# Poultry Science Association

# Annual Meeting Abstracts

115th Annual Meeting

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# ORAL PRESENTATIONS

## Friday, July 10, 2026

### Student Competition: Extension and Instruction

**206 (E-F) (MTCC)**

**1 Chicken tracks:poultry programs as high-impact vehicle to superior youth development**

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Poultry Youth Programming (PYP) is an integrated educational model that builds skills beyond traditional Poultry Science competencies. While many educational settings struggle to develop the "soft skills" essential for professional success, PYP addresses this gap by using poultry-based learning as an accessible, real-world platform for holistic youth development. The program also functions as a leadership incubator through a tiered competitive structure spanning county, district, state, and national levels. Participants strengthen competencies such as oral reasoning, analytical decision-making under pressure, and the effective use of constructive feedback. Advancement opportunities include quiz bowl, poultry judging, and egg, chicken, and turkey culinary events, which reinforce both subject competence and professional skill development. This report will examine this from the perspective of youth participating in the Craven County 4-H Program, highlighting how engagement in PYP translates into tangible personal and professional growth from a local to national level. By exploring participant experiences across competitive and educational events, the report will assess how youth develop communication, leadership, and decision-making skills. It will also consider how mentorship, competition, and program accessibility contribute to confidence, adaptability, and goal-setting, providing insight into PYP's effectiveness as a youth development model. In North Carolina, participants further enhance these skills by mentoring and teaching younger members, supporting peers, and assisting with program delivery. Through a combination of in-person, virtual, and hands-on learning, youth gain experience adapting to diverse learning styles while reinforcing their own knowledge. By integrating technical poultry knowledge with leadership development and peer mentorship, PYP cultivates confident, articulate, and resilient individuals prepared for the demands of the modern workforce supporting PYP as a model for holistic youth development.

**Keywords:** youth development; poultry program; 4-H; life skills; workforce readiness

**2 Identifying 4-H Poultry Programming Needs in Iowa**

Cameron F. Hall*[1GS], Dawn Koltes[1], Amy Powell[1] [1]*Iowa State University, Animal Science, Ames, Iowa, United States*

Research in workforce development has demonstrated that experiences as youth shape their possible career paths, and lack of exposure can inhibit interest in a particular path. Therefore, it is critical in developing poultry workforce to increase avenues of poultry exposure to youth. Many youth gain poultry experience through 4-H programming. However, the state and national resources for poultry programing in 4-H might be a limiting factor for 4-H leaders to incorporate poultry in their local clubs. Therefore, the purpose of this project is to identify the 4-H poultry program needs by characterizing current educational opportunities provided, reference sources used, and the interest level in the development of a statewide poultry specific interest (SPIN) club. To complete these objectives, a survey was developed and distributed to country youth coordinators (CYC) in the Iowa State University Extension and Outreach which resulted in 100 distributed surveys. This survey had 72 respondents (72% response rate) which was evenly distributed across the state of Iowa. The average number of poultry interested youth as indicated by the CYCs was 25 youth per county, with a minimal of 10 youth and one county indicating over 150 youth interested in poultry. The CYCs indicated that over 59% (38/64) of the face-to-face educational opportunity for poultry are either not poultry specific or they are not provided. When asked about poultry-based resources, the CYCs indicated that the primary resource for poultry education are "adult volunteers" with either "non-Iowa based or national 4-H material" or "no material used" being the second ranking resource. When asked about poultry topics of interest, the CYCs indicated topics of interest by rank were "show preparation", "show selection", "nutrition", "health" and "chick care". Lastly, 95.8% of CYCs indicated if a virtual 4-H poultry SPIN club were developed they would have youth interest in attending. Based on this outcome, a virtual SPIN club was developed for the summer of 2025. The SPIN club had 30 youth participating in the program with the champion and reserve champion at the Iowa State Fair Special Broiler division having been participants in the program. With the start of the 2026 SPIN club, it has expanded beyond broilers and has 57 participants. These data indicated the strong need to increase support for resources and education of poultry to the CYCs to foster youth 4-H interest in poultry as possible career.

**Keywords:** youth; workforce development; education

**3 Identifying barriers and facilitators to the adoption of biosecurity practices by rural women in Punjab, Pakistan**

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Backyard poultry production in low- and middle-income countries (LMICs), such as Pakistan, is primarily managed by rural women. In many cases, these small-scale operations improve household nutritional outcomes and provide a source of income. However, due to poor biosecurity measures and absence of effective disease control strategies, backyard poultry systems are often particularly susceptible to disease outbreaks. These challenges can be further exacerbated by an overreliance on antibiotics to prevent or control bacterial infections. Improving biosecurity in backyard poultry systems in LMICs requires a better understanding of existing biosecurity and poultry management practices among small-scale, female poultry producers in order to identify knowledge and action gaps. This study applied behavior theory to identify the barriers and facilitators to adopting of biosecurity practices among rural female poultry producers in Pakistan. Three focus groups discussions (each comprising 9-12 partctipants) were conducted with rural women from different regions of Punjab, Pakistan. Data collection and analysis were based on the COM-B (capability, opportunity, motivation) model in combination with the theoretical domain framework (TDF). The analysis demonstrated that while women involved in backyard poultry production in Punjab were aware of biosecurity practices and demonstrated a basic understanding of them, they struggled with multiple challenges in implementing these practices effectively. Participants reported adopting certain precautionary measures during disease outbreak, partcilualry Newcastle disease in nearby areas. However, lack of opportunity to implement biosecurity practices (e.g., limited access to resources and financial constraints to purchase feed, medicine etc.) was identified as a major barrier. Additionally, participants noted limited access to biosecurity and animal health training led by women, which is necessary to align with cultural norms in rural Pakistani communities. As such, participants noted that they rely

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on traditional practices and knowledge shared within their communities. These findings demonstrate the need for effective context-specific interventions, including targeted education and training programs, and applied biosecurity strategies. This study also identified data-informed interventions and specific policy strategies to that could facilitate adoption of enhanced biosecurity practices in this audience, including education, training, enablement, incentivization and environmental restructuring.

**Keywords:** biosecurity; rural women; behavioral science; COM-B; TDF

### 4 Predictive modeling tools utilizing artificial intelligence can aid in digestible lysine requirement estimation for turkeys on a phased feeding program

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The ideal protein ratio ensures dietary amino acids (AA) align with metabolic needs. Lysine is the reference AA because it is exclusively used for tissue protein synthesis and is easy to analyze in feeds and feedstuffs. Lysine is fixed at 100% and other AA are expressed as percentage of the lysine requirement. As we consider a future where technology can assist in diet formulation, it is important to consider modern technological advancements, such as predictive modeling using artificial intelligence (AI). In this work, data were collected from 12 tom turkey production studies over 6 years from 1,289 pens containing 8,589 birds. Each study varied in length from 4-20 weeks. This database will become more robust over time. Average pen performance was compiled into 1,289 body weight data points and was used to train the model. While models can be manipulated to predict digestible lysine requirement based on the integrator's primary performance metric (feed conversion ratio, bodyweight (BW), average daily gain), this tool was trained to estimate digestible lysine requirements for phased feeding programs based on target BW. These AI models were trained using a 10-fold cross validation approach to evaluate metrics using a 75/25 ratio to split data into training and testing sets. The predictive modeling tool provided Mean Average Percentage Error (MAPE) of 1.71±0.2% and Coefficient of Determination ($R^2$) of 0.89±0.01 on the unseen testing set, considering average lysine values across all feeding phases. The current model utilizes turkey production data provided by the Pennsylvania State University; however, the model can utilize an integrator's historical data, allowing for customizable predictions based on individual operations. In conclusion, this predictive modeling tool using AI can aid nutritionists, live production managers, and feed mill operators in optimizing diet formulations that align with feeding efficiency and operational logistics to meet production goals.

**Keywords:** Prediction; Lysine; Performance; Artificial Intelligence; Formulations

## Student Competition: Immunology, Health and Disease - Health and Disease

**205 (A-D) (MTCC)**

### 5 Upper respiratory microbiota signatures associated with resilience to H7N1 high pathogenicity avian influenza in broiler chickens

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The objective of this study was to identify respiratory microbiota signatures linked to host resilience against H7N1 high pathogenicity avian influenza (HPAI) and to characterize how microbial communities influence disease variability. To this end, a longitudinal multi-omic analysis was conducted in a Ross 308 chicken model of H7N1 HPAI. Birds were grouped by clinical outcome: early mortality (M1), late mortality (M2), and resistance (RS). Fifteen-day-old chickens were challenged intranasally with H7N1 HPAI virus (A/Chicken/Italy/5093/1999) under BSL-3 conditions. Choanal swabs, nasal turbinates, and blood samples were collected at 0, 2, 4, and 10 days post-inoculation (DPI). We integrated choanal/nasal 16S rRNA sequencing with local (nasal turbinate) and systemic (blood) transcriptomics, plus blood proteomics. Paired samples were used to correlate microbial shifts with host immune and metabolic changes. Spearman's rank correlations assessed microbial-host expression links. Group differences were evaluated using FDR-adjusted $p < 0.05$. Most chickens died between 2 and 4 DPI, enabling classification into early mortality (M1, 2-4 DPI), late mortality (M2, >4 DPI), and resistant survivors (RS, 10 DPI). At baseline (0 DPI), microbial profiles suggested that pre-existing community compositions may influence host resilience. Although *Turicibacter* showed a modest association with survival, higher *Hungatella* levels were observed in birds that died late or survived, suggesting a link with the avoidance of acute mortality. During the acute phase (2-4 DPI), infection was associated with the downregulation of host mitochondrial respiratory genes in both M2 birds and RS birds. Notably, *Hungatella* abundance correlated inversely with mitochondrial expression and positively with host citrate synthase, suggesting a microbial role in supporting energy metabolism during infection. In contrast, late mortality was preceded by an ecological shift towards *Burkholderia_Caballeronia_Paraburkholderia*. Survivors were characterized by early *Klebsiella* peaks, which correlated with mucosal interferon-stimulated genes at 10 DPI. The persistence of *Pseudomonas* at 10 DPI may reflect the establishment of a stable post-infectious microbial state. Overall, resilience to H7N1 HPAI appears to involve pre-existing microbial signatures and host-metabolic interactions in the upper respiratory tract. Taxa such as *Hungatella* could serve as indicators of resilience, offering targets for precision probiotics to enhance poultry resilience to viral pathogens.

**Keywords:** H7N1 HPAI; Respiratory microbiota; Hungatella; Resilience; Metabolism

### 6 Computational Identification of Natural Inhibitors Targeting Fiber Proteins of FAdV-1 and FAdV-4 Through Integrated Virtual Screening and Molecular Dynamics Simulations

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Fowl adenoviruses (FAdVs) represent a major threat to poultry health, with serotypes FAdV-1 and FAdV-4 causing adenoviral gizzard erosion (AGE) and hepatitis-hydropericardium syndrome (HHS), respectively. A wide variety of afflicted birds, including chicken, pigeon, and psittacine species, have been reported to carry aviadenoviruses. The disease is highly contagious and spreads rapidly between flocks and farms through vertical and horizontal transmission. In this study, we implemented a multi-stage computational drug-discovery pipeline to identify natural inhibitors of the viral fiber proteins for both FAdV-1 and FAdV-4. A curated library of 7523 natural compounds from the African Natural Products Database (ANPDB) and the South African Natural Compounds Database (SANCDB) was subject-

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ed to ADMET-based filtering, molecular docking, ADMET prediction, and 500 ns molecular dynamics simulations against four structural targets: Fiber-1 and Fiber-2 of FAdV-4, and the Short and Long Fibers of FAdV-1. Three ligands, ANPDB_6449 (−10.3 kcal/mol), ANPDB_2908 (−10.2 and −10.0 kcal/mol), and SANCDB_245 (−9.2 kcal/mol), consistently emerged as strong candidates across the entire computational workflow. While ANPDB_2908 demonstrated notable multi-target capability by binding to fiber proteins from both FAdV-1 and FAdV-4, ANPDB_6449 and SANCDB_245 exhibited strong serotype-specific potential, supported by stable interaction profiles and favorable drug-likeness characteristics. Together, these compounds highlight promising natural scaffolds for the development of targeted antiviral interventions against pathogenic FAdV serotypes.

**Keywords:** FAdV; Fiber protein ; ADMET; ANPDB; SANCDB

**7 Impact of cold stress on immune responses, production performances and colibacillosis transmission in cage-free layer chickens**

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Avian colibacillosis caused by Avian Pathogenic *E. coli* (APEC) poses a significant threat to the poultry industry, causing economic losses and animal health concerns. Additional stressors, like cold stress, may further compromise production efficacy. We hypothesized that, exposure to APEC and cold stress would negatively impact chicken performance, immune response and increase the risk of horizontal transmission among layer hens. Cage-free layer hens underwent a two-phase experiment. In phase one, 162 birds (37 weeks old) were divided into 4 treatments: thermoneutral control (TNC) and thermoneutral infected (TNI) maintained at 70°F, and cold stress control (CSC) and cold stress infected (CSI) maintained at 40°F. Birds in TNI and CSI groups were orally challenged with APEC (3.28 x $10^{10}$ CFU/ml/bird). At 1 and 7 days post-infection (dpi), 8 birds per group were sampled for cecal bacterial loads. Additionally, peripheral blood mononuclear cells (PBMCs) and ileal intraepithelial lymphocytes (IEL) were isolated for flow cytometry analysis. In phase two, to investigate transmission dynamics, 77 hens (40 weeks old) per group TNI and CSI were placed at a stocking density 1.5 $ft^2$/bird in floor pen. Within each group, 30% of birds were challenged with the APEC and remaining were unchallenged. At 3, 7, 10, and 28 dpi, 8 unchallenged birds were sampled for cecal bacteriological analysis. Egg production and quality were monitored in both phases. Data were analyzed using unpaired *t*-tests, one-way ANOVA or the Kruskal–Wallis test (flow cytometry) as appropriate in GraphPad PRISM and significance set at $p < 0.05$. In phase one, no significant differences ($p > 0.05$) were observed among groups in body weight, egg production, egg quality and egg weight. At 1 dpi, the numbers of $TCR\Gamma\Delta^+$ and $TCR\Gamma\Delta^+CD8^+$ IEL subsets were significantly increased in TNI ($p < 0.05$) compared to TNC, while other IEL subsets remained unchanged. By 7dpi, The TNI maintained an increased number of $TCRAB^+CD8AA^+$ IEL. No significant difference was detected in PBMC subsets at 1 dpi; however, at 7dpi, heterophils and $TCRAB^+CD8^+$ cells were significantly higher in CSI group ($P < 0.05$) compared to TNC group. In phase two, no significant differences were also observed in production parameters. Bacterial colonies were detected in unchallenged birds in both groups at 3 dpi, although the levels were not statistically significant. By 28 dpi, bacterial recovery markedly declined, likely reflecting the development of effective immune response. Our results suggest that concurrent exposure to APEC and cold stress modulates the host immune response without significantly affecting egg production parameters and APEC transmission dynamics under the conditions of this study.

