linear Model.

Results

There were no health challenges or mortalities, and thus no antimicrobial treatments required. Over the total treatment period, all parameters improved with probiotic supplementation, including significant reductions in feed conversion rate (FCR; 1.843 vs. 1.794; P < 0.05) and the number of broken eggs (0.64 vs. 0.16; P < 0.05). The hypothesis that probiotics aid digestion is supported by the reduction in the number of dirty eggs (0.78 vs. 0.45; P = 0.0619 overall, statistically significant in the last two production phases at P < 0.05). In terms of egg quality, the probiotic group recorded significantly increased shell weight and albumen height (7.5 vs. 7.8 and 7.62 vs. 8.13, respectively; P < 0.05).

Conclusions

Although the focus of probiotic application has been on broilers, there are good reasons to examine the benefits they can bring to layer operations. This does not mean probiotics registered for broilers can always be extrapolated to layers: it remains good practice to evaluate the available research in layers first. For the probiotic at hand, this trial clearly shows the benefits of Clostridium butyricum during lay with improvements in FCR, broken eggs, dirty eggs, shell weight and albumen height.
Keywords: Layers; Huvepharma; Clostridium butyricum; Top Gut; Probiotic

5170P Nutritional and Immunological Impacts of Garlic Leaf Extract Supplementation in Broiler Diets

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The poultry industry has increasingly sought natural feed additives as alternatives to synthetic growth promoters to enhance productivity and animal health in a sustainable manner. Garlic and its bioactive compounds have been widely recognized for their antimicrobial, antioxidant, and immunomodulatory properties, yet limited information is available regarding the efficacy of garlic leaf extract (GLE) in broiler diet. This study evaluated the effects of GLE as a natural dietary additive on growth performance, intestinal morphology, immune response, and gut microbiota composition in broiler chickens. A total of 160 one-day-old broilers were randomly assigned to four dietary treatments supplemented with 0, 125, 250, or 500 mg/kg of GLE for 35 days. Results revealed that dietary inclusion of 125 mg/kg GLE significantly increased (P < 0.05) final body weight and average daily gain compared with the control group. Supplementation with GLE also improved (P < 0.05) villus height in the duodenum and ileum, with the 125 mg/kg group exhibiting the most favorable villus height-to-crypt depth ratio, suggesting enhanced nutrient absorption and intestinal integrity. The GLE 125 treatment group exhibited the highest (P < 0.05) serum IgA concentration, which was significantly greater than that observed in the other experimental groups. In contrast, the concentrations of IgM and IgY did not differ significantly (P > 0.05) among treatments. Analysis of 16S rRNA gene sequences indicated that dietary GLE significantly altered the composition and structure of the intestinal microbiota, characterized by an increased (P < 0.05) relative abundance of Firmicutes, a reduction in Bacteroidetes, and enrichment of beneficial genera such as Lactobacillus. Principal coordinate and LEfSe analyses confirmed these compositional shifts. In conclusion, dietary inclusion of garlic leaf extract, particularly at 125 mg/kg, effectively enhanced growth performance, intestinal morphology, immunity, and gut microbial balance, supporting its potential as a natural feed additive in broiler production.
Keywords: garlic peel extract; broiler chicken; immune response; Gut Health; Gut microbiota

5171P Comparison of a conventional coccidiostat and a botanical feed additive in broilers

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To prevent coccidiosis and its negative impact on animal welfare and performance, ionophore coccidiostats are commonly added to broiler feed in Switzerland. In addition to improving health, their use may also enhance growth. In view of possible coccidiostat resistance, plant-based alternatives could become increasingly

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relevant. The aim of the present study was to investigate the performance and welfare of broilers fed a botanical feed additive (BA) based on thymol, saponin and carvacrol, compared to a negative control (NC, without any additives) and a positive control (PC, with standard coccidiostats).
In total, 5600 Ross 308 broilers were housed in a research barn under Swiss commercial conditions. The birds were allocated to 20 pens (20 m2, 280 birds each), which were randomly assigned to treatments (NC n=6, PC and BA n=7). All birds were fed the same standard broiler diet in 3 phases (starter, grower, finisher) supplemented either with AviPower®2 (BA), Narasin (PC) or nothing (NC). At days 10, 21, 28 and 36 (day of slaughter), body weight, feed efficiency and mortality were recorded. As an indicator for welfare, litter quality and the incidence of foot pad dermatitis and hock burns were assessed on day 28 and 35. Birds were not artificially infected with Coccidia, but the natural coccidial load was assessed on day 22 (per treatment) and day 35 (per pen), using Eimeria oocysts counts (OPG) in fresh droppings. Continuous data was analysed using a linear model with treatment as a fixed factor, while discontinuous data were analysed using either a generalized linear model with binomial family or a multinomial log-linear model. For OPG, the best-fitting model was a linear model with log-transformed data. The Tukey correction was applied for post-hoc multiple comparisons.
Body weight and feed efficiency developed similarly during the starter and grower phases ($p > 0.05$), but differed on day 36 (body weight: $p = 0.019$; feed efficiency: $p = 0.003$). A higher body weight was found in PC compared to NC, though BA did not differ from PC or NC. Feed efficiency was lower in PC compared to NC as well as BA. Mortality did not differ between treatments ($p > 0.05$). Litter encrustation was lower in PC compared to NC and BA on day 28 ($p = 0.013$) and day 35 ($p = 0.019$). However, footpad and hock health were not affected ($p > 0.05$). On day 22, OPG was highest in NC, followed by PC and BA with the lowest levels (descriptive). Pen-level OPG assessment on day 35 showed no treatment effect ($p > 0.05$). In conclusion, performance and litter quality were improved in PC compared to NC under natural coccidial exposure, while BA showed some, though limited, beneficial effects.
Keywords: Broiler; Coccidiostat; Botanicals; Feed Additives; Eimeria

5172P Cost effective approach of uding unconventional phytobiotics for boosting growth and beneficial gut microbiota with low ammonia emission

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Farmers often incorporate various feed additives to enhance animal performance without adequately considering feed cost. Feed prices have risen sharply due to the increased use of costly synbiotics, driven by high demand and the ban on antibiotics for prophylactic use. However, cost effective of uncoenvention phyotobiotics on broilers are severely lacking in Bangladesh. Therefore, this study evaluates the cost effectiveness of different unconventional local phytobiotics and commercial synbiotics supplementation on broiler performance, gut microbiota and mitigating ammonia (NH3). A total of 160 day-old Lohmann Meat broiler chicks were reared for 28 days and divided into five treatments, each treatment consist of 32 broilers: a control group (T0) and four treatment groups (T1–T4) receiving varying phytobiotics such as moringa leaf meal (MLM), kalmegh leaf meal (KLM), papaya leaf meal (PLM) and synbiotics (2g, 2g, 2g, and 2g perliter of water, respectively). These phytobiotics were aseptically collected, washed, dried and powdered before supplying with drinking water. Similar iso-nitrogenous and iso-caloric starter (1 to 14 days) and grower (15 to 28 days) diet were used. Parameters such as feed intake, live weight (LW), feed conversion ratio (FCR), dressingpercentage (DP), environmental conditions (ammonia levels, litter pH), microbial profiles and economic viability assessed.Data were analyzed in One way ANOVA with SPSS statistical method version 26. Results indicated that T3 (2g PLM/L of water) significantlyachieved ($P<0.000$) the highestLW (1999.19 g), lowest FCR (1.28), and highest DP (76.38%),alongside significant reductions ($P<0.007$) in NH3 levels (20.50 ppm) and litter pH($P<0.000$) (8.21) .T3 also improve gut microbiota by increasing Lactobacillus counts ($P<0.000$)while reducing pathogenic Salmonella and E. coli. ($P<0.000$). Economically, T3 yieldedthe highest benefit-cost ratio (1.30) ($P<0.007$), demonstrating improved profitability. The study concludes that phytobiotics, particularly at 2g PLM, optimize growth, health, and environmental sustainability by mitigating NH3 and increasing nutrient utilization in green broiler production.
Keywords: Phytobiotics; Feed additive; Ammonia; Environment; Cost-effectiveness

5173P Susceptibility of Bacillus pumilus Probiotic Strain with common Coccidiostats in Poultry Feed tested in a New in vitro Model

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Probiotics offered to farm animals are gaining exponential interest as a feed additive to enhance gut health and improve zootechnical performance. The efficacy of a direct-fed microbial in poultry nutrition depends on its viability and metabolic activity once delivered to the host. However, the combination of a microbial additive with other additives, such as chemical compounds, could harm the microbial cells' viability. Besides in vitro official methods determining the minimum inhibitory concentration of an active with the probiotic strain, there is also a field interest in having the viability tested under conditions more representative of the practical exposure of both additives. This study investigated the in vitro compatibility of Bacillus pumilus NCIMB 30362 (BP, commercialized as EFFIXIENCE) with six commonly used coccidiostats at their recommended dose: Robenidine, Lasalocid, Narasin, Narasin/Nicarbazin, Diclazuril, and Nicarbazin. Compatibility was assessed through two approaches: (1) stability in dry feed over six months, and (2) viability in a simulated digestive environment at 41°C using a rehydrated feed model. Total viable counts (TVC) and total spore counts (TSC) were monitored on TSA media. Data from this trial were analyzed using non-parametric models (SPSS v31.0), with significance set at $P < 0.05$.
Results indicate that BP maintained high viability under all tested conditions, and the presence of coccidiostats had minimal impact, as no significant differences were observed between BP alone and BP combined with coccidiostats ($P>0.05$). Spore-to-vegetative cell transformation was confirmed at 30°C and 41°C, indicating favorable conditions for probiotic activity in the gut.
These findings demonstrate that the probiotic BP is fully compatible with the tested coccidiostats, ensuring its stability and efficacy in poultry feed formulations. This compatibility supports its use in integrated feed strategies aimed at improving animal health and performance.
Keywords: Probiotic; Coccidiostats; Compatibility; Bacillus pumilus

5174P Gut health benefits of protected organic acids and essential oils in commercial laying hen production

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Gastrointestinal functionality and health are closely linked to the productive performance of laying hens. To support laying persistence, maintaining microbiota homeostasis is essential. This study aimed to evaluate the effects of dietary supplementation with Protected Organic Acids and Essential Oils - P(OA+EO) in laying hens raised under commercial conditions. A total of 208,000 laying hens at 2-weeks-old were assigned to one of two treatments for 20 weeks: T1: Control, commercial diet, and T2: Diet supplemented with P(OA+EO) at 300 g/t (Jefo Nutrition). A completely randomized design with 2 treatments and 12 replicates of 1 hen/replicate, was used. At weeks 6, 12 and 21, 12 hens/treatment were sampled for blood collection and necropsy. Birds were inoculated with FITC-d (fluorescein-isothiocyanate labelled dextran), and blood samples were collected after 1.5 hours later to evaluate intestinal permeability. Lower FITC-d blood levels indicate reduced intestinal permeability. The ISI - I See Inside Scoring System was used to assess organ alterations: overall health (sum of the scores given for macro analysis of the intestine, liver, proventriculus, annex glands, locomotor and respiratory system), macroscopic intestinal health (sum of the scores assigned to the duodenum, jejunum, ileum and cecum) and, histologic intestinal health were evaluated in the ileum. Lower ISI index represents better health status. Data were subjected to one-way ANOVA and T test was used for parametric and Kruskal Wallis for non-parametric data (XLSTAT software). P-values <0.05 were considered significant. Jefo P(OA+EO) significantly reduced (P<0.001) FITC-d levels in the blood of laying hens, indicating improved intestinal integrity and less risk of bacterial translocation from the intestinal lumen to bloodstream. Hens supplemented with Jefo P(OA+EO) showed better (P<0.05) overall health status at weeks 6 and 12, improved (P<0.05) macroscopic intestinal health at weeks 6 and 21 and improved (P<0.05) histologic intestinal health at all evaluated time points. Additionally, hens on P(OA+EO) did not present Eimeria oocysts, whereas the control group did (P<0.001). In conclusion, commercial laying hens supplemented with Protected Organic Acids and Essential Oils had better intestinal and overall health status, which may contribute to more persistent productive performance.
Keywords: Essential oil; Intestinal health; Intestinal integrity; Laying hens; Organic acids

5175P A blend of organic acids and essential oils reduces Salmonella challenge in a chicken intestinal organoid model

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Chicken intestinal organoids, also referred to as 'mini-intestines', are 3D in vitro models developed to evaluate the effects of feed additives and pathogen exposure on avian gut health. This new technology offers a promising alternative to in vivo trials, potentially reducing the use of live animals. This study aimed to determine the effects of a blend of Organic Acids + Essential Oils (OA+EO) on Salmonella Typhimurium invasion and viability, immune response, and protein phosphorylation in a Salmonella-infected intestinal organoid model. Chicken intestinal organoids were generated from 18-19 day-old Hy-Line embryos. Organoids and Salmonella were pretreated with 0, 0.25, and 0.50 mg/mL of OA+EO (Jefo Nutrition) for 48h and 24h, respectively. Then, 500 CFU/ Salmonella (pretreated or not) was added to the corresponding treated organoid. Salmonella viability was measured 24h after exposure to OA+EO. Salmonella invasion was assessed 3h post-infection in the organoids. Innate immune responses were analyzed via qPCR, and protein phosphorylation was evaluated using a kinome array to measure changes in immune signal transduction pathways under different treatments. Statistical analyses were conducted using GraphPad Prism 9 or GenEx v6 and P-values<0.05 were considered significant. The OA+EO reduced Salmonella viability by 28% (average of the two doses) compared to the untreated group. The treatment with 0.25 or 0.50 mg/mL of OA+EO showed a significantly lower (P<0.001) number of organoids invaded by Salmonella. Untreated organoids exposed to Salmonella exhibited increased (P <0.05) expression of pro-inflammatory markers like cytokines and chemokines, compared to those treated with OA+EO. In contrast, OA+EO completely downregulated (P<0.05) these inflammatory responses induced by Salmonella infection. As expected, the induction of pro-inflammatory gene expression by Salmonella infection elicited the activation of signaling pathways in the kinome array analysis. The data indicated significant changes in protein phosphorylation, with OA+EO treatment reducing (P<0.05) phosphorylation in inflammatory pathways during Salmonella challenge, aligning with decreased inflammatory gene expression. In conclusion, OA+EO reduced Salmonella viability and invasion, downregulated the inflammatory responses, and decreased protein phosphorylation in chicken intestinal organoids. These results support the effectiveness of Organic Acids and Essential Oils in controlling Salmonella challenge when delivered in the intestine.
Keywords: Essential oils; Intestinal organoid; Intestinal inflammation; Organic acids; Salmonella

5176P The effect of dietary omega-3 polyunsaturated fatty acids (PUFA) on performance and antibody titers in response to immune challenge of pullets induced by vaccination against avian encephalomyelitis (AE)

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Omega-3 polyunsaturated fatty acids (PUFA) have been shown to modulate avian immune function, particularly humoral immunity. Avian encephalomyelitis (AE) is a viral disease affecting young chickens and other poultry species worldwide, leading to economic losses in the poultry industry. However, limited information exists on whether dietary supplementation with different types of omega-3 PUFA can enhance antibody responses to vaccination against AE compared with a control diet. This study aimed to compare the effects of a control pullet diet and diets enriched with different omega-3 PUFA sources on antibody titers of pullets vaccinated against AE.

A total of 320, day-old Dekalb White pullets were randomly assigned to one of four diets formulated to meet their nutritional requirements: a corn-soybean meal-based Control diet (A); a diet with 0.4% α-linolenic acid (ALA) from flaxseed oil (B); a diet with 0.4% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil (FO) (C); and a diet with 0.4% DHA from algal DHA (D). Each treatment consisted of 16 cages, with 5 birds per cage. On the first day of week 11, all birds were vaccinated with a live AE virus (AEV) vaccine via intracutaneous wing-web

injection and monitored for adverse reactions. Blood samples were collected at week 9 (baseline) and from weeks 12–16 from 5 birds per treatment (each from different cages) and analyzed for AEV antibody titers using ELISA. Feed intake (FI) and body weight (BW) were recorded weekly, and data were analyzed using the MIXED procedure of SAS.

Diet significantly affected body weight gain (BWG) during the starter phase ($P<0.01$), with pullets fed the 0.4% algal DHA diet showing the highest BWG. Significant effects of diet were also observed on the feed conversion ratio (FCR) during the starter phase ($P<0.01$) and overall ($P<0.05$). Pullets fed the Control diet and the 0.4% algal DHA diet achieved better FCR compared to those fed other diets in both phases. Antibody titers were negative at weeks 9, 12, and 13 but became positive from weeks 14–16. Significant effects of both diet and week were detected for sample-to-positive (S/P) ratios. Pullets fed the 0.4% EPA+DHA FO diet had higher S/P ratios than those fed the control diet ($P < 0.05$), with S/P ratios increasing from week 14 to 16 ($P<0.05$).

Dietary supplementation with fish oil rich in EPA and DHA enhanced antibody titers following AE vaccination compared with a control diet. Future work will examine the persistence of this effect during the pre-lay and laying periods and evaluate cellular immune responses.

Keywords: Omega-3 PUFA; Vaccination; Immune challenge; Antibody; Pullets

5177P Comparative In Vitro Antioxidant Activities and Chemical Characterization of Chestnut Tannins for Poultry Nutrition

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Antioxidants are essential in poultry nutrition to counteract oxidative stress, enhance immune function, and improve product quality. Among phytogenic antioxidants, hydrolysable tannins from chestnut wood have shown promising bioactivity. This study evaluates the in vitro antioxidant capacity and chemical composition of a French chestnut extract (TAN_H1, Kingbrown®) compared to other tannin sources, including condensed tannins (Quebracho, Acacia, Grape pomace) and hydrolysable tannins from Italy and Slovenia.

Antioxidant activities were assessed using three standardized assays: TEAC (DPPH), ABTS, and ORAC, with results expressed in µmol Trolox equivalents per gram. TAN_H1 exhibited the highest antioxidant potential (TEAC: 7057; ABTS: 7321; ORAC: 3014 µmol Trolox/g), outperforming other tannin sources and reference antioxidants (Vitamin C, BHT) ($p<0.05$). Chemical analysis revealed TAN_H1 contains over 90% hydrolysable tannins, with high levels of ellagitannins (vescalagin, castalagin) and ellagic acid, distinguishing it from condensed tannins rich in proanthocyanidins.

The elevated antioxidant activity of TAN_H1 is likely linked to its unique phenolic profile, supporting enhanced free radical scavenging and metal chelation. These findings highlight the importance of tannin source and composition in designing effective antioxidant strategies for poultry nutrition. While in vitro results are promising, further in vivo studies are needed to confirm physiological benefits, determine optimal dosages, and refine application protocols.

This research supports the use of chestnut tannins as a natural, functional feed additive to improve poultry health and performance in a sustainable manner.

Keywords: Chestnut tannins ; antioxidant activity; polyphenols; ellagitannins

5178P Temperature–diet interactions alter cecal butyrate capacity and barrier function in broilers

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Heat stress has been shown to limit broiler production by inducing cecal dysbiosis, reducing the production of short-chain fatty acids, and weakening intestinal epithelial integrity. We hypothesized that temperature–diet interactions modulate gut microbiota composition, butyrate-producing capacity, and intestinal barrier function. Accordingly, we assessed Butyryl-CoA:acetate CoA-transferase (butCoAT) as a functional marker of butyrate production, secretory immunoglobulin A (sIgA), fluorescein isothiocyanate–dextran (FITC-dextran) permeability, and jejunal barrier-related genes. A 2×3 factorial design was employed with temperature (constant 25 °C or late-phase heat stress at 35°C) and diet (basal, probiotic, or antibiotic). Cecal digesta and jejunal mucosa were collected to enumerate lactic acid bacteria and Escherichia coli (log CFU/g), quantify butCoAT gene copies, assess barrier-related gene expression by qPCR, and measure cecal sIgA (ELISA) and serum FITC-dextran permeability. Data were analyzed by two-way ANOVA with Benjamini–Hochberg false discovery rate (FDR) correction. Heat stress reduced the Lactobacillus:E. coli ratio by 92% ($P < 0.05$) and decreased butCoAT copy number by 80% ($P < 0.05$). Tight-junction genes (ZO-1, occludin, claudin-1) were down-regulated ($P < 0.05$). Functionally, sIgA declined by 60%, whereas FITC-dextran increased by 131% ($P < 0.05$), indicating compromised intestinal barrier integrity. Under heat stress, probiotic and antibiotic diets were associated with higher Lactobacillus and lower E. coli counts ($P < 0.05$), increased butCoAT abundance (basal 1.44 ×108, probiotic 5.30 ×108, antibiotic 2.50 ×108 copies/g, $P < 0.05$), normalized barrier gene expression ($P < 0.05$), elevated sIgA (basal 23.7, antibiotic 38.2, probiotic 50.3 µg/g, $P < 0.05$), and reduced FITC-dextran (basal 9.7, antibiotic 7.2, probiotic 5.1 µg/mL, $P < 0.05$). The probiotic diet produced stronger effects than the antibiotic diet. These results demonstrate that temperature–diet interactions reshape the broiler cecal microbiota–butyrate axis and intestinal barrier function in heat-stressed broilers, supporting dietary strategies to preserve gut integrity under thermal challenge.

Keywords: Broiler; Butyrate; Heat stress; Microbiota; Probiotics

5179P Impact of Dried Apple Pomace on Antioxidant Activity and Gut Morphology in Broiler Chickens

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Dietary incorporation of apple pomace (AP), a polyphenol-rich fruit residue, in broiler chickens' diets may modulate physiological mechanisms that influence antioxidant capacity and intestinal integrity in broiler chickens. This study evaluated the effects of dietary inclusion of AP dried using three different methods on antioxidant activity and gut integrity of broiler chickens. 300 one-day-old Ross 308 chicks were randomly allocated to ten dietary treatments arranged in a 3 x 3 + 1 factorial design. These included a control diet (0% AP) and diets with 3, 6, or 9% of hot air dried (HAD), freeze dried (FD), or electrohydrodynamic dried (EHD) AP. Each treatment had six replicate cages, with five birds per cage. The feeding trial lasted 21 days. On d 21, blood and jejunal tissue samples from 2 birds per cage were collected to assess plasma antioxidant parameters and gut morphology. Data were analyzed using a completely randomized design with the MIXED procedure of SAS. Total antioxidant capacity (TAC) and plasma malondialdehyde (MDA) concentrations were not significantly affected by drying method, inclusion level, or their interaction ($P>0.05$). Jejunal morphometric parameters (villus height and crypt depth) were significantly influenced ($P < 0.01$) by drying method, inclusion level, and their interaction, with both parameters decreasing as inclusion level increased. However, villius height to crypt depth ratio did not differ significantly among treatments. Overall,

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increasing inclusion level of AP to 9% led to reduced villus height and crypt depth, suggesting potential negative effects on intestinal absorptive capacity at higher inclusion levels. In conclusion, incorporating 3 and 6% dried apple pomace (DAP) preserved normal antioxidant activity and intestinal structure in broiler chickens. However, higher levels might affect gut integrity.
Keywords: apple pomace drying method; broiler chicken; oxidative status; jejunal morphology; sustainable feed

5180P Probiotic fermented Mentha arvensis hydro-distilled residue as potential natural feed additive

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The aim of this study is to improve the bioactivity and bioavailability of M. arvensis hydro-distilled residue by probiotic fermentation and evaluate the antioxidant and anti-inflammatory effects of fermented M. arvensis residue (FMAR) supplemented in broiler diet. Lactobacillus plantarum, Bacillus velezensis, or Entercoccus faecium ferment for 48, 72, or 96 hours with 70% or 80% initial moisture were test for suitable fermentation condition. In vitro antioxidant activity was test by DPPH scavenging ability and ferric reducing antioxidant power. Phenolic compounds of FMAR including rosmarinic acid, luteolin, apigenin, and the flavonoid glucosides were analyzed by HPLC-UV. The FMAR with relatively stronger antioxidant activity and higher flavonoid aglycone content was chosen for further in vivo experiments. 320 1-day-old Arbor Acres broilers were randomly allocated into 4 groups, which were control group fed basal diet and 3 experimental groups fed basal diet supplemented with 0.5%, 1%, or 2 % of FMAR. The results showed that L. plantarum fermentation with 70% initial moisture for 96 hours (LFMAR) had the highest DPPH scavenging ability and higher content of flavonoid aglycones ($P < 0.05$). Supplementation of 1% LFMAR significantly increased the 21d body weight ($P < 0.05$). LFMAR significantly decreased the serum protein carbonyl, 1% and 2% supplementation increased the jejunal catalase and glutathione peroxidase activity respectively ($P < 0.05$). LFMAR also increase the jejunal mRNA expression of SOD in all experimental groups and GCLC in 1% and 2% groups. The protein expression of jejunal IL-6, IL-8, and IL-1β were significantly lower in 2% group in blood serum ($P < 0.05$). In conclusion, L. plantarum fermentation effectively improve the bioactivity of the residue by converting the flavonoid glycosides into aglycones, 1% LFMAR supplementation exhibited higher antioxidant effect while 2% LFMAR showed greater anti-inflammatory effect in broiler feed.
Keywords: Broiler; Fermentation; Phytogenic

5181P POST-PEAK PRODUCTION BENEFITS OF A FEED SUPPLEMENTATION WITH A TRIPLE-STRAIN BACILLUS-BASED PROBIOTIC IN DYSBIOSIS-CHALLENGED LAYERS

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Layer producers have been navigating through a range of challenges stemming directly from the genetic progress with hens now laying for longer periods and being more feed efficient. Those improvements do come with a few trade-offs with hens being more sensitive to pathogenic infections and dysbacteriosis problems that will negatively affect gut integrity and therefore productivity. The aim of the study was to assess the effect of a feed supplementation with a commercial triple-strain Bacillus-based probiotic on productivity and egg quality of dysbiosis-challenged hens throughout a period of 30 days. 288 Lohman Brown hens of 40 weeks of age were randomly allocated to 6 pens of 48 layers (one pen per treatment group). The challenge consisted in inducing dysbiosis in the gut of layers by applying a 10 days antibiotic treatment (based on Enrofloxacin at 10mg/kg feed and Amoxicillin at 20mg/kg feed) through the diet as from D10. The birds were all on the same diet and allocated to 6 pens: a negative control group (NC) not challenged, a positive control challenged, a probiotic group (PRO30) challenged and continuously supplemented with a triple strain Bacillus-based probiotic (1.6x10E6 CFU/g finished feed) from D0 to D30, another probiotic group (PRO10) challenged but supplemented with the same probiotic from D20 to D30 only, a phytogenic group (EO30) challenged and fed with a combination of essential oils and organic acids and another phytogenics group (EO10) challenged but fed with the same combination as EO30 but only during the last 10 days of the experiment. The statisitical analysis was performed using SAS 9.2. Antibiotic administration affected intestinal morphology but did not affect intestinal permeability nor the counts of enterobacteria. PRO supplementation to laying hens led to significantly ($p<0.05$) higher intestinal villus length-to-crypt depth ratio compared to negative and positive controls: at D30 of the experiment in the duodenum, at D10 , D20 and D30 in the jejunum and at D20 and D30 in the ileum . Proportion of dirty eggs, being one of dysbiosis markers in layers, was relatively high but was significantly lower in PRO30 birds compared to all treatment groups ($p<0.05$) except NC. Laying rate was also improved in PRO30 ($p<0.05$) compared to EO and PRO10. A significant reduction on average daily feed intake was found in hens in PRO and EO compared to hens in NC, PC and EO10 groups ($P = 0.01$). In conclusion, continuously feeding layers with the probiotic, starting 10 days before the dysbiosis challenge, did enhance post-peak egg production and feed efficiency while improving the cleanliness of eggs through improved gut morphology and greater surface area for nutrient absorption.
Keywords: Layers; gut integrity; laying rate; digestibility; Probiotic

5182P Effect of feed Supplementation with Muramidase on productivity of commercial hens during the first phase of the laying cycle

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In the early phase of the production cycle for commercial layers, there is a critical window that lays the groundwork for the flock long-term's productivity and health. During this initial stage, producers face unique challenges – from ensuring proper nutrient intake and uniform growth to managing early stressors that can impact future laying rates. Addressing these early-phase hurdles is key to optimizing overall flock performance and egg quality down the line. Muramidase (EC 3.2.1.17) which is an enzyme that hydrolyzes bacterial cell wall peptidoglycans, has gained increasing attention for its role in improving gut functionality and nutrient utilization. Previous studies have shown that muramidase supplementation can improve feed efficiency and productivity in broilers or turkeys. Muramidase has now been evaluated in layers. The aim of the study was to evaluate the effect of dietary muramidase supplementation on the performance of Isa Brown laying hens during early production. A 140-day randomized complete block design was conducted with 210 Isa Brown hens (22 weeks old), allocated to 42 cages (5 hens/cage, 21 replicates/treatment). Two treatments were compared: control (CON; basal diet) and muramidase (MUR) at 30,000 LSU (F)/kg feed. Productive parameters data were analyzed using a General Linear Model (GLM) once the test con-

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ditions were checked and the least squares corrected means were separated with Tukey's post-hoc comparison test (SPSS v27.0). The cage was the experimental unit. Statistical significance was set at P≤0.05. MUR supplementation significantly increased laying index (95.1% vs. 90.9%, P=0.0051) and egg mass (57.3g/hen/d vs. 55.0g/hen/d, P=0.0132) compared to control. Feed conversion per kg egg (2.17 vs. 2.27, P=0.0189) and per dozen eggs (1.57 vs. 1.65, P=0.0062) were also improved in layers fed with MUR. No significant differences were observed in egg weight, feed intake, or mortality. By supporting feed efficiency, supplementing the diet with a specific muramidase shows great potential to sustainably optimize performance of hens from point of lay up to peak production and beyond.

Keywords: Muramidase; Laying hens; laying rate; egg quality; feed efficiency

5183P Deposition of lutein and zeaxanthin in egg yolk in four different layer breeds

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The xanthophylls lutein and zeaxanthin are important natural compounds due to their role in human eye health and their ability to increase the orange color of the egg yolk in layers and the skin in broilers. A study was conducted to investigate if the deposition of these two pigments in egg yolk is affected by the genetic commercial breed. A total of forty 23-week-old laying hens from the Babcock Brown, Lohmann Brown, Hy-line Brown and Isa Brown breeds (10 birds per breed) were used. A completely randomized arrangement of treatments was used as the experimental model with each individually housed hen as the experimental unit. To reduce the lutein and zeaxanthin reserves in the birds before starting the trial, a diet devoid of xanthophylls (white corn/soybean) was given for 21 days. After 21 days they were fed a yellow corn/soybean diet for 28 days (diet 1) followed by the same type of diet supplemented with 40 mg/kg lutein (diet 2) for 28 days and finally a third diet containing 80 mg/kg lutein for another 28 days (diet 3). Response variables measured included performance parameters (feed intake, egg production, egg weight, egg mass and feed conversion), egg quality parameters (yolk color as determined by a Nabel 6500 Egg Tester Analyzer, Haugh units, eggshell thickness and eggshell strength, among others) and lutein and zeaxanthin content in eggs and feed by high-performance liquid chromatography (HPLC).

Yolk color intensity increased progressively with each diet reaching a plateau at 7, 10 and 11 units with diets 1, 2 and 3, respectively. Mean lutein plus zeaxanthin concentrations in yolk were 1.5, 4.0 and 5.5 µg/100 g for the diets 1, 2 and 3, respectively. These concentrations correspond to an approximate content of 100, 600 and 850 µg per egg, respectively. No significant differences (P>0.05) were observed among the different laying hen breeds for any of the performance or egg quality parameters measured, including egg yolk color and xanthophyll content.

It was concluded that the dietary supplementation of 40 or 80 mg/kg lutein increases the egg yolk color and lutein content without affecting production parameters or egg quality. However, in all breeds, the efficiency of deposition decreased as the lutein concentration in the diet increased. Doubling the amount of lutein in the diet from 40 to 80 mg/kg increased the yolk color by only one unit (11 vs 10) and the xanthophyll content by 41%. These findings suggest that moderate amounts of lutein are more cost-efficient as a means of supplementing eggs with lutein and no differences in deposition should be expected among laying hen breeds. More studies are needed to further determine the ideal level of lutein supplementation in laying hens.

Keywords: lutein; zeaxanthin; layers; egg yolk color

5184P Sugarcane yeast cell wall alone or in combination with phytogenic additive reduces Escherichia coli airsacculitis and mortality in challenged broiler chickens

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Escherichia coli remains a major pathogen in broiler production, associated with airsacculitis, immunosuppression, increased mortality, and impaired growth. However, limited controlled studies have assessed the efficacy of sugarcane-derived yeast cell wall (YCW) with or without phytogenic feed additives (PFA) under E. coli challenge. This study evaluated whether a sugarcane YCW, rich in β-glucans (recognized for their immunomodulatory properties), and mannan-oligosaccharides (MOS; capable of binding Gram-negative bacteria, promoting pathogen gut clearance), alone or combined with a PFA blend with anti-inflammatory and antimicrobial essential oils, could mitigate E. coli-induced airsacculitis and mortality in broilers challenged with avian pathogenic E. coli (APEC). A total of 1,200 broilers were distributed into 24 pens with 50 birds each (n=8 replicates/treatment). Birds were assigned to one of the three dietary treatments: T1, negative control (no additive); T2, YCW (IMW50, 0.5 kg/mT; Immunowall, Saccharomyces cerevisiae cell wall composed of 30% β-glucans and 20% MOS; ICC Animal Nutrition); and T3, YCW (460 g/mT) + PFA (140 g/mT; Alterna Poultry, a blend of thyme, clove, and cinnamon oils; Probiotech International). Birds were reared on reused litter, coarse-sprayed at 24 d with NDV and IBV vaccines, and at 29 d with APEC (X-7122 Curtiss) following Glisson et al. (2004). Samples for intestinal integrity (FITC-d) and cytokine response were collected on d35, and liver samples for gene expression were obtained on d14 and 35. Data were analyzed using ANOVA and the T-Test ($P < 0.05$). Airsacculitis-related mortality was significantly reduced in T3 (3.50%) compared with the control (6.25%), while T2 showed intermediate values (5.00%; $P<0.05$). Lesion scores were not significantly different among treatments ($P>0.05$). Although performance was not statistically altered, YCW alone (T2) resulted in the highest numerical body weight on d35. IL-10 mRNA expression on d14 was upregulated in T2 and T3 compared to T1 ($P < 0.05$), indicating an enhanced anti-inflammatory response. On d35, T2 birds showed increased IL-1, LITAF, and IFN-γ expression ($P<0.05$), suggesting stronger innate immune activation. The APEC challenge successfully induced airsacculitis and mortality, validating the model. The combination of YCW and PFA significantly reduced mortality, while YCW alone enhanced immune activation and numerically improved growth performance. These findings demonstrate that sugarcane YCW, particularly when combined with phytogenic oils, provides effective immunomodulatory and protective effects against E. coli challenges in broilers.

Keywords: airsacculitis; Escherichia coli; β-glucans; MOS; phytogenic feed additive

5185P Effect of different levels of olive leaf hydroalcoholic extracts on performance, egg quality characteristics, and blood indices in commercial laying hens during early production

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Considering global population growth and limited resources for food, it is essential to find alternative feed ingredients and increase the quantitative and qualitative efficiency of poultry production. The fresh leaves of the olive tree, as an agricultural residue after harvesting, contain about 10% polyphenolic compounds and have the highest antioxidant activity and free radical scavenging power among different parts of the olive tree. This research aimed to evaluate the effect of varying levels of hydroalcoholic extracts from olive leaves (HEO) on yield, egg quality characteristics, and blood indices of commercial laying hens during peak production. A total of 440 25-week-old Hy-Line laying hens were randomly assigned to 11 treatments (n=5 replicates/treatment with 8 hens per replicate) consisting of control (0 mg/kg HEO) and both aqueous and alcoholic HEO (200, 400, 600, 800, and 1000 mg/kg) for 28 days. At the end of the experimental period, two eggs from each replicate were collected for measurement of internal (color index, % yolk, % albumen, Haugh unit) and external (specific gravity, % shell ((Shell weight/egg weight)*100), shell thickness, shell strength qualities. Blood was collected from the jugular vein during euthanasia for the biochemical measurement of blood serum. Data were analyzed with the general linear model in SAS, and the means of experimental groups were compared using the Tukey method at a 5% probability level The results showed that birds consuming 1000 HEO had the highest egg mass and the lowest feed conversion compared to the control treatment (P<0.05). During peak, hens consuming 1000 HEO had the highest percentage of egg production compared to the control treatment (P<0.05). Different levels of HEO increased Haugh units compared to the control treatment, with the 1000 HEO level showing the greatest significant difference (P<0.05). Using 1000 mg/kg HEO caused a significant decrease in circulating triglycerides and cholesterol and a significant increase in high-density lipoprotein (LDH) (P<0.05). The highest level of HEO used in bird feed increased the total antioxidant index and decreased malondialdehyde in egg yolk compared to the control treatment (P<0.05). In this experiment, 1000 mg/kg of HEO improved performance (% egg production), and aspects of internal (decreased LDH) and external egg quality (% egg shell). It is suggested that they be proposed as compounds to enrich eggs and improve quality and marketability.
Keywords: olive leaf; Haugh unit; yolk color index; specific gravity

5186P Effects of natural feed additives on reproductive performance, nutritional lipid profile and egg quality dynamics in brown hens

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Natural feed additives offer sustainable alternatives to synthetic growth promoters by enhancing nutrient utilization and reproductive efficiency in poultry. This study evaluated the effects of three natural feed additives Vernonia amygdalina (bitter leaf), Ocimum gratissimum (clove basil), Syzygium aromaticum (clove) and their combinations on reproductive performance and egg quality in brown hens. A total of 315 NovoGen® brown pullets were randomly assigned to seven treatments in a completely randomized design with three replicates of 15 birds each. Treatments were T1 (control), T2 (0.5% V. amygdalina), T3 (0.5% O. gratissimum), T4 (0.5% S. aromaticum), T5 (0.25% V. amygdalina + 0.25% O. gratissimum), T6 (0.25% V. amygdalina + 0.25% S. aromaticum), and T7 (0.25% O. gratissimum + 0.25% S. aromaticum), diets meet layer requirements, while hen-day production (HDP), egg weight, egg mass, feed intake and feed conversion efficiency (FCE) were recorded for 56 weeks. Egg quality indices such as shell weight, thickness, albumin weight/index, haugh unit, yolk color and lipid profile were accessed at pre-peak, peak, and post-peak stages. Data were analyzed using ANOVA and Duncan's test to separate means at 5% level of significance. The HDP was significantly higher (P<0.05) in hens on T6 (81.33%) and T7 (79.99%) when compared to control (65.00%); while T2, T3, T5 showed no variation (P>0.05) on treatment effects. There seem to be a greater impression on egg mass in T6 (43.80g/bird/day) and T7 (41.68g/bird/day) than control (37.15g/bird/day) with no pre-peak differences (P>0.05) in mean values. Feed intake was within the range (115–120g/bird/day) in treated groups as against control (125.84g/bird/day), improving FCE in T6 and T7, with control having FCE values of 2.26. Comparative differences in haugh unit was statistically higher (P<0.05) in treated groups compared to control. Yolk color improved across treatments with significant (P<0.05) impression in yolk index in T6 (50.23%), control (45.31%) and T3. Yolk cholesterol and triglycerides were lower (P<0.05) in T2, T5, T6, T7 as against control groups. Corresponding values of HDL were higher across treatments, while LDL had lower values across treated groups with clear variation in mean values. Therefore, combinations of V. amygdalina or O. gratissimum with S. aromaticum at 0.25% each (T6, T7) optimally enhanced laying performance, egg quality, and yolk nutritional lipid profile due to synergistic bioactive interaction that improved nutrient digestibility and ovarian function, while reducing oxidative stress.
Keywords: Haugh unit; cholesterol, egg quality; performance; yolk index; chicken Hens

5187P The effects of a chestnut-quebracho tannins blend on the growth performance of broilers fed with a reduced nutrients level

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Strategies that allow for lower dietary nutrient levels without compromising productivity are of great importance for improving economic efficiency and environmental sustainability. Plant-derived bioactive compounds from chestnut and quebracho extracts have shown potential to enhance poultry gut health and digestive efficiency through modulation of gut microbial composition, optimisation of intestinal motility, and antioxidant properties. Yet their ability to sustain broiler performance under reduced nutrient diets remains insufficiently validated. This study was conducted to investigate the effects of chestnut (Castanea Sativa) and quebracho (Schinopsis Balansae) extracts mixture (Silvafeed® Nutri P, Silvateam S.p.A.) on the growth performance of broilers fed with reduced levels of metabolizable energy, crude protein and digestible lysine. A total of 192 as-hatched Ross 308 broilers were received from a commercial hatchery, weighed, and randomly distributed into 16 floor pens with 12 birds each (8 pen replicates per treatment). The two dietary treatments consisted of a corn-wheat diet formulated to meet broiler nutrient requirements (CON; with on average AMEn=3037 kcal/kg, CP=210g/kg and digestible lysine=12.2 g/kg) and a reformulated corn-wheat diet with moderately reduced nutrient levels [AMEn: -45 kcal/kg; CP: -3.6 g/kg; digestible lysine: -0.17g/kg as compared to CON] and supplemented with 1 kg/ton of Nutri P (TAN). The feeding program consisted of three phases: starter (0 to 10d), grower (11 to 24d), and finisher (25 to 35d). Growth performance was determined cumulatively (from 0

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to 35 days of age), and uniformity was measured at 35 days of age. Data on growth performance was analyzed by ANOVA using the MIXED procedure of SAS (SAS Ins. Inc., 2019) with the diet fed to birds as the main source of variation. No significant differences were observed between treatments for initial body weight (46.9 g in both groups; P=0.95) and body weight at day 35 (2348g in CON group, and 2356g in TAN group; P=0.85). Likewise, there was no significant differences between treatments for average daily gain and mortality-adjusted feed conversion ratio (respectively 66 g/day and 1.6 in both groups; P>0.30) from 0 to 35 days of age. The uniformity at 35 days was similar between CON and TAN groups (11.6 and 10.6%, respectively; P=0.39). In conclusion, these findings support that a chestnut-quebracho tannins blend could be an effective strategy for optimizing feed formulation and economic efficiency while maintaining productive performance and flock uniformity in broilers.

Key words: chestnut; quebracho; polyphenol; optimizing feed formulation
Keywords: chestnut; quebracho; polyphenols; feed cost

5188P Association of Moringa oleifera, cassava meal, Acromia aculeata in layers feeding and its effects on serum analytes

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Introduction: Native plants have been studied as potential sources for replacing traditional feeds in poultry production. As anti-nutritional factors may be present, it is important to assess physiological reactions provoked when trying new sources of food. In this context, blood parameters can be of great help in monitoring birds.
Purpose: We aimed to assess the impact of the combination of cassava whole meal, Moringa oleifera leaves and Acromia aculeata pulp at different levels as substitute for commercial feed on the serum analyses of cage free layers.
Methods: We measured serum concentrations of uric acid (UA) and activity of aspartate aminotransferase (AST) in 3 groups of layers during the weeks 20, 24, 28, 32, 45 and 64:
Group 1 (Control): diet with soja meal and corn.
Group 2 (G2): substitution with 4% Acromia aculeata, 18% wholemeal manioc flour, 4% Moringa leaves
Group 3 (G3): substitution with 6% Acromia aculeata, 24% wholemeal manioc flour, 6% Moringa leaves
Results: The peak laying period interfered with the concentrations of the analytes between the different collection periods, especially albumin. UA values: G1 = 4,39a ± 0,36; G2 = 4,71a ± 0,94; G3 = 5,32a ± 0,83, with ANOVA p value of 0,053,
Kruskal Wallis p value 0,16 and blocked p value <0,01. AST values: G1 = 117,20 ± 21,03; G2 = 113,59 ± 23,94; G3 = 115,31 ± 10,05
and ANOVA p value of 0,87, Kruskal Wallis p value 0,77 and blocked p value 0,51.
Conclusion: The diets did not cause significant changes in markers of kidney (uric acid) and liver (aspartate aminotransferase) damage. Moringa leaf meal, cassava bran and bocaiuva pulp can be used as alternative sources in laying hens'diets without significantly altering the birds' serum analyses.
Keywords: diet; hens; native plants

5189P In-vivo anticoccidial activity of botanical compounds against Eimeria spp

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Growing societal concerns to replace coccidiostats in poultry farms lead to the use of alternatives as plant bio-actives. This trial assessed the effect on feed utilisation of different botanical compounds against coccidiosis. 4 plant materials were selected to perform this study: two non-volatile active molecules (a lectins rich extract- PE1 and a lactones rich extract - PE2) and two volatile compounds (organosulfur microcapsules - PE3 and a phenols rich oil - PE4). The 4 extracts were tested at the same concentration to achieve 40 ppm in the feed. A total of 80 Ross 308 broilers were randomly allocated to one negative control group and four experimental groups, each with 2 replicates and 8 birds per cage. All birds received a common starter (d 0-7) diet and an experimental diet (d 8-35). At day 14, 3 types of Eimeria were inoculated (E. Acervulina, E. Maxima, E. Tenella) on 4 experimental groups and one negative control group. On day 26, oocysts per gram feces (OPG) were measured according to Mc Master protocol. 6 birds/group were sacrified at day 26 for intestinal lesion scoring according to Johnson and Reid system (scale 0 to 4) and intestinal histologic samples were collected for villi measurements. Feed consumption and live weight were measured per cage at day 8 and day 35 to calculate the feed conversion ratio (FCR) throughout the experiment. Results showed that all these 4 botanicals reduced the oocysts per gram feces (OPG) excretion by 81%, 88%, 97% and 93% for PE1, PE2, PE3, PE4, respectively ($p<0.05$). Lesion scoring was also reduced (lesion score <1 vs. 2.82 for control group). The feed conversion ratio was reduced by -8%, -6%, -4%, -12% for PE1, PE2, PE3, PE4, respectively. The PE groups have a higher duodenal VH/CD ratio : +46%, +44%, +47%, +75% respectively. All selected extracts proved beneficial to manage coccidiosis development and incidence on broiler performances.
Keywords: Broilers; Coccidiosis; Botanical compounds

5190P Evaluation of 25-hydroxyvitamin D3 and phytase on growth performance, carcass yield, and bone characteristics of broiler chicken

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The objective of this study was to evaluate the effects of dietary supplementation with 25-hydroxycholecalciferol (25-OH-D3) and a high dose of phytase on the growth performance, carcass yield and bone characteristics of broiler chickens from 1 to 40 days of age. A total of 750 one-day-old male Cobb 500 chicks were allocated to 30 floor pens in a commercial poultry farm, following a completely randomized design with three dietary treatments, 10 replicate pens per treatment, and 25 birds per pen. The corn–soybean meal–based dietary were as follows: (1) Control (C), diet formulated to meet commercial nutrient levels and containing 1,500 phytase units (FYT)/kg; (2) C + 25OHD3, supplemented with 25-OH-D3 at a dose of 69 µg/kg feed (equivalent to 2,760 IU of vitamin D3 activity) from 1 to 24 days; and (3) C+25OHD3+High Phytase (C+25OHD3+HP), same 25-OH-D3 supplementation as treatment 2 and increased phytase to 3,000 FYT/kg from 1 to 40 days. All diets included a nutrient matrix equivalent to 0.19% calcium and available phosphorus for phytase. Basal diets provided 5,160; 5,160; 4,300; 4,300; and 3,440 IU of vitamin D3 per kg of feed in the pre-starter, starter, grower 1, grower 2, and finisher phases, respectively. Feed intake (FI), body weight (BW), BW gain, and feed conversion ratio (FCR) were measured weekly from 1 to 40 days. On day 40, tibia samples were collected from two

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birds per pen for tibia breaking strength, color and ash analysis. On day 41, carcass and commercial cut weights and yields were evaluated. Data were subjected to one-way ANOVA and treatment means were compared by the Tukey test ($P \leq 0.05$). Contrasts were applied to assess the effects of feed additives compared to the Control diet. Performance parameters were similar among dietary treatments, and no differences were observed between C+25OHD3 and C+25OHD3+HP for any evaluated variable ($P > 0.05$). Nevertheless, carcass weight increased by 3.0% in the C+25OHD3 group compared with the Control ($P \leq 0.05$). Broilers fed the C+25OHD3 diet had higher breast fillet and thigh weights than the Control, with breast yield increasing by 1% ($P \leq 0.05$). The C+25OHD3 diet also improved tibia breaking strength and reduced tibia b* (redness) values compared with the Control diet ($P \leq 0.05$). Broilers fed C+25OHD3+HP showed higher tibia ash content compared with the Control diet ($P \leq 0.05$). In conclusion, dietary inclusion of 25-OH-D3 until 24 days of age enhanced carcass yield, and bone quality, in broiler indicating that 25-OH-D3 supports improved bone integrity and growth performance under commercial conditions.