**Keywords:** immune response; cage free layer; egg production; cold stress; APEC

**8 NetB+ *Clostridium perfringens* strain has more pathogenicity and toxin production than NetB+/TpeL+ strain in embryonated chicken eggs**

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Poultry meat is an affordable source of animal protein and is central to global food security. Restricted antibiotic use in the industry in response to bacterial resistance has led to a resurgence of poultry diseases, including necrotic enteritis (NE). Among several toxinotypes, *Clostridium perfringens* (CP) type G is prevalent in NE outbreaks. While NetB, a pore-forming cytotoxin, plays a key role in NE development, TpeL, a glucosylating toxin, can enhance severity. This study investigated the (1) in ovo pathogenicity and (2) NetB toxin expression of two NE outbreak strains in chickens [strain 1 (S1): netB+/TpeL-; strain 2 (S2): netB+/TpeL+]. Two experiments were conducted to determine the in ovo pathogenicity and toxin expression of CP strains individually or in combination. In each experiment, 60 one-day-old embryonated eggs were incubated (37 °C, 55-60% relative humidity) in four groups [negative control (NC), S1, S2, co-culture of both strains (S1+S2)]. Eggs were candled from day 7 onwards, and fertile eggs were inoculated with CP ($10^7$ CFU/100 ML) or toxin (2-13 pg/100 ML) on day 10 in the allantoic cavity. Eggs inoculated with PBS served as NC. Sampling was done on day 13. Characteristic black colonies of CP were enumerated on Tryptose Sulfite Cycloserine agar supplemented with egg yolk plates and expressed as mean log10 CFU/mL. An indirect ELISA was used to quantify NetB toxins in filter-sterilized allantoic fluid samples. Embryo weights (g) were determined after gross embryo lesion scoring (scale 1-4). Embryo vitality was assessed as percentage mortality. All assays included at least 4 biological replicates and were repeated twice. Data were analyzed using ANOVA, followed by Tukey's HSD for embryo weights and CFU counts. Percent mortality and lesion scoring were analyzed using Fisher's exact test with pairwise comparisons, and Kruskal–Wallis test followed by Dunn's post hoc test, respectively. In the in ovo pathogenicity testing, all groups showed significant bacterial recovery ($P < 0.05$) compared with NC, and S1 and S2 produced more severe lesions ($P < 0.05$) than NC. S1 caused significantly higher mortality, lower embryo weights, and higher lesion scores than NC ($P<0.05$). In the in ovo toxin expression study, S1 produced significantly more NetB toxins than other groups ($P < 0.05$). The S1+S2 combination exhibited intermediate NetB toxin levels compared with the individual strains. The study indicates that S1 is a more pathogenic and NetB toxin-producing strain than S2 or their combination, and confirms that the NetB+ strain, not the NetB+/TpeL+ strain, has greater pathogenicity and toxin production and needs to be controlled in poultry production systems.

**Keywords:** Necrotic enteritis; Clostridium perfringens; NetB toxin; Tpel toxin; In ovo

**9 Necrotic enteritis challenge alters egg quality, body composition, and bone microarchitecture in laying hens**

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Necrotic enteritis (NE) is an enteric disease caused by *Clostridium perfringens* (CP). There are lots of predisposing factors. *Eimeria maxima* (EM) is one of them. NE is known to impair intestinal health and nutrient utilization in poultry. However, its effects on egg production and skeletal health in laying hens is not fully understood. The objective of this study was to evaluate the impact of NE challenge on egg production, egg quality, body composition, and bone microarchitecture in laying hens. A total of 144 Hy-line W-36 hens (32 weeks) were assigned to four treatments with six replications. The treatments include 1) non-challenge control (NC); 2) CP challenge at $1\times10^9$ colony forming unit; 3) EM Challenge at 20000 sporulated oocysts; and 4) EM+CP challenge. Birds were orally inoculated with EM at 32 weeks of age, and the day of inoculation is considered 0 days post infection (DPI), followed by Net B+ *Clostridium perfringens* Del-1 strain on 4 and 5 DPI. Intestinal lesion scores and CP counts in jejunal and cecal tissues and contents were determined to confirm NE challenge, with lesion scoring performed using a standardized 0–3 scale. Egg quality and production were measured throughout the study. Body composition was assessed using dual-energy X-ray absorptiometry (DEXA), and bone microarchitecture was analysed using micro-computed tomography (micro-CT). Data were analyzed in JMP Pro 19 using one-way ANOVA, and means were separated using Tukey's test, with significance set at $P < 0.05$. NE challenge increased the intestinal lesion scores and CP counts in challenged groups with EM+CP having the most severity ($P < 0.05$). Egg shell thickness was reduced in the challenged groups on 7, 14 and 21 DPI ($P < 0.05$). Shell weight was decreased in the challenge groups on 21 and 28 DPI ($P < 0.05$). Haugh unit showed a reduction in EM and EM+NE group compared to NC ($P < 0.05$) on 14 DPI. Hen day egg production was reduced during 0 to7 DPI and 7 to 14 DPI ($P < 0.05$) in all the challenged groups. In addition, DEXA scan revealed decreased bone mineral density and bone mineral content ($P < 0.05$), with the group EM+NE having the highest reduction. Further, Micro-CT analysis revealed decreased bone volume fraction and trabecular architecture in the EM and EM+NE groups on 14 DPI ($P < 0.05$). These results indicate that NE challenge impairs not only intestinal health but also the egg production and quality. In addition, it also compromises bone integrity in laying hens, increasing the risk of skeletal fractures leading to reduced productivity and significant economic losses.

**Keywords:** Necrotic enteritis; Laying hens; Clostridium perfringens; Bone health; Gut health

### 10 *In vitro* assessment evaluating the effect of auranofin, a thioredoxin reductase inhibitor, on *Histomonas meleagridis*

Taylor Schubauer*[1GS], Evy Hafer[1], Aaron Forga[1], Kasey A. Matusik[1], Karen Echevarria[1], Melissa Martinez[1], Juan D. Latorre[1], Zhicheng Dou[2], Danielle Graham[1] [1]*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States;* [2]*Clemson Univerisity, Biological Sciences, Clemson, South Carolina, United States*

Currently, there is no effective treatment to control histomonosis, the disease caused by *Histomonas meleagridis,* in commercial poultry flocks. Auranofin has been shown to effectively reduce the growth of other protozoal parasites, *in vitro*, because of its ability to act as a thioredoxin reductase inhibitor. The purpose of this study was to assess the efficacy of a commercially available compound, auranofin, against *H. meleagridis* (PHL 2017 strain) *in vitro*. Treatment groups included: 1) non-treated control, 2) DMSO (1%) control, 3) auranofin 1µM, 4) auranofin 10µM, and 5) auranofin 100µM. Auranofin was suspended in DMSO, so a DMSO control, representative of the highest inclusion, was utilized to differentiate effects of auranofin and DMSO on histomonads. Three assays were conducted evaluating all treatments, except for the auranofin 1µM treatment, which was only evaluated in two assays. Effect of treatment was evaluated following a 48-hour incubation period (40°C; n=4 replicates/treatment/assay). In all assays, histomonad concentration (Log10 *H. meleagridis* cells/mL) was determined using a hemocytometer. To determine viability, treated cells (2 replicates/treatment) were transferred to individual 25cm$^2$ tissue culture flasks containing fresh medium. Flasks were visually monitored for *H. meleagridis* growth up to three times from d3-7 post-passage using an inverted microscope. In assay 3 only, *Escherichia coli* and *Klebsiella pneumoniae* recovery (Log10 CFU/mL) was assessed by drop plating onto CHROMagar Orientation agar. Plates were incubated aerobically at 37°C overnight. ANOVA was used to determine significant differences ($P<0.05$) between treatment groups. Tukey's HSD was used to further separate means where appropriate. In all assays, T4 and T5 significantly reduced histomonads compared to T1. T4 and T5 only differed significantly in assay 3, as there was a greater reduction in histomonads following T5 treatment. More importantly, no viable histomonads were recovered post-treatment with T5. In assay 3, there was no effect of treatment on *E. coli* or *K. pneumoniae* recovery. These results suggest that treatment with 100µM auranofin diminishes *H. meleagridis* viability without affecting the bacterial population and may be effective in downstream *in vivo* studies.

**Keywords:** Histomonas meleagridis; auranofin; turkey; histomonosis; protozoa

### 11 Cecal microbiome profiles in directly inoculated and laterally exposed turkey poults during *Histomonas meleagridis* challenge

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Histomoniasis, caused by *Histomonas meleagridis* (HM), is a protozoal poultry disease that causes severe cecal and liver damage, high mortality, and significant economic losses. A 35-day (**d**) floor-pen study was conducted to assess the effects of histomoniasis on the cecal microbiome of turkey poults. Day-old female poults (n=264) were raised in the same room until d 14, then assigned to a non-inoculated control group (**NC**) and a challenged group (6 replicate pens, 20-24 birds/pen) reared in separate rooms. Within the challenged group, 12 poults per pen were individually wing-tagged and intracloacally inoculated with ~150,000 HM histomonads and designated as directly inoculated (**DI**). The remaining 12 poults from each pen were designated as non-directly inoculated (**NDI**) via lateral transmission of the parasites, resulting in 3 treatment groups: NC, DI, and NDI. All birds received the same modified starter diet in mash form throughout the study period. On d 21 and d 28, cecal scrapings were collected from 4 birds per treatment group for sequencing the V3-V4 region of the 16S rRNA gene and analyzed using QIIME2 (v2025.10). Alpha diversity was evaluated using Kruskal-Wallis tests with FDR correction, beta diversity using PERMANOVA, and differential abundance using ANCOM-BC2 with FDR-adjusted pairwise comparisons. Results were considered significant at $q < 0.05$. At d 21, Faith's phylogenetic diversity (PD) was higher in DI and NDI compared to NC ($q = 0.03$). Cecal microbial community composition differed among treatment groups based on Bray-Curtis, Jaccard, and weighted UniFrac distances ($q < 0.05$) on d 21, but not on d 28. Also on d 21, DI group was enriched in *Escherichia-Shigella*, *Candidatus* Actinomarina, *Sediminibacterium*, *Diaphorobacter*, *Prochlorococcus*_MIT9313, and *Cloacibacterium*, whereas NDI had enrichment in *Ruminococcus* and *Oscillospiraceae Incertae Sedis*, and NC in Turicibacter and Butyricicoccaceae *Incertae Sedis*. On d 28, NDI was enriched in *Bacteroides* ($q < 0.05$) compared to NC birds. Direct inoculation and lateral exposure to HM produced a distinct cecal microbiome profile at d 21, with DI poults showing enrichment of potentially pathogenic taxa, suggesting that the route of exposure shapes early cecal dysbiosis

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**Keywords:** histomoniasis; microbiome; turkey; Histomonas meleagridis; lateral transmission

### 12 Evaluation of the effect of wheat-based diets on the intestinal microbiome of turkey poults infected with *Cochlosoma anatis*

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*Cochlosoma anatis* is a flagellated protozoan and is the causative agent of cochlosomiasis. Anecdotal evidence suggests that dietary inclusion of wheat during growout may improve performance in *C. anatis* affected flocks. The purpose of the present study was to evaluate dietary wheat inclusion on the intestinal microbiome of turkey poults infected with *C. anatis*. On D0, 41-day old turkey hens (n=226) were moved from a commercial flock exhibiting clinical signs of cochlosomiasis to the Poultry Health Laboratory where they were reared on fresh litter and a stocking density lower than commercial standards (512 $in^2$/turkey). These poults were individually neck tagged and assigned to one of three treatment groups (n=8 pens/group): Group 1) standard corn/soy diet, Group 2) mid wheat diet (400lbs/ton), Group 3) high wheat diet (800lbs/ton). Each diet was formulated to meet or exceed the nutritional requirements. On D14, D31 and D42, n=8 poults from each group were culled to obtain ileal and cecal mucosa samples, as well as cecal contents for microbiome evaluation. Genomic DNA was extracted and 16S rRNA V3-V4 regions were amplified for sequencing. Alpha diversity was discerned at amplicon sequence variant level by leveraging observed richness (OR), Shannon diversity, inverse Simpson index, and Faith's phylogenetic diversity. Generalized Linear Mixed Models were fit with diet, sampling time and sample type as the fixed effects, with random intercepts specified for pen nested within diet and for pen x time nested within diet to account for longitudinal repeated measures and within-timepoint subsampling of sample type. Beta diversity (BD) at the community structure level was evaluated using Bray-Curtis, weighted and unweighted UniFrac distance matrices. PERMANOVA was performed using 9,999 permutations constrained by Diet × Pen, and the effects of diet, sampling time, sample type and their interactions were assessed using a separate-model approach to enable marginal testing of interaction terms, in which main effects were tested without interaction terms and interaction effects were evaluated while controlling for all corresponding main effects. Statistical significance was set at $P<0.05$. OR was significantly affected by sampling time and sample type and significant diet × time and time × sample interactions were detected. For BD, across distance metrics, diet, time, and sample type were significant, with small effect sizes for diet and larger effects for sampling time and type. Significant diet × time interactions and time x sample type were detected for all distance metrics. Further investigation would be warranted to evaluate the relationship between wheat inclusion and *C. anatis* infection.

**Keywords:** Wheat; turkey; Cochlosoma; microbiome; Cochlosomiasis

## Student Competition: Metabolism and Nutrition - Amino Acids I

**203 (B-D) (MTCC)**

### 13 Laying hens' responses to graded dietary crude protein reduction in a conventional cage system

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Dietary protein represents a major cost component in laying hen production and a key constraint in the face of growing global demand for animal-derived protein and heightened volatility in corn and soybean meal prices. 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, reared under conventional cage housing system. A total of 240 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 8 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 strength, 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 or the proportion of saleable and unmarketable eggs. Among the quality parameters, egg weight and egg mass showed a significant effect ($p=0.001$ and $p=0.004$, respectively), with the highest values obtained at 16, 17 and 18% CP. Eggshell thickness and yolk color decreased linearly ($p<0.05$). Independently of dietary treatment, eggs stored under refrigeration exhibited higher albumen height and Haugh unit values across all evaluation periods ($p<0.05$). In the hematological assessment, relative and absolute monocyte counts, as well as eosinophils, differed significantly among treatments, with monocytes reaching their highest proportion at 16% crude protein ($p=0.009$). It is concluded that supplying dietary protein levels equal to or greater than 16% optimizes egg weight and egg mass in commercial laying hens reared in conventional systems, outperforming the responses observed at lower protein levels.

**Keywords:** Laying Hens; Crude protein; Performance; Egg quality; Blood parameters

### 14 Re-evaluating digestible lysine requirements in modern male turkey poults from 0-6 weeks of age

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While digestible amino acid (AA) requirements are well established for broilers, comparable data for turkeys remain limited, particularly during early growth. Given the rapid rate of lean tissue accretion in modern turkeys, precise AA nutrition is critical. Lysine, as the reference AA, provides a foundation for defining these requirements. Therefore, this study aimed to determine the digestible lysine (dLys) requirements of male poults from 0-6 weeks of age. A total of 1,920 Nicholas Select male poults were fed diets containing graded concentrations of dLys (1.30, 1.40, 1.50, 1.60, 1.70, 1.80, and 1.90%), formulated using AA-to-lysine ratios above genetic supplier recommendations. A separate control diet (CON; 1.70% dLys) was formulated based on genetic supplier-recommended AA ratios. Poults were allocated to 96 pens (20 poults/pen) in a randomized complete block design. Weekly performance parameters, including feed intake (FI), body weight (BW), live weight gain (LWG), mortality-corrected feed conversion ratio (FCRm), and dLys intake, were evaluated from 0-6 weeks of age. On d 42, individual bird weights were collected to determine flock uniformity and coefficient of variation (CV). Data were analyzed using one-way ANOVA in JMP Pro 18, with means separated using Fisher's LSD ($P<0.05$). Quadratic polynomial regression was used to estimate dLys requirements at 95% of the vertex. Increasing dLys from 1.30 to 1.60% improved FI, LWG, and BW ($P<0.001$), with responses plateauing by d 21. Birds fed ≥1.60% dLys performed similarly thereafter. The FCRm improved with increasing dLys ($P<0.05$) and was optimized at ≥1.60%, with no addi-

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tional benefit at higher inclusion levels. Birds fed ≤1.50% dLys exhibited reduced growth and poorer feed efficiency across all periods. Birds fed the CON treatment performed similarly to those fed 1.50% dLys, indicating that AA ratios used in the graded diets were more suitable for performance than breeder-recommended ratios. Quadratic regression indicated that dLys requirements decreased with age, with estimates (based on BW and LWG) of 2.09% (0-7 d), 1.87% (0-14 d), 1.74% (0-21 d), 1.71% (0-28 d), and 1.70% (0-42 d). No differences were observed for CV and uniformity (P>0.05); however, birds fed 1.30% dLys numerically exhibited greater variability. Overall, dLys requirements of modern turkey poults aligned with genetic supplier recommendations, although higher AA ratios supported improved performance. Increasing dLys during the first two weeks appears critical to support early growth, and maintaining appropriate AA ratios relative to lysine remains essential for optimizing performance.

**Keywords:** amino acids; precision feeding; turkey poults; lysine optimization; digestible

### 15 Determination of Amino Acid Digestibility in Conventional and Low Oligosaccharide Soybean Meal Using Cecectomized Roosters, Broilers, and Turkeys

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The objective of the current study was to evaluate the amino acid (**AA**) digestibility of high protein low oligosaccharide soybean meal (**UHP-LO-SBM**) compared with conventional soybean meal (**C-SBM**) using 3 bioassays. In Experiment 1, a precision-fed rooster assay was conducted using cecectomized Leghorn roosters to determine standardized AA digestibility. Roosters were fasted for 26 h and precision-fed (crop intubated) 25 g of UHPLO or C-SBM. Excreta were then quantitatively collected for 48 h. In Experiments 2 and 3, standard ileal digestibility (**SID**) of AA digestibility was determined using ad libitum-fed male broiler chickens (Experiment 2) and female turkey poults (Experiment 3). Birds were provided a nutritionally complete corn-soybean meal starter diet until 18 d-of-age. Birds were then fasted overnight and placed their respective dietary treatments from 19 to 22 d-of-age. Dietary treatments were semi-purified cornstarch-dextrose-based diets where the test ingredient was the sole source of AA. Both diets were formulated to contain the same protein content and titanium dioxide was used as an indigestible marker. On day 22, ileal digesta were collected. There were 6 replicate pens of 1 individually caged rooster in Experiment 1, and 8 replicates pens of 5 broiler chickens or 8 turkey poults in Experiments 2 and 3, respectively. Data from all experiments were analyzed using a 1-way ANOVA for a completely randomized design. The significance value for all analyses was $P < 0.05$. Total AA content was approximately 6 percentage units higher for UHPLO-SBM. In Experiment 1 with precision-fed cecectomized roosters, AA were highly digested for both meals, where the overall mean AA digestibility was approximately 91%. Few differences in standardized AA digestibility were observed between meals ($P > 0.05$). In Experiment 2 with ad libitum-fed broiler chickens, no differences were observed between meals for SID of AA ($P > 0.05$). Mean SID of AA was approximately 88% for both meals. In Experiment 3 with ad libitum-fed turkey poults, no differences were observed between meals for SID of AA ($P > 0.05$) and mean SID of AA was approximately 94%. Due to the higher total AA content in UHPLO-SBM and similar AA digestibility values, the digestible AA content in UHPLO-SBM was greater compared with C-SBM in all 3 bioassays for all AA except Cys ($P < 0.05$). Overall, AA digestibility values were in good agreement among bioassays and both SBM had high AA digestibility values, with little difference between the two meals. The UHPLO-SBM had greater digestible AA content compared with the C-SBM, thus indicating improved nutritional value for poultry.

**Keywords:** soybean meal; amino acid digestibility; low-oligosaccharide; broiler; turkey

### 16 In ovo feeding of monosodium glutamate improved early cardiac development in broiler chicks

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Improving resilience in newly hatched chicks is critical to ensure survival, welfare, and optimal growth of broiler chickens. Potential shortages of energy and amino acids may hinder embryonic development, particularly in the late stages before hatching. Glutamic acid (Glu), an energy substrate and the most abundant amino acid in broiler eggs, may offer a potential solution through in ovo feeding (IOF). This study, supported by the AgriFutures Chicken Meat Consortium (PRO-016111), aimed to improve broiler embryonic development. We hypothesized that monosodium glutamate (MSG) in ovo administration would improve the late stages of embryonic development and the early post-hatched broiler growth in a dose-dependent manner. Ross 308 eggs were assigned to 6 treatments (n = 50/treatment), control (0.9% saline), and MSG at 5, 10, 15, 20, 25 mg/egg doses. On embryonic day 17.5, 1 mL of treatment (pH 6.56–7.23) was injected into the amniotic fluid. Hatchability, chick quality (deformity, navel score, body weight (BW) and length), organ weights (OW) (n=10; yolk, heart, liver, intestine, gizzard and proventriculus) were recorded. Chicks without deformities were selected for rearing (4 pens/treatment, 7 birds/pen) following Aviagen Ross 308 guidelines and fed ad libitum with a starter diet (2975 kcal/kg ME, 23% CP). On day 7, 2 birds/pen (n=8) were slaughtered, and OW (as at day of hatch) were measured. Data were analysed using SAS 9.4: PROC LOGISTIC for Hatchability and chick quality, PROC GLM for BW, length, and OW (egg weight, BW and day 7 gender as covariates), and dose and dose$^2$ as linear and quadratic terms in regression modelling. Tukey's test was used for multiple comparisons ($P < 0.05$). Hatchability, deformity, navel score, BW, length, and OW at hatch were not affected by MSG IOF compared to the control ($P > 0.05$). The navel score was higher in the 20 mg/egg group compared with the 5 mg/egg IOF groups: 1.93 vs 1.44 (P=0.043). Day 7, BWG, FI, FCR, and OW were not affected by MSG IOF compared to control ($P > 0.05$). A linear and quadratic relationship was observed with the relative heart weight at day 7 (linear: P=0.005, quadratic: P=0.004, $R^2$=0.45). This may indicate enhanced early cardiac development by MSG IOF up to 15 mg/egg doses. The highest relative heart weight was observed in the 10 mg/egg group, which was significantly different from the 5 and 25 mg/egg IOF groups (1.52 vs 1.29 and 1.27; P=0.01). In conclusion, MSG doses at the levels tested showed a positive potential for IOF. Molecular mechanisms underlying cardiac development require further study to optimise MSG IOF applications.

**Keywords:** Monosodium glutamate; In ovo feeding; Heart; Broiler; Hatchlings

### 17 Comparative effects of guanidinoacetic acid and L-citrulline on growth performance, breast meat yield, and plasma amino acid profiles of broiler chickens fed arginine deficient diets and exposed to cyclic heat stress

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This study aimed to determine whether guanidinoacetic acid (GAA) or

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L-citrulline (Cit) can mitigate the negative effects of an arginine deficient diet on growth performance, carcass yield, and plasma amino acid profiles of broiler chickens subjected to cyclic heat stress (HS). There were five treatments evaluated including a positive control (PC; arginine balanced with 0.06% supplemental L-arginine), negative control (NC; PC without L-arginine), NC+GAA (0.06%), NC+Cit (0.06%) under HS, plus a thermoneutral PC (TN-PC). Each treatment was replicated 12 times with 8 birds per replicate. Broilers were fed experimental diets across starter, grower, and finisher phases and reared under TN to d 24, then exposed to cyclic HS (33 ± 1 °C, 09:00–17:00) from d 24 -35, or maintained under TN (22 ± 1 °C). Growth performance was measured; carcass yield was assessed on day 35 (3 birds/replicate), and plasma amino acids were measured on d 33 (1 bird/replicate). Data were analyzed by one-way ANOVA with Tukey's test used to separate treatment means at P < 0.05. The results showed that from d 10-24, NC reduced BW and increased FCR vs PC (P < 0.05), whereas NC+GAA restored BW comparable to PC levels and improved FCR vs NC (P < 0.05). Cit did not improve BW gain or FCR compared with NC (P > 0.05). From d 24-35 and overall d 10-35, TN-PC achieved the highest BW and feed intake (P < 0.05). Within HS treatments, GAA improved FCR compared with HS-NC and HS-NC+Cit (P < 0.05) and improved BW-corrected FCR. Breast meat yield was highest in TN-PC (P < 0.05); under HS, GAA partially mitigated reductions in breast meat yield relative to other HS groups (P < 0.05). Plasma amino acid profiling showed that Cit supplementation markedly increased plasma Cit (P < 0.05) and restored plasma arginine from the lowest level observed in HS-NC to TN-PC concentrations (P < 0.05). Heat stress and all diet treatments also influenced other amino acids, where plasma asparagine was higher in TN-PC compared with HS groups (P < 0.05). Plasma tyrosine was higher in HS-NC + GAA and TN-PC than HS-PC and HS-NC, and plasma aspartate was lower in TN-PC than HS treatments (P < 0.05). Among essential amino acids, plasma isoleucine and leucine were higher in TN-PC and generally improved with GAA relative to HS-NC (P < 0.05). In conclusion, under cyclic HS, arginine deficiency depressed growth and feed efficiency; GAA addition (0.06%) improved feed efficiency and partially alleviated carcass yield losses, whereas Cit primarily shifted circulating Cit and arginine with comparatively less performance benefit.

**Keywords:** Heat stress; Arginine; Guanidinoacetic acid; Citrulline; Plasma amino acids

### 18 Responses of broilers to increasing amino acid density from soybean meal or feed-grade amino acids

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The growth performance and meat yield responses of male Ross YP × 708 broilers to increasing amino acid density (**AAD**) provided by soybean meal (**SBM**) or feed-grade amino acids (**FGAA**) were evaluated. A reduced AAD diet served as a basal diet (treatment 1) and 6 additional treatments were based on 3 increasing AAD concentrations formulated with either a static SBM inclusion and higher FGAA inclusions (2-4) or higher SBM inclusions (treatments 5-7). For the diets containing higher AAD from SBM, only feed-grade Lys, Met, and Thr were used, whereas Met, Lys, Thr, Arg, Val, Ile, Trp, and His were used in diets with static SBM and higher FGAA inclusion. Amino acid density was increased by approximately 4% at each step, and diets were maintained across starter (0 to 17 d), grower (17 to 30 d), finisher 1 (30 to 40 d), and finisher 2 (40 to 49 d) phases. For the starter phase, AAD diets based were based on digestible Lys levels of 1.23 (basal), 1.28, 1.33, and 1.38%. Digestible amino acid ratios, energy, and other nutrients were close to breeder recommendations within each phase. Treatments were replicated with 10 pens of 27 birds, with 2 birds per pen removed for tissue sampling at 30 d, and all remaining birds minus mortalities processed at 49 d. Statistical analysis was based on comparing linear responses to AAD provided by either FGAA or increasing SBM, and the reduced AAD diet was set as the common intercept for each slope. Data were also analyzed by 2-way ANOVA with AAD level and amino acid source (SBM or FGAA) as the main effects after excluding the reduced AAD basal treatment. For the overall period (0 to 49 d), BWG and FCR responded positively to increasing AAD, regardless of AA source, though there was a tendency (slope contrast: $P < 0.10$) for a greater linear reduction in FCR when AAD was increased with FGAA. Carcass yield increased linearly with AAD from both AA sources, though the slope was steeper for birds fed FGAA (slope contrast: $P < 0.05$). Increasing AAD also decreased relative fat pad weight, with a greater reduction observed for birds fed the increasing SBM diet than for birds fed with FGAA. Total breast meat yield also linearly increased ($P < 0.05$) with AAD, with no influence of AA source. Prevalence of severe footpad dermatitis (score 3) increased in birds fed higher AAD from SBM but not when higher BP was provided by FGAA (slope contrast: $P < 0.05$). In conclusion, these data demonstrate that growth performance and carcass characteristics respond positively to increasing AAD provided by both SBM and FGAA, though the magnitude of responses and the effects on foot pad quality can differ with amino acid source.

**Keywords:** amino acid; broiler; soybean meal; balanced protein; yield

### 19 Effects of dietary protein source and limestone level on gizzard digesta pH in broiler chickens

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This study evaluated whether the effect of dietary limestone (calcium) on gizzard pH differed among protein sources in broiler chickens. A total of 540 one-day-old Ross 708 male broilers were allocated to 90 metabolic cages (6 birds/cage) in a completely randomized design (n=9). Birds were fed a commercial starter diet from d 1 to 13 and experimental diets from d 14 to 21. Experimental diets were corn starch-based semi-purified diets formulated to be isocaloric and isonitrogenous at 15% digestible crude protein with each test ingredient serving as the sole source of dietary protein. The diets were arranged as a 2 × 4 factorial with 2 calcium levels (1 % and 4.4% Ca) and 4 protein sources: soybean meal (SBM), canola meal, meat and bone meal (MBM), and corn DDGS. Birds had free access to feed and water throughout the experimental period, and feed intake was recorded. On day 21, 6 birds/cage were euthanized, and their gizzard digesta were collected for pH measurement. Data were analyzed using PROC GLIMMIX in SAS with diet as a fixed effect and cage as the experimental unit. There was a significant diet × calcium level interaction for gizzard pH ($P < 0.001$), indicating that the effect of limestone inclusion on gizzard acidification was dependent on the dietary composition. Specifically, increasing calcium from 1.0 to 4.4% increased gizzard pH by 43.6% in canola meal (2.79 to 4.00), 32.1% in corn DDGS (3.27 to 4.33), 24.6% in SBM (3.55 to 4.42), and 8.2% in MBM (4.09 to 4.43). Thus, the gizzard pH response to limestone was greatest in canola meal and least in MBM, showing that dietary calcium did not exert a uniform effect across protein-source diets. Feed intake also showed a protein source × calcium level interaction ($P = 0.0132$). The high-calcium canola meal diet supported the greatest intake (70.58 g/d), followed by high-calcium DDGS (65.08 g/d), whereas MBM and low-calcium SBM diets supported the low-

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est intake (47.89 and 49.74 g/d). The feed intake and pH patterns were partly similar, in that canola meal and DDGS generally showed stronger responses than MBM. However, they also differed in that, MBM maintained relatively high gizzard pH despite low feed intake. In conclusion, limestone-derived calcium increased gizzard pH in a protein source-dependent manner, and the significant interaction indicates that the gastric pH response to calcium was influenced by the physical and chemical characteristics of the diets rather than calcium concentration alone. The dissimilarity between feed intake and pH patterns further suggests that variation in feed consumption did not fully explain the observed pH response.