Keywords: broiler; carcass yield; performance; tibia ash; vitamin D metabolite

5191P Effect of bio-available silicium on footpad dermatitis scores in broiler chicken

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Footpad dermatitis (FPD) scoring is a welfare assessment method primarily used in poultry—particularly broilers and turkeys—to evaluate the health and condition of their footpads. It reflects the extent of lesions, ulcers, or inflammation that may result from wet litter, poor hygiene, or inadequate housing conditions. A low FPD score (near 0) signifies good welfare and health conditions. Bio-available silicium supports bone, tendon, and skin health by promoting collagen synthesis, thereby improving the structural integrity of broiler legs and having potential benefit for lowering FPD score. The aim of this study was to evaluate the effect of bio-available silicium (Poulsil®) supplementation on footpad dermatitis (FPD) scores in broiler chickens. A total of 250 broiler day-old chicks were randomly distributed into one control and four treatment groups. Each group included five replicate pens with ten chicks per pen, maintained under uniform management and nutritional conditions. The control group (T01) received no silicium supplementation, while the treatment groups (T02–T05) were provided with varying formulations and concentrations of silicium from day one to day 41 via drinking water. Birds in groups T02 and T03 received Silicium–orthosilicic acid–vanillin complex (Si-OSA VC) at dilution levels of 1/1000 and 1/200, respectively, whereas groups T04 and T05 were supplemented with Silicium–monomethylsilanetriol (Si-MMST) at the same respective dilutions. The resulting silicium concentrations in drinking water were 0 mg/L, 0.87 mg/L, 4.33 mg/L, 0.30 mg/L, and 1.48 mg/L for T01, T02, T03, T04 and T05 respectively. Moreover, birds in all groups were reared on humidified bedding material to simulate bad housing conditions and induce mild locomotive and skin complications. FPD scores for all groups were evaluated on a 0–2 scale on day 41, with values of 1.31, 1.09, 0.98, 0.90, and 1.04 recorded for T01, T02, T03, T04, and T05, respectively. Data were analysed with R (version 3.2.5). The treatment groups were compared to the untreated challenged group as reference. Mean scores were added. Statistical significances were assessed at $P \leq 0.05$. Result showed that Poulsil® supplementation resulted in a reduction of footpad dermatitis (FPD) scores across all treatment groups. The reduction was significant at the higher concentration of the first formulation (Si-OSA VC; $p = 0.044$) and at the lower concentration of the second formulation (Si-MMST; $p = 0.01$). Thus, it can be concluded that supplementation with bio-available silicium (Poulsil®) reduces footpad dermatitis (FPD) scores in broilers, indicating its potential to enhance bird welfare and overall leg health.

Keywords: bio-available silicium; broiler; foot pad dermatitis score; welfare; poulsil

5192P Synergistic effects of a phytogenic feed additive with coccidiostats on performance, intestinal microbiota and resilience in broilers challenged with coccidiosis

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Coccidiosis impairs intestinal integrity and performance in broiler production. Phytogenic feed additives are being evaluated as natural tools to stabilize the intestinal microbiome and enhance resilience against enteric pathogens. Anta®Phyt NXT, a phytogenic formulation containing hops and licorice, combines antimicrobial and immune system-supporting properties. This study investigated the effects of Anta®Phyt NXT, alone or combined with coccidiostats, on growth performance, faecal quality, oocyst excretion and gut microbial diversity in broilers under coccidiosis challenge.

A 35-day trial was conducted with 631 Ross 308 broilers (8 replicates; 19–20 birds per pen) allocated to: (1) vaccinated control (Paracox-5), (2) Maxiban + Monensin, (3) Maxiban + Monensin + Anta®Phyt NXT 200 g/t, and (4) Anta®Phyt NXT 400 g/t. Initial pen weights and chick numbers were homogeneous across groups, with the smallest variation in groups B and C, confirming comparable starting conditions. On day 17 all birds were exposed to reused litter containing Eimeria acervulina, E. maxima and E. tenella. Performance, faecal quality and oocyst output were recorded, and cloacal samples were analyzed by 16S rRNA sequencing for microbial diversity. All data were analysed using T-test with dietary treatments as grouping factor.

The results clearly showed that a combination of Anta®Phyt NXT with coccidiostats delivers the best results, outperforming both the use of coccidiostats alone and the use of phytogenic feed additives alone. The group treated with Anta®Phyt NXT plus coccidiostats achieved the highest final body weight (≈ 2.16 kg) and best corrected feed conversion ratio (1:1.46), with reduced oocyst excretion and improved faecal consistency compared with the vaccinated control. Microbiome profiling showed higher α-diversity and evenness in Anta®Phyt-supplemented groups, while β-diversity revealed no significant inter-group clustering (PERMANOVA $p = 0.54$). The intestinal community remained dominated by Lactobacillus, Limosilactobacillus, Corynebacterium and Streptococcus, indicating a stable lactic-acid bacterial core.

In conclusion, Anta®Phyt NXT, particularly when combined with coccidiostats, improved performance, faecal quality and microbial diversity in broilers challenged with coccidiosis. These synergistic effects occurred under balanced starting conditions, supporting Anta®Phyt NXT as a valuable nutritional strategy to enhance intestinal health and reduce reliance on conventional anticoccidials.

Keywords: Phytogenics; Cocciciosis; Performance; Microbiota; Microbial diversity

5193P Using a Nonlinear Support Vector Mathematical Model to Evaluate Growth Performance, Blood Biochemical Indexes, and Immunity of Broilers Fed with Capparis Spinosa L. Fruit Powder and Comparing It with a Third-Order Linear Model

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This experiment was conducted to investigate the effect of consuming levels of Capparis Spinosa L. Fruit powder on growth performance, carcass and blood biochemical indices of broiler chickens evaluated by nonlinear support vector regression model and third-order linear model. In this study, 300 one-day-old Ross 308 broiler chicks were used with 6 experimental treatments including: different levels of 0, 3, 6, 9, 12, and 15 percent Capparis Spinosa L. fruit powder in five replications (each replication included 10 chicks) in a completely randomized design. During the rearing period, the chicks had free access to water and feed. Growth performance traits, carcass and blood biochemical indices were fitted with nonlinear support vector regression (SVR) and cubic linear models. The results of this study showed that adding Capparis spinosa L. fruit powder to the diet of broiler chickens did not have a significant effect on most performance indicators, but certain patterns were observed at different levels. The highest feed consumption was recorded at 6% in the growth, final and total periods, and at 9% in the initial period. Body weight showed a decreasing trend during the initial and growth periods, while it increased to 6% in the initial period and to 3% throughout the entire period, and then decreased. The highest body weight gain was achieved in the initial period at 6% and in the growth and final periods at 3%. The feed conversion ratio index reached the lowest value in the final periods and the entire period for levels of 3 and 9 percent. Among carcass components, the highest coefficient of determination (0.94%) was for relative breast weight in the nonlinear support vector model. Also, in blood biochemical indices, the nonlinear support vector model showed higher accuracy than the cubic linear model, and the highest coefficient of determination (0.98%) was obtained for cholesterol and LDL. The results of comparing the goodness-of-fit indices of the models showed that the nonlinear support vector regression model, with a higher coefficient of determination, lower mean square error, and lower sum of squares of the nonlinear support vector regression model, had a better estimate of growth performance, carcass percentage, and blood biochemical indices of broiler chickens compared to the cubic linear model. On the other hand, the results of this study indicated that the nonlinear support vector regression model provided more accurate results for performance, carcass, and blood biochemical indices during the entire rearing period compared to the cubic linear model.

Keywords: Broiler chicken; Capparis Spinosa L; Cubic linear model; Growth performance; Support vector regression

5194P Effects of dietary inclusion of oat hulls, wheat straw, and sunflower hulls as a structural fibre ingredient on total tract nutrient retention and energy utilization in laying hens

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The objective of this study was to evaluate the effects of structural fibre ingredients (SFI) on nutrient utilization in laying hens, with a particular focus on the coefficient of total tract apparent retention (CTTAR) of nutrients and apparent metabolizable energy (AME). A total of 84 ISA Brown hens (57 weeks of age, 12 per treatment) were used. Three SFI sources (oat hulls, wheat straw, and sunflower hulls) were included at 3.5 or 7.0% in a mash control diet (CON; 2700 kcal/kg AME, 150 g/kg crude protein) without phytase or NSPase, replacing wheat. After 8 weeks of feeding, digestibility was measured during a 4-day excreta collection period. Data were analyzed using Dunnett's test to compare each treatment with CON. In all SFI diets, CTTAR of DM was reduced compared with CON ($P < 0.001$), with wheat straw at 3.5% showing the smallest reduction (−2.36%) and wheat straw at 7% the largest reduction (−6.93%). Starch retention was not affected ($P = 0.235$). Protein retention was improved ($P < 0.001$) by oat hulls at 7% (+10.2%), wheat straw at 3.5% (+4.81%), and sunflower hulls at 3.5% (+5.25%), while sunflower hulls at 7% tended ($P < 0.10$) to increase CTTAR of protein (+4.04%). AME values were lower in all SFI diets ($P < 0.001$) compared with CON (2920 kcal/kg analyzed), with the smallest reduction observed for oat hulls at 3.5% (−69 kcal/kg) and the greatest reduction for wheat straw at 7% (−307 kcal/kg). Similar patterns were observed for AME corrected for nitrogen, except for oat hulls at 3.5%, which showed only a tendency ($P < 0.10$) to decrease (−64 kcal/kg). Inclusion of SFI in layer diets therefore consistently reduced AME utilization relative to the control diet baseline, while starch retention remained unaffected. Improvements in protein retention warrant further investigation of ileal digestibility responses. Overall, replacing wheat with SFI in diets without exogenous enzymes compromises dietary energy availability in laying hens.

Keywords: Structural fibre ingredients; Laying hen; Nutrient digestibility; Apparent metabolizable energy

5195P Effect of 2 doses of α-monolaurin in broilers during a necrotic enteritis challenge

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Necrotic enteritis is a major and growing concern in poultry industry as responsible for performance reduction and high mortality rate. Intestinal lesions are observed and can be caused by several factors (bacterial toxins, mycotoxins…). One route of this disease is caused by intestinal overgrowth of Clostridium perfringens associated with predisposing factors in young animals. α-monolaurin, recognized for its potent antimicrobial and immune-modulatory properties, seems to be a novel preventive treatment against C. perfringens-induced necrotic enteritis in broiler chickens. This study was conducted with a total of 576 one-day old chicks (Ross 308). The broilers were randomly assigned to one of the 4 experimental groups (18 birds/pen, 8 pens/group) over a 35 days period (d0-d35). Treatments consisted in a non-challenged group (Negative Control) fed with a basal diet and 3 groups inoculated with Clostridium perfringens at d21 after being predisposed with Eimeria Maxima at d16. For these challenged groups, 3 doses of α-monolaurin have been compared all through the experimental period: 0 (Positive Control), 0.4 (GML0.4) and 0.8 kg/T (GML0.8). Growth performance has been recorded on a pen basis and intestinal lesions have been scored at day 22 on 16 euthanized animals per treatment. Statistical analysis was performed with ANOVA, the treatment being considered as fixed effect and the block as random effect. Lesion scores and mortality were assessed using the chi-squared independence test. The overall mortality of chicks remains low (3.5% from d0 to d35) and was not significantly affected by the dietary treatment. During the pre-inoculation period (d0-d16), growth performance didn't differ significantly among the 4 groups. From d16 to d35, the inoculation tended to increase feed conversion ratio: 1.51 for NC vs. 1.55 for PC ($P=0.06$). Feed efficiency recorded for the 2 challenged groups fed α-monolaurin was intermediate between the control groups (NC & PC). Both Clostridium ($P<0.0001$) and Eimeria ($P=0.06$) scores have been affected by the dietary treatments. Proportion of birds without intestinal lesions was significantly lower for the group PC compared to the non-challenged animals (NC). For the groups GML0.4 and GML0.8, the

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proportion of birds without intestinal lesions was intermediate between the 2 control groups. In a context of light Necrotic Enteric challenge, a supplementation with 0.4 kg/T of α-monolaurin minimized the reduction of feed efficiency and the severity of intestinal lesions.

Keywords: alpha-monolaurin; broilers; necrotic enteritis; intestinal health; challenge

5196P Effects of Yeast Cell Wall Supplementation on Broiler Performance Under Chronic Heat Stress and Avian Pathogenic E. coli Challenge

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Avian colibacillosis, caused by avian pathogenic Escherichia coli (APEC), remains a major concern in broiler production, especially under environmental stress conditions such as heat stress. Yeast cell wall components have been proposed as functional feed additives to support gut health and immune response.

The objective of this study was to investigate the effects of dietary supplementation with a commercial yeast cell wall product (YCWP) on the performance of broilers exposed to chronic heat stress and challenged with APEC.

Four hundred forty one-day-old male Cobb broilers were allocated to four groups in a 2 × 2 factorial design: YCWP supplementation (1 kg/t starter; 500 g/t grower/finisher) vs. no YCWP, and APEC challenge vs. no challenge. Birds were reared under chronic heat stress (+32 °C) until 42 days. Two groups were orally challenged with APEC at day 14, 15, 16. Body weight (BW) was recorded weekly, and feed intake (FI) was measured per feeding phase to determine the average daily gain (ADG), average daily feed intake (ADFI) and Feed Conversion Ratio (FCR). Bacterial translocation (E. coli and Salmonella enumeration in liver) was assessed at days 14 and 21. Serum IgA and E. coli lipopolysaccharide (LPS) levels were measured at day 42. Data were analyzed using SPSS (v 24.0). A two-way analysis of variance (ANOVA) was performed using a linear model to assess the effects of dietary treatment (Control vs. YCWP), APEC challenge (Yes vs. No), and their interaction on performance parameters.

APEC challenge significantly reduced growth performance (final BW: 2565 g vs. 2679 g), ADFI (−2.7%), and increased FCR (+0.03 unit). Mortality was 1.8 times higher in challenged birds, and the European Efficiency Factor (EEF) was significantly reduced (367.4 vs. 401.7). YCWP supplementation significantly improved final BW (2659 g vs. 2585 g), reduced mortality (1.82% vs. 7.27%), and increased EEF by 27 units. A tendency for interaction was observed for ADFI, with the challenged control group showing the lowest intake. On day 21, APEC challenge significantly increased bacterial translocation, while YCWP significantly reduced both E. coli and Salmonella counts in the liver under challenge conditions. On day 42, LPS levels were numerically lower in challenged birds receiving YCWP. Serum IgA rose with APEC but was reduced by YCWP under challenge, suggesting moderated immune activation due to lower pathogen translocation.

This study shows that YCWP supplementation improves broiler performance and survival. It also mitigates the negative impact of APEC challenge on feed intake and enhances resilience through reduced pathogen translocation and moderated immune activation.

Keywords: Yeast cell wall; APEC; translocation

5197P In-feed antibiotics and in-ovo probiotics show limited effects on broiler performance, meat quality, and caecal fermentation under well-managed rearing conditions

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As antimicrobial resistance continues to rise worldwide, the poultry industry faces increasing pressure to reduce reliance on antibiotic growth promoters, prompting interest in probiotics as sustainable and biologically compatible alternatives. This study evaluated whether in ovo administration of probiotic strains could serve as an alternative to bacitracin by matching its effects on growth performance, meat quality, and caecal fermentation in broilers raised under standardized environmental and biosecurity conditions. Study design had two stages: in ovo treatment and post-hatch dietary intervention. Ross 308 fertile eggs were allocated to three in ovo treatments, with sufficient eggs injected to obtain 3240 hatched chicks (84% hatchability): a control group (T, n = 1620 chicks), a probiotic group injected in ovo with Bacillus subtilis and Enterococcus faecium (Pb1, n = 810 chicks, 1.5 × 107 CFU, 1:1), and a second probiotic group injected with E. faecium, Pediococcus acidilactici, Bifidobacterium animalis, and Lactobacillus reuteri (Pb2, n = 810 chicks, 1.5 × 107 CFU, 1:1:1:1). Upon hatching, the in ovo control (T) was subdivided into two post-hatch groups: one receiving bacitracin-supplemented feed (T+, 0.5 kg/ton) and one receiving a bacitracin-free basal diet (T-). Both in ovo probiotic groups (Pb1 and Pb2) were maintained on the bacitracin-free basal diet. This resulted in four final experimental groups (T+, T-, Pb1, Pb2), utilizing 18 replicate pens per group with 45 chicks per pen. Growth performance, feed efficiency, breast meat yield and quality (n = 36 samples/group), and caecal volatile fatty acids (12 samples/group) were measured. Data were analyzed using linear mixed-effects models with treatment and sex as fixed effects, and significant differences were identified using Tukey's test ($P < 0.05$). Overall growth performance did not differ among treatments. At d10, caecal butyrate and acetate concentrations were higher ($P < 0.05$) in Pb2 than in T+, whereas at d35 acetate concentrations were lower ($P < 0.05$) in Pb1 and Pb2 than in T- but not T+. Pb1 birds exhibited greater ($P < 0.05$) breast meat yield than T+ but not T-, and ultimate pH was higher ($P < 0.05$) in Pb1 than in both T+ and T-. Breast meat from Pb2 showed higher ($P < 0.05$) lightness (L*) and yellowness (b*) than T+ and T-, while redness (a*) did not differ. In conclusion, under well-managed conditions, neither bacitracin nor in ovo probiotics consistently improved broiler performance relative to an antibiotic-free diet, suggesting limited benefits in optimized production systems.

Keywords: Broiler; in-feed antibiotics; in-ovo probiotics; volatile fatty acids; growth performance

5198P Determination efficacy of polyherbal mixture against Eimeria in a sporozoite viability assay

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Coccidiosis is one of the most economically damaging diseases in global poultry production, driving substantial losses due to impaired performance, mortality, and the high cost of preventive measures. In this sense, the continuous appearance of resistance to traditional anticoccidials, the interest for natural alternatives has risen. This study evaluated the in vitro anticoccidial efficacy of a polyherbal mixture (PHM, PeptaSanTM contining 15% saponins and 1.0–1.5% tannins), against Eimeria tenella and Eimeria maxima using a sporozoite viability test (SVT). The test was conducted in Poulpharm (Belgium), where freshly excystated E. tenella and E. maxima sporozoites were brought to a final concentration of 1.4×106/mL and aliquoted in 4 replicates per treatment. The treatments were: negative control with phosphate-buffered saline (NC-PBS), solvent control NC-PBS with 1% Dimethyl sulfoxide

(DMSO), Salinomycin at 12 mg/kg in 1% DMSO in PBS (SAL), and PHM at 500 mg/kg in PBS (PHM). Treatments were added to the sporozoite suspension in a 1:1 ratio, and sporozoite concentration was determined using a Neubauer chamber. Subsequently, sporozoites were incubated for 1 h at 41○C, followed by an incubation at 37○C for 71 h. Sporozoite viability was assessed at 24, 48, and 72 hours post-induction (HPI), and reduction percentages were calculated relative to baseline. Data of reduction percentage of sporozoites in the in vitro trial were analyzed by a one-way ANOVA with Dunnett's multiple comparison test in Graphad Prism (v9). In vitro results showed that PHM increased ($P < 0.001$) the Eimeria sporozoite reduction percentage. A reduction in the amount of sporozoites was considered biologically relevant from 30% onwards. Treatment with PHM reduced the amount of E. tenella sporozoites in a similar manner to SAL with a mean reduction of approximately 54, 97, and 100 % at 24, 48, and 72 HPI, respectively. The effect of PHM on E. maxima sporozoites appeared to be even stronger compared to the effect of SAL, as PHM was able to completely reduce the amount of sporozoites within 24 HPI, whereas SAL induced a sporozoite reduction of 34 % at the same time point. SAL completely reduced the amount of sporozoites at 48 HPI. In conclusion, based on this in vitro assay, the supplementation with 500 mg/ kg of Peptasan™ reduced the viability of E. tenella and E. maxima sporozoites, showing an anticoccidial effect.

This result suggests that this polyherbal product, which contains a high amount of saponins, directly inhibited Eimeria life cycle, suggesting its potential use as a natural dietary strategy for controlling coccidiosis in poultry production systems.

Keywords: polyherbal supplementation; in vitro; coccidiosis; sporozoites viability assay

5199P Anticoccidial Efficacy Trial Report of Myristic Acid-Based Preparation

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This study aimed to evaluate the anticoccidial activity of a myristic acid-based preparation (commercially designated as "Gutmyria"). In the first trial, 50 twenty-day-old Rudong grass chickens with similar body weights were randomly divided into five groups: positive control (untreated-infected), negative control, and three treatment groups receiving Gutmyria at 200 g/t, 300 g/t, and zoalene at 125 g/t. At 22 days of age, all birds were inoculated via the crop with 8.5×10^4 sporulated oocysts of Eimeria tenella (Nantong strain). The trial ended on day 8 post-infection, during which fecal blood scores, survival rates, relative weight gain, cecal lesion scores, and oocyst counts were recorded, and Duncan's new multiple range test was used for significant analysis of average weight and average lesion score for each experimental group. According to the Anti-Coccidial Index (ACI), values above 180 were considered highly effective, values between 160 and 180 were considered moderately effective, and values between 120 and 160 were considered low effective. ACI was calculated following Merck's method: ACI = (Survival rate + Relative weight gain) - (Lesion score + Oocyst value).

Results showed that the positive control group had an ACI of only 101.54, whereas Gutmyria demonstrated significant dose-dependent anticoccidial activity, with ACI values of 128.70 and 141.38 for the 200 g/t and 300 g/t groups, respectively. Compared to the positive control, Gutmyria significantly improved weight gain, reduced cecal lesion scores, and decreased oocyst output. The 300 g/t Gutmyria group performed slightly better than the zoalene group. A repeat trial was conducted using 50 birds of the same age and breed, assigned to five groups including maduramicin- and Gutmyria-treated groups (300 g/t and 500 g/t). The positive control ACI was 107.3, while Gutmyria at 300 g/t and 500 g/t achieved ACI values of 144.87 and 162.51, respectively, showing significant improvements in all evaluated parameters. The 500 g/t Gutmyria group exhibited efficacy comparable to medium-efficacy anticoccidial drugs and outperformed maduramicin.

In conclusion, dietary supplementation with 300–500 g/t of the myristic acid-based preparation Gutmyria achieved ACI values comparable to those of synthetic anticoccidial drugs, suggesting its potential as a natural alternative to antibiotic coccidiostats. Further validation under field conditions is recommended.

Keywords: myristic acid; anticoccidials ; ACI(Anticoccidial Index); poultry; coccidiosis

5200P Benefits of using monensin and nicarbazin in combination compared with individual usage

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Objective: Demonstrate synergy when using monensin and nicarbazin together compared with using both products separately at a higher concentration.

Experimental design: An anticoccidial sensitivity trial was conducted using Ross 308 male chickens (six replicates per treatment with five birds per cage). The birds were fed a non-medicated starter diet from day 1 until day 12 followed by a grower diet supplemented with coccidiostats from day 12 until day 21 (except for both control groups which were fed a non-medicated grower diet). On day 14, the birds were challenged with an inoculum containing 28,797 E. acervulina and 20,853 E. maxima sporulated oocysts via gavage. The following treatments were included in the trial; an infected untreated control group (IUC), an uninfected untreated control group (UUC), a monensin group (100 ppm), a nicarbazin group (80 ppm) and a monensin/nicarbazin group (40/40 ppm).

Materials and method: The variables considered in this study were lesion scoring results of both E. acervulina and E. maxima according to the Johnson and Reid scoring system.

Statistical analysis: Species specific lesion scores were studied using ordinal regression models with treatment as the fixed effect. Models were not fit for species with low lesion scores. The UUC group was not included in the analysis to allow model fit.