**Keywords:** Broiler chicken; gizzard pH; protein source; acid-binding capacity; limestone inclusion

**20 Assessing the interactive effects of alanine, glycine, and proline in male broilers fed reduced crude protein diets from 15 to 35 days**

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Broiler responses to non-essential amino acid (NEAA) supplementation in reduced CP diets remain poorly characterized. Previous data from our laboratory indicated that NEAA supplementation was required to maintain broiler performance when dietary CP was reduced by 40 g/kg. Using a Plackett-Burman design, Ala, Gly, and Pro were identified as significant predictors of live performance and carcass yields; however, further experimentation is necessary to determine whether these effects were independent or interactive. Therefore, the objective of this experiment was to evaluate the effects of supplementing L-Ala, Gly, and L-Pro, and their potential interactions in a reduced CP diet on broiler live performance and carcass yields. Male 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, 70 pens with 11 birds each (0.10 $m^2$/bird) were fed 10 treatment diets from 15 to 35 d of age (7 replicate pens/treatment). To create dietary treatments (T), a positive control diet (T1; 212 g/kg CP, 3,000 kcal/kg ME and 11.6 g/kg digestible Lys) and a negative control diet (T2; 172 g/kg CP, 3,000 kcal/kg ME and 11.6 g/kg digestible Lys) were formulated to contain a 40 g/kg difference in CP. Minimum constraints were placed on all essential amino acid ratios. To create T3-9, L-Ala, Gly, and L-Pro, and were added in various combinations to equal T1 levels at the expense of an inert filler following a 2×2×2 factorial design. A CP control (T10) was created by supplementing L-Glu to contribute the same g/kg CP as the combined addition of L-Ala, Gly, and L-Pro in T9. Treatments were assigned in a randomized complete block design with pen as the experimental unit and pen location as the blocking factor. Live performance was assessed from 15 to 35 d and processing yields (4 birds per pen) at 36 d of age. Treatments 2-9 were subjected to a three-way ANOVA and means were separated using a Tukey's HSD test when appropriate. Pre-planned orthogonal contrasts were used for comparisons of T1 vs T2 and T9 vs T10. All data were analyzed using SAS 9.4 with significance considered at $P \leq 0.05$. Reducing dietary CP by 40 g/kg compromised ($P < 0.05$) BW gain, FCR, peritoneal fat yield, and breast filet yield by 6, 10, 28, and 8%, respectively. Supplementing Gly ameliorated ($P < 0.05$) live performance and breast filet yield losses. L-Ala, Gly, and L-Pro individually reduced ($P < 0.05$) peritoneal fat yield. Substituting L-Glu for L-Ala, Gly, and L-Pro negatively affected ($P < 0.05$) FCR and breast filet yield. In conclusion, supplementation of NEAA in reduced CP diets warrants further investigation.

**Keywords:** broiler; non-essential amino acid; alanine; glycine; proline

## Student Competition: Metabolism and Nutrition - General Nutrition I

**201 (A-F) (MTCC)**

**21 The apparent metabolizable energy and standardized ileal amino acid digestibility of low-protein soybean meals in broiler chickens**

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We previously compared the standardized ileal amino acid digestibility (SIAAD) values of a soybean meal (480 g/kg crude protein, CP) and a low-protein soybean meal (LPSBM, 460 g/kg CP) generated by inclusion of soy hull (SH) in the SBM. We hereby investigate the effect of further inclusion of SH into SBM on AME (Expt. 1) and SIAAD (Expt. 2) of the resultant LPSBM, as these have not been previously reported. A total of 210 and 180 male broiler chickens were used in Expt. 1 and 2, respectively. The chickens were allocated to metabolism cages with 7 and 5 treatments, respectively, each with 6 replicates. All birds were fed a standard corn-SBM diet until d 14, and the experimental diets from d 14 to 21. In Expt. 1, diets included a corn-SBM reference diet (RD) and six test diets in which 250 or 500 g/kg of each of LPSBM1 (440 g/kg CP), LPSBM2 (420 g/kg CP), or LPSBM3 (400 g/kg CP) partly replaced the energy-yielding feedstuffs in the RD. The AME of LPSBM was determined by regressing feedstuff-associated ME intake (kcal) against feedstuff intake (kg). The slope of the linear regression (kcal/kg) represented the AME of each feedstuff. In Expt. 2, diets included LPSBM1, LPSBM2, LPSBM3, and standard SBM (SSBM, 460 g/kg CP) as the sole sources of N in the four semi-purified diets, plus a N-free diet. LPSBM1, LPSBM2, and LPSBM3 were generated by adding SH at rates of 50, 110, and 170 g/kg, respectively, to the SSBM. On d 21, excreta and ileal digesta were collected from Expt. 1 and 2, respectively. Data were analyzed by one-way ANOVA using JMP. For Expt. 2, orthogonal polynomial contrasts were also performed to compare the means for SIAAD values. The AME values for LPSBM1, LPSBM2, and LPSBM3 were not significantly different, and the derived AME values for diets containing each LPSBM were similar to the analyzed values. For Expt. 2, SIAAD decreased as SH inclusion increased ($P < 0.05$), primarily in LPSBM3. In conclusion, the incorporation of additional SH into the SSBM only marginally affected AME but adversely affected SIAAD at the high (170 g/kg) SH inclusion level.

**Keywords:** Low-protein soybean meal; soyhull; metabolizable energy; standardized amino acid digestibility; crude protein

**22 Nutritional modulation of gut function during *Eimeria maxima* challenge: role of dietary fiber in broiler chickens diets**

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The 35-day study investigated the effects of wheat bran (WB) on growth performance, nutrient digestibility, intestinal permeability, plasma nitric oxide (NO), intestinal lesion score, jejunal tissue gene expression, immune responses, and cecal microbial metabolites in broilers challenged with *E. maxima*. A total of 704 male broiler chicks at 0 d old were allocated to four treatments, with eight replicates in a randomized complete block design with a 2×2 factorial . The diets were corn-soybean meal (SBM) based, and the treatments were: (1)non-challenge (NCH), (2) NCH-WB, (3) challenge (CH), (4) CH-WB. On d12, the birds in the CH group were orally gavaged with a solution containing 25,000 sporulated oocysts of *E. maxima* suspended in 1 ml of distilled water. Data were analyzed using a 2×2 factorial and the Kruskal-Wallis test for lesion score in JMP Pro 18.

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Birds and feed were weighed at d 0, 8, 12, 15, 19, 27, and 35. No significant treatment effects were observed during the starter (d0-8) and early grower (d8-12) phases. At the end of the acute phase of infection (d19), a significant interaction effect was observed for BWG and FCR: CH-WB birds gained more weight and had lower FCR than CH birds (P < 0.05). During the overall period (d0-35), CH birds had lower BWG and higher FCR (P < 0.05). On d27, birds fed WB-containing diets had lower digestibility of DMD, N, and AA (P < 0.05). *Eimeria* challenge increased intestinal permeability on d 19 (P < 0.001). No interaction or main effects were observed on d 27 and d 35 for gut permeability and plasma NO. The CH birds had higher lesion scores (jejunum and ileum) irrespective of feed type (P < 0.001). On d19, CH decreased the expression of CLDN, PepT1, GLUT1, and SGLT1 (P < 0.05), and birds fed WB also had lower SGLT expression (P = 0.046). A trend was observed for anti-inflammatory cytokine (LITAF), where WB-fed birds had higher expression (P = 0.073). On d27, a trend was observed for CLDN, with CH-WB showing higher levels than the CH group (P = 0.077). On d19, a significant diet × *Eimeria* interaction was observed, with CH and CH-WB showing greater cecal isobutyrate and isovalerate concentrations (P < 0.05). At the end of the compensatory growth phase (d35), a tendency toward higher butyrate was observed, with WB-fed groups showing higher concentrations, irrespective of CH (P = 0.0916). In conclusion, under the conditions of the present experiment, although the inclusion of dietary WB reduced nutrient digestibility, it improved BWG and FCR during the acute phase. WB inclusion also showed immunomodulatory effects, with moderate effects on LITAF and CLDN expressions, suggesting a role in improving intestinal integrity and immune responses in challenged birds.

**Keywords:** Wheat bran; Eimeria; Growth performance; Short-chain fatty acids; Intestinal permeability

### 23 Evaluating the effects of full-fat mealworm larvae meal inclusion on Ross 308 male broiler performance and apparent ileal amino acid digestibility

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Mealworm Larvae (Tenebrio molitor) Meal (MLM) may serve as a sustainable alternative protein source in poultry diets due to its digestible amino acid (AA) profile. The AA digestibility and optimal inclusion rates for broilers are not well documented. This study explored the effects of graded MLM inclusion on apparent ileal amino acid digestibility (AIAAD) and d1-21 broiler performance. A total of 450-d-old Ross 308 male broilers were randomly allotted to one of 45 cages (10 birds/cage). Dietary treatments were formulated to be isocaloric and isonitrogenous**.** The five dietary treatments (0%, 2.5%, 5%, 7.5%, 10% MLM) were arranged in a randomized complete block design (RCBD) with nine replicate cages per treatment. Live weight gain (LWG), body weight (BW), feed intake (FI), and mortality-corrected feed conversion ratio (mFCR) were determined from d1-21. Upon recording d21 BW, broilers were provided with their respective dietary treatment, which contained titanium dioxide (TiO2), for six hours. Thereafter, three birds/cage were euthanized for distal ileal digesta collection. Pooled digesta samples per cage were frozen and later freeze-dried. Feed and digesta were analyzed for TiO2 and AA content to calculate AIAAD. Data were analyzed using the GLM procedure in SAS by one-way ANOVA, and significant treatment effects were further separated using Fisher's LSD. A quadratic polynomial regression was performed with the estimate set at 95% of the vertex to predict the optimal inclusion of MLM to maximize BW and LWG. Control-fed birds (0%) had the lowest BW, LWG, FI, and highest mFCR. Birds fed 5% or 10% MLM had the greatest BW, LWG, and FI. Birds fed 5% MLM had the lowest mFCR. Those receiving 2.5% or 7.5% MLM had intermediate performance (P < 0.001). The quadratic polynomial regression predicted an optimal MLM inclusion of 6.6% (at 95% of vertex) to maximize BW and LWG. Digestibility coefficients of Met, His, Ala, Cys, Gly, Ser, and Tyr improved when MLM was included (P<0.05). The digestibility coefficient of Thr was lowest at 0% MLM and highest at 5% and 7.5% MLM inclusion (P<0.001). The Trp and Pro digestibility coefficients increased incrementally as MLM inclusion increased to 10% (P<0.001). In conclusion, a clear improvement in broiler performance was supported by the enhancement in AIAAD coefficients of several essential and non-essential AA when MLM was included between 5 and 10%. Within the parameters of this 21-d study in battery cages, MLM can be used as an alternative protein source that optimizes broiler performance when included at 6.6%. Further evaluation of MLM in pelleted broiler diets provided throughout grow out is needed to confirm practicality in the broiler industry.

**Keywords:** Insect; alternative Protein; Sustainability; Digesta

### 24 Starch–protein digestive dynamics drive energy partitioning and nutrient deposition in broilers fed reduced crude protein diets

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Attempts have been made to comprehend the mechanisms underlying increased fat deposition in broiler chickens offered reduced crude protein (RCP) diets. Several contributing factors have been proposed, including excessive starch, rapid starch digestion, and increased starch-to-crude protein (CP) disappearance ratios. Nevertheless, it remains unclear how these factors impact energy partitioning and nutrient deposition in broilers. Therefore, this study examined how starch and CP digestibility, and their disappearance rates contribute to energy partitioning, and protein and fat deposition. The study comprised a 2 × 2 × 2 factorial arrangement with two net energy (NE) levels (standard vs. 75 kcal/kg reduction), two starch digestion rates (rapidly vs. slowly digestible starch), and two CP levels (standard vs. 30 g/kg reduction). Standard NE and CP during the grower and finisher periods were 2350 and 2400 kcal/kg, and 205 and 190 g/kg, respectively. Field peas (200 g/kg) were added as slow digestible starch source. Each dietary treatment had 7 replicates with 6 birds each, totaling 336 off-sex male Ross 308 broilers over a feeding period of 11-35 d. Ileal digestibility, disappearance rates, body composition, and energy partitioning in the broilers were determined. Disappearance rates were calculated from nutrient intake and digestibility coefficients, while body composition was determined via the comparative slaughter technique. The CP digestibility of the birds negatively correlates with protein (r = -0.370, P = 0.005) and fat (kF: r = -0.312, P = 0.020) deposition per unit intake of the broilers. While the starch disappearance rate related positively with energy retained as fat (ERf: r = 0.460, P < 0.001) and kF (r = 0.689, P < 0.001), the CP disappearance rate positively correlated with protein deposition (Pd: r = 0.407, P = 0.002), but negatively with ERf (r = -0.352, P = 0.008) and NE for maintenance (NEm: r = -0.653, P < 0.001). The starch:protein disappearance rate ratio correlated negatively with Pd (r = -0.428, P = 0.001) and positively with ERf (r = 0.508, P < 0.001) and NEm (r = 0.644, P < 0.001). These relationships indicate that rapid starch digestion may promote more fat deposition than protein deposition and increase maintenance metabolic costs, thereby highlighting the potential of slow starch digestion to reduce fat deposition. It also

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shows that when CP supply is diluted or mismatched relative to starch digestion, lean tissue deposition declines, and energy shifts towards fat deposition. Acknowledgments: Damilola Kareem's PhD is supported by the University of Sydney International Stipend and Tuition Fee Scholarships, and the Christian Rowe Thornett Supplementary Scholarship.

**Keywords:** Body composition; Energy retention; Fat deposition; Low protein diet; Protein deposition

### 25 Determination of phosphorus availability in conventional distillers dried grains with solubles and corn fermented protein with and without phytase use during fermentation at the ethanol plant

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The objective was to determine the impact of phytase use during fermentation at ethanol plants on P availability in dry-grind corn co-products. Three types of feedstuffs were evaluated: conventional distillers dried grains with solubles (CDDGS), corn fermented protein (CFP), and fractionated DDGS (FDDGS; sample remaining after CFP recovery). One sample of CFP and FDDGS were obtained from an ethanol plant using phytase and not using phytase, whereas CDDGS could only be sourced from a plant using phytase (5 samples total). In Experiment 1, a precision-fed rooster assay was conducted using Single Comb White Leghorn roosters to determine the phytic acid (myo-inositol 1,2,3,4,5,6; InsP6) disappearance. Roosters were fasted for 26 h prior to being precision-fed (crop intubated) 25 g of sample. Excreta were quantitatively collected for 48 h post-feeding. In Experiment 2, bioavailability of P based on bone ash (RBV) was determined in ad libitum-fed broiler chickens from 7 to 21 d-of-age using the slope-ratio method. Diet 1 was a P-deficient corn-soybean meal-based diet. The remaining diets were the same as diet 1, except 2 increasing levels of KH2PO4 or the test ingredients were added in place of cornstarch and dextrose. The RBV of P was determined by regressing bone ash content (mg/tibia) on supplemental P intake. In Experiment 3, standardized ileal digestibility (SID) of P was determined using ad libitum-fed broiler chickens from 18 to 21 d-of-age. Birds were provided cornstarch-dextrose-based diets, where the test ingredients were the sole source of P. Diets were formulated to contain 0.75% Ca, 0.21% total P, and titanium dioxide was used as a digesta marker. Experiments contained 5 to 6 replicates per treatment (individual rooster or pen of broiler chickens). Data were analyzed using a one-way ANOVA and regression analyses were conducted for Experiment 2. The significance value for all analyses was $P < 0.05$. Samples from ethanol plants using phytase had less InsP6. In Experiment 1, InsP6 disappearance was greater ($P < 0.05$) for samples from ethanol plants using phytase. There was an increase of up to 40 percentage units for FDDGS and CFP by phytase. In Experiment 2, regression of tibia ash content vs. supplemental P intake yielded an RBV value of 91% for CDDGS ($R2 = 0.93$). Further, RBV of P was affected by phytase use at ethanol plants, where phytase increased RBV of P by up to 46 percentage units. In Experiment 3, the SID of P in CDDGS was 88%. Further, phytase improved the SID of P in FDDGS and CFP by up to 38 percentage units. In conclusion, there was good agreement among the 3 bioassays. Furthermore, utilization of phytase at ethanol plants improved P availability in the co-products for poultry.

**Keywords:** Phytase; Distillers Dried Grains with Solubles; CFP; Phosphorus digestibility; Phosphorus bioavailability

### 26 In Ovo A-Ketoglutarate Modulates Plasma and Hepatic Antioxidant Defence in Broilers Under Cyclic Heat Stress

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Accelerated global warming has led to rising ambient temperatures, a critical abiotic stressor for rapidly growing, high-yield broiler chickens. Hence, this study was conducted to investigate the effects of in ovo administration of alpha-ketoglutarate (AKG) on heat resistance in broiler chickens. Accordingly, 350 fertile eggs laid by 40-week-old Arbor Acre breeder hens were injected with 0.6 mL of 0% AKG (140 eggs), 0.5% AKG (70 eggs), 1% AKG (70 eggs), and 1.5% AKG (70 eggs) in the amniotic sac at 17.5 embryonic days. At hatch, 36 chicks per treatment were randomly distributed into 6 replicates of 6 birds each and reared under standard conditions for 4 weeks. During 29 – 31 days of age, broilers were exposed to cyclic heat stress (31 ± 1°C, 6 hrs/day; HS) or maintained at normal temperature (21 ± 1°C; NT). Hence, the birds were assigned to the following five treatment groups: (i) 0AKG-NT, (ii) 0AKG-HS, (iii) 0.5AKG-HS, (iv) 1.0AKG-HS, and (v) 1.5AKG-HS. At the end of HS, 6 birds per treatment (1 per replicate) were slaughtered to collect plasma, liver, and jejunal samples. Rectal temperature (RT) was recorded at the start and end of the HS period. The data were subjected to one-way ANOVA (all groups) and polynomial regression (excluding the 0AKG-NT group), followed by Tukey's post hoc test if significant differences were observed ($p < 0.05$). Under HS, 0AKG-HS showed the lowest plasma DPPH-RSA%, indicating significantly reduced antioxidant capacity ($p = 0.001$). MDA concentration decreased linearly with increasing AKG doses and was significantly lower in the 1.5AKG-HS group compared to the 0AKG-HS group ($p = 0.037$). While initial RT did not differ among treatments, RT increased in HS groups ($p = 0.001$). As the AKG dose increased, the change in RT decreased linearly ($p = 0.019$). Hepatic nuclear factor erythroid 2-related factor (NRF2) expression was higher in the 0.5AKG-HS and 1.0AKG-HS groups ($p = 0.032$), while NADPH oxidase1 (NOX1), NOX4, and heat shock protein 70 (HSP70) ($p = 0.001$) were highest in the 1.5AKG-HS group, as compared to 0AKG-NT. Cluster analysis revealed a strong positive correlation between hepatic NOXs and HSPs gene expression. Jejunal mRNA expressions were not significantly affected. In conclusion, in ovo administration of AKG activated antioxidant and stress-response genes (NOX1, NOX4, HSP70), primarily in the liver, and improved plasma antioxidant capacity and thermoregulation under heat-stress conditions.

**Keywords:** in ovo feeding; AKG; gene expression; heat stress; broilers

### 27 Further evaluation of amino acid digestibility among different soybeans varying in protease inhibitors

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Six experiments were conducted to determine standardized amino acid digestibility (SAAD) in 9 new soybean (SB) lines varying in trypsin inhibitor units (TIU) and 3 different cooking treatments (raw uncooked, or intermediate and more extensive cooking by autoclaving at 121 degrees Celcius for 25 and 30 min, respectively ).TIU were determined (method Ba 12a-2020; AOCS, 2020) by Eurofins Scientific Inc. The first group of 5 SB were CS and Lee5 parent lines and 3 crosses of those parent lines. The CS line overexpresses O-acetylserine sulfhydrylase, a sulfur assimilatory enzyme with a Maverick cultivar background, which results in increased sulfur AA. The Lee5 is a high protein Korean soybean. The second group of SB were a KTi-3 mutant (LG17) and HBK-WF and HBK-PF that were developed by crossing a beta conglycinin alpha subunit mutant (allergen) SB and the KTi-3 mutant SB to produce SB with reduced TIU. A Maverick conventional SB line was also included as a control SB. Raw uncooked

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SB ranged from 28 to 118 TIU/mg, with HBK-WF and HBK-PF being lowest. Intermediate cooking reduced TIU to a range between 3.2 and 16.5 TIU/mg, with HBK-WF and HBK-PF again being lowest. More extensive cooking reduced TIU to 2 TIU/mg or less. Precision-fed rooster assays were conducted to determine SAAD in cecectomized roosters. For each experiment, individually caged White Leghorn roosters were fasted for 26 hours, then 4 or 5 roosters were assigned to each SB and crop-intubated with 25 g. Excreta were collected 48 hours post-feeding. Data were analyzed as a one-way ANOVA for a completely randomized design with each individually caged rooster as the experimental unit. Differences among treatment means were considered significant at ($P < 0.05$) using the least significant difference test. Results for the raw uncooked SB indicated that HBK-WF and HBK-PF had SAAD that was higher ($P < 0.05$) than the other SB lines. Intermediate cooking of SB increased SAAD, particularly for the highest TIU SB. For the more extensive cooking, most SAAD values further increased to between 85 and 92%. Overall, the results of these experiments indicated the lower TIU SB lines generally had higher AA digestibility values than higher TIU SB lines when fed raw or moderately cooked, whereas little or no difference among SB lines was observed when they were more extensively cooked. Consequently, many of the new SB lines had increased concentrations of digestible AA when more extensively cooked due to their high digestibility combined with their higher CP and AA content, particularly sulfur AA.

**Keywords:** soybean; rooster; digestibility; trypsin; amino acid

### 28 Apparent metabolizable energy (AME) of soybean soapstock in chickens.

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Soapstock is a by-product of the soybean crushing industry that is gaining traction as an addback to soybean meal as a method of disposal. Soapstock consists of soaps, neutral oil, phosphatides, water, and unsaponifiables. Soybean soapstock has a fatty acid profile similar to that of soybean oil and does not contain substantial protein or carbohydrate factions. However, it is unknown whether or not birds are able to derive any energy from the soapstock or if it is just acting as a dilutant to the soybean meal. Therefore, the AME of soy soapstock in chickens was determined and compared to soy oil using slope-ratio analysis. In total, 420 male chicks from a female broiler breeder line were allocated to 15 replicate cages of 4 birds for each of the 7 treatments. The chicks were fed a common diet until D 16 before being switched to experimental diets: control without oil addition, control + 3% soy oil (SO), control + 6% SO, control + 9% SO, control + 3% soapstock (SS), control + 6% SS, or control + 9% SS. Experimental diets were generated using a basal diet with the addition of 0.35% titanium dioxide used as an indigestible dietary marker. Data were analyzed using JMP with significance set at $P \leq 0.05$ and if significance was noted, means were separated using Student's t-test. Chick body weight on day 16 was 572 g across all pens without differences among treatments ($P = 0.13$). Excreta samples were collected on D 23 following a 7-day adaptation period. Apparent metabolizable energy of the soapstock and oil was calculated using the ratio of dietary AME v. oil inclusion. The AME value of SO was estimated at 8,170 kcal/kg and the AME of SS was estimated at 3,670 kcal/kg. Using slope-ratio analysis to compare the AME of soy oil and soapstock, the AME of soapstock was found to be 0.449 when compared to soy oil. Soybean soapstock can contribute metabolizable energy when added to soybean meal, but soybean meal crude protein should be reduced by the proportion of added soapstock as it does not contain appreciable crude protein.

**Keywords:** soybean meal; soapstock; AME

### 29 Effects of dry fractionation and multienzyme supplementation on non-starch polysaccharide depolymerization and fiber digestibility of barley and its fractions in broiler chickens

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A better understanding of the functional properties of barley B-glucan has shifted its perception from an anti-nutritional factor to a beneficial, fermentable dietary fiber. Particularly, its physiological effects related to digesta viscosity are partly affected by its molecular weight (MW); however, viscosity's limits can be mitigated effectively with exogenous enzymes. Moreover, enzymatic hydrolysis of barley's non-starch polysaccharides (NSP) generates glycobolites that exert prebiotic properties and enhance fiber digestibility in broiler chickens. Dry fractionation of barley generated six fractions (F1-F6) differing in particle size, fiber and B-glucan contents and characteristics. The chemical composition of the grain and fractions and in vitro depolymerization of NSP by a multi-carbohydrase (xylanase, B-glucanase, and cellulase) were evaluated. On a DM basis, barley grain contained 21.3% total NSP, 7.2% water-soluble NSP, 3.9% B-glucan, 18.7% NDF, and 12.6% CP. Fractions represented hull-rich (F1, F2), endosperm-rich (F4, F5), intermediate (F3), and fine material (F6). Composition varied among fractions, with NSP ranging from 12.9 to 39.2%, NDF from 9.7 to 44.5%, and CP from 9.3 to 14.9%. Fractions 1-6 contain 1.62, 1.34, 3.31, 3.95, 5.93 and 2.26% B-glucan, on a DM basis, respectively. The degree of NSP depolymerization, determined by changes in NSP content, was significant in the whole grain and all its fractions treated with the enzyme ($P<0.05$; analyzed with t-test, R Studio). In total, 756 Ross 308 AP as-hatched broilers were assigned to 14 dietary treatments in a randomized complete block design (9 replicates; 6 birds per pen). Birds were fed a corn-soybean meal starter diet to day 14, followed by experimental diets (days 14–21) containing 40% barley or individual fractions, with or without multi-carbohydrase supplementation. Excreta were collected on days 17–20 to determine apparent NDF digestibility. Enzyme supplementation increased ($P<0.05$) NDF digestibility in whole barley and the F1, from 5.03 to 10.6% and from 8.0 to 14.6%, respectively. Although not statistically significant, enzyme supplementation numerically increased NDF digestibility in fractions F2 to F5, but not in F6. This appears to be associated, at least in part, with the MW of B-glucan, as determined by high-performance size-exclusion chromatography. The B-glucan extracted from F6 had a greater MW than that from F3 to F5, while whole barley had the lowest MW among all tested ingredients. Overall, the strategic use of enzyme supplementation can increase the nutritive value of barley and its fractions by promoting fiber degradation, potentially leading to the generation of beneficial glycobolites.

**Keywords:** barley; B-glucan; dry fractionation; multi-carbohydrase; non-starch polysaccharides

### 30 The effects of corn particle size and a 15 x coccidial vaccine challenge on 21 to 42 day hen poult performance and nutrient digestibility

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An experiment was conducted to determine the effects of corn particle size (CPS) and 28 d coccidial challenge on turkey hen poults. Response criteria included growth performance, intestinal permeability (IP), nutrient digestibility, and litter moisture. A total of 1,600 Hybrid hen poults

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were sourced from a local hatchery and raised until 21 d of age when 1,472 poults were allotted to floor pens to ensure similar initial BW among treatments (0.57 kg/bird; P = 0.96; SEM = 0.005). The poults were fed one of two pelleted and crumbled starter 2 diets as part of a 2 x 2 factorial including two corn particle sizes (597 µm and 1,049 µm), with and without a 15x dose of coccidiosis vaccine provided to the poults orally on 28d. In total, 16 replicate pens of 23 turkey poults per treatment were used. Pellet quality and mill operations were measured during feed manufacture. Body weight gain (BWG), feed intake, and FCR were measured from D21 to 42. Nutrient digestibility and IP were measured on D35. Litter moisture was measured on D21, 28, 35, and 42. All performance data, litter moisture, and nutrient digestibility were analyzed as a 2 x 2 factorial with CPS and coccidial challenge as main effects and feed manufacture and pellet quality data were analyzed using ANOVA in JMP Pro 18 with each of the three manufacture runs as the experimental unit in JMP Pro 18 ($P \leq 0.05$), and if significant, means were separated using Student's t-test. Pellet quality was not influenced by CPS ($P > 0.05$), although the coarse corn had a higher mill load ($P < 0.01$). There were no interactions between CPS and coccidial challenge from 21 to 42 d. A main effect occurred for oocyst shedding where the challenged birds had a higher number of oocysts on d 5 to 7 post vaccination ($P \leq 0.01$) with no difference in IP ($P = 0.20$). Hen poults fed coarse corn resulted in reduced FI and BWG ($P \leq 0.01$) with no difference in FCRm ($P = 0.60$). Hen poults challenged with coccidiosis on D28 had reduced performance for FI, BW, FCRm, and relative gizzard weight ($P \leq 0.01$). Birds consuming 1,049 µm corn had an increase in energy digestibility ($P \leq 0.01$); whereas challenged birds had reduced crude fat digestibility ($P = 0.02$). Day 35 litter moisture was increased with the challenge ($P = 0.02$). Unlike previous data, larger particle corn (1,049 µm) reduced overall poult performance and suggests caution should be used to move to larger particle size corn with starter poults. Additionally, a single dose of 15x coccidial vaccine was shown to reduce poultry performance and alter the digestion of nutrients.

**Keywords:** turkey; particle size; digestibility; coccidiosis; performance

### 31 Amino acid digestibility and metabolizable energy content in western Canadian faba bean (*Vicia faba*) for laying hens

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There is growing interest in faba bean (*Vicia faba*, FB) as a feedstuff for poultry in western Canada to reduce reliance on imported soybean meal. There is relatively little information about the nutrient and energy digestibility in domestically-grown FB cultivars. A digestibility trial compared the standardized ileal digestibility (SID) of amino acids (AA) and AME content among 25 unique FB specimens collected from across western Canada. In a 7-day experiment, 72-wk-old laying hens (n=1080; 50:50 Lohman Brown:Lohmann LSL Classic) in conventional battery cages were fed either an N-free diet, a basal diet, or one of 25 test diets in a randomized complete block experiment with 5 replicate test cages per treatment. All diets contained titanium dioxide as an indigestible marker, and test diets were comprised of a 70:30 blend of the basal diet and one of the 25 FB specimens. Excreta was collected over a 36-h period preceding collection of ileal digesta on the morning of d 7, which was pooled within cage. Digesta collected from birds fed the N-free diet was used to correct apparent ileal digestibility coefficients to obtain SID coefficients. The SID of AA and AME in the basal and test diets were calculated using the marker method, and the same were calculated for individual FB specimens using the difference method. For the statistical analysis, FB specimens were classified according to flower colour, which serves as a crude proxy for tannin content (FC; white/low tannin vs. coloured/high tannin), and vicine/convicine (VC; low vs. normal) phenotypes. The calculated SID of AA and AME in the FB specimens were analyzed as generalized linear mixed models using the GLIMMIX procedure (SAS Institute; Cary, NC, USA). Models included fixed effects of FC and VC phenotype and the two-way interaction, with block included as a random effect. The significance level for all statistical analyses was 5% (A = 0.05). There was no significant interaction between FC and VC phenotype for SID of any AA or AME ($P > 0.21$). The SID of Lys (91.8% vs. 86.7%), Met (92.4% vs. 76.1%), Thr (89.5% vs. 77.5%), Arg (92.9% vs. 87.0%), Val (87.8% vs. 74.8%), and most other AA was higher in white- compared with coloured flower (i.e., tannin-containing) FB specimens ($P < 0.01$), but did not differ between VC phenotypes ($P > 0.10$). Conversely, FC phenotype did not affect AME ($P > 0.16$), but AME was significantly higher in low- compared with normal VC FB specimens (2725 vs. 2511 kcal/kg; $P < 0.01$). In conclusion, our data support the need to use separate SID coefficients and AME values for FB based on FC and VC phenotype when formulating diets for poultry.