Results: On day 19 lesion scoring was done on half of the birds. The score in the UUC group was 0.0 and 0.4 for E. acervulina and E. maxima, respectively, compared to 2.67 and 2.33 in the IUC group ($p < 0.001$). When comparing the groups supplemented with coccidiostats, the lesion scores for E. acervulina and E. maxima were respectively 2.07 and 1.67 for the monensin, 1.87 and 1.53 for nicarbazin and 1.87 and 1.20 for the monensin/nicarbazin supplemented group. The E. maxima score of 1.2 in the monensin/nicarbazin group was the only score that was significantly lower than the 2.33 score of the IUC group ($p < 0.001$). On day 21 the other half of the birds were scored. The following distribution of E. acervulina and E. maxima scores were found: 0.13 and 0.53 in the UUC group, 1.8 and 2.87 in the IUC group, 1.47 and 1.93 for the monensin, 1.47 and 2.13 for nicarbazin and 1.27 and 1.53 in the monensin/nicarbazin supplemented group. On day 21 the only significant differences with a p value < 0.001 were noted in the UUC group (both species) and in the monensin/nicarbazin treatment group (E. maxima).

Conclusions: The study demonstrated that combining monensin and nicarbazin resulted in a significantly lower score for E. maxima compared with using the products separately at a higher concentration.

Keywords: coccidiostats; AST; synergy; parasitology; monensin/nica-

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5201P A comparative study of technical performance parameters in broilers under two different coccidiosis control programs

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Objective: Determine the effect of different coccidiosis control programmes on performance indicators of broiler chickens under floor pen conditions with natural coccidiosis challenge.
Experimental Design: A floor pen study was conducted in the Netherlands in 2025 using 288 Ross 308 male chickens. There were 2 treatments, 8 replicates per treatment with 18 birds per replicate. All animals included in the study were housed in one compartment and subjected to similar ambient conditions including artificial heating, cooling or ventilation. The birds were fed a starter diet from day 0 till day 14, a grower diet from day 14 till day 28 and a finisher diet from day 28 till the end (day 35). On day 14 litter from a commercial broiler farm was added to all floor pens to simulate a natural coccidiosis challenge. Group A received monensin/nicarbazin in the starter diet at 50/50 ppm and salinomycin at 70 ppm in the grower and finisher diets. Group B received narasin/nicarbazin at 50/50 ppm in the starter diet and narasin at 70 ppm in the grower and finisher diet.
Materials and Methods: The variables which were considered in this study were live weight at day 0, 14, 28 and 35, feed intake per feeding phase, daily mortality, daily weight gain and FCR per feeding phase. Additionally, a commercial profit calculation was done.
Statistical Analysis: Performance data (BW, DWG, FCR) were analysed using a two-sample t-test (Excel). Pen was experimental unit, and significance was set at $p < 0.05$. Mortality was evaluated descriptively.
Results: The mortality rate of both groups was identical (4.86%). The body weight of group A and B on the different ages (day 0, 14, 28 and 35) were respectively 43.01 vs 43.05, 610.46 vs 607.83, 1846.56 vs 1841.72 and 2632.59 vs 2602.52. The FCR per feeding phase (starter, grower and finisher) for group A and B are respectively 0.96 vs 0.97, 1.46 vs 1.46 and 1.69 vs 1.76. the overall (day 0-35) difference in DWG and FCR is 73.11 vs 72.24 and 1.41 vs 1.42. The difference in DWG and FCR makes Group A 10 points higher EPEF compared to group B (493 vs 483). Additionally, the economic margin per bird was calculated as slaughter revenue minus the cost of the day-old chicken and feed. For group A the economic margin was1.48€ while group B had a benefit of 1.44 €.
Conclusions: The study demonstrated a general benefit for group A (monensin/nicarbazin followed by salinomycin) compared to group B (narasin/nicarbazin followed by narasin) on DWG and FCR. When evaluating the benefit per feeding phase it is clear that the biggest benefit is seen at the end of the trial which implies that changing the ionophore within the shuttle programme (monensin to salinomycin for group A) is beneficial.

Keywords: coccidiostats; performance; floor pen study; monensin/nicarbazin

5202P Characterization and Acute Toxicity Assessment of Chili and Amla Extract-Loaded Chitosan Nanoparticles in Broiler Chickens

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Extracts of chili and amla are of growing interest in broiler production but are limited by irritant properties, poor bioavailability, and rapid elimination. This study investigated the physicochemical characteristics and acute toxicity of chili- and amla-loaded chitosan nanoparticles in broilers. Nanoparticles were prepared via ionic gelation by mixing chitosan with sodium tripolyphosphate and subsequently loading with 2% chili or amla extract under continuous stirring. They were characterized for particle size, zeta potential, morphology, stability, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. Acute toxicity was evaluated in 24 broilers (42 days old), individually caged and assigned to eight treatments (n = 3 per treatment) in a completely randomized design: control (distilled water), 2% chili extract (CE), 2% amla extract (AE), 2% mixed extract (ME), unloaded chitosan nanoparticles (UN), 2% chili-loaded nanoparticles (CN), 2% amla-loaded nanoparticles (AN), and 2% mixed-loaded nanoparticles (MN). Treatments were administered orally for seven days. Hematological parameters and hepatic enzymes—alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP)—were measured, and data analyzed using ANOVA with Tukey's or Kruskal–Wallis tests. CN and AN had mean particle sizes of 133.9 and 313.2 nm, zeta potentials above +30 mV, PDI 0.2–0.4, nearly spherical morphology, and stability up to three weeks. Both CN and AN showed higher TPC and TFC than crude extracts ($P < 0.05$), while AN exhibited the highest antioxidant activity ($P < 0.05$). No adverse effects on body weight or liver and kidney function were observed ($P > 0.05$). Broilers fed mixed extracts, in crude or nano forms, had significantly lower ALT levels than controls (7.67 ± 1.20 and 8.00 ± 1.15 U/ml; $P < 0.05$). These results indicate that chili- and amla-loaded chitosan nanoparticles are safe and promising feed supplements for poultry production.

Keywords: Chili extract; Amla extract; Chitosan nanoparticles; Physicochemical characteristics; Toxicity

5203P Effects of Dietary Supplementation with Aqueous and Alcoholic Extracts of Rose Hip (Rosa canina) Fruit on Growth Performance and Carcass Characteristics in Broiler Chickens

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This study tested the hypothesis that supplementing broiler diets with aqueous or alcoholic extracts of rose hip fruit would enhance growth performance and improve carcass characteristics compared to an unsupplemented control diet. A completely randomized design (CRD) was used in this 42-day study. A total of 280 one-day-old male Ross 308 broiler chicks (average initial weight 45 ± 5 g) were randomly allotted to 7 dietary treatments with 4 replicates of 10 birds each. The experimental diets included: 1) a basal control diet, and diets supplemented with 2) 150, 3) 300, or 4) 450 mg/kg of aqueous rose hip extract, and 5) 150, 6) 300, or 7) 450 mg/kg of alcoholic rose hip extract. Growth performance parameters were measured weekly. Data were analyzed using one-way ANOVA in the General Linear Model procedure of SAS. Treatment means were compared using Tukey's test at $P < 0.05$. At 42 days, birds fed the diet containing 300 mg/kg of aqueous rose hip extract showed significantly higher ($P < 0.05$) final body weight and total weight gain compared to the control group. The highest feed intake was observed in birds receiving 150 mg/kg of alcoholic extract ($P < 0.05$). The lowest (most improved) feed conversion ratio was found in the 300 mg/kg aqueous extract group, and all extract-supplemented groups had significantly better ($P < 0.05$) feed conversion ratios than the control. No significant differences were observed among treatments for carcass yield or relative weights of breast, thighs, back, neck, or abdominal fat. Dietary supplementation with rose hip extracts, particularly 300 mg/kg of aqueous extract, effec-

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tively improved growth performance and feed efficiency in broiler chickens without altering carcass characteristics.
Keywords: Broiler; Performance; Feed Conversion Ratio; Carcass Yield; Rose Hip

5204P Metabolite postbiotic derived from Ligilactobacillus ingluviei C37 improves growth performance and attenuates intestinal injury in broilers challenged with lipopolysaccharides

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This study aimed to evaluate the efficacy of a metabolite postbiotic derived from Ligilactobacillus ingluviei C37 (M-LIC37) on growth performance, intestinal barrier function, and inflammation in lipopolysaccharide (LPS)-induced broiler chickens. One hundred forty-four healthy male broilers Ross 308 were randomly allocated to 4 treatments with 6 replications each in a CRD as follows: 1) control (CON), basal diet without LPS challenge; 2) negative control (NC), basal diet with LPS challenge; 3) positive control (PC), basal diet with bacitracin methylene disalicylate and LPS challenge; and 4) postbiotic group, basal diet with M-LIC37 and LPS challenge. From days 1 to 21, birds in the CON, NC, and PC groups received daily oral gavage of sterile PBS (1 mL/bird), while the M-LIC37 group received the M-LIC37 via oral gavage at the same volume. Starting on day 16, the CON group received intraperitoneal injections of PBS, while the NC, PC and M-LIC37 were challenged with Escherichia coli LPS (O55:B5) at 0.5 mg/kg body weight on days 16, 18, and 20. On day 21, growth performance parameter was recorded, and jejunal mucosa samples were collected for analysis of gut dysfunction and inflammation marker gene expressions. All data were analyzed using SPSS software, and mean comparisons among treatments were performed using Tukey's test. Differences were considered statistically significant at $P < 0.05$. The results revealed that orally administered M-LIC37 enhanced body weight and feed intake in LPS-challenged broilers and improved FCR to levels comparable to the unchallenged CON group. Additionally, M-LIC37 alleviated intestinal barrier dysfunctions by upregulating tight junction and mucin genes (MUC2, OCLD, and CLND) and attenuated inflammation by downregulating inflammation mediators (TLR4, LITAF, and NF-κB) in LPS-challenged birds. Furthermore, mRNA expression of proinflammatory cytokines, including IL-6 and TNFα, was reduced in the postbiotic group to levels similar to both Con and PC groups. These findings suggest that metabolite postbiotic M-LIC37 effectively improves growth performance, strengthens intestinal barrier function, and reduces inflammation in broilers under immunological stress conditions. M-LIC37 holds significant promise as a functional feed additive to support gut health and productivity, offering a viable alternative to in-feed antibiotics.
Keywords: metabolite; postbiotic; lipopolysaccharide; gut health; broiler

5205P Phytogenic Additive agProFito Mitigates Wooden Breast and Enhances Meat Quality in Broilers

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Global concern is increasing over the widespread use of antimicrobials in food-producing animals. Phytogenic additives have emerged as promising alternatives to conventional growth promoters, owing to their antimicrobial and antioxidant properties and their capacity to enhance digestibility, intestinal morphology, and meat quality (Windisch et al., 2008; Ren et al., 2025). agProFito (Agroceres Multimix) is a commercial phytogenic additive containing essential oils, plant extracts, and prebiotics. It is designed to modulate the intestinal microbiota, enhance gut integrity, improve nutrient utilization, and potentially contribute to better meat quality. This study aimed to evaluate the effects of agProFito supplementation in broiler diets on the incidence of wooden breast and the water-holding capacity of chilled breast fillets. Meat samples from 126 chickens distributed across three supplementation treatments: group A (control, no agProFito), group B (1 kg/t agProFito), and group C (2 kg/t agProFito). After chilling, breast fillets were weighed and scored for wooden breast on a four-point scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe), following Kuttappan et al. (2016), and subjected to drip loss analysis (Northcutt et al., 1994). The incidence of severe wooden breast scores was expressed as a percentage for each group and analyzed by Fisher's exact test. Water-holding capacity data were expressed as means ± standard error (SE), and statistical differences were evaluated using a one-way ANOVA followed by Tukey's post-hoc test. The level of significance was accepted at $p < 0.05$. Brazilian legislation updated the criteria for condemning woody breasts in 2017. It was proven that this myopathy is not related to microbiological agents and does not compromise public health. Therefore, only woody breasts classified as severe are condemned due to their repugnant appearance. For this reason, only the percentage of severe myopathies were considered as effective production losses. Broilers supplemented with agProFito showed a lower ($p < 0.05$) incidence of breasts classified as severe wooden breast. The data indicate a dose-dependent reduction, with losses of 7.14% in group A (no additive), 4.76% in group B, and 2.44% in group C. Breasts from groups B and C exhibited lower drip loss (4.82 ± 1.1 and 4.99 ± 1.3%, respectively; $p < 0.05$) compared with the control group (5.84 ± 1.2%). These findings suggest that agProFito may have exerted an antioxidant effect at the muscle-cell level, reducing cellular damage and, consequently, the intracellular water loss, the severity of myopathy and reducing meat production losses.
Keywords: essential oils; myopathy; antioxidant properties; plant extracts; water-holding capacity

5206P Hydrating Probiotic Gel Reduces Transport-Induced Weight Loss in Day-Old Broiler Chicks

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Transport conditions in the poultry production chain pose major challenges, particularly related to heat stress and hydration in day-old chicks. Due to their limited thermoregulatory capacity, chicks are highly susceptible to dehydration and associated declines in health and quality during transport from the hatchery to farm. This study evaluated the efficacy of a spherical hydrating probiotic gel composed of probiotics, prebiotics, vitamins, electrolytes, and essential and non-essential amino acids (Agroceres Multimix) in mitigating transport-induced weight loss, compared with a group that did not receive the gel. A total of 44 boxes of day-old chicks (22 replicates per treatment, n = 100 per box; average initial weight = 45.29 g) were assigned to two treatments: a group receiving 2 g of gel per chick during transport (gel-supplemented group); and a group receiving no gel (non-gel treatment group). All of the gel provided to the gel-supplemented group in the hatchery was fully consumed before the chicks were transported to the farm. Transport occurred under controlled environmental conditions over 572 km for approximately 8 hours. Body weight and bird counts were recorded at departure and arrival. Data were subjected to one-

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way ANOVA after confirming normality and homoscedasticity, and the means were compared using test t ($p < 0.05$). Chicks in the non-gel treatment group lost 5.21 ± 0.11% of the initial body weight, whereas supplemented gel- treatment chicks lost 4.10 ± 0.15 %, representing a 21% reduction in weight loss between the two groups ($p < 0.001$). These findings indicate that the evaluated gel supplementation during transport is effective in minimizing dehydration-related weight loss, thereby improving early body condition and chick quality. Further studies should investigate different transport durations, second-grade chick batches, and additional early performance indicators.

Keywords: body condition; chick quality; hydration; electrolyte supplementation; uniformity

5207P Effect of Muramidase Supplementation on Laying Hens: 1. Production Performance

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Objective: Peptidoglycan (PGN), which forms bacterial cell wall, has been reported to remain in the intestinal tract as cellular debris or accumulate on villi after bacterial growth or cell death. PGN is known not only to obstruct nutrient absorption by acting as cellular debris in the gut, but also to induce inflammatory responses by acting as an immune stimulant alongside lipoproteins. This is considered an opportunity for energy loss due to an excessive inflammatory response, which may negatively impact production performance in laying hens. Therefore, we evaluated whether supplemented muramidase, an enzyme that degrades PGN, could improve production performance of laying hens.

Experimental design: This trial used 3,400 Lohmann LSL hens aged 38 weeks, divided into two groups: a control group (CON) fed a diet of ME 2,800 kcal/kg, CP 14.67%, and a muramidase group (MUR) fed the CON diet supplemented with 30,000 units/kg feed of muramidase. The rearing environment was a curtain-sided poultry house, enabling ventilation by both natural airflow and exhaust fans. The housing system was conventional cages, with 8 birds per cage. The trial lasted 14 weeks, from 38 to 52 weeks of age. Throughout the experimental period, HD egg production, egg weight, feed intake, and other standard production indicators were evaluated weekly. Serum and egg yolk carotenoid levels and egg yolk color were compared at the start and end of the trial as indicators of improved nutrient absorption. Data were analyzed using two-sample t-tests, and all procedures complied with the ethical guidelines of Nippon Veterinary and Life Science University.

Results: No notable differences were observed four weeks after the start of the trial. However, from week 8 onwards, a tendency toward reduced feed intake was seen in MUR, while HD production and egg weight were maintained. MUR tendency outperformed CON regarding cumulative feed intake (104.7 vs 107.5 g/day), egg mass (57.9 vs 57.6 g/day), and FCR (1.81 vs 1.87) throughout the trial period. Serum carotenoids in MUR showed a tendency to be higher (2.0 vs 1.5 mg/kg; $p<0.1$). MUR improved carotenoid absorption, yielding significantly higher yolk levels at trial end (22.9 vs 16.7 mg/kg; $p<0.01$). Yolk Fan score also tended to improve by 0.9 points.

Conclusion: These results suggest that muramidase supplementation enhanced nutrient absorption and likely improved nutrient utilization in laying hens. The reduced feed intake with maintained egg production observed in this study indicates that muramidase may be a useful tool for controlling feed costs in commercial egg production.

Keywords: Muramidase; Peptidoglycan; FCR; Egg yolk color; Laying hens

5208P Effect of Muramidase Supplementation on Laying Hens: 2. Gastrointestinal Environment

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Objective: Muramidase supplementation has been shown to improve growth performance in broilers by degrading peptidoglycan (PGN), reducing gut debris, and enhancing nutrient absorption. Similar benefits, such as improved egg mass and feed conversion ratio, are anticipated in laying hens. Our previous study observed reduced feed intake without affecting egg production, though the underlying mechanism remains unclear. Therefore, this study investigated the effects of muramidase on intestinal villus morphology and on short-chain fatty acids (SCFAs) and organic acids (OAs) in cecal contents of laying hens.

Experimental design: A total of 3,400 Lohmann LSL hens aged 38 weeks were assigned to two dietary treatments for 14 weeks: a control diet (2,800 kcal/kg ME, 14.67% CP) or the same diet supplemented with muramidase at 30,000 units/kg feed (MUR). At 52 weeks of age, eight birds per group were randomly selected for sampling. Samples of duodenum and ileum were collected, fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin. Morphological features were then observed using hematoxylin and eosin staining for morphological evaluation. Cecal contents were also collected for profiling of SCFAs and OAs using precolumn-derivatized LC-MS/MS. Target analytes included lactic acid, beta-hydroxybutyric acid, acetic acid, propionic acid, isobutyric acid, butyric acid, 2-hydroglutaric acid, succinic acid, isovaleric acid, valeric acid, pyruvic acid, 2-oxobutyric acid, alpha-ketoglutaric acid, glycolic acid and malic acid. The data was calculated using arbitrary values. Group differences were analyzed using t-tests, and all procedures complied with the ethical guidelines of Nippon Veterinary and Life Science University.

Results and Discussion: No morphological changes due to MUR were observed in the duodenum or ileum; villus length, crypt length, and their ratio remained unchanged ($p > 0.05$). Analysis of cecal SCFAs and OAs revealed a significant decrease in lactic acid (1.25 vs 3.5; $p <0.01$) and a tendency toward increased alpha-ketoglutaric acid (0.54 vs 0.31; $p < 0.1$) in MUR. The reduction in cecal lactic acid and the increase in alpha-ketoglutaric acid observed in this study may indicate enhanced utilization of these metabolites as substrates for gluconeogenesis and/or amino acid mobilization in laying hens.

Conclusion: These findings suggest that muramidase supplementation does not adversely affect intestinal morphology in laying hens and may enhance the absorption or utilization of SCFAs and OAs. The observed metabolic changes in cecal contents indicate potential shifts in energy metabolism, including gluconeogenesis and amino acid–derived energy supply.

Keywords: Muramidase; Peptidoglycan; Intestinal morphology; Cecum SCFAs and organic acids; Laying hens

5209P Effects of Muramidase Supplementation on Laying Hens: 3. Plasma TCA Cycle-Related Metabolites and Central Melanocortin System

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Objective: Muramidase, an enzyme that hydrolyzes peptidoglycan (PGN) in bacterial cell walls, may improve nutrient absorption and reduce excessive immune response by degrading PGN in the gut. In laying hens, dietary muramidase has been associated with lower feed intake while maintaining egg production and improving egg mass and feed efficiency; however, the physiological mechanisms remain unclear. This study investigated the effects of muramidase on plasma TCA cycle-related metabolites and on hypothalamic melanocortin system regulation, a central regulator of energy homeostasis in laying hens.
Experimental design: A total of 3,400 Lohmann LSL hens at 38 weeks of age were assigned to two dietary treatments for 14 weeks: a control diet (2,800 kcal/kg ME, 14.67% CP) or the same diet supplemented with muramidase at 30,000 units/kg feed (MUR). At 52 weeks of age, eight birds per group were randomly selected for sampling. Plasma was isolated for targeted metabolite analysis by precolumn-derivatized LC-MS/MS, focusing on TCA cycle intermediates and related molecules including lactic acid, beta-hydroxybutyric acid, acetic acid, propionic acid, butyric acid, succinic acid, valeric acid, maleic acid, glyoxylic acid, pyruvic acid, 2-oxobutyric acid, alpha-ketoglutaric acid, glycolic acid, malic acid, malonic acid, and oxaloacetic acid. The hypothalamus was collected for quantitative real-time PCR analysis of AgRP, POMC, and melanocortin receptors MC3R, MC4R, and MC5R. Differences between groups were assessed with t-tests, and all procedures complied with the ethical guidelines of Nippon Veterinary and Life Science University.
Results: Muramidase supplementation significantly increased alpha-ketoglutaric acid and oxaloacetic acid concentrations ($P<0.05$), with tendencies for higher butyric acid, succinic acid, and malic acid ($P<0.1$). In the hypothalamus, POMC expression tended to be lower in MUR and AgRP expression tended to be higher in MUR, although neither difference reached significance ($P>0.1$). MC4R expression showed a decreasing trend ($P<0.1$), whereas MC3R and MC5R were unaffected.
Conclusion: These results suggest that muramidase supplementation may modulate systemic energy metabolism by increasing the availability of TCA cycle-related metabolites to meet energy demands in laying hens. The observed changes in melanocortin-related gene expression further indicate that central energy regulatory pathways may respond to muramidase. Taken together, muramidase supplementation may contribute to improvements in production efficiency through coordinated effects on metabolic status and hypothalamic regulation of energy balance.
Keywords: Muramidase; Plasma metabolites; TCA cycle; Melanocortin system; Laying hens

5210P Investigation of a Non-Invasive Method for Evaluating Protein Nutrition in Chickens Using Excreted Creatinine as an Indicator

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Objective: We investigated an evaluation method using excreted creatinine (Cre) as an indicator, which allows for a simple assessment of the protein/energy (P/E) balance during production capacity evaluation. First, the consistency between Cre excretion levels and growth or laying requirements was confirmed using broiler chicks and laying hens. Furthermore, to determine whether partial excretion of creatinine could indicate optimal P/E status, the ratio of Cre to uric acid in general nitrogen excretion was compared between chickens of different ages and birds with different diets, during both night and day periods. At last, effect of Muramidase Supplementation on Laying Hens on Cre/UA were evaluated.
Materials and Methods: In Experiments 1 and 2, 8-day-old broilers and 200-day-old White Leghorns were fed three levels of dietary protein for a 7-day-feeding trial. Cre levels in excreta and liver arginine-glycine amidinotransferase (AGAT) activity were measured during the final 3 days. In Experiment 3, excreta from 8-day-old broilers, 200-day-old laying hens (White Leghorn), 200-day-old laying Japanese quail, and zebra finches were collected under normal housing conditions together with samples of their respective diets. Excreta were collected twice daily, at 7:00 and 19:00. In Experiment 4, 3,400 Lohmann LSL chickens aged 38 weeks were raised for 14 weeks until 52 weeks of age in two groups: a control group (CON) fed a diet of 2,800 kcal/kg ME and 14.67% CP, and a muramidase group (MUR) fed the CON diet supplemented with 30,000 units of muramidase per kg of feed. At the end of the trial at 52 weeks of age, a portion of excreta was collected from each of the three replicates. The excreta were analyzed for Cre and uric acid (UA) content using standard methods. Data obtained were treated with one way or two way ANOVA and Turky's multiple range test all procedures complied with the ethical guidelines of Nippon Veterinary and Life Science University.
Results: In Experiments 1 and 2, both excreted Cre and hepatic AGAT activity decreased to optimal levels with increasing dietary protein content and then increased thereafter. In Experiment 3, the nighttime Cre/UA ratio was higher than the daytime ratio in broiler chicks, but no difference was observed in other bird species. The relationship between Cre and UA remained constant within each species when the same feed was provided. In Experiment 4, the Cre/UA ratio decreased in MUR compared to CON.
Conclusion: These results suggest that the excreted Cre/UA ratio is useful as a criterion to enable evaluation of whether a feed's P/E ratio was appropriate for chickens in a quick and simple manner.
Keywords: Chickens; Protein/energy status; Excreted creatinine; Excreted uric acid; Feed evaluation