**Keywords:** faba bean; digestibility; amino acids; AME; laying hens

### 32 Effect of extra arginine and glutamine supplementation in reduced-protein diets on growth performance, plasma metabolites, and amino acid metabolism in *Eimeria*-challenged broiler chickens raised on clean or reused litter

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The objective of the study was to evaluate the effects of extra Arg and Gln supplementation in reduced-protein (RP) diets on growth performance, plasma metabolites, and the expression of Arg and Gln metabolism genes in Eimeria-challenged broilers raised on clean or reused litter. On d0, 1,320 male broilers were allocated to either clean wood shavings or reused Eimeria-contaminated litter with six blocks in a randomized complete block design. On d7, birds within each litter condition (main plot) were subdivided into 5 treatments (subplot) in the split-plot design: (1) non-challenged standard protein diet (NCH-SP), (2) challenged standard protein diet (CH-SP), (3) challenged RP diet (CH-RP), (4) challenged RP diet supplemented with Arg and Gln at 150% of standard level (CH-AA), (5) challenged RP diet with coccidiostat (CH-CSTAT). On d15, birds in challenged groups were orally inoculated with mixed Eimeria spp. Growth performance, gut permeability, plasma metabolites, and gene expression were evaluated during the prepatent (d15-18), acute (d18-22), recovery (d22-29), and finisher (d29-35) phases. Data were analyzed using two-way ANOVA, where litter condition, treatment, and their interaction were fixed effects and litter within block was included as a random effect. No significant interactions were observed throughout experiment. During the starter phase, birds raised on reused litter showed lower ($P<0.05$) weight gain (WG) and higher feed conversion ratio (FCR), and this effect persisted through prepatent phase (d18). However, litter condition had no significant effect during later phases. From acute phase onward, NCH-SP group showed the highest ($P<0.05$) WG and final body weight, followed by CH-CSTAT, whereas CH-SP, CH-RP, and CH-AA were lower ($P<0.05$) than the others. During recovery phase, WG in the CH-AA group was comparable to NCH-SP and CH-CSTAT group, whereas WG in the CH-RP group was lower ($P<0.05$). Challenged groups had greater ($P<0.05$) gut permeability on d21 than the NCH-SP group, consistent with higher plasma nitric oxide (NO) concentrations on d22 and 29. On d29, CH-AA had the lowest ($P<0.05$) total glutathione concentration, whereas CH-SP had the highest. In addition, CH-RP and CH-AA had the greatest ($P<0.05$) relative expression of GDH2, ODC, and OAT among the treatments. Relative expression of NOS2 in the CH-AA was the highest ($P<0.05$) among the treatments. In conclusion, supplementation of Arg and Gln at 50% above standard level in RP diets improved growth recovery during the recovery phase following Eimeria challenge, irrespective of litter condition. This response may be associated with modulation of NO production,

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polyamine synthesis, and glutamate catabolism for energy production.

**Keywords:** Eimeria; Reduced-protein; Arginine; Glutamine; Broiler

### 33 Allometric organ growth and tibia development of two modern broiler genotypes and a heritage meat-type chicken

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This study compared organ and tibia development of two modern broiler genotypes (Ross YP × Ross 708 and Ross × Ross 308) with a heritage genotype that has been unselected since the 1950s (New Hampshire × Columbian; NHC). Male chicks (n = 944) were placed in floor pens (n = 8/genotype). Forty birds were placed per pen for the modern genotypes and 38 were placed for the NHC line, providing at least 0.11 $m^2$ per bird throughout the trial. Ross genotypes were reared to 56 d, whereas NHC birds were reared to 63 d to achieve at least 2 kg final BW. All groups were fed the same 4-phase feeding program consisting of starter (0 to 14 d), grower (14 to 28 d), finisher 1 (28 to 42 d), and finisher 2 (42 to 63 d) phases. Organ weights were determined weekly (1 bird/pen), whereas tibia traits were determined every other week (1 bird/pen). Measured organs included heart, spleen, liver, crop, proventriculus, gizzard, pancreas, small intestine, and ceca. Tibia traits included length, diameter, breaking strength, and ash content. Data were analyzed by one-way ANOVA ($P < 0.05$), and growth was modeled using Gompertz and allometric equations with F-tests to compare curves. For digestive organs (crop, proventriculus, gizzard, small intestine, and ceca), modern broilers achieved greater ($P < 0.05$) absolute weights and faster maturation rates. Specifically, liver and pancreas reached maximum growth 5–10 days earlier than in NHC birds. Allometric analysis showed higher B values for crop, proventriculus, ceca, liver, and pancreas in Ross 308 compared with Ross 708, indicating faster growth as a function of BW ($P < 0.05$). Both modern genotypes had heavier ($P < 0.05$) small intestine, liver, and pancreas weights as a function of BW than the heritage line. On the other hand, NHC had higher B values for gizzard compared with the modern genotypes ($P < 0.05$). In contrast to the digestive organs, spleen and heart followed a different developmental pattern. NHC showed greater spleen weight relative to BW ($P < 0.05$), whereas heart growth differed among modern genotypes, with Ross 308 scaling similarly to NHC at a common BW of 2 kg, but greater than Ross 708. Allometric analysis of tibia growth showed larger parameters (A and B) for NHC birds than both modern genotypes, reflecting heavier, longer, and thicker bones relative to BW ($P < 0.05$), although modern broilers had greater absolute values ($P < 0.05$). However, modern broilers had higher breaking strength ($P < 0.05$) as a function of BW compared with NHC from 14 d post-hatch onward. In conclusion, these data indicate that genetic selection has led to earlier visceral and skeletal maturation to support rapid muscle accretion.

**Keywords:** broiler; heritage; allometry; organs; tibias

### 34 Effects of ExPress® Soymeal on Growth Performance and Feed Conversion Efficiency in Broiler Chickens

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Soybean meal is the primary protein source in broiler diets; however, processing methods may influence nutrient availability and energy utilization. ExPress® soymeal (E-SM) retains residual oil and may alter feed efficiency compared with conventional solvent-extracted soybean meal (SE-SBM). The objective of this study was to evaluate growth performance and feed conversion ratio (FCR) of broilers fed diets containing E-SM versus SE-SBM diets formulated with supplemental oil. A total of 360 Ross 708 broilers were reared to 38 d of age in floor pens (0.91 x 1.52 m; 12 birds/pen). Chicks were feather-sexed at placement to ensure a 50:50 male-to-female ratio. Birds were randomly assigned to three dietary treatments with 10 replicate pens per treatment: (1) SE-SBM with conventional soybean oil (TRT1), (2) SE-SBM with ExPress® soybean oil (TRT2), and (3) E-SM without supplemental oil (TRT3). Diets were formulated to be isoenergetic and isonitrogenous across starter (d 0–10), grower (d 10–25), and finisher (d 25–37) feeding phases. Birds were weighed weekly to monitor growth, and feed intake was recorded by pen for each phase. Pen served as the experimental unit, and data were analyzed using one-way ANOVA. Starter feed intake differed among treatments ($P < 0.001$), with birds fed TRT3 consuming less feed than those fed TRT1 and TRT2. Mean starter feed intake per pen was 4.37, 4.37, and 4.02 kg for TRT1, TRT2, and TRT3, respectively. Consequently, starter phase weight gain differed among treatments ($P < 0.05$). Final body weight and average daily gain, however, were not significantly affected by dietary treatment ($P > 0.10$). Mean final bodyweight per bird was 2.84, 2.82, and 2.75 kg for TRT1, TRT2, and TRT3, respectively. Feed conversion ratio differed among treatments ($P < 0.01$), with TRT3 showing improved FCR compared with TRT1 and TRT2 (1.415 vs. 1.461 and 1.459, respectively) Phase analysis indicated that treatment effects on feed efficiency were primarily associated with the grower period ($P < 0.01$), whereas starter and finisher FCR were not significantly affected ($P > 0.10$). Body-weight-adjusted analysis confirmed that the improved feed efficiency observed in TRT3 was not attributable to differences in final body weight. These results indicate that broiler diets containing ExPress® soymeal can improve feed conversion efficiency compared with conventional solvent-extracted soybean meal diets, without significantly affecting final body weight or average daily gain.

**Keywords:** broiler chicken; solvent-extracted soybean meal; high-shear dry extruded-mechanically pressed soymeal; feed efficiency; feed conversion ratio

## Student Competition: Microbiology and Food Safety I

**202 (A-D) (MTCC)**

### 35 Farm to Processing Microbial Population Dynamics in a Non-Conventional Poultry Operation

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Farm environments, management practices, and seasonal variation shape poultry microflora and determine what persists through processing. Understanding changes in these microbial communities from the farm to final products could help processors implement better strategies to reduce food safety risks. This study profiled microbial populations and its shifts across key processing stages of flocks from diverse small, non-conventional farms processed at a single facility. Two flocks (F1 and F2) from eight farms processed at a small plant were sampled, with one flock per visit (n=16) over a 6-month period. Farm samples (n=10) using bootsocks and whole carcass rinses (WCR) from hot-rehang (RH=20) and post-chill (PC=20) were collected during each visit, resulting in a total of 800 samples. Samples were analyzed for *Salmonella* and *Campylobacter* by culture methods, followed by quantification with BAX®. Aerobic count (AC) and Enterobacteriaceae (EB) were enumerated from WCR. The

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data was analyzed using ANOVA and Tukey's HSD at $p \leq 0.05$ in JMP® 19. *Salmonella* prevalence in F1 (82%) and F2 (78%) were similar. There was a downstream reduction in F1 from Farm (91%) to PC (65%) and F2, from 100% (Farm) to 59% (PC). Four farms in F1 and three in F2 showed reduced prevalence along the Farm-PC continuum. In contrast, *Campylobacter* was more prevalent in F1 (77%) than F2 (37%), and it reduced from Farm (F1=100%, F2=78%) to PC (F1=50%, F2=14%), with more farms exhibiting reduction in F2 than F1. In both flocks, *Salmonella* counts were low and similar between Farm and PC samples, showing persistent behavior, with a slight decrease from RH to PC (0.2 (F1) and 0.4 (F2) log10 CFU/ml). Contrarily, *Campylobacter* loads were high with a 0.2 log CFU/ml reduction from RH to PC in F1 and an increase by 0.7 log CFU/ml in F2. A total of 19 serotypes were isolated, five (Indiana, Thompson, Muenchen, Cerro, Uganda) were unique to F1 and three (Altona, Teddington, Muenster) to F2. *C. jejuni* was the most isolated *Campylobacter* in both F1 (22%) and F2 (49%), with a reduction along Farm-PC continuum, while *C. coli* increased in both flocks. Farms in F1 (5.2 log CFU/ml) had higher AC than F2 (4.6 log CFU/ml) and reductions in AC from RH to PC were similar between flocks (F1=2.6 log CFU/ml, F2=2.3 log CFU/ml). Likewise, EB was significantly higher in F1 (4.5 log CFU/ml) than F2 (3.7 log CFU/ml) with better RH to PC reduction in F1 (3.2 log CFU/ml) than F2 (2.7 log CFU/ml). This study showed greater flock-level diversity across all populations, except *Salmonella*, whose loads remained consistent. Despite location-specific shifts, persistent microbial patterns indicate the need for targeted interventions and management strategies.

**Keywords:** Salmonella; Campylobacter ; non-conventional farm; poultry processing; food safety

### 36 Sanitation-linked microbial ecology and diversity on equipment in pastured poultry processing

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Poultry processing equipment can harbor microorganisms that promote cross-contamination, particularly in non-conventional operations where incoming flocks carry diverse microbiota. While sanitation remains a vital mitigation tool, its role in shaping microbial dynamics of processing surfaces requires attention, for an effective food safety plan. This study evaluated the impact of sanitation on microbial ecology of a processing plant by characterizing their composition and succession before and after sanitation. A pasture processing plant was visited twice monthly for 11 months (a flock/visit). Samples collected include eight equipment swabs pre- and post-sanitation (N=352) at processing stages A (pre-evisceration: bleeder, scalder, and picker), B (evisceration: tank, conveyor, and cutter), and C (second processing: debone cone and table). Samples were analyzed for *Salmonella* and *Campylobacter* by culture methods and quantified by BAX®. Aerobic count (AC) and Enterobacteriaceae (EB) were enumerated using Petrifilm™, while the microbial community was characterized by 16S rRNA sequencing. Data was analyzed using one-way ANOVA in JMP and R-Studio. Pathogen prevalence on equipment reduced significantly ($p<0.001$) across all processing stages after sanitation and was similar for both pathogens. Stage A had a higher pre- and post-sanitation prevalence, decreasing from 63% to 31% (*Salmonella*) and 91% to 24% (*Campylobacter*) compared to stages B (51% to 5% and 80% to 3%) and C (27% to 3% and 9% to 0%) for *Salmonella* and *Campylobacter*, respectively. Pathogen load reduced from an average of 0.14 log10 CFU/mL (*Salmonella*) and 1.93 log CFU/mL (*Campylobacter*) to below detection limits post-sanitation. Of the seven *Salmonella* serotypes identified, *S*. Kentucky dominated (73%) and persisted at all stages, but reduced by 26% post-sanitation, while *S*. Indiana increased by 19%. *C. jejuni* also dominated (48%), followed by mixed species *coli/jejuni* (31%), which were eliminated with sanitation. AC and EB loads followed a significant ($p<0.001$) trend across the stages. AC counts were reduced by 1.4, 1.3, and 1.7 log CFU/mL and EB counts by 1.3, 2.0, and 1.6 log CFU/mL at stages A, B, and C, respectively. Furthermore, sanitation significantly reduced bacterial diversity across all stages, and the main genera before sanitation were *Pschrobacter*, *Pseudomonas*, and *Escherichia*, while *Lysinibacillus*, *Bacillus*, and *Clostridium* dominated after sanitation, especially at stage C. The abundance and succession of these bacteria show their ability to persist on surfaces. Our results show that sanitation can alter the microbial composition of processing surfaces, allowing persistence of pathogenic, spoilage, and opportunistic populations.

**Keywords:** Salmonella; Campylobacter ; poultry processing; microbiome; sanitation

### 37 Evaluation of airborne bacterial recovery using three bioaerosol sampling approaches in a broiler house

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Accurate quantification of airborne bacteria in broiler houses is essential for assessing exposure risks to workers and animals, as well as for developing informed sanitation and surveillance strategies. Numerous sampling methods and equipment with differing collection physics, sampling parameters, processing, and handling have been developed in order to collect and quantify bacteria within bioaerosols. The objective of this study was to conduct a field comparison of three commonly used air sampling methods, including a cyclonic liquid impinger, a single-stage impactor, and gelatin filters housed in two-piece cassettes. Sampling was performed in an active broiler house using a balanced design (n = 6, k = 3, days = 5, N = 90) across five days (broiler ages D37, D38, D39, D40, and D44), with variation across sampling days evaluated descriptively. Liquid impinger and gelatin filter samples were collected from 100 L of air, whereas impactor samples were collected from 10 L to maintain countable colony densities. All counts were standardized to log10 cfu/$m^3$ before statistical analysis. Sampler type was found to have a statistically significant effect on bacteria recovery ($P < 0.05$), with method effects evaluated independently for total aerobic bacteria and *Staphylococcus* spp. cultured on tryptic soy agar and mannitol salt agar using a linear mixed-effects model that accounted for day level and block level variability. MacConkey agar results yielded frequent non-detections, with only sporadic recoveries on 100 L impactor samples, indicating that Gram-negative bacteria including coliforms were present at concentrations near or below method specific limits of detection. Although differences between samplers were modest compared to variability between days (accounting for 34 to 51% of total estimated variance across media), the impactor consistently yielded the highest recoveries across both tryptic soy agar and mannitol salt agar (4.47 and 4.18 log10 cfu/$m^3$, respectively), with recoveries from the liquid impinger and gelatin filters overlapping across media. These results indicate that sampler choice and associated processing can produce statistically significant but modest shifts in culturable airborne bacterial estimates that should be treated as a controlled methodological factor when interpreting and comparing field data. However, given the relative comparability between methods, ease of use and intended sampling parameters should guide sampling method selection rather than expectations of differing bacterial recovery.