5211P Effects of Supernatant of Fermented Maize (Omidun) on Haematological Parameters in Broiler Chickens

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Hypothesis / Objective
This study evaluated whether the supernatant of fermented maize (Omidun) containing naturally occurring lactic acid bacteria (LAB) could serve as a probiotic and influence haematological parameters in broiler chickens.
Experimental Design
A total of 150 day-old Cobb500 broilers were assigned to five groups (A–E) of 30 birds each, in triplicate. Group A served as control; Group B received commercial probiotics; Groups C, D, and E received standardized Omidun at 10, 5, and 2.5 mL/L respectively for six weeks.
Materials and Methods
Omidun was prepared by steeping yellow maize in water for three days. For microbial isolation, 0.1 ml of the standardized inoculum (0.5 McFarland; 150000000CFU/ml) was plated on De Man, Rogosa and Sharpe (MRS) agar and incubated at 37 °C for 24 h. Colony morphology was recorded, and isolates were subjected to Gram staining and catalase testing. Further biochemical identification included IMViC reactions, triple sugar iron (TSI) tests,

hydrogen sulphide and gas production, and carbohydrate fermentation (glucose, sucrose, lactose). The pH of raw and standardized Omidun was measured (range: 4–6). Haematological parameters (PCV, Hb, RBC, WBC, and differential counts) were determined using standard laboratory procedures. Weekly data were analyzed using one-way ANOVA, and significant differences ($p < 0.05$) were separated with Tukey's test.
Statistical Analysis
Weekly haematological data were analyzed by one-way ANOVA, with significant differences ($p < 0.05$) compared using Tukey's test. Data are presented as mean ± SE. All procedures were approved by the Faculty Animal Research Ethics Committee, Usmanu Danfodiyo University, Sokoto (AUP No: 2019/RO-1).
Results
All Omidun samples consistently yielded Lactobacillus spp., with colonies that were Gram-positive, catalase-positive, and short to medium rod-shaped. Biochemical tests showed negative results for indole, methyl red, Voges–Proskauer, citrate utilization, and gas production, while glucose, sucrose, and lactose were fermented. The pH of raw and standardized Omidun ranged from 4 to 6. Significant differences ($p < 0.05$) were observed in PCV, Hb, RBC, and monocyte counts at various weekly intervals, mainly between probiotic-treated groups and the control, whereas heterophil-to-lymphocyte ratios showed no significant variation.
Conclusions
Omidun contained viable Lactobacillus spp. and produced selective improvements in weekly haematological indices of broilers, comparable in some cases to commercial probiotics. These findings suggest Omidun may serve as a low-cost probiotic alternative in broiler production.
Keywords
Omidun; Broilers; Probiotics; Lactobacillus spp.; Haematology
Keywords: Omidun; Broilers; Probiotics; Lactobacillus spp; Haematology

5212P Combined effects of ionophores, enramycin, and plant extracts on broiler growth performance and carcass yield

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In commercial broiler diets, ionophores and/or chemical coccidiostats are typically added to control coccidiosis, while antibiotic growth promoters such as enramycin target Clostridium perfringens, preventing necrotic enteritis. These compounds are primarily intended to control pathogenic microorganisms, but they do not fully support gut health or immune function. A synergistic blend of plant extracts based on oregano, cloves, and cinnamon (FP) (Fytera Perform, Trouw Nutrition, The Netherlands) has been shown to improve gut health and reduce inflammation and may complement the effects of these conventional additives in broiler chickens. The present study evaluated the effects of combining coccidiostats (a 50:50 mix of nicarbazin and narasin), enramycin, and FP on broiler performance and carcass yield. A total of 960 straight-run Ross-308 broilers were assigned to four dietary treatments with 12 replicates per treatment: T1 – ionophores; T2 – ionophores + enramycin; T3 – ionophores, enramycin, and FP for 42 days; and T4 – ionophores, enramycin, and FP for 28 days, followed by FP alone for the remaining 14 days. Birds were reared in floor pens with fresh rice husks as bedding material. Performance indicators, including body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and mortality, were measured throughout the 42-day trial, while carcass traits were assessed on day 42. Data were analyzed using the MIXED procedure in SAS, and treatment means were compared using Tukey's test ($P < 0.05$).
Results indicated that both T3 and T4 significantly improved growth performance, showing higher BW and ADG ($P < 0.0001$) compared to T1, and exhibited the lowest FCR and abdominal fat percentage at day 42 ($P < 0.05$). Additionally, T3 and T4 had significantly higher dressing percentages than T1 and T2 ($P < 0.0001$), with no significant differences in mortality. Compared to T2, T4 significantly increased BW and ADG ($P < 0.05$), whereas the effects of T3 on these parameters were intermediate.
In conclusion, supplementing broiler diets with ionophores, enramycin, and FP (T3 and T4) enhanced growth performance and carcass yield compared to ionophores alone. The improved growth rate observed with T4 relative to T2 demonstrates the added benefits of FP when administered alone during the final phase, further enhancing broiler productivity.
Keywords: poultry; plant extracts; growth performance; carcass yield; ionophores

5213P INTERACTION BETWEEN LEVELS OF GARLIC AND CINNAMON EXTRACT AND LIPID INCLUSION ON THE PERFORMANCE OF BROILER CHICKENS

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The use of natural extracts as phytogenic additives has gained prominence in broiler chicken nutrition due to their antimicrobial and antioxidant potential. Concomitantly, different levels of lipid inclusion can alter the energetic utilization of the diet. Thus, the present study aimed to evaluate the effects of including garlic and cinnamon extracts and low and high soybean oil inclusion in the diet of broiler chickens on zootechnical performance parameters. The experiment was conducted at the Federal University of Grande Dourados (UFGD), Brazil, in an experimental broiler house. A total of 1,440 one-day-old male Ross TM4® chicks were housed and evaluated from 1 to 42 days of age. The birds were distributed in a completely randomized design, in a 2 x 3 factorial arrangement, with two levels of soybean oil (low and high) and three levels of garlic and cinnamon extract (0; 0.250 and 0.500 kg/ton), totaling six treatments, with six replicates and 40 birds per experimental unit. Zootechnical performance evaluations were performed at 7, 21, 35, and 42 days of age. Data were analyzed using the MIXED procedure of SAS, and means were compared by Tukey's test at 5% significance. There was no significant interaction between soybean oil inclusion level and garlic and cinnamon extract for any variable studied. However, from 1 to 21 days, an isolated effect of the extract on live weight ($P = 0.0038$) was observed, with the 0.500 kg/ton level providing the highest weight. In the total period (1 to 42 days), an isolated effect of the extract on viability ($P = 0.0444$) also occurred, which showed a linear increasing behavior, indicating improvement as inclusion increased. It is concluded that supplementation with garlic and cinnamon extracts represents a viable nutritional strategy for broiler chickens, as it does not compromise performance and can favor indicators such as live weight and flock viability. The levels of soybean oil inclusion did not influence performance, suggesting that adjustments in dietary energy density can be made without prejudice to the birds' development.
Keywords: Phytogenic additives; Performance; Zootechnical response; Soybean oil; Natural extracts

5214P Ileal fatty acid digestibility and energy utilization of MCFA-rich oils in grower–finisher broilers

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Medium-chain fatty acids (MCFAs) sources are rapidly absorbed and can influence digestive tract function. Black soldier fly oil (BSFO), palm kernel oil (PKO) and palm kernel fatty acid distillate (PKFAD) are some of the sources considered rich in MCFA, with PKFAD being a by-product of PKO refining that contains very high free fatty acid levels (FFA > 60%). Although these fats share a high lauric acid (C12:0) content, they differ substantially in both lipid class profile and FFA concentration. The aim of the present study was to evaluate the effects of incorporating these MCFA-rich fats into grower–finisher diets on ileal fatty acid (FA) digestibility and apparent metabolizable energy (AME) at 36 days of age. A total of 128 female Ross 308 broilers were assigned to four dietary treatments with eight replicates of 4 birds each. From day 21 to 36, birds were fed wheat–soybean meal basal diets supplemented with 6% added fat. The experimental fats were soybean oil (SO; 61.66% PUFA, 0.35% FFA), BSFO (36.18% MCFA, 1.51% FFA), PKO (48.79% MCFA, 2.87% FFA), and PKFAD (49.48% MCFA, 65.64% FFA). Ytterbium oxide was included as an inert marker. At 36 days of age, ileal digesta were collected to determine apparent FA digestibility and AME. Data were analyzed by one-way ANOVA, and means were separated using Tukey's test ($P \leq 0.05$). Broilers fed MCFA-rich diets (BSFO, PKO, and PKFAD) showed significantly higher digestibility of MCFAs, particularly lauric acid (C12:0), than those fed SO ($P < 0.001$), whereas SO promoted the highest digestibility of polyunsaturated FA (PUFA), mainly linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3), compared with all MCFA-rich diets ($P < 0.001$). No differences were detected among SO, BSFO, and PKO for long-chain saturated FA (LCSFA) and monounsaturated FA (MUFA). However, for total FA (TFA), SO and PKO presented the highest digestibility values relative to BSFO and PKFAD ($P < 0.001$). Notably, BSFO and PKO also showed similar digestibility profiles for most FA, yet PKFAD showed reduced ileal digestibility of TFA, LCSFA, and MCFA when directly compared with PKO. Consistently, AME was significantly lower in broilers fed PKFAD than in those receiving the other dietary treatments ($P < 0.001$), while SO, BSFO, and PKO did not differ in AME. Overall, ileal digestibility indicates that the elevated FFA concentration of PKFAD impaired digestion across multiple FA classes and reduced dietary energy recovery.

Keywords: Broiler chicken; MCFA-rich oils; ileal fatty acid digestibility ; apparent metabolizable energy; free fatty acids

5215P IMPACT OF NATURAL EXTRACTS AS SUBSTITUTES FOR ANTIMICROBIALS ON MEAT QUALITY IN BROILER CHICKENS

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The use of natural extracts as alternatives to antimicrobial growth promoters has gained prominence in modern poultry farming. Among them, garlic (Allium sativum) and cinnamon (Cinnamomum zeylanicum) exhibit antimicrobial and antioxidant properties that can positively influence the physicochemical and technological characteristics of meat. Therefore, this study aimed to evaluate the effects of including garlic and cinnamon extracts in the diet of broiler chickens on meat quality parameters at 42 days of age. The experiment was conducted at the Federal University of Grande Dourados (UFGD), Brazil, in an experimental broiler house. A total of 1,200 one-day-old male Ross TM4® chicks were housed and evaluated from 1 to 42 days of age. The birds were distributed in a completely randomized design with five treatments: T1 – Positive control (Bacitracin); T2 – Negative control (without antimicrobial and without additive); T3 – Inclusion of 0.250 kg/ton of garlic and cinnamon extract; T4 – 0.500 kg/ton; T5 – 0.750 kg/ton. Each treatment had six replicates and 40 birds per replicate, totaling 30 experimental units. At 42 days, two birds per replicate were weighed and slaughtered for evaluation of meat quality parameters. Data were subjected to analysis of variance using the MIXED procedure of SAS. At 42 days, all inclusion levels of garlic and cinnamon extract showed a significant and higher result for the fillet weight variable ($P = 0.0195$) when compared to the positive control (inclusion of Zinc Bacitracin). It is concluded that supplementation with garlic and cinnamon extracts has the potential to improve cut yield, without compromising the physicochemical and technological characteristics of broiler chicken meat. Thus, their use represents a viable alternative to traditional antimicrobials, contributing to more sustainable production systems aligned with the demand for more natural, safe, and consumer well-being-oriented foods.

Keywords: Garlic and cinnamon extracts; sustainable alternative; fillet weight; Allium sativum; Cinnamomum zeylanicum

5216P Productive performance of broiler chickens supplemented with two different essential oils

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This study evaluated the performance of broiler chickens supplemented with two different commercially available essential oil additives, compared with a non-supplemented control, under late-cycle heat stress. A total of 39 replicates (3 treatments; 13 per treatment; 30 birds each) of Hubbard Efficiency Plus broilers were raised for 42 days without AGP. All birds received maize–soybean meal-based basal diets formulated to be nutritionally equivalent across treatments. Three dietary treatments (T1–T3) were compared: T1 (Control) – basal diet with no additive; T2 – basal diet + 25 g/ton of a synergistic essential oil blend (oregano, clove, and cinnamon); and T3 – basal diet + 50 g/ton of a Macleaya cordata extract. In week 2, a colibacillosis outbreak occurred but was controlled in all groups, after which mortality return to normal levels. During the final two weeks, birds were exposed to acute heat stress (>36 °C). All performance data were analyzed by one-way ANOVA in a completely randomized design, and treatment means were compared using Tukey's test (significance at $P < 0.05$). Final BW tended to differ among treatments ($P=0.0650$), with Treatment 2 averaging 3.188 kg, exceeding Control (3.057 kg; +131 g) and the Treatment 3 (3.081 kg; +107 g). Daily weight gain showed a similar trend ($P=0.0649$), with Treatment 2 reaching 75.9 g/day versus 72.8 g/day in Control and 73.4 g/day in Treatment 3. The FI did not differ statistically ($P=0.3868$). FCR was not significantly different ($P = 0.3320$) among treatments. Total mortality was not statistically different among treatments ($P=0.1882$). The European Production Efficiency Factor (EPEF) was highest for Treatment 2 (471.6 points; $P=0.025$), outperforming both the Control (442.7) and the Treatment 3 (437.4). These findings indicate that the oregano–clove–cinnamon essential oil blend (T2) improved overall

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broiler performance under heat stress conditions relative to both the unsupplemented control and the Macleaya cordata extract, supporting its effectiveness as a phytogenic strategy to enhance production efficiency.
Keywords: Essential oil; Chicken broiler; Performance

5217P Effect of two mycotoxin detoxifiers on weight variability and physiological responses in broiler chickens raised on two litter types from 0 to 21 days of age
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This study evaluated the effect of two commercial detoxifiers (included at 1500 ppm) on body weight variability and physiological responses in broilers raised on new (N) or reused litter (R) from 0 to 21 days. Detoxifier 1 (D1) composed by aluminosilicates, inactive yeast, and biological biotransforming agents, whereas Detoxifier 2 (D2) contained modified clay minerals and yeast cell walls. A 2×2×3 factorial design was used, including litter type (N or R), diets without (WM) or with mycotoxin contamination (M; contamination achieved with 5% high-mycotoxin corn residue), and detoxifiers (C, D1, D2). A total of 1,920 Ross 308 chicks were assigned to 12 treatments with eight replicates. Individual body weights were recorded weekly. At day 21, serum parameters (ALT, AST, globulins, total protein and albumin) and intestinal morphometry were evaluated. Statistical analysis was performed using a GLM in RStudio, and pairwise comparisons were carried out using Tukey's test ($P \leq 0.05$).
Weight variability showed significant triple interactions at 7 and 14 days (P-value = 0.006). At 7 days, the lowest CV (14.79%) occurred in NxMxD1, followed by NxWMxC (15.28%) and RxWMxD2 (15.50%). At 14 days, the best uniformity (17.70%) was observed in NxWMxC, while D2 produced intermediate CVs (18.95–19.27%). At 21 days, only main effects were significant: litter (P-value = 0.038), dietary mycotoxins (P-value = 0.005) and detoxifier (P-value = 0.016). The lowest CV values were observed in N (17.5), WM (17.2), and D2 (16.9), indicating that the greatest uniformity at this age occurred under NxWMxD2. Intestinal morphometry showed reductions in total intestinal length (LT) (P-value = 0.009), jejunum/LT (P-value = 0.017) and ileum/LT (P-value = 0.030) in birds receiving D1. ALT showed a triple interaction (P-value = 0.030), globulins a litter × feed interaction (P-value = 0.041), and AST was influenced by litter type (P-value = 0.007), while total protein and albumin were unaffected.
These findings indicate that early weight variability in broilers is influenced by intestinal development and hepatic–immune responses, and that the best uniformity at 21 days is achieved under NxWMxD2.
Keywords: broilers; mycotoxins; detoxifiers; weight variability; physiological responses

5218P Evaluation of Hydroxy Trace Minerals on Post-Storage Egg Quality, Flock Uniformity, and Bone Mineral Retention in Lohmann LSL Laying Hens
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Trace mineral supplementation plays a relevant role in the performance and physiological integrity of commercial laying hens. Besides participating in eggshell formation and bone metabolism, the chemical form and absorption efficiency of trace minerals can influence flock uniformity, nutrient utilization, and egg quality. Hydroxy-based minerals offer higher stability and bioavailability than inorganic sources, potentially reducing excretion and improving physiological responses. This study evaluated different sources and inclusion levels of trace minerals on flock uniformity, body weight gain, post-storage egg quality, and tibia mineral retention in Lohmann LSL hens across five production phases. The trial was performed at CIANA (Jalisco, Mexico) using 1,040 hens at 21 weeks of age, randomly assigned to four dietary treatments with 13 replicates of 20 birds each. Treatments included: inorganic minerals (Control group), a group with 50% of inorganic minerals replaced by organic and hydroxy sources (IntOpt50), a group with 100% replacement (IntOpt100), and a group in which only inorganic Cu, Zn, and Mn were replaced by hydroxy minerals (IB100). Diets were isocaloric, isoproteic, based on corn and soybean meal, and provided ad libitum. Body weight and flock uniformity were assessed at weeks 31, 42, 52, 65, and 83. Egg quality after 21 days of room-temperature storage was evaluated through shell strength and internal quality using a DET 6000 analyzer. At 83 weeks, tibias from four hens per treatment were collected for ash and mineral analysis following AOAC methods. No significant differences were observed in eggshell strength after storage ($p>0.05$). Yolk color was significantly affected by dietary treatment, with IB100 producing the most intense pigmentation ($P<0.05$). The highest uniformity was recorded in hens fed IB100 (90.7%), with statistically significant differences observed between treatments ($p<0.05$). However, a reduction in BW was observed between weeks 65 and 83, with an average loss of 34.7 g across all treatments ($p<0.05$). Tibia ash content did not differ significantly among treatments ($p>0.05$). Phosphorus content was highest in the control group (7.44%) and in hens fed IntOpt50 (7.03%), with no significant differences detected ($p > 0.05$). Calcium content in tibia was significantly lower in hens fed IB100 (17.38%) compared to the Control group (23.27%), while intermediate values were observed in the IntOpt100 (15.66%) and IntOpt50 (19.42%) groups ($p < 0.05$). In conclusion, trace mineral source influenced flock uniformity, yolk pigmentation, and tibia mineral levels across the laying cycle.
Keywords: Bioavailability; Egg storage; Trace Minerals

5219P Effects of Zn- and Cu-Methionine Hydroxy-Analogue Chelates on Skin Integrity and Industrial Quality of carcass and cuts Broilers Under Commercial Conditions
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Broiler economic production efficiency is defined not only by growth performance but also by carcass and cut quality, as visual defects reduce processing yield and final product quality and therefore must be considered in nutritional strategies. Organic minerals such as Zn–Methionine Hydroxy-Analogue Chelate (Zn-MHAC) and Cu–Methionine Hydroxy-Analogue Chelate (Cu-MHAC) offer greater bioavailability and have the potential to enhance tissue integrity. The objective of this study was to evaluate the effect of supplementation programs with Zn-MHAC alone or in combination with Cu-MHAC on carcass and cut skin quality under commercial conditions. The trial was conducted in the subtropical Southern region of Brazil using a block design by production units with continuous monitoring over a three-month period. Broilers received diets according to each unit's nutritional program: T1 – 30 ppm Zn-MHAC, T2 – 40 ppm Zn-MHAC, and T3 – 40 ppm Zn-MHAC plus 30 ppm Cu-MHAC. Analyses were performed to assess skin quality through breaking force, energy to rupture, and elasticity. In parallel, industrial yield evaluations included

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skin and skin-on cuts (deboned thighs, wings, skin-on breasts, drumsticks, and thighs), all examined for skin integrity and visual defects that disqualify products from export markets. Statistical analyses demonstrated significant effects of nutritional programs on mechanical parameters ($p < 0.001$), while industrial data were compared based on percentage variation between programs. Results showed that the 30-ZnMHAC program had the lowest skin resistance, whereas both 40-ZnMHAC and 40-ZnMHAC + 30-CuMHAC increased breaking force (2.3 vs. 3.01 vs. 2.98 kg) and energy to rupture (7.48 vs. 8.83 vs. 8.86 kg/mm), respectively. In industrial evaluations, 40-ZnMHAC + 30-CuMHAC achieved the highest proportion of normal export skin (90.3% vs. 79.6% in 30-ZnMHAC and 83.4% in 40-ZnMHAC). Normal export skin was defined as skin presenting no visible lesions, discoloration, or defects, meeting international export standards. This program also reduced severe defects (4.6% vs. 9.2%). For deboned thigh, 40-ZnMHAC improved the proportion of normal pieces (86.4%), indicating greater mechanical resistance during processing. In conclusion, supplementation with Zn-MHAC improves broiler carcass skin mechanical resistance and industrial quality indicators, while the combination of Zn-MHAC + Cu-MHAC further enhances these effects by reducing defects, increasing the proportion of export-grade cuts, and strengthening tissue integrity on a large scale. These findings highlight the strategic role of organic minerals in optimizing skin quality, industrial efficiency, and competitiveness in broiler production.

Keywords: carcass skin quality; organic minerals; skin resistance; processing performance; broilers

5220P Comparing the efficacy of different organic zinc sources on growth performance of broiler chickens

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Mineral nutrition plays a fundamental role in poultry health and performance. Organic minerals, which are minerals bound to carbon-containing molecules, generally exhibit higher bioavailability and lower environmental excretion compared to inorganic sources. However, organic mineral sources are not equivalent, and their efficacy depends on multiple factors. This study evaluated the effectiveness of different organic zinc (Zn) sources on the growth performance of broiler chickens. A total of 2,000-day-old chicks were assigned to 10 treatments in a 5 × 2 factorial design (5 Zn sources × 2 Zn levels) with 10 replicates per treatment (20 birds/pen) and were reared for 42 days. Two diets were used: starter (1–21 days) and grower–finisher (21–42 days). Treatments differed by Zn source and inclusion level (40 or 80 mg/kg). One inorganic Zn source (Zn sulfate) and four organic sources (Zn proteinate, Zn glycinate, Zn methionate, and Zn amino acids chelate) were tested. Birds were housed in floor pens (1 m × 2 m) equipped with tube feeders and nipple drinkers, with ad libitum access to mash feed and water. Growth performance was recorded by pen during 1–21, 21–42, and 1–42 days. Statistical analysis was performed using PROC GLM (SAS), with data analyzed within each Zn level. Comparisons among Zn sources were conducted using two-way ANOVA and Duncan's test ($p < 0.10$). Broilers fed zinc amino acid chelates showed the greatest improvement in feed efficiency, with a reduction in feed conversion ratio of 2 points (NS) at 40 mg Zn/kg and 5 points ($p = 0.0346$) at 80 mg Zn/kg compared with broilers receiving other zinc sources. These birds also achieved greater final body weight at 42 days, with gains of +42 g at 40 mg Zn/kg (NS) and +57 g at 80 mg Zn/kg ($p = 0.0936$). Feed intake did not differ among Zn sources, except for Zn methionate (+170 g), which resulted in poorer FCR at 80 mg/kg. These findings highlight substantial differences in efficacy among Zn sources—both inorganic and organic. Even when selecting an organic mineral, rigorous evaluation is necessary to ensure precise and sustainable mineral nutrition in broiler production.