**Keywords:** air sampling; bacterial recovery; bioaerosol; broiler house; method comparison

### 38 Assessing the Impact of Feed and Water Withdrawal in Poultry Production Processes Through Litter, Carcass, and Gastrointestinal

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**Microbiome**

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Feed withdrawal (FW) is a common preharvest practice in poultry production, while water withdrawal (WW) often occurs during transport and is largely unavoidable. Although these practices are intended to support processing, their broader effects on the gastrointestinal tract (GIT), carcass, and litter microbiome remain incompletely understood. The objective of this study was to compare a 12-h FW and 6-h WW treatment with a non-feed withdrawal (NFW) control in broilers. Birds (N = 14; n = 7/treatment) were raised on the same litter to 42 d of age. The NFW group remained on litter with continued access to feed and water for 12 h, whereas the FW group remained on litter without feed for 6 h and was then transferred to transport crates for an additional 6 h without water. At the end of treatment, litter, carcass rinsates, and GIT samples were collected for 16S rRNA gene sequencing of the V4 region using the Illumina MiSeq platform. Bioinformatic analyses were conducted in QIIME2-2025.7, with significance declared at P ≤ 0.05 and Q ≤ 0.05. FW carcasses exhibited greater microbial richness and evenness than NFW carcasses before scalding and plucking (P = 0.004, Q = 0.004; P = 0.048, Q = 0.048, respectively), and Enterobacteriaceae was more abundant on FW carcasses before and after processing. Within the GIT, the proventriculus and jejunum showed significant treatment-associated differences in microbial phylogenetic composition (Q < 0.05), including depletion of Lactobacillus in the proventriculus, whereas the ceca showed a trend toward community differences and reduced Lactobacillaceae abundance under FW. Litter from FW birds also exhibited greater richness and evenness, with significant differences in community structure (Q < 0.05). Overall, these findings indicate that feed and water withdrawal altered microbial ecology across the bird, litter, and carcass rather than affecting only gut fill, with selective reductions in *Lactobacillus*-associated taxa suggesting shifts in microbial stability that may be relevant to bacterial persistence and contamination risk. Further research is needed to determine the functional and food-safety significance of these changes.

**Keywords:** Microbiome; Broilers; Gastrointestinal Tract; Feed Withdrawal

**39 Impact of Peanut Skin Supplementation and *Salmonella* Challenge on Gut Microbiota of Broiler Chickens**

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*Salmonella*, a major foodborne pathogen in poultry, poses a human health risk. It has been demonstrated that peanut skins (PS) contain antioxidants and antibacterial properties, and our previous work demonstrated that feeding PS during challenge reduced *Salmonella* colonization. However, there is limited research on how PS affects the gut microbiota in chickens, though it likely influences *Salmonella*'s ability to colonize and expand. Therefore, this study evaluated the outcomes of the presence of PS (5%) in the conventional diet on ileal microbiota in *Salmonella* Typhimurium-challenged birds. A 2 x 2 design was used to randomly assign 180 Ross 308 chicks into four treatments: CON (control diet, non-challenged), PS (peanut skin diet, non-challenged), CONS (control diet with *Salmonella*), and PSS (peanut skin diet with *Salmonella*). At the end of the trial (day 44), ileal contents were aseptically collected from 12 birds/treatment for microbiota analysis. Sequencing of the V3-V4 hypervariable region of the 16S ribosomal RNA was performed using the Illumina MiSeq platform, and the data were analyzed using QIIME2 software for diversity metrics and LefSe for differential abundance analysis. Alpha diversity statistical analysis was performed using nonparametric tests, with a significance level of $p < 0.05$. Evenness did not differ significantly ($p > 0.05$). However, Richness, Faith's Phylogenetic Diversity (PD), and Shannon Index were significantly reduced in *Salmonella* challenged birds in both diets ($p < 0.05$). In addition, pairwise comparison revealed significant differences between CON and PS groups in Richness and Faith's PD metrics ($q < 0.05$). Unweighted UniFrac based PERMANOVA showed distinct microbiota clustering in non-challenged groups compared to the challenged groups ($p = 0.001$) and differences ($q < 0.05$) between CON and PS groups in pairwise comparison. Differential abundance analysis comparing challenged vs non-challenged birds within diet revealed 20 unique differentially abundant genera within CON and just 5 unique genera differences in PS fed birds (adjusted $p < 0.1$). This suggests a more widespread challenge effect in CONS than in PSS group. Considering our previous findings where *Salmonella* colonization was reduced in PSS birds, these results indicate that differences in microbiome composition under PS supplementation may influence selective microbial populations either through its antimicrobial activity or by altering the gut environment. However, *Salmonella* challenge was a dominant factor of overall microbial shifts. Future study is needed to fully understand the mechanisms by which PS interact with *Salmonella* and the gut microbiota in improving intestinal health in poultry production.

**Keywords:** Salmonella; Peanut Skin; Gut Microbiota; Broilers; Feed Additive

**40 Egg barn microbiome influence on biofilm formation, sanitizer effectiveness and S*almonella* persistence**

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**Objectives:** (1) To characterize the aviary and enriched layer barn microbiome before and after sanitation, (2) detect environmental biofilms, and (3) assess how microbial community interactions influence *Salmonella* persistence. **Experimental Design**: Environmental samples were collected from multiple locations in aviary and enriched layer barns at the Glenlea Research Station (Winnipeg, Canada). Sampling was conducted in December 2024, February 2025, and May 2025 to capture temporal variation in the barn microbiome. In May 2025, samples were collected at three defined stages: before cleaning (following depopulation), after cleaning, and after sanitation. Presumptive biofilms were detected using BioDetect®, a non-specific screening assay for biofilms (Sani-Marc, Quebec, Canada), then cultured and sequenced. **Materials and Methods:** For microbiome characterization, DNA was extracted from environmental samples and sequenced with the Illumina NovaSeq X. Kraken2 and Bracken on Galaxy Europe, were used for taxonomic classification and estimation of relative abundance. Thirty-six lactic acid bacteria (LAB) isolates obtained from the aviary and enriched barns were cultured, and cell-free supernatants (CFS) were used to test for inhibition effects on *Salmonella*. **Statement of Statistical Analysis:** Microbial community differences before and after sanitation were evaluated using alpha and beta diversity metrics and principal coordinates analysis (PCoA) of metagenomic sequencing data. **Results:** Metagenomic analysis revealed distinct microbial communities between aviary and enriched housing systems. Several bacterial taxa associated with biofilm formation were detected in the barn envi-

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ronment. Before cleaning, the aviary barn microbiome was dominated by *Staphylococcus equorum* (27.14%), followed by *Brachybacterium faecium* (7.46%) and *Lactobacillus crispatus* (4.79%), indicating lower community evenness. In contrast, the enriched barn exhibited a more evenly distributed community, with *Aerococcus urinaeequi* (9.34%), *Bifidobacterium pullorum* (9.30%), and *L. crispatus* (7.61%) as the most abundant species. After sanitation, *S. equorum* decreased to 12.95% but remained dominant in the aviary barn, while the enriched barn maintained a more even microbial community. Of the 25 LAB isolates, seven strains produced cell-free supernatants that exhibited bacteriostatic activity against *Salmonella*. **Conclusions**: These findings suggest that the egg barn microbiome may influence *Salmonella* persistence through microbial interactions and biofilm formation. Environmental LAB isolates exhibited inhibitory effects against Salmonella, suggesting that beneficial microbiota may play a key role in controlling pathogens in poultry production systems.

**Keywords:** Microbiome; Metagenomics; Layer Barn; Salmonella; Biofilms

### 41 Assessment of transport coop bacteriota in response to disinfection

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Poultry transport coops are an overlooked fomite for the transmission of foodborne and poultry specific pathogens. Understanding the microbial composition in response to microbial intervention strategies can provide evidence of how pathogens persist in certain areas after intervention, helping tailor protocols that target specific taxa and mitigate risk. The objective of this study was to use long-read sequencing to evaluate the impact of three disinfectants on transport coop microbiota. Four coops were presoaked for 15 min, mechanically power washed for 120 s, disinfected with garden sprayer application for 90 s, left for a 10 min contact, then rinsed for 60 s. Treatments used were a tap water rinse (WR), a glutaraldehyde (GLT), a quaternary ammonium compound (QAC), and a GLT – QAC blended product (SYN). Pre- and posttreatment samples were collected from each coop compartment before and after disinfectant application using sterile gauze in 10 mL of PBS (N = 80, n = 10, k = 4, time = 2) transported to the lab on wet ice. Genomic DNA was isolated from each sample collected and submitted for 16S amplicon sequencing targeting the full-length V1–V9 region using an Oxford Nanopore platform. Data were clustered and classified using NaMeco, and the diversity and taxonomy were assessed with QIIME2.2026.1. Due to the inability to account for coop differences in the statistical models, treatments were assessed individually. Alpha diversity increased with rinsing but decreased with QAC or SYN treatment ($P < 0.05$). Beta diversity was altered by SYN treatment and rinsing; however, the rinsing may have been driven by dispersion ($P < 0.05$). Treatment with QAC enriched the family Micrococcaceae and the genera *Kocuria* and an unclassified Micrococcaceae. Treatment with SYN depleted the families Aerococcaceae, Staphylococcaceae, and Bacillales DSM-18226, while the genera *Staphylococcus* and *Jeotgalibaca* were depleted. These taxa are typically found in the environment on various animal skins, with *Kocuria* and *Staphylococcus* previously implicated in harboring antibiotic resistance genes. Reductions in taxa associated with skin or environmental reservoirs suggest broad susceptibility to disinfectants, while enrichment patterns likely reflect relative survival within the residual community rather than directional ecological shifts. Given the established role of environmental microbiomes in pathogen transmission and antimicrobial resistance in poultry production, these results underscore the need to assess sanitation beyond quantitative reductions in microbial loads.

**Keywords:** Microbiome; Transport coops; Food Safety; Biosecurity; Disinfection

### 42 Probiotic formulations of *Lactobacillus paracasei* reduce *Salmonella* colonization in laying hens and egg contamination

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Introduction: Egg contamination with *Salmonella* Enteritidis (SE) is a major cause of foodborne illness in the United States. Effective preharvest interventions are limited. Probiotics are promising alternatives due to their ability to inhibit pathogen colonization. In our previous work, in-feed supplementation of *Lacticaseibacillus paracasei* DUP-13076 (LP) as a fresh culture significantly reduced SE contamination in eggs. However, efficacy across different formulations remains unclear. Hypothesis: In-feed LP supplementation in different formulations reduces SE colonization in laying hens and subsequent egg contamination. Methods: Two independent experiments were conducted using 19- and 40-week-old Lohmann Lite hens assigned to 4 dietary treatments: control (no probiotic) or feed supplemented with LP (9 log CFU/kg) as fresh culture, encapsulated beads, or lyophilized powder. Each treatment had 2 replicate pens (10 birds/pen) for 60-d. Birds were orally challenged with SE (10 log CFU/bird). Eggs were collected daily for 5 wk and analyzed for SE on outer shells (OS) and internal contents (IC). Cloacal swabs were collected weekly. At d 60, ceca and oviduct were sampled for SE enumeration. Data were analyzed using one-way ANOVA with significance at $P \leq 0.05$. Results: LP supplementation across formulations significantly reduced SE colonization in the cecum ($P \leq 0.001$), with ~2 log CFU/g lower counts in treated birds compared to controls. This reduction was associated with increased cecal *Lactobacillus* populations (~10–11 vs. ~8 log CFU/g), suggesting competitive exclusion. No SE was recovered from the oviduct in LP groups, whereas control birds consistently showed SE presence, suggesting reduced translocation to the reproductive tract. Cloacal shedding of SE was significantly reduced across probiotic treatments ($P < 0.01$), reflecting decreased fecal shedding and horizontal transmission. These effects reflected significant reduction in egg contamination over the 5-week period ($P \leq 0.01$), with >29% reduction on OS and >4% in IC. In 19- and 40-week, 10.01–19.67% and 9.14–12.81% of OS samples were SE-positive in LP groups compared to 50.26% and 41.75% in controls, respectively. Approximately 4% of IC samples were SE-positive in controls, whereas no IC contamination was detected in LP-treated hens ($P \leq 0.001$). Among formulations, encapsulated LP showed the greatest numerical reduction. Conclusions: In-feed supplementation of *L. paracasei* formulations effectively reduced SE colonization, translocation, shedding in laying hens and subsequent egg contamination. Comparable efficacy suggests alternative delivery forms can be used to mitigate SE transmission and improve egg safety under practical production conditions.

**Keywords:** Salmonella control; probiotic formulations; laying hens; egg safety; competitive exclusion

### 43 Effect of a short- and medium- chain monoglycerides blend on *Salmonella* colonization, translocation and growth performance in broilers.

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Short- and medium-chain fatty acids and their derivatives monoglycerides (SCFA-/MCFA-MG) have been used as antimicrobial agents against pathogenic bacteria by lowering luminal pH, regulating immune function, and improving nutrient utilization in broilers. This study was designed to evaluate the effects of a dietary SCFA-/MCFA-MG blend on performance and *Salmonella* colonization in broilers. A total of 600 Ross 708 male broilers were randomly assigned to 24 floor pens with 25 birds each on the day of hatch. Three dietary treatments with 8 replicate pens include: Control, Control + 0.15% MG blend (MG-Low), Control + 0.3% MG blend (MG-High). Growth performance was measured weekly. Birds were orally challenged with a nalidixic acid-resistant *Salmonella* Typhimurium strain at ~5 log CFU/bird on d 7. Cecal, liver, and spleen samples were aseptically collected from two birds per pen on d 14, 21, 35, and 42. Boot swab was collected from each pen weekly post-inoculation. On d 43, broilers were processed through the standard procedure. Two carcass rinsate samples per pen were collected at the post-pick stage. *Salmonella* concentration in the ceca was enumerated, while prevalence in the liver, spleen, boot swab, and carcass rinsate were assessed. Data was analyzed using PROC GLM in SAS, with Tukey's test for post-hoc comparisons. Polynomial contrasts were used to evaluate dose effects. Differences in *Salmonella* prevalence were analyzed using the chi-square test. Significance level was set at $P \leq 0.05$. Dietary supplementation of MG linearly improved FCR by 4 points (1.13 vs 1.09) during d 0-14 ($P = 0.03$). Boot swabs remained positive during the whole post-inoculation period. On d 14, MG supplementation linearly decreased ($P < 0.01$) cecal *Salmonella* concentration. The MG-High group showed a 2 log CFU/g reduction on *Salmonella* concentration compared to the Control: 1.51 vs 3.54 log CFU/g ($P = 0.02$). The MG-Low group numerically reduced the *Salmonella* concentration to 2.50 log CFU/g. Broiler carcass rinsates from the Control group had a *Salmonella* prevalence of 100%, while MG-Low group had a lower prevalence at 71%, and MG-High supplementation further reduced the prevalence to 44% ($P < 0.01$). In conclusion, supplementation of SCFA-/MCFA-MG blend improved the feed efficiency of broilers at an early age and reduced *Salmonella* colonization, effectively lowering pathogen prevalence on carcasses at processing.