Keywords: Mineral nutrition; Zinc source; Bioavailability; Performance; Sustainability

5221P Circulating Hormonal Profile of Heat-Stressed Broilers Divergently Selected for Water Efficiency

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Climate change and increasing water scarcity are placing growing pressure on poultry production systems, particularly in fast-growing broilers that are especially vulnerable to heat stress (HS). Improving internal water-use efficiency has become a key priority for sustaining growth and performance under such environmental challenges. However, the physiological mechanisms that support water-use efficiency, especially under chronic HS, remain poorly understood. In this study, we examined how chronic HS influences circulating hormone levels and gene expression in broiler lines divergently selected for high (HWE) and low (LWE) water efficiency. From day 29 to 49, birds were raised under thermoneutral conditions (25°C) or exposed to daily heat stress (36°C for 9 hours) in a 2x2 factorial design. On day 49, blood was collected for ELISA-based hormone analysis, and qPCR and Western blot were used for gene and protein expression. Data were analyzed by two-way ANOVA and Tukey multiple comparison test and significance was set at $P < 0.05$. The results illustrate that plasma corticosterone and cortisol levels were significantly higher in heat-stressed LWE birds compared to their TN- and HS-HWE counterparts. indicating a greater physiological stress response. Under HS conditions, plasma angiotensinogen levels were numerically higher in HWE birds, however, insulin levels were higher in LWE birds. Cholecystokinin levels were higher in LWE birds under thermoneutral conditions. Gene expression of adiponectin, adiponectin receptor 2, and orexin receptor 2 was significantly upregulated in LWE birds compared to their HWE counterparts. In summary, the present study identified differential gene expression and hormonal profile in the blood of HWE and LWE birds and provide new insights on systemic HS response.

Keywords: Heat stress; Water efficiency; Hormonal profile; Gene Expression; Broiler

5222P Computational hemodynamics of poultry: first application of CTA–CFD to elucidate vascular adaptation and heat stress injury

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Background: Hemodynamics is central to cardiovascular physiology and pathology, with blood flow velocity, vascular resistance, pressure, and wall shear stress (WSS) serving as key indicators of vascular function. Although computed tomography angiography–based computational fluid dynamics (CTA–CFD) has been extensively applied in human and large-animal studies, its feasibility and interpretive value in poultry remain largely unexplored. Species-specific cardiovascular anatomy and technical limitations have constrained the acquisition of dynamic, functional hemodynamic data in avian models.

Method: In this pilot, proof-of-concept study, CTA–CFD was employed to evaluate the feasibility of non-invasive hemodynamic assessment in poultry. High-resolution CTA data were obtained from a healthy adult rooster, and three-dimensional reconstructions

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of the major arterial vasculature were generated for CFD simulations of blood flow velocity, pressure distribution, and WSS under baseline physiological conditions and experimentally induced heat stress.
Result: The analysis revealed that the poultry aorta exhibits a tapering geometry that accelerates blood flow and promotes preferential energy delivery to major arterial branches. Under physiological conditions, spatially organized WSS patterns were consistent with endothelial stability. In contrast, heat stress produced pronounced increases in flow velocity and pressure gradients, with regions of elevated WSS expanding by approximately 148%. These extreme WSS values are suggestive of endothelial perturbation and pro-inflammatory signaling, providing a plausible hemodynamic mechanism linking heat stress to increased risks of thrombosis, organ dysfunction, and mortality.
Conclusion: As a first-in-poultry, proof-of-concept investigation, this study demonstrates the technical feasibility and translational potential of CTA–CFD for avian cardiovascular research. Despite the limited sample size, the findings offer preliminary mechanistic insight into heat stress–associated vascular injury and establish a methodological foundation for future large-scale validation studies, precision breeding strategies.
Keywords: hemodynamics; poultry; computational fluid dynamics (CFD); numerical simulation; heat stress

5223P Comparison of the trace elements in eggshells from the developing male- and female-chick eggs
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As eggshells are mobilized to provide minerals for the growth of chick embryonic skeleton during incubation, it is interesting to investigate the difference of trace elements in eggshells between the male- and female-chick eggs for the possible indicators of in ovo sexing. Hence, this study aimed to identify the differences of trace elements in the eggshells collected from the fertilized eggs on Days 7, 9 and 13 of incubation. The fertilized eggs were purchased from Fortune Breeders Farm, which primarily supplies the LOHMANN layer strain. The gender of the embryos was identified by PCR while the eggshells were collected. Eggshells from the fertilized eggs were sampled at four distinct regions: the blunt end, the middle A side, the middle B side, and the sharp end. Trace mineral concentrations in each region were then determined semi-quantitatively using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results showed that the female eggshells had higher levels of Chromium (Cr) and Plumbum (Pb) than the male ones, and significant differences between sexes were observed on Day 9 of incubation. Lithium (Li), Chromium (Cr), and Tungsten (W) showed significant differences across Days 7, 9, and 13 in the female eggshells, and the highest levels were found on Day 9. The male eggshells exhibited significant differences in Argentum (Ag) levels on position 1 and position 2 on Day 9. In summary, the trace element profiles of eggshells exhibited distinct differences between male and female chick eggs, indicating their potential application in the development of an in ovo sexing technique.
Keywords: eggshell; in ova sexing; trace element

5224P Is a commercial ELISA suitable for the determination of steroid hormones in feces of poultry?
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The welfare of poultry kept in human care is gaining increasing importance in research, animal welfare, and practical applications. A well-established method for assessing stress and well-being is the measurement of stress hormones. However, the collection of saliva or blood samples can, due to the necessary handling of the animal, influence the samples and lead to elevated concentrations of the respective stress hormones. Therefore, many studies have investigated metabolites in excretions.
In these studies, using a commercial ELISA (IBL International, Hamburg, Germany), not the metabolites of the respective stress hormones were analyzed, but the primary hormones themselves. Since many species secrete both corticosterone and cortisol as active stress hormones, the direct detection of these hormones enable the determination of their ratio to one another. For example, during their first weeks of life, chickens (Gallus gallus) produce both corticosterone and cortisol as active stress hormones. Following a similar protocol, another steroid hormone, estradiol-17β, was also determined using an ELISA from IBL International. Estradiol-17ß plays a crucial role in the initiation of laying and in egg production performance. For each hormone, a dilution series in quadruple determination (N=40) was applied to the ELISA plate after drying, extraction, and resuspension in phosphate-buffered saline. The measured concentrations for corticosterone and cortisol ranged between 25 and 150 ng/ml, while concentrations for estradiol-17β ranged between 1200 and 1700 pg/ml. All results are within the detection limits of the kits.
The results show that the respective steroid hormones can be determined within physiological concentrations and within the detection limits of the ELISA kits used. Therefore, the commercial ELISA kits represent a suitable tool for detecting these hormones in the feces of poultry.
Keywords: feces; corticosterone; Cortisol; estradiol-17β; steroid hormones

5225P Cosinor-based rhythmometry reveals that heat stress disrupts the circadian rhythm of core temperature in broilers
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In response to the global increase in ambient temperatures, heat stress (HS) has emerged as a significant concern for livestock production. Core body temperature (CBT) has been a reliable indicator of HS in broilers. However, most research has focused on single-time-point measurements, thereby limiting our current understanding of thermoregulation in broilers. Consequently, the objective of this study was to conduct a longitudinal trial to evaluate the effect of HS on the diurnal pattern of CBT in finishing broilers. To this end, n = 96 mixed-sex chicks were divided into two groups: thermoneutral group (CON; 21°C, 49% relative humidity (RH)) and cyclic HS group (CHS; 33°C, 53% RH, from 10:00h to 16:00h) from 34 to 40 days of age. Each group consisted of 8 replicates of 6 birds.
Both environmental temperature and CBT were recorded every 5 min using a thermohygrometer and an ingested telemetry-based sensor, respectively, throughout the trial. Data were analyzed using both linear mixed models and cosinor-based rhythmometry. The models included the random effect of replicate and the fixed effects of thermal treatment, sex, time (or period for the cosinor model), as well as their interactions. The results indicated that HS significantly ($P < 0.01$) modulated the hourly CBT of broilers for 7

consecutive hours. The HS group had higher CBT from 12:00h to 16:00h but lower CBT at 18:00h and 19:00h compared to the CON group. However, no significant difference in the hourly average of CBT between male and female broilers was found, irrespective of thermal treatment. Additionally, cosinor-based rhythmometry highlighted that rhythmicity parameters were significantly ($P = 0.022$) influenced by thermal challenge. The HS group exhibited a slightly higher mesor (41.19°C vs. 41.00°C), greater amplitude (0.66°C vs. 0.21°C), and an expedited acrophase (13:52h vs. 15:25h), compared to the CON group. Besides, the CON male broilers exhibited a slightly higher amplitude ($P < 0.01$) compared to their female counterparts. Overall, the current study demonstrated that CHS disrupts the circadian rhythm of CBT in broilers by increasing their mesor and amplitude and synchronizing their acrophase to the time of the peak environmental temperature. Furthermore, broilers might adapt to cyclic HS by substantially decreasing their basal CBT during the cooler phase of the day.

Keywords: heat stress; climate change; chicken; circadian rhythm; rectal temperature

5226P Thyroid hormones modulate the reproductive axis during egg production in turkey hens

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In avian species, reproductive activity is regulated by multiple neuroendocrine axes, including the gonadotropic axis, the lactotropic axis, and the thyrotropic axis, which are key to enhancing egg production in the turkey industry. The objective of this study was to determine the influence of circulating thyroid hormone (TH) levels on the reproductive axis during peak egg production. Sixty average egg producing turkey hens (0.65–0.75 eggs per day) were assigned to 3 treatment groups: control (C, no treatment), the TH synthesis inhibitor Lugol's Iodide (LI; 0.1%), and synthetic thyroxine (T4; 1 ppm), dosed in the water for 2 weeks starting at 35 weeks of age (n=20 hens/treatment). At week 37, plasma samples were collected to measure progesterone, estradiol, and TH. Tissue samples were collected for RT-qPCR analysis of mRNA levels for candidate genes (n=16 hens/treatment). Statistical analyses were conducted using one-way ANOVA with the MIXED procedure in SAS, with $P<0.05$ considered significant. The results showed that, among the plasma hormones measured, only T4 levels were elevated by the T4 treatment. Gene expression analysis revealed that T4 and LI treatments affected both the gonadotropic and thyrotropic axes. In the gonadotropic axis, T4 decreased hypothalamic GNRH and pituitary GNRHR mRNA levels. Hypothalamic PGR and ESR1 increased slightly in the T4-treated group, while ESR2 dramatically decreased. T4 elevated ESR1 expression 3-fold in the pituitary, whereas ESR2 expression decreased dramatically. In the thyrotropic axis, pituitary TSHB expression markedly increased in the LI group but was reduced 13-fold in the T4-treated group, showing a similar pattern to TSHR expression in the thyroid gland. THRB expression in the thyroid decreased 5-fold following T4 treatment. Moreover, THRA expression was significantly increased in the liver of the LI group. T4 increased DIO3 levels 10-fold in the hypothalamus but reduced pituitary DIO1 and DIO2. Levels of TSHR, TPO, and TG in the thyroid gland were all dramatically reduced by T4 treatment. Liver TTR expression was elevated by T4 treatment. These findings demonstrate that TH can modulate both the gonadotropic and thyrotropic axes, potentially playing a central role in coordinating reproductive and metabolic regulation during peak egg production in laying turkey hens.

Keywords: egg production; hen; reproductive axes; thyroid hormones; turkey

5227P Effect of water deprivation on hypothalamic and renal water homeostasis gene expression in chickens selected for water efficiency

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Poultry production faces increasing challenges due to water scarcity driven by climate change, making genetic selection for water efficiency vitally important. This study aimed to investigate the effects of water deprivation (WD) on gene expression profiles related to hypothalamic and renal water homeostasis in high water conversion ratio (HWCR) and low water conversion ratio (LWCR) chicken lines. Birds from each line were raised with free access to feed and water in six pens per line (15 birds/pen). At 32 days of age, chickens from each line were assigned to two groups (3 pens/line/group): a control group with free access to water (0 h of WD) and an experimental group subjected to 12 h of WD, starting at 2:30 AM before the lights came on. Six birds per group per line were humanely euthanized by cervical dislocation, and hypothalamic and kidney samples were collected for gene expression analysis. Total RNA was isolated, and target genes associated with water homeostasis were quantified using reverse transcription-quantitative PCR (RT-qPCR). Data were analyzed using ANOVA with the mixed models procedure of SAS. In the hypothalamus, mRNA levels for arginine vasotocin (AVT), mesotocin (MT), ATPase Na+/K+ transporting subunit alpha 1 (ATP1A1), aquaporin 1, 2, and 3 (AQP1, AQP2, AQP3), nitric oxide synthase 2 (NOS2), neuropeptide Y (NPY), proopiomelanocortin (POMC), orexin (ORX), and pro-melanin concentrating hormone (MCH) were quantified. Levels of ATP1A1 mRNA decreased in response to WD in both HWCR and LWCR lines. Interestingly, AQP2 and AVT mRNA levels decreased following WD in the HWCR line but not in the LWCR line. In contrast, WD increased mRNA levels of AQP3 in the LWCR line but not in the HWCR line. In the kidney, mRNA levels for AQP1, AQP2, AQP3, AQP4, AQP7, AQP11, ATP1A1, ATP1B1, AVT, AVT receptor 1B (AVTR1B), AVT receptor 2 (AVTR2), MT, MT receptor (MTR), chloride voltage-gated channel Ka (CLCNKA), renin (REN), and angiotensinogen (AGT) were quantified. WD decreased mRNA levels for ATP1A1 and CLCNKA in both lines. Interestingly, AQP7, AVT, and AVTR2 mRNA levels decreased by WD in the LWCR but not in the HWCR line. In contrast, AVTR1B and MTR mRNA levels increased in the HWCR line but not in the LWCR line following WD. MT mRNA levels increased in the HWCR but decreased in the LWCR due to WD. Genetic selection for WCR altered mRNA levels for AVT, AQP2, and AQP3 in the hypothalamus, as well as mRNA levels for AQP7, AVT, AVTR2, AVTR1B, MT, and MTR in the kidney in response to WD. These differential responses might explain a portion of the WCR differences between the HWCR and LWCR genetic lines.

Keywords: Broiler; Gene expression; Hypothalamus; Kidney; Water efficiency

5228P Potential of interspecific hybrids for avian genome conservation through PGC incorporation

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Preservation of the female avian genome poses a significant challenge, as avian oocytes and embryos are not suitable to cryopreservation. To overcome this limitation, our research focused on the use of primordial germ cells (PGCs) as alternative carriers of genetic material. In this study, we investigated the potential of crossbred hybrids between guinea fowl (Numida meleagris) and domestic fowl (Gallus gallus domesticus) as recipient hosts for PGC transplantation.

Initially, we evaluated the viability and developmental competence of hybrid embryos for germ cell incorporation. Encouraged by preliminary results, GFP-expressing PGCs—isolated from transgenic White Leghorn chicken embryos—were injected into the bloodstream of 3 day-old hybrid embryos. Following injection, the eggs were incubated at 37.8 °C and 70% relative humidity.

Out of 156 injected embryos, twelve died before hatching (perinatal death), and five successfully hatched. Two hybrid individuals reached sexual maturity. Comparative analyses of testicular development revealed a markedly slower maturation rate in hybrids relative to domestic fowl, presumably attributable to impaired hormonal regulation. Histopathological examination of both mature hybrids confirmed extensive colonization of testicular tissue by GFP-labelled donor PGCs. In one specimen, spermatogonia, spermatocytes, and mature spermatozoa at various developmental stages were observed, while the 4 weeks younger hybrid exhibited colonization without fully developed spermatozoa. In contrast, control individuals were sterile and lacked detectable gametogenic activity.

Despite of crossing chicken and guinea fowl results only male offspring, these interspecific hybrids may serve as viable surrogate hosts for male and – with lower efficiency - for female PGCs, because previous studies reported that avian PGCs can differentiate into functional germ cells in the gonad of the opposite sex. To increase the efficiency of this method, our future research aim to use male and female mule ducks as sterile hybrid recipients.

Acknowledgement: HUSK/2302/1.2/018.
Keywords: interspecific hybrids; primordial germ cells; avian preservation; guinea fowl; domestic fowl

5229P Evaluation of the effects of grape seed and –skin micro powder enriched extender on the quality parameters of cryopreserved rooster semen

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Objective: The inbalance between ROS and antioxidant defence system of spermatozoa can be disrupted by the stress effects of cryopreservation, causing irreversible damage to cell functions. The antioxidant system of sperm can be supported by the use of various antioxidants. The blue grape seed micro-powder contain a complex of polyphenols, so their effect was tested on the results of rooster semen freezing using three different concentrations.

Materials and methods: Semen was collected with dorso-abdominal massage method twice a week from 15 individually placed one year old Black Transylvanian Naked Neck males. The collected pooled semen was divided into four equal parts. The control samples were diluted with Lake's extender, while the sperm diluent used for the experimental samples supplemented with 10, 50, and 100 µg/ml of blue grape seed and skin micro-powder. After 30 minutes of pre-cooling at 5°C, 6% DMA (dimethylacetamide) was added to the samples as a cryoprotectant. Freezing was performed in 0.25 ml straws 5 cm above the surface of liquid nitrogen for 15 minutes, followed by 1 cm for another 15 minutes. The samples were thawed in 5°C water bath for 1 minute. Motility was examined using the SCA® CASA system; while the live/dead cell ratio and the ratio of morphological abnormalities were determined using aniline blue-eosin staining. The TUNEL assay was carried out to examine DNA fragmentation. For the statistical analysis were used one-way ANOVA and Tukey HSD post-hoc test.

Results: The ratio of total motile cells did not increase using blue grape seed and skin extract moreover, when applied at a concentration of 10 µg/ml, it decreased (9.6% vs. 18.2%) compared to the control. In contrast, the proportion of live, intact cells gradually increased with increasing concentrations of grape seed extract, and was significantly ($p \leq 0{,}05$) higher at 100 µg/ml (14.9% vs. 21.2%), while the incidence of morphological abnormalities decreased (20.9% vs. 12.3%). The rate of DNA fragmentation increased more than twofold in the control frozen/thawed samples compared to the fresh (1.5% vs. 0.6%), while it did not change significantly in the experimental samples frozen/thawed with applied blue grape seed and skin micro-powder at the highest concentration (0.6% vs. 0.8%).

Conclusion: Based on in vitro studies blue grape seed and skin micro-powder did not affect sperm motility, but had a beneficial effect on the proportion of live, intact cells, live morphologically abnormal cells, and DNA fragmentation.

This experiment was supported by the Interreg VI-A Slovakia-Hungary Cooperation Programme (HUSK/2302/1.2/018) and Bock Winery.
Keywords: poultry; sperm; blue grape; cryopreservation; polyphenol

5230P Evaluation of Productive Parameters in Light and Medium Breed Male Chickens

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The culling of male chicks is common in the Mexican poultry industry, especially in egg production, since males of laying breeds are not profitable for meat. Although this has been highlighted in Europe, it also occurs in Mexico. Hypothesis: The productive performance of light and medium breed laying chickens will differ. Materials and Methods: The development of Bovans White and Dekalb Brown males fed a corn-soybean diet was compared. Three hundred birds (150 of each breed) were used, divided into two treatments and 10 replicates of 15 chickens each: 1) Bovans White light and 2) Dekalb Brown medium-heavy. There were two feeding phases: initiation (days 1-21) and completion (days 22-42). Water was free-choice until day 10; from day 11 to 42, daily consumption was recorded. Statistical analysis: The experimental design was completely randomized.

Where: Yij is the response observed in the i-th treatment in the j-th replicate, k-th repetition; µ is the overall mean; ti is the effect of the i-th treatment; Replica j is the effect of the j-th replicate; εijk is the random error. Estimators, confidence intervals, hypothesis tests, mean comparisons, correlations, and model generation were performed using SAS Studio (SAS OnDemand for Academics, SAS Institute, Cary, North Carolina, USA). Results: No statistically

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significant differences (P>0.05) were detected between treatments with respect to weight gain, feed intake, or feed conversion ratio over a 42-day period. Bovans strain demonstrated significantly higher yellow skin pigmentation than the Dekalb strain at 42 days of age (b*, P<0.05). No significant differences were found in lightness (L*) or red hue (a*). This assessment utilized reflectance colorimetry (Minolta, CIE L*a*b* system) following a dietary supplementation of 100 ppm yellow xanthophylls. Conclusion: Consequently, both strains showed equivalent productive performance during the first six weeks, although Bovans showed better skin pigmentation.
Keywords: Rearing of unwanted male chicks; Productive performance; Growth and efficiency; Light and semi-heavy layers strains; Aklternative to chick culling

5231P Incorporation of mealworm (Tenebrio molitor) and forages (Bituminaria bituminosa and Morus alba) in diet of Lohmann Dual hens

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The increasing global demand for eggs has driven the expansion of intensive livestock systems based on specialized layer genotypes, often at the expense of more extensive and environmentally sustainable production models. Nevertheless, growing attention has been directed toward alternative systems that integrate animal welfare and sustainability objectives, such as the use of dual-purpose breeds. A major challenge within these systems lies in feed formulation, particularly in meeting protein requirements, which constitute both an essential and costly component. Therefore, the present preliminary field study aimed to evaluate the inclusion of sustainable protein sources—specifically insect meal and locally available forages—on the productive performance of Lohmann Dual hens under practical free-range conditions. A total of 40 birds were randomly allocated to two groups (20 hens per group) and fed from week 27 to week 35 under different dietary treatments: T1 (commercial layer diet) and T2 (diet supplemented with 5% Bituminaria bituminosa, 5% Morus alba, and 5% Tenebrio molitor larvae powder). Both diets were isoproteic (16.5% crude protein) and with similar nutritional balance. At 33, 34 and 35 weeks of age, 10 eggs were randomly collected from each pen (30 eggs in total/diet) to perform egg trait measurements within the next 48 h after oviposition. Given the limited number of experimental units, statistical analyses were intended to provide an exploratory assessment of treatment effects. Data were analyzed using an independent-samples t-test. The results showed that hens from experimental group showed significantly lower feed intake (67.2 g/d) and laying rate (45.2%) compared to those from control group (78.2 g/d; 52.4%). However, no significant differences were detected for feed conversion ratio and egg weight between treatments. Likewise, yolk, albumen and eggshell proportions did not differ significantly. Yolk color analysis revealed a lower lightness (L*=61.2) and redness (a*=4.3) values, but a higher yellowness (b*=45.0) index in the experimental group respect to control group (L*=63.5; a*=5.7; b*=39.0). Finally, there were not significant differences for eggshell strength. The present findings indicate that incorporating insect meal and locally available forages into Lohmann Dual hen diet affected egg production and quality attributes. Although preliminary, these results provide useful field-based information, and longer-term studies with higher replication will be necessary to support the potential of these ingredients as sustainable protein sources in alternative poultry production systems.
Keywords: insect meal; mulberry forage; tedera forage; egg quality

5232P Comparative effects of conventional free-range and shrubby legume foraging systems on laying performance and egg physicochemical characteristics

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The incorporation of locally available, high-protein fodder species such as tagasaste (Chamaecytisus proliferus ssp. palmensis), an endemic leguminous shrub of the Canary Islands, into the diet of laying hens may represent a sustainable strategy to optimize feed resource utilization. Its inclusion in free-range production systems can enhance local feed autonomy, improve the use of regional protein sources, and reduce both feed costs and the overall carbon footprint, while contributing to the production of eggs with superior nutritional quality and lower environmental impact. The objective of this preliminary study was to evaluate the egg production performance and egg physicochemical traits of Lohmann Brown hens reared under different free-range systems. Three experimental groups, each consisting of 25 Lohmann Brown hens, 30 weeks of age, were used over a 12-week period. The first group was fed exclusively with a commercial layer diet (Control), the second group received the same diet supplemented each day with cut tagasaste forage (Trial 1), and the third group was allowed to graze on a tagasaste plot in addition to receiving the layer feed (Trial 2). Forty eggs were randomly collected from each pen during the last two weeks of the experimental period, and egg trait measurements were performed within 48 hours after oviposition. Given the limited number of experimental units, statistical analyses were conducted to provide an exploratory comparison among treatments. Diet effect was evaluated using a multiple comparison test by the Tukey's post hoc method. The results showed that there were no significant differences in hen body weight, which averaged approximately 1.82 kg across the three experimental groups. Nevertheless, the hens of the Trial 2 (6.42 eggs/hen/wk) had a higher egg production respect to the hens of the Control group (5.96 eggs/hen/wk). Likewise, the average egg weight of hens in Trial 2 (57.84 g) was higher than that of the Control group (55.48 g). However, no significant differences were detected in yolk, albumen, or eggshell proportions among treatments. Regarding yolk color, the lightness (L*), redness (a*), and yellowness (b*) indices of eggs from Trial 1 and Trial 2 were significantly higher than those of the Control group. Finally, no significant differences were observed in eggshell strength, which showed a mean value of 47.45 N. The present findings suggest that legume foraging on tagasaste may enhance egg production and quality traits in free-range systems. Although not derived from a fully replicated controlled experiment, these field-based results provide useful information, and further studies with greater replication are needed to confirm these trends.
Keywords: alternative system; tagasaste foraging; egg quality; Lohmann hen

5233P Effect of egg weight on fertility, hatchability and chick weight of local chickens in selected communities in the Dormaa Central Municipality of Ghana

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The rearing of indigenous chicken plays several important roles in the livelihood of rural households in Ghana. The objective of this study was to determine the effect of egg weight on the hatchability and chick weight of indigenous chicken in the Dormaa Central Municipality of the Bono region of Ghana. A total of one hundred and seventy-two (172) indigenous chicken hatching eggs were obtained from selected households in three communities (Dwen, Aboabo No. 1 and Ahomeka) in the Municipality. The eggs were selected based on shape and shell type and stored at room temperature for seven days. The eggs were individually weighed, marked and purposely grouped into light (33- 40 g), medium (41-48 g) and heavy (49-56 g) weight categories. Fifteen (15) eggs from each weight category were set in an incubator and replicated at three different periods due to the difficulty in obtaining large quantities of eggs (60 eggs per group) within a period of seven days. A one-way analysis of variance statistical method was used to determine the effect of egg weight on fertility and hatchability of eggs and day-old chick weight. The overall mean weight of the indigenous chicken eggs was 40.84 g. The distribution of light weight eggs in the study area was higher than those of medium and heavy weight categories. The mean percentages fertility, hatchability on set and hatchability on fertile eggs were 54.86%, 47.29% and 84.96%, respectively. Egg weight category had significant effect ($p<0.05$) on percentages fertility, hatchability on set and hatchability on fertile eggs. The medium weight eggs recorded the highest percentage fertility (65.75±4.5%). The medium weight eggs also recorded the highest percentage hatchability on set (62.18 ± 4.58%) whilst light weight eggs recorded the highest percentage hatchability on fertile eggs (94.79± 2.74%). The heavy weight eggs produced significantly heavier ($p<0.05$) day-old chick weight (33.22 ± 1.00 g) compared to medium (29.20 ± 0.61 g) and light weight (23.93 ± 0.75 g) egg categories. It can be concluded that indigenous chicken egg weight affects percentage fertility and hatchabilities hence egg weight should be considered in incubation of indigenous chicken eggs.

Keywords: chicken eggs; fertility; hatchability; chick weight; egg weight

5234P Multigenerational Analysis of Hybrid Vigor in Sasso × Local Chicken Crosses: Growth performance, resilience, and implications for sustainable breeding strategies in tropical poultry systems

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This study evaluated hybrid vigor between imported Sasso broilers and indigenous Togolese chickens using a two-generation breeding design. The first cross (G1) involved 75 Sasso hens and 25 local roosters to produce F1 hybrids, while the second generation (G2) resulted from reciprocal crosses between F1 chickens (F1 × F1). Purebred Sasso (SS) and local Togo ecotype (LL) served as control groups. A total of 110 chicks per genetic group (3 replicates) were reared in floor pens. Growth metrics included weekly body weight and daily feed intake, while resilience was assessed through daily mortality records and physiological stress indicators (panting scores) during peak dry-season heat (≥35°C). Statistical analysis used GraphPad Prism 5 with one way Anova, supplemented by Tukey's post-hoc tests. The results showed significantly higher egg weight ($p<0.005$) in SS groups compared to other genetic lines (SS > F1 > F2 = LL), with no differences between F2 and LL groups. Day-old chick weight and subsequent body weights were significantly higher ($p<0.005$) in F1 hybrids compared to F2 crosses, though Sasso chicks outperformed all groups while LL chicks showed the lowest weights. Feed intake and daily weight gain were lowest in LL groups, with no significant differences between F1 and F2 groups. The highest feed conversion ratio (FCR) occurred in SS groups, while LL groups showed the lowest FCR, with no differences between F1 and F2. Panting scores and mortality rates were significantly elevated ($p<0.005$) in SS groups compared to other genetic lines. It can be concluded that first-generation hybrids (F1) demonstrate substantial heterosis for growth traits while maintaining intermediate thermotolerance, offering a viable compromise between exotic broiler productivity and local breed adaptability. However, the rapid dissipation of hybrid vigor in F2 generations underscores the need for continuous crossbreeding strategies rather than intergenerational propagation.

Keys words: hybrid vigor, sustainable, poultry, resilience, tropical
Keywords: hybrid vigor; sustainable; poultry; resilience; tropical

5235P Effect of Calcium Propionate during the breeding stage of laying hens in a cage-free system

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An innovative strategy is the inclusion of natural additives in the diets of layer pullets. Organic acids (OA) are recognized as antioxidants, antimicrobials, and growth promoters in poultry nutrition. Calcium Propionate (CP) is an organic salt and acts as a gluconeogenic precursor, increasing glucose availability and helping maintain metabolic balance during periods of high energy demand or stress. In this context, the use of CP is proposed as an alternative to improve energy availability and mitigate metabolic stress in young birds. The purpose of the study was to evaluate the effect of calcium propionate supplementation in the diet on growth parameters during the breeding stage of laying hens in a cage-free system.

The experiment was conducted at the Unidad Académica de Producción Avícola located in Torreón, Coahuila, Mexico. The birds were housed in a cage-free system with a stocking density of 15 birds per m2. The study was conducted with 120 birds aged 28 days to 20 weeks, spanning 16 weeks, and distributed into two treatments with four replicates each. A Completely Randomized Design (CRD) was applied to evaluate the effects of the treatments. CP was added to the commercial basal diet at 1 %. Productive variables—including weight gain, feed intake, tarsus length (TL), and feed conversion ratio (FCR)—were recorded weekly. Both TL and body weight (BW) were measured in live birds. TL was determined as the distance between the intertarsal and metatarsophalangeal joints using a digital caliper. BW was recorded to two decimal places using a precision digital scale to ensure data accuracy. The data obtained were analyzed using mean comparison (Student's t-test) with a p-value<0.05. Statistical analysis was performed using SPSS 28 software.

The average BW at week 20 was 999.48 g in the treated group, compared to 989.42 g in the control group. The results showed no significant differences among treatments ($p > 0.05$). Similarly, TL was 52.38 mm in the control group and 52.81 mm in the treated group. On the other hand, feed intake in the treated group was 67.78 g in week 5, 76.68 g in week 11, and 123.67 g in week 20; whereas the control group consumed 76.61 g, 108.07 g, and 83.05 g, respectively, showing a significant difference between treatments. Finally, no significant difference in FCR was observed between the treated and control groups.

Supplementation with CP during the breeding stage of laying hens in a cage-free system showed no negative effects on body weight, tarsus growth, or feed conversion ratio during the initiation stage.

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Although differences in feed intake were observed between treatments in specific weeks, these variations were not consistent across the 16-week study.
Keywords: Additives; Cage-Free ; Laying Hens; Organic Acids; Metabolism

5236P Molecular characterization of Newcastle disease virus and the disease controlling interventions for chicken at farmer's level

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Backyard chicken play a vital role in our national economy, meet-up protein demand, building rural economy and women empowerment. Newcastle disease (ND) is one of the highly contagious and considered as the cause of the highest economic loss in village chickens worldwide. ND had the highest proportional mortality rate and annual loss in Bangladesh is 288.49 million US dollar. Therefore, the study was carried out 3 years (2022-2025) to unveil the status of ND in backyard chickens of a community and to monitor the immunity of ND after mass ND vaccination with some technical interventions to develop a ND controlling model village. A baseline survey was completed over 100 (n=70 female; n=30 male) backyard poultry households at Nowapara village under Bhanga Upazila, Faridpur, Bangladesh. Disinfection, deworming, demonstrations, training and enhancing bio-security programme were implemented there. Molecular identification of the NDV through one step RT-PCR with F gene sequencing and sero surveillance of ND before and after vaccination were done using Haemagglutination (HI) assay (β method). A total of 71 samples were collected from clinically suspected birds tested with one-step RT-PCR and sequenced after isolation. Sero surveillance of ND titer before and after (BCRDV, RDV) vaccination of total 133 birds (44 serum samples for pre-vaccinated and 101 samples for post-vaccinated) were done using Haemagglutination (HI) assay (β method). The survey result showed that the distribution pattern of total existing poultry population (n=1151) were as 58% (n=667) , 25% (n= 288) and 17% (196) for indigenous chicken, duck, pigeon. Farmers were faced different type of diseases. Only 6% farmer's knew about anthelmintics, 91% farmers are disposing slaughtering waste and carcasses in environments. Out of 71 samples, 25.35% (n=18) samples were detected for NDV. Among them 9 samples were isolated in the specific pathogen free embryonated chicken eggs and 3 samples were sequenced. The amino acid sequence of the F-protein cleavage site of three samples was 112R-R-Q-K-R-F117. The characteristics of the F gene of the isolates were velogenic, belonged to the class II, genotype VII, sub-genotype VII.2.i. The results of Ab titer level of HI test showed <8 log2 in 87.5% for pre-vaccinated birds whereas >8 log2 in 78% for post-vaccinated birds (after 21 days) in the first year. In second and third years, >8 log2 (protection level) in 80% of birds in post vaccination. Hence, vaccination significantly improved the birds' immune status and reduced the ND prevalence rate and mortality rate. Therefore, it is need to be continued all above mentioned activities for developing the Nowapara village as ND controlling model village.
Keywords: Backyard chickens; Newcastle disease; antibody; molecular characterization; interventions

5237P Establishment of primordial germ cell lines for germplasm preservation in the indigenous Oravka Chicken

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The domestic fowl represents a key avian model for research in developmental biology and reproductive physiology. In contrast to mammals - where reproductive technologies such as somatic cell nuclear transfer and embryo cryopreservation are routinely applied - avian species have biological limitations that prevent these approaches. Consequently, avian genetic conservation strategies rely predominantly on primordial germ cells (PGCs), the progenitors of sperm and oocytes. During early embryogenesis, PGCs can be isolated, and in the domestic chicken, maintained and expanded in vitro while retaining full germline competence. These cultured PGCs serve as a vital biological resource for germplasm preservation and can be reintroduced into recipient embryos to generate germline chimeras capable of producing donor-derived offspring. In the present study, we isolated, propagated, characterized, and cryopreserved PGCs from the Slovak indigenous chicken breed Oravka to establish a reference gene bank collection supporting the long-term conservation of this local genetic resource. A total of 64 embryonic blood samples were collected individually from Oravka embryos, and then cultured in vitro. Based on cell yield and quality, selected cultures were cryopreserved for inclusion in the gene bank. In total, 12 female and 6 male PGC lines were successfully established. Following comprehensive characterization - including immunohistochemistry, gene expression analysis, karyotyping, proliferation assays, and in vivo migration studies - 108 cryopreserved samples were added to the gene bank. This work represents the first successful derivation, culture, and cryopreservation of PGCs from the Oravka breed for genetic conservation purposes.

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Keywords: primordial germ cell; gene bank; cryopreservation; avian biotechnology; Oravka chicken breed

5238P Examination of alternative opportunity in the production of foie gras

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The force-feeding used in the production of foie gras is generating increasing concern, primarily due to animal welfare and ethical considerations. In order to preserve tradition and heritage, it is essential to explore alternative and innovative approaches to goose liver production. These alternatives must effectively meet economic, technological, and animal welfare requirements. The goal of the industry should be to keep making foie gras in a way that is sustainable, moral, and competitive. The aim of our study was to examine whether, with the right husbandry and feeding technology, it is possible to produce foie gras without force-feeding, using natural methods based on the animals' own feeding habits.
The rearing period lasted eight weeks. Individuals were divided into two groups: one group was force-fed (FF) for two weeks, and the other group was fattened without force-feeding (WFF) for six weeks. In the force-fed group, the geese were fed twice on the first day, three times on the following two days, and then four times a day at six-hour intervals. During the last four days, birds were

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fed every five hours. The initial feed quantity (100 g/bird/meal) was incrementally increased to 400 g/day. The diet consisted of premix (2%), maize semolina (70%), and grain maize (28%). In the group without force-feeding, the geese were fed twice a day for the first two weeks, three times a day for the next two weeks, and four times a day for the last two weeks. During the initial four weeks, the feed portions were increased by 200, 400, and 600 grams, respectively. In the final two weeks, the animals received an additional 400 grams of feed. Production parameters (mortality, live body weight, feed conversion, consumption, liver, breast, and thigh weights) and liver quality (colour and stiffness) were measured. The Shapiro–Wilk test for normality and two-tailed t-test were carried out by the R software package. The final live weight did not differ between groups (FF: 6345 g, WFF: 5937 g). There was no difference in feed conversion, consumption, thigh weight, liver stiffness and mortality parameters. Significantly higher breast weight was detected in the group without force-feeding. Liver weight was significantly raised in the FF group. The L* and b* values significantly increased in the FF group; oppositely, the a* value was enlarged in the WTH group. Self-steatosis is currently still in the experimental stage, but our test results and the knowledge we have gained since the experiment clearly show that it is possible to produce fattened livers without force-feeding, based on the natural metabolism of waterfowl.

Keywords: force feed; goose

5239P The effect of 25-hydroxyvitamin D3, plant based polyphenol vitamine E replacer and selenomethionine on the reproductive performance of breeding geese in indoor keeping system

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In recent years, animal health problems affecting poultry breeding have posed major challenges to goose breeders. Geese are most suited to extensive and semi-intensive farming; however, the development of indoor keeping systems and related research has become increasingly important. Animals kept in closed farming systems may be subjected to increased stress levels. To counteract this and improve production, feed supplements that reduce these negative effects should be used in future studies. In this study, we examined the effects of 25(OH)D3, plant-based polyphenol vitamin E replacer, selenomethionine (SeMet), and their combination on reproductive parameters when add to feed.

The study was carried out using the INTEGRAL MB 09 breed during the first production cycle. Based on our preliminary results, groups were established with a stocking density of 1.2 animals/m2 and a sex ratio of 1:3. The control group (n=210) received commercial feed for breeding geese, whereas in the experimental Group1 (n=247), half of the vitamin D source was replaced with 25(OH)D3. Group 2 (n=232) was supplemented with a plant-based polyphenol vitamin E replacer and SeMet, and Group 3 (n=240) received a combination of treatments applied in groups 1 and 2. During the production period, data on egg production and candling were collected. Sperm transport was assessed in 15 eggs per group (a total of 480 eggs) by determining the OPVL sperm counts every two weeks. Moreover, fecal corticosterone levels were measured in each group using ELISA. For the statistical analysis, ANOVA and Tukey HSD post-hoc tests were applied for normal distributions, whereas Kruskal Wallis ANOVA and Kolmogorov-Smirnov two-sample tests for not normal distribution.

Egg production in the control group (33.1 eggs/female) was significantly lower ($p \leq 0.05$) than in the other groups (41.0, 42.3, and 39.4 eggs/female).

No differences were observed between the groups in OPVL sperm count and candling results. The average fertility ranged from 92 to 94.6%. The proportion of eggs containing dead embryos ranged between 5.5 and 6.1% in the experimental groups. The average hatching rate varied from 71.3% to 79.7% among the groups, and values of the control group were significantly ($p \leq 0.05$) lower than those of groups 1 and 2 (71.3% vs. 79% and 79.7%, respectively). Fecal corticosterone levels did not differ between the groups; however, hormone levels increased in all groups compared with the beginning of production.

Keywords: goose; indoor keeping; reproduction; polyphenol

5240P Determining the effects of Para grass on meat quality parameters of local geese in Bangladesh

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Geese (Anser anser) are herbivorous waterfowl. Their gizzards are large and strong, allowing them to break down high-fiber roughages with great force and to consume enormous volumes of forages. The goal of this study was to evaluate the effect of adding Para grass (Brachiaria mutica) with commercial feed on carcass and breast meat quality traits of local Bangladeshi white and grey geese varieties. A semi-intensive production system was used to raise 144 adult geese of both white and grey kinds, at Bangladesh Livestock Research Institute, Headquarters, Savar, and other three Rrgional staion- Jashore, Rajshahi and Saidpur which were slaughtered at 14 weeks of age. They were distributed into four groups at random, each consisting of three replicates with three geese each in above mentioned four locations. Maize soya-based diet was considered as control diet (CL) (2700ME/Kcal, 16% CP) where T1 & T3 group were given 200 g of CL diets in white and grey variety respectively and other diets included T2 (White variety)- 140 g of para + 160 g of CL diet, T4 (Grey variety)- 140 g of water spinach + 160 g of CL diet. During rearing, the geese were individually weighed every week from 4th week to 14th weeks of age. As no significant differences were observed among the four regions, data were pooled and analyzed using one-way ANOVA in SPSS (23 version) following a random arrangement. There was no significant difference between groups for the growth. Statistical analysis was performed on 13 slaughter traits, which were separated into two groups: measured traits and calculated traits. There were no significant differences between groups for these traits neither the quality traits like- drip loss, cooking loss, pH, water holding capacity, proximate analysis, texture quality, TBARS value of breast meat except the redness value (a*) that was higher for T2 group ($p<0.05$). Replacing partly concentrate with forage reduced feed cost ($1.85/bird/14th week rearing period) and had no negative effects on the growth, carcass composition and quality traits of breast meat of geese.

Keywords: Geese; para grass; Meat quality; carcass traits

5241P Comparative analysis on productive performance, carcass characteristics and meat quality attributes of different varieties of turkey at marketing age

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The study aimed to compare growth performance, carcass characteristics, physio-chemical traits and proximate composition of meat among different turkey varieties raised up to marketing age. A total of 200 turkeys from different varieties were reared in floor pens and provided with balanced recommended ration (Starter diet: ME 2950 kcal/kg, CP 23% for initial 8 weeks; Grower diet: ME 2950 kcal/kg, CP 16% for 9-20 weeks). Proper vaccination schedule and bio-security were followed. Growth traits such as body weight, weekly gain, feed intake was recorded weekly. A 10-h fasting period was applied prior to slaughter and only water was provided during this period. Furthermore, the body weights of the birds were determined before slaughter. Then, abdominal fat content, edible organ weights and carcass weights were weighed following slaughter. Carcasses were cut into parts according to standard method. Weight was taken for all carcass parts. Meat color (L*, a*, b*) values were determined using Color Reader CR8 and meat pH were determined 24 hours of slaughter using pH meter (Hach, USA). Drip loss, cooking loss and water holding capacity were also determined according to standard method. The chemical composition of meat was analyzed on skinless breast and thigh meat stored at minus eighteen-degree Celsius. Dry matter, crude protein, crude fat, and ash contents were determined. Data were analyzed using one-way analysis of variance (ANOVA). Statistical analyses were performed using Statistical Analysis System (SAS) Institute Inc., Cary, NC, USA, (version 9.4M7, 2020) at a significance level of 5% followed by Duncan's multiple range tests. No significant differences were observed among turkey varieties in body weight, weekly weight gain. Bronze variety had numerically higher body weight (3231.60 g) at 20 weeks. No discernible difference was observed in carcass characteristics among four varieties of turkey. However, Bronze variety had numerically the highest dressing percentage (64.83%). Moreover, there was no noticeable difference found in physio-chemical parameters except drip loss and water holding capacity However, Black variety had the lowest drip loss (7.03%) and White variety had the highest water holding capacity (81.01%). Furthermore, proximate composition showed no variation ($p>0.05$) in moisture, crude protein and ether extract contents among varieties. Bronze had numerically higher crude protein percentage. Bronze demonstrated the best overall performance, while White and Black showed advantages in meat quality. These results support genetic selection for improved productivity and consumer-preferred turkey meat.
Keywords: Turkey; growth performance; carcass traits; meat quality; proximate composition

5242P Integrative multi-omics analysis identifies key genes and pathways underlying meat quality in geese

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Background
Meat quality in geese is a polygenic trait influenced by both genetic and environmental factors, but its genetic basis remains insufficiently characterized because systematic analyses remain limited. Clarifying its key genes and underlying pathways is critical for enhancing breeding efficiency and product quality.
Methods
To explore the genetic basis of meat quality variation, this study integrated genomic and transcriptomic approaches. A total of 255 individuals from five Chinese local breeds were evaluated for drip loss, shear force, pH, intramuscular fat (IMF) content, and meat color (L*, a*, b*). Two-way ANOVA was used to assess the effects of breed and sex. Two breeds with contrasting meat quality profiles, Sichuan White goose and Youjiang Gray goose, were selected for strand-specific RNA-seq of pectoral muscle samples (three biological replicates per sex per breed). Gene Set Enrichment Analysis (GSEA) was performed to infer meat quality-related pathways and GO terms. In parallel, a genome-wide association study (GWAS) using the FarmCPU model was conducted under dual significance thresholds (5e-7 and 1e-5) to minimize false negatives, identifying 223 candidate genes for enrichment analyses.
Results
Integrated analyses revealed multiple biological processes underlying meat quality variation, notably extracellular matrix (ECM) organization, MAPK signaling, focal adhesion, actin-cytoskeleton regulation, lipid metabolism, and autophagy. ADAMTS10 was closely related to ECM remodeling and postmortem pH regulation, indicating its role in water-holding capacity. DGKD and MOGAT2 were enriched in glycerolipid metabolism, suggesting involvement in lipid turnover and meat color formation. EGF and GADD45A participated in MAPK-mediated energy-stress responses that influence postmortem acidification and color stability. PTK2B, BCAR1, FLNB, and SYDE2 contributed to cytoskeletal organization and adhesion, affecting drip loss and shear force, whereas ATG7 was linked to mitochondrial autophagy and pH maintenance. Notably, the use of a relaxed GWAS threshold ($P < 1e-5$) successfully identified these candidate genes, and their biological relevance was further substantiated by convergent evidence from GSEA and PPI networks, which also revealed distinct sex-biased expression patterns.
Conclusion
This study provides integrative genomic and transcriptomic evidence linking key genes and pathways to meat quality variation in geese. The findings highlight molecular targets for functional validation and offer a framework for precision breeding aimed at improving meat quality in waterfowl.
Keywords: Goose ; Meat quality; Multi-omics; Key candidate genes; Molecular basis

5243P Reducing bacterial load on quail eggshells after washing with an essential oil–based solution

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The storage of quail eggs outside of refrigerated environments, whether in supermarkets or at home, accelerates the growth of bacteria on the eggshell. The more bacteria present on the eggshell, the greater the risk of contamination of the internal contents, which could render it unsafe for consumption. This study aimed to evaluate the antibacterial efficacy of a solution based on Eucalyptus globulus essential oil for washing quail eggs. 1,8-Cineole was the main compound identified in the essential oil by gas chromatography. The oil was subjected to antibacterial analysis using the disk diffusion method. In this analysis, each bacterial strain (Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922) was standardized to a turbidity equivalent to the 0.5 McFarland standard, and 100 µL of each suspension were subsequently inoculated onto Mueller–Hinton agar plates. Sterile filter paper discs, with or without 10% essential oil, were placed on the agar surface. After incubation of the plates at 36 °C for 24 hours, the inhibition zones were measured to determine the presence or absence of antibacterial activity. The assay was performed in triplicate. Twenty

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eggs were equally divided into two groups: washed and unwashed (n = 10 per group). A 10% essential oil solution with alcohol was prepared and applied to quail eggs, which were then left to dry at room temperature for 24 hours. After this period, the eggshell bacterial count was performed. All data were analyzed by ANOVA (PROC GLM), followed by Tukey's test at a 5% significance level, using SAS Studio University Edition (SAS Institute Inc., Cary, NC, USA). The essential oil of Eucalyptus globulus at 10% inhibited the growth of Staphylococcus aureus and Escherichia coli in the in vitro test, with similar efficiency ($P > 0.05$) against both bacteria. Quail eggshells washed with a solution containing 10% Eucalyptus globulus essential oil and stored at room temperature for 24 hours showed a 56.31% reduction in mesophilic bacterial counts ($P < 0.05$) compared to unwashed eggshells. No bacteria from the Enterobacteriaceae family ($P < 0.05$) were detected on eggshells washed with essential oil. A 10% Eucalyptus globulus essential oil-based solution can be used to control bacteria present on quail eggshells.

Keywords: Bacterial contamination; Eggs; Eucalyptus globulus ; Natural product

5244P A higher feed intake after pullet's transfer thanks to a botanical supplementation

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Pullet transfer to the laying house represents a significant source of stress, exposing birds to handling and vehicle vibrations, which compromise their welfare (Carlisle, A.J.,1998). A smooth transition from pullets to the laying phase is essential for lifetime productivity. Standardized botanicals may help facilitate this transition by alleviating the transfer stress through rosmarinic acid anxiolytic-like activity (Pereira, P., 2005). This study aimed to assess the effect of dietary supplementation with a standardized Melissa officinalis (Lemon Balm) extract combined with natural magnesium chloride and sulfates (CareZen) to alleviate transfer stress in pullets and to measure its impact on zootechnical performance.

Pullets of the same source from the same commercial caged house were allocated to a control (n = 6,912) or supplemented group (n = 5,774). Birds in the supplemented group were given CareZen via drinking water (2 L/1,000 L) for 6 days (3D before and 3D after transfer). 30-second videos were taken in each group after a third of the pullets had been transferred to assess stress-related feeding behavior in the new environment qualitatively. Over the four weeks following transfer, feed intake, egg production, and mortality were monitored at the group level.

Over the 4 weeks after transfer, feed intake, egg production, and mortality were monitored in each group. Data on daily egg production and weekly feed intake per hen were analyzed using a Mann–Whitney–Wilcoxon test, as repeated-measures analysis could not be robustly estimated due to the lack of replication at the experimental-unit level.

Birds in the supplemented group had higher feed intake than those in the control group (p-value < 0.05). The mean feed intake per hen per day was 96g in the control group and 114g in the supplemented group. No statistically significant differences were observed between groups for daily egg production or mortality ($p > 0.05$). Feeding behavior was assessed in the supplemented group, whereas it was not observed in the control group in the videos of visible birds. Visible control pullets also tend to stay at the back of the cages, away from feed.

The results suggest that supplementation with Melissa officinalis extract and magnesium may reduce stress while improving feed intake during the post-transfer adaptation period.

Carlisle, A.J, et al. (1998). Physiological responses of broiler chickens to the vibrations experienced during road transportation. British Poultry Science, 39(sup001), pp.48–49. doi:https://doi.org/10.1080/00071669888340.

Pereira, P. et al. (2005). Neurobehavioral and genotoxic aspects of rosmarinic acid. Pharmacological Research, 52(3), pp.199–203. doi:https://doi.org/10.1016/j.phrs.2005.03.003.

Keywords: Pullet; Transport stress; Melissa officinalis; Welfare; Feed Intake

5245P Validation of a tracking software to assess locomotor deviations in broiler chickens

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Lameness in broiler chickens is a common welfare problem, likely causing pain and effects on production. Environmental enrichment (EE) is an alternative practice to improve welfare, but there are a few quantitative methods to evaluate its results. The gait score (GS) is the gold standard for on-farm and research assessments. In search of objective measures to assess the onset of leg pathologies that could lead to poor GS, digital methods have been used to evaluate locomotor problems in broiler chickens.

Objective: To validate the use of Tracker software Modeling Analysis Tool for gait assessment in broilers reared under different EE conditions and to compare its use with GS on the 21 and 49 days of age (doa).

Methods: 300 1-day-old Cobb 500 chickens were used and separated into three groups (n = 100/group), divided into four treatments (n = 25/treatment): control, single-level perch (Perch L1), double-level perch (Perch L2), and platform. Video recordings were made on 21 and 49 doa. The broiler chickens were placed on a one m-long runway and recorded in a left-to-right and right-to-left sequence. The walking movement was divided into two moments (initial swing (IS) and terminal swing (TS)) for assessment of intertarsal joint angulation changes using the Tracker Video Analysis and Modeling Tool (version 6.2.0, USA). The GS was conducted as instructed in the Welfare protocol Quality, ® Assessment protocol for poultry.

The statistical analysis was conducted using a chi-square test of independence for gait score, and for the angle evaluation, a repeated-measures test with a Tukey post hoc test, both with an alpha of 0.05.

Results: The control group showed less average angulation in IS than the experimental groups at 21 doa (134.91° vs. mean 144.08°; $p<0.05$). Meanwhile a decrease in the average IS angulation was observed between 21 and 49 doa (mean 141.25° to 117.74°; $p<0.05$). During the TS, the broiler chickens in the PerchL2 group showed a decrease in angulation between 21 and 49 doa (138.87° to 127.62°; $p>0.05$). The GS did not identify differences between groups or age ($p=0.35$); exhibiting no lameness (90 % above GS 0) at 21 doa and variable levels of lameness at 49 doa (70% birds with a GS of 1-3).

Conclusion: The angulation tracker software was able to pinpoint joint angle alterations during walking movements at different doa and treatments, while the GS determine a general qualitative lameness. The use of EE can likely cause changes in motor capacity in

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broiler chickens as their age and body weight increase.
Keywords: Animal welfare, broiler chicken, gait score, tracking, environmental enrichment.
Keywords: Animal-welfare; Broiler-Chicken; gait-score; trackging ; environmental-enrichment.

5246P Overview of chicken transportation in Nigeria; need for humane education and policy enforcement

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The efficient and humane transportation of poultry remains a vital component of sustainable poultry production systems worldwide. In Nigeria, however, chicken transportation practices continue to pose significant welfare, economic, and public health challenges. Information on the overview of current chicken transportation methods in Nigeria, identifying critical welfare concerns and highlighting the need for humane education and effective policy enforcement is of topmost importance. Field observations and stakeholder interviews reveal widespread issues including overcrowding, inadequate ventilation, rough handling, and prolonged travel durations. These factors contribute to elevated stress, injury, and mortality rates among transported birds, resulting in economic losses and compromised meat quality. The study emphasizes that many transporters and traders lack awareness of animal welfare principles, while enforcement of existing animal transport regulations remains weak or inconsistent. Out of the 50 batches of broilers observed during transportation from the farm to the slaughter house, 80.00% journeyed over 50 kilometers at an average duration of 140 minutes because of bad roads. Average death in transit was 2.50% while haemorrhagic carcass and fractured wing was 12.50%. One-on-one interaction with 40 stakeholders (20 transporters and 20 fowl suppliers) revealed that 100.00% are not aware of any information on the standard stocking density for broilers during transportation and also on the existence of policies on humane livestock transportation in Nigeria.

To address these gaps, this research advocates for targetted humane education programs for poultry stakeholders and the integration of welfare standards into national poultry transport policies. It further calls for capacity-building initiatives and stronger institutional collaboration between government agencies, veterinary authorities, and industry stakeholders. Improving chicken transportation practices through education and policy enforcement will not only enhance animal welfare and product quality but also support Nigeria's alignment with global standards for ethical and sustainable poultry production.

Keywords: chickens; transportation; stress; welfare; humane

5247P Nest use patterns in laying hens: Individual preferences, "hot-spot" boxes and implications for nest capacity

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Electronic nest boxes with RFID identification provide high-resolution phenotyping of laying behavior, yet quantitative evidence for individual nest preferences and box-level "hot-spots" is scarce. This study aims to quantify individual nest preferences and nest box-level "hot-spots" in three local breeds using RFID-based electronic nests. Understanding these patterns is important, as consistent nest preferences can influence nest occupancy, production, breeding strategies, social dynamics, and welfare-related aspects such as frustration and competition.

We analyzed 86,213 nest visits of three local breeds (Bielefelder n = 100, Ramelsloher n = 96, Altsteirer n = 103) using RFID leg tags. Birds were housed in six pens (two replicate groups per breed), within a floor housing system. Each pen contained eight electronic nest boxes arranged in a 2x4 layout. For each nest visit, hen ID, nest ID, egg signal, bodyweight and visit duration were recorded. Chi-square tests were used to assess deviations from uniform nest use, and Kruskal-Wallis tests were applied to analyze individual differences in visit durations. Correlations between mean visit duration and body weight were evaluated using Pearson's r. Nest usage preferences were markedly non-uniform ($\chi2(47)=2,793.05$, $p<0.001$). Within compartments, some nest boxes consistently operated as "hot-spots" while others remained rarely used. Hens showed a clear preference for the upper row of nests, with the top left nest emerging as the main "hot-spot" in five of the six pens. These patterns were highly consistent across breeds.

At the individual level, nest visit durations differed strongly between hens ($n=243$, $H=8,636.02$, $p<0.001$, $\varepsilon2=0.424$), indicating consistent differences in how long individual birds stayed in the nests. When analyzed within each breed, the same pattern emerged (all $p<0.001$), demonstrating that these individual differences occurred across all breeds and were not primarily explained by breed-level variation. Tests for non-random nest use revealed clear deviations from uniformity: nearly all hens showed significant preferences (238/238 hens), and with 135 hens concentrating ≥35% of their egg visits in a single box. Further, mean nest visit duration correlated positively, but weakly, with body weight ($n=257$, $r=0,179$, $p=0.004$), suggesting that heavier hens tended to stay slightly longer inside the nest.

The consistent occurrence of "hot-spot" nests and strong individual preferences suggests that both environmental and individual factors shape laying behavior. Understanding these patterns may support more targeted nest design, placement and capacity planning, and provides a basis for welfare-oriented housing decisions and breeding strategies.

Keywords: nest use; RFID; individual behavior; local chicken breed; nest capacity

5248P Effect of classical conditioning on broiler chickens exposed to an amino acid-deficient diet

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Reducing crude protein in broiler diets requires maintaining an adequate supply of essential amino acids (EAA) to sustain performance and welfare. When EAA are deficient, birds exhibit increased exploratory and feeding-seeking behaviors, suggesting a capacity for nutritional self-regulation. Classical conditioning may enhance this adaptive ability by allowing a neutral stimulus, such as the color of a drinker, to acquire biological relevance when paired with a nutritional source. Based on this premise, this work hypothesized that broilers exposed to a classical conditioning process would be able to select conditioned stimuli associated with protein when subjected to dietary EAA deficiency. The objective was to evaluate the effect of an EAA-deficient diet on productive performance and classical conditioning responses in broiler chickens. A total of 128 male Ross 308 broilers were distributed in 32 floor pens (4 birds/pen) under a 2×2 factorial design: diet (standard protein, SP; amino acid–deficient, AA-D) and conditioning (applied or not), resulting in four treatments with eight replicates

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each. Conditioned groups underwent eight days of training in which visual cues (green or yellow drinkers) were associated with 3% sucrose or 3% protein solutions based on a commercial plant concentrate. The associative response was later evaluated through 96-h double-choice tests, where both drinkers contained only water to assess preference for the protein-conditioned stimulus (CS+). Intake was adjusted by metabolic body weight, and preference (%) was calculated. Data were analyzed by two-way ANOVA (diet, conditioning, and interaction). The diet had a significant effect on all performance variables ($p < 0.001$), with lower body weight, average daily gain, and average daily feed intake in birds fed the AA-D diet compared to those on the SP diet. Classical conditioning did not significantly affect growth performance ($p > 0.05$), but it improved the feed conversion ratio in conditioned birds relative to unconditioned ones ($p < 0.03$). The interaction between diet and conditioning was also significant ($p < 0.003$), suggesting behavioral modulation under nutrient restriction. In conclusion, dietary EAA restriction negatively affected broiler performance, whereas classical conditioning improved feed efficiency, possibly by influencing motivation or feeding behavior. These findings suggest that associative learning may complement nutritional strategies aimed at enhancing adaptation to amino acid–deficient diets.
Keywords: amino acid deficiency; broiler chicken; classical conditioning; growth performance

5249P The effect of dietary moisture level on the short-term feeding preference of laying hens

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Feed intake in laying hens, particularly those housed in alternative systems, can be challenging for the industry. Birds have a well-developed sense of taste, which influences their selection and acceptance of feed ingredients. In other species, increasing feed moisture has been shown to enhance feed intake. In fact, it is a common strategy in pet food and has also shown positive results in pigs. However, this approach remains scarcely explored in poultry nutrition. This study aimed to assess whether short-term feeding preference in laying hens varies with dietary moisture content. Forty-eight 79-week-old Bovans Brown laying hens were individually housed in enriched cages. After a 7-day training period, four experimental diets were prepared by adding 5, 10, 15, or 20% drinking water to a commercial mash feed. Each of these diets was compared against the dry control feed in a series of double-choice tests. Two identical feeders were placed in front of each cage, offering the control diet versus one of the water-added diets in a complete block design. Feed intake was recorded for two hours, twice daily, following a one-hour fasting period. Consumption was expressed as a preference index (percentage of test diet intake relative to total intake) and compared to the random choice value of 50% using Student's t-test. To minimize potential side or cage-location effects, the feeders were alternated between left and right positions in the two daily comparisons. A control comparison (dry feed vs. dry feed) was also included to validate the experimental design and, as expected, showed no significant differences ($P > 0.05$). In all cases (5, 10, 15, and 20% moisture), hens significantly preferred the water-added diets over the control feed ($P < 0.001$). Preference increased with higher moisture levels, reaching mean values of 25% for the control diet vs 75% for the 20% water-added diet. These results demonstrate that dietary moisture markedly influences feed preference in laying hens, which consistently favor moister diets. This finding highlights a promising opportunity for the poultry industry to improve feed acceptance. However, routine implementation would require careful evaluation of possible sanitary and logistical challenges associated with handling and distributing moister diets.
Keywords: Water; Moisture; Palatability; Preference; Double-choice

5250P Impact of bio-available silicium supplementation on resting behavior in broiler chickens with experimentally induced gouty arthritis

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Mean resting behavior in broiler chickens is commonly assessed through repeated observations at fixed time intervals and expressed as the proportion or percentage of birds at rest. This behavior is an important indicator of animal welfare, comfort, and health status. Prolonged resting is often associated with lameness or reduced mobility, whereas insufficient resting may reflect stress, discomfort, or environmental disturbance. In the present study, resting behavior was evaluated using video recordings, with 12 observations conducted at 10-minute intervals, recording birds exhibiting closed eyes. Lameness was experimentally induced in challenged control and treatment groups by intra-articular injection of 0.2 mL sodium urate solution (30 mg/mL) to induce gouty arthritis and increase resting behavior. The objective of this study was to evaluate the effect of bioavailable silicium (Poulsil®) on mean resting behavior in broiler chickens. A total of 144 day-old broiler chicks were randomly assigned to six experimental groups: one untreated challenged control (UCC; T01), four treatment groups (T02–T05), and one untreated unchallenged control (UUC; T06). Each group consisted of three replicate pens with 24 birds per pen, maintained under uniform management and nutritional conditions. The control groups received no silicium supplementation, while treatment groups were provided with different formulations and concentrations of silicium via drinking water. Groups T02 and T03 received a silicium–orthosilicic acid–vanillin complex (Si-OSA VC) at 1/1000 and 1/200 dilutions, respectively, whereas T04 and T05 received silicium–monomethylsilanetriol (Si-MMST) at the same dilutions. Corresponding silicium concentrations in drinking water were 0, 0.87, 4.33, 0.30, 1.48, and 0 mg/L for T01, T02, T03, T04, T05, and T06, respectively. On day 25, gouty arthritis was induced in the challenged groups, after which mean resting behavior was assessed across all groups. Behavioral data obtained from video recordings were analyzed using R (version 3.2.5). Treatments were compared against the UUC (negative control) and UCC (positive control), with statistical significance set at $P \leq 0.05$. Compared to the UCC group, mean resting behavior was numerically lower in T02 and T03 (not statistically significant), significantly reduced in T05, and highly significantly reduced ($P \leq 0.001$) in the UUC group. These findings indicate that supplementation with bioavailable silicium (Poulsil®) can reduce excessive resting behavior in broiler chickens, suggesting a potential benefit for improving leg health and overall welfare.
Keywords: bio-available silicium; broiler; mean resting behavior; welfare; poulsil

5251P Optimizing Electrical Stunning Parameters in Red-Feather Native Chickens

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Red-feather native chickens differ from fast-growing broilers in physiology and slaughter age, and may therefore require distinct electrical stunning parameters. This study evaluated the effects of voltage, age, sex, and physiological traits on stunning efficiency to identify optimal conditions for humane slaughter.
Male and female chickens at 9 weeks, 10–12 weeks, and 16 weeks were stunned individually in a pulsed direct-current water bath for 7 seconds using 120 V, 140 V, or 160 V (n = 25–30 per voltage per group). Stunning current, unconsciousness duration, and behavioural reflexes (spontaneous blinking, corneal reflex, wing flapping, tonic-clonic activity, neck tension, rhythmic breathing, and pain response) were recorded. Body weight, abdominal fat weight, and abdominal fat percentage (AFW%) were measured for each bird.
Pearson correlations and hierarchical regression models were used to evaluate the contributions of sex, voltage, body weight, and AFW% to unconsciousness duration. Group differences across voltages and ages were analysed using MANOVA and one-way ANOVA.
Across all age and sex groups, 140 V produced the most reliable stunning performance, yielding higher initial unconsciousness and significantly longer unconscious durations than 120 V ($p < 0.05$). Although 160 V generated higher current, its stunning outcomes were less consistent. AFW% was positively associated with unconscious time ($r = 0.149$, $p < 0.01$), whereas body weight showed no meaningful correlation ($r = -0.005$). Hierarchical regression confirmed AFW% as the only significant physiological predictor of unconscious duration (standardised coefficient = 0.179, $p = 0.003$; increase in explained variance = 0.017). Birds with higher abdominal fat percentage remained unconscious longer, indicating that internal body composition rather than body weight influences stunning response.
A 140 V, 7-second water-bath stunning protocol provides consistent and age-robust unconsciousness in red-feather native chickens. Abdominal fat percentage is an important determinant of unconscious duration and should be considered when refining slaughter parameters for slow-growing poultry. These findings provide essential evidence for improving humane slaughter guidelines in native chicken production systems.
Keywords: Red-feather native chicken; electrical stunning; unconsciousness duration; voltage; abdominal fat percentage

5252P Behavioral Expression and physiolocical stress in broilers as affected by stocking density levels
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Stocking density is a critical environmental factor influencing broiler welfare, behavior, and physiological stress responses. This study evaluated the effects of stocking density on broiler chickens by integrating welfare and behavioral indicators collected from a commercial high-density farm and an animal-welfare-certified low-density farm in Korea. Broilers were reared under two stocking densities: a low density of 16.7 birds/m2 (animal welfare certified farm; n = 32,000; initial body weight = 42.1 ± 0.32 g) and a high density of 20.3 birds/m (commercial farm; n = 32,155; initial body weight = 42.9 ± 0.31 g). All birds were of the Arbor Acres strain and were managed under typical commercial housing conditions with floor litter systems and standard feeding and lighting programs. Welfare indicators included growth performance, litter moisture, footpad dermatitis (FPD), feather cleanliness, ammonia gas emissions, and plasma corticosterone concentrations. Behavioral assessment was performed using instantaneous scan sampling at 2-min intervals, recording the frequency of standing, exploration, sitting, locomotion, feeding, and drinking behaviors. Statistical differences between treatments were analyzed using least-squares means comparisons, with the PDIFF option applied alongside t-tests to determine significance. Broilers reared at lower stocking density exhibited significantly greater body weight gain and feed intake during the grower phase, along with an improved feed conversion ratio. Welfare outcomes showed clear density-dependent differences: low-density flocks had reduced litter moisture, lower ammonia emissions, and significantly improved footpad and feather cleanliness scores by day 29. Corticosterone concentrations were 2.35% lower in birds housed at low density, indicating reduced physiological stress. Behavioral observations further demonstrated that increased space availability promoted higher levels of feeding, drinking, and locomotor activity, whereas prolonged sitting behavior—often associated with discomfort or restricted mobility—was more prevalent under high-density conditions. Overall, these findings demonstrate that reduced stocking density supports both behavioral expression and physiological stability in broilers. Compliance with lower stocking density standards required by animal welfare certification programs contributes to improved welfare by facilitating natural behaviors, reducing contact dermatitis, and lowering stress hormone levels. This integrated commercial-scale evaluation provides evidence-based support for the expansion of welfare-friendly production systems within the broiler industry.
Keywords: Animal welfare; Broiler; Stock density; FPD; Behvior

5253P Egg laying is a team event – social dynamics in nest visits of laying hens
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Nests are critical infrastructure for laying hens, including breeders, and therefore not only important for the economic outcome in terms of valuable eggs but also represent a resource-dependent welfare indicator reflecting the need for an undisturbed laying process. Despite the clear importance of nests as limited resources, little is known about how hens temporally coordinate access to nests or form consistent social partnerships during egg laying. In this context, RFID-based electronic nests offer high-resolution data that allow social network analysis to capture individual dynamics of nest use. The aim of this study was to characterize patterns of simultaneous nest use and assess whether hens show non-random social structure during egg laying, with implications for nest design and flock management.
Here we analyzed 19,534 egg laying events of three local breeds (Bielefelder n = 100, Ramelsloher n = 96, Altsteirer n = 103) kept in six separate pens (two per breed) equipped with 8 electronic nest boxes each in a floor housing system. Each hen was individually identifiable by RFID during the observational period of 25 weeks. For each nest visit, nest ID, hen ID, egg signal, and visit duration were recorded. Egg-laying events were defined as nest visits associated with an egg signal. For network construction, edges were created when two hens occupied adjacent nests (nest ± 1) with ≥ 5 min temporal overlap, representing synchronous laying. Edge weights therefore reflect the frequency of repeated synchronous laying events between a pair.
Across all six pens, a total of 2,176 synchonous dyads (≥5 min temporal overlap in adjacent nests) were detected. The frequency of repeated synchrony varied markedly between breeds. Biele-

felder hens formed the strongest and most frequent synchronous pairs (maximum edge weight = 12, with several dyads reaching ≥ 10 repetitions), followed by Ramelsloher (max = 9) and Altsteirer (max = 5). Notably, strong dyads (weight > 5) occurred almost exclusively in Bielefelder and Ramelsloher pens, whereas Altsteirer networks were dominated by low-weight, infrequent synchrony. Variation in individual nest-visit duration and daily laying routines is discussed as a methodological consideration, as it may influence overlap probability and therefore network structure. Overal, RFID-derived social networks provide a powerful tool to detect consistent synchrony patterns and potential social partnerships during egg laying. Future work will integrate genetic relationships to explore whether synchrony in nest use is associated with kinship.

Keywords: nest use; nest capacity; social network; RFID; local chicken breed

5254P Evaluation of egg production with enriched cages in Hy-Line W80

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Habitat enrichment is important for improving animal welfare; in egg-producing poultry systems, it is especially relevant due to the common production systems that involve large volumes of birds in reduced spaces. The objective of this study was to evaluate the effect of enrichment in caged production in Hy-Line W80 hens, 28 weeks of age, housed under two production systems: Enriched Cage (EC) and Conventional Cage (CC). Each treatment consisted of 10 replicates with 20 hens each, and the duration of the project was 8 weeks. Materials and Methods.

This study was conducted at the facilities of the Teaching, Research and Extension Center in Poultry Production of the Faculty of Veterinary Medicine and Animal Science at the National Autonomous University of Mexico. A naturally ventilated poultry house was used, where the birds were housed during the laying stage. The EC system used in this study corresponds to a housing model known as "Poultry Cage," with 261.56 in2 per hen. In contrast, the CC system had 139.50 in2 per hen. A repeated-measures linear model was used to evaluate the hens' productive characteristics and internal and external egg quality over the eight-week period. Results.

Regardless of time, egg mass, laying rate, average egg weight, clean eggs, and dirty eggs showed no significant differences between treatments (P>0.05); however, feed conversion ratio (higher in CC), feed intake (higher in CC), and broken eggs (higher in EC) showed statistically significant differences (P<0.01). With the exception of broken eggs (P>0.09), all variables studied changed over time regardless of treatment, due to the evaluation period coinciding with changes in the hens' production weeks. Conclusions.

The repeated-measures evaluation allowed extracting and analyzing the effect of the treatment. Egg production and production efficiency improved with enriched housing.

Keywords: mixed-effects linear models; repeated measures; egg quality

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