**Keywords:** broiler; Salmonella; organic acids; monoglycerides; food safety

### 44 Non-invasive detection of *Salmonella* contamination in SPF White Leghorn eggs via VOC profiling at cold storage temperatures

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This study aims to characterize volatile organic compound (VOC) emissions from eggs stored at low temperatures as early biomarkers of microbial contamination. Specifically, we targeted microbial VOCs (mVOCs) produced by *Salmonella enteritidis* (SE) in eggs stored under standard hatchery conditions (17°C) versus commercial storage conditions (4°C). 120 Specific Pathogen Free (SPF) White Leghorn eggs were randomly assigned to eight treatment groups (control vs. SE; yolk vs. albumen; 17°C vs. 4°C). The eggs were then inoculated with Phosphate Buffered Saline (PBS) or PBS containing nalidixic acid-resistant SE ($10^3$ CFU/mL) and stored at the assigned temperatures for three days. VOCs were collected daily from the same eggs using a sorption extraction device in sterile glass jars for 90 minutes. The collected VOCs were analyzed via 2D gas chromatography–mass spectrometry (GC×GC–MS), with identification and alignment through the NIST 2017 spectral database. Ground truth data were collected by performing daily subset breakouts for each treatment group. Egg contents were diluted at a 1:1 ratio in 1% Buffered Peptone Water (BPW) and then subjected to serial dilutions in PBS. The PBS dilutions were plated on Brilliant Green Sulfa Agar (BGS) containing 100 ppm Nalidixic Acid and incubated for 48 hours to determine SE colony counts, which served as the ground truth data for VOC profiles. For samples with no detectable SE after 48 hours, enrichment was performed, followed by re-plating on BGS to confirm the absence of SE. Principal Component Analysis (PCA) was applied to VOC data, and one-way ANOVA was used to analyze SE recovery counts. Results indicate that temperature is a key driver of VOC profile differentiation. At 17°C, SE-positive yolk samples produced VOC signatures that were distinct from controls, as confirmed by PCA. At 4°C, VOC intensities were low and variable, with no consistent differences between SE and control groups, suggesting limited SE metabolic activity and weak VOC outputs. At 17°C, overall VOC production increased in both SE and control samples. Despite this general increase, SE-positive samples still exhibited VOC patterns that were distinct from the background VOCs produced by the egg matrix. Although additional replicates are needed, these preliminary results highlight the potential for mVOC profiling as a non-invasive tool for early detection of contamination. This method carries the potential for reducing pathogen transmission risk and enhancing hatchery biosecurity and the safety of the final product.

**Keywords:** Egg; Salmonella; VOC; Non-invasive; Detection

### 45 Effect of a multi strain *Bacillus* probiotic on *Salmonella* colonization in broiler chickens

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Modern broiler production faces increasing pressure to maintain gut integrity and microbial balance under intensive rearing conditions. The objective of this study was to evaluate whether a multistrain *Bacillus* probiotic could reduce cecal, environmental, systemic, and carcass *Salmonella* recovery in broiler chickens under a controlled challenge model. To test this, we used a blend of three *Bacillus* strains: *Bacillus velezensis* NRRL B-67647, *Bacillus pumilus* NRRL B-67648, and *Bacillus licheniformis* NRRL B-67649 (BS; Phileo by Lesaffre, France) on *Salmonella* recovery in broiler chickens. A total of 900 day-old male chicks (Ross 708) were assigned to 3 dietary treatments in a RCBD with 12 replicate pens of 25 birds each: (1) positive control (PC; challenged with nalidixic acid resistant *Salmonella Typhimurium* and *S. Infantis* at $10^5$ CFU/bird on day 0), (2) PC + bacitracin methylene disalicylate (BMD; 100 mg/kg feed), and (3) PC + *Bacillus* strains (BS; 500 mg/kg feed). Negative control was included as an unchallenged reference group. Diets were formulated to meet or exceed Ross 708 nutrient requirements for starter (d0-14), grower (d15-28), and finisher (d29-35) phases. Ceca, boot swabs, liver, and spleen were aseptically collected at d7, 14, 21, 28, and 35. Broilers were processed under a standard procedure on d36, and carcass rinsates were taken at post pick stage (n=24 per treatment). Log CFU counts were analyzed by ANOVA with Tukey's HSD, and prevalence outcomes were evaluated using Chi-square, Fisher's exact tests, and Bonferroni adjusted pairwise proportion tests. Significance was set at $P<0.05$, and tendency was taken at $P<0.1$. BS did not differ from PC or BMD at

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d7 (P=0.20) but reduced cecal *Salmonella* at d14, 21, and 28 (P<0.001), with consistently lower counts than both PC and BMD (P<0.01). At d35, most counts were near or below detection. Boot swab *Salmonella* was lower in BS than BMD at d7 (P=0.011), but from d14 to 28, BS, BMD, and PC did not differ (P>0.10). At d35, boot swab *Salmonella* counts were lower in BS than PC (P=0.036), with a trend versus BMD (P=0.053). Systemic prevalence was high at d7 (liver 67–75%; spleen 38–54%) in all challenged treatments. From d14 onward, liver and spleen *Salmonella* prevalence did not differ among PC, BMD, and BS (P>0.90). Carcass rinsate prevalence remained high at d36 (PC 100%, BMD 79%, BS 83%), with no reduction relative to PC (P>0.10). The multistrain *Bacillus* probiotic reduced cecal *Salmonella* at mid and late growout and produced occasional improvements in environmental shedding but did not mitigate systemic infection or carcass contamination under challenged conditions.

**Keywords:** Bacillus probiotic; broiler; Salmonella; colonization; carcass prevalence

### 46 Effects of dietary organic acids supplementation on broiler growth performance and *Salmonella* colonization

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Formic acid has been used for improving feed hygiene, promoting growth performance, and supporting gut health in poultry. This study evaluated the effects of a formic acid-based blend (FB) on growth performance and the colonization and translocation of *Salmonella*. A total of 600-day-old Ross 708 male chicks were randomly distributed among 24 floor pens (25 birds/pen) and assigned to 1 of 3 dietary treatments for 42 d: Control, Control + 0.3% FB (FB-Low), and Control + 0.6% FB (FB-High). On d 7, all birds were orally inoculated with 5 log CFU/bird of nalidixic acid-resistant *Salmonella* Typhimurium. Growth performance was measured on d 7, 14, 21, 28, 35, and 42. On d 14, 21, 35, and 42, 2 birds per pen were euthanized for aseptic collection of liver, spleen, and ceca. Boot swabs were collected from each pen weekly post inoculation. On day 43, 2 birds per pen were processed and carcass rinsates were collected post-pick. Data was analyzed using PROC GLM in SAS, with Tukey's test for post-hoc comparisons. Polynomial contrasts were applied to evaluate dose effects, and linear contrasts were used to assess responses in growth performance including body weight (BW). Differences in *Salmonella* prevalence were analyzed using the chi-square test and significance level was set at $P \leq 0.05$. Supplementation with FB-High significantly increased ($P < 0.05$) BW gain compared to the Control during both the 0-28 d and overall (42 d) periods. Boot swabs remained positive throughout the trial post inoculation. Furthermore, FB supplementation reduced *Salmonella* prevalence in carcass rinsates from 100% (Control) to 64% (FB-Low) and 69% (FB-High) treatments, respectively ($P = 0.017$). In conclusion, both low and high doses of the formic acid-based blend reduced *Salmonella* contamination in post-pick carcass rinsates. The high dose provided additional benefits by improving BW gain and reducing cecal *Salmonella* concentrations prior to processing.

**Keywords:** Organic acids; formic acid; Salmonella; growth performance; food safety

### 47 Synergetic effect of chemical stabilizers on peracetic acid and resulting antimicrobial ability

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Peracetic acid (PAA) is utilized in the immersion chilling process to ensure food safety with its antimicrobial properties, but its chemical lifetime can be limited by the harsh conditions of chillers. Our previous work identified two chemicals which proved effective at extending PAA lifetime: 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) and ethylenediaminetetraacetic acid (EDTA). That study determined PAA's half-life was most extended by the synergetic effect of 15 ppm HEDP with 200 ppm EDTA. This optimal combination was then implemented for an antimicrobial study to determine PAA's efficacy with an extended half-life. Each experiment used wings, drumettes, and drums, which were inoculated by submerging (for 10 seconds) into a *Salmonella* Typhimurium ($10^5$ CFU/mL) PBS solution. The parts were drained, placed in a bag, and incubated at 37 C for 60 minutes, allowing for surface attachment. After incubation, parts were randomly distributed into four groups with n=15 per group: control, PAA only, PAA plus 15 ppm HEDP with 100 ppm EDTA (¬Ω conc.), and PAA plus 15 ppm HEDP with 200 ppm EDTA (full conc.). Each part was exposed to its assigned condition by submerging for 15 seconds. PAA was maintained at 600 ppm with pH adjusted to 9.0 using 4M NaOH. After exposure, samples were individually placed in bags containing 50 mL neutralizing buffered peptone water (nBPW) to deactivate residual PAA. *Salmonella* was recovered by hand shaking each bag for 60 seconds and all samples were serially diluted in PBS. Two dilutions per sample were plated (duplicates per dilutions) on 3M aerobic Petrifilm®. Plates best fitting the counting range of 10-150 CFU/mL were selected from the two dilutions per sample. Dilution factors were accounted for to calculate average final counts and were log transformed. This experiment was replicated three times. The PAA only group had an average log reduction of 0.712; ¬Ω conc. group had an average reduction of 0.879; and full conc. group had an average reduction of 0.870. A One-Way ANOVA was performed to compare conditions: $F$ (3,56) = 18.344, $p$= 0.05; indicating significance. Post hoc analysis was then conducted using Tukey Kramer HSD to compare all possible pairs of conditions and determine which comparisons were significant. Results showed significance existed between: control and PAA group (mean difference= 0.713, q= 7.314); control and ¬Ω conc. group (mean difference= 0.879, q= 9.018); control and full conc. group (mean difference= 0.870, q= 8.932). No significance was found between treatment groups. This identified that each PAA treatment was similarly effective at reducing *Salmonella,* showing that extended lifetime does not interfere with antimicrobial ability.

**Keywords:** Peracetic acid; Decay Kinetics; Lifetime; Synergy; Antimicrobial

### 48 Microbial Recovery of Layer Hen Carcasses Treated with a Poultry Mortality Dehydrator

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Poultry carcass disposal must effectively contain pathogens to maintain biosecurity while minimizing environmental impacts. While sustainable mortality management strategies aim to balance pathogen control, microbial inactivation through thermal dehydration has not been well defined. The objective of this study was to evaluate the use of thermal processing in the disposal of layer hen mortalities. Fifteen independent runs were conducted, in which a commercial poultry mortality dehydrator processed 357.4–430.9 kg loads of layer hen carcasses. Processing cycles lasted 8.6–10.9 h and reached peak temperatures of 79.7–84.6 °C, resulting in approximately 56–58% carcass mass reduction. For each run, five replicate samples were collected before and after heat treatment. At each

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time point, samples were stomached, 1:10 serial diluted, and cultured using plating and enrichments for total aerobic loads, *Staphylococci,* and coliforms. Data were reported as log10 cfu/g. Samples with no colony growth with positive enrichment were assigned counts of 1.5 log10 cfu/g for statistical analyses. The pre- and post-treatment mean counts were analyzed using Welch's unpaired t-test. Thermal treatment reduced all evaluated microbial populations ($P < 0.001$). Prior to treatment, total aerobes, *Staphylococci*, and coliforms were 8.99, 8.43, and 8.63 log10 cfu/g, respectively. After treatment, total aerobes, *Staphylococci*, and coliforms were 3.19, 2.32, and 1.43 log10 cfu/g, respectively. These results demonstrate that commercial thermal dehydration produces consistent and substantial reductions in culturable microbial populations in layer hen mortalities. Reductions were consistent across independent runs, supporting thermal dehydration as a reliable approach for mortality management.

**Keywords:** mortality management; Biosecurity; Pathogen control; Thermal processing; Bacterial inactivation

## Student Competition: Immunology, Health and Disease - Feed Additives

**206 (A-D) (MTCC)**

### 49 Effects of black soldier fly larvae oil on growth performance, gut permeability, and *Clostridium perfringens* colonization in broiler chickens under Necrotic enteritis challenge

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A study was conducted to evaluate the effects of black soldier fly larvae oil (BSFLO; EnviroOil, EnviroFlight LLC, USA) on growth performance, intestinal permeability, and *Clostridium perfringens* colonization in broiler chickens under necrotic enteritis (NE) challenge. A total of 448 one-day-old broilers were allocated to four treatments with eight replicates of 14 birds: non-challenged control (NC; 3% soy oil), challenged control (CC0; 3% soy oil), CC1.5 (1.5% soy oil + 1.5% BSFLO), and CC3 (3% BSFLO). Birds were challenged with *Eimeria maxima* and *Eimeria acervulina* on day 14 as predisposing factors, followed by *Clostridium perfringens* ($1 \times 10^9$ CFU) on days 18 and 19. Growth performance was evaluated during d 0–14 (pre-challenge) and 0–7 and 0–14 days post-inoculation (dpi). Intestinal permeability and jejunal *C. perfringens* counts were measured. Data were analyzed using one-way ANOVA with Tukey's HSD test and orthogonal contrasts for treatment effects. During the pre-challenge period (d 0–14), FI and FCR were not affected ($P > 0.05$), but BWG increased linearly with increasing replacement of soy oil by BSFLO ($P = 0.023$), with CC3 showing approximately 15 g greater BWG than CC0. After challenge, growth performance declined in all challenged groups compared with NC. At 0–7 dpi, BWG and FI decreased and FCR increased relative to NC ($P < 0.001$), with no differences among challenged treatments. Over 0–14 dpi, partial or complete replacement of soy oil with BSFLO improved BW and BWG relative to CC0 and increased FI ($P = 0.003$). Intestinal permeability was not affected during the pre-challenge period but increased in challenged groups at 6 dpi ($P < 0.001$). At 7 dpi, intestinal permeability decreased linearly with increasing replacement of soy oil with BSFLO ($P = 0.017$), with CC3 showing the lowest values among challenged treatments. Jejunal *C. perfringens* counts were highest in CC0 and decreased linearly with increasing replacement of soy oil by BSFLO at 6 and 7 dpi ($P = 0.043$). In conclusion, replacement of soy oil with BSFLO improved BWG during the pre-challenge period and partially mitigated growth reduction during 0–14 dpi while reducing intestinal permeability and *C. perfringens* colonization. These results support BSFLO as a functional lipid source for improving resilience to enteric challenge in broiler chickens.

**Keywords:** black soldier fly larvae oil; broiler; growth performance; gut permeability; Clostridium perfringens

### 50 Exploratory meta-analysis of antibiotic alternatives (probiotics and prebiotics) on growth performance in broiler chickens before, during, and after experimental necrotic enteritis infection

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Health challenges reduce voluntary feed intake reductions, affecting weight gain and sometime feed conversion in broilers. The influence of antibiotic alternatives on performance response is unclear. This meta-analysis evaluated probiotic and prebiotic effects on feed intake reduction (ΔADFI) and their relationship with body weight gain reduction (ΔADG) in broilers subjected to experimental necrotic enteritis models. Data from 12 peer-reviewed papers (2012–2026; 160 observations) were analyzed. Data on feed intake and weight gain were collected from treatments: positive control (PC - non-challenged; no additives), negative control (NC – challenged; no additives), antibiotic (ATB), probiotic (PROB), and prebiotic (PREB). The ΔADFI and ΔADG were calculated for the NC, ATB, PROB, and PREB treatments relative to PC: ΔRti= (Rti - RPC)/RPC, where ΔRti is the relative response (ΔADFI or ΔADG) of one of the treatments (ti: NC, ATB, PROB, or PREB) and RPC is the response (ADFI or ADG) of the PC. Based on the time of infection, the data were separated into periods: pre-infection (before inoculation), infection (immediately after inoculation), and post-infection (more than 7 days post-infection). Data were analyzed using a mixed-effects model with ΔADG as the response, fixed effects for ΔADFI, treatment, and interaction treatment×ΔADFI, and random effects for study and study×ΔADFI. To evaluate the influence of ΔADFI on ΔADG, a regression analysis was performed to compare the slope between treatments: ΔADGti= a + bti x ΔADFIti. No significant study effect or interaction with ΔADFI was observed. ΔADFI significantly influenced ΔADG during pre-infection (P=0.049) and infection (P=0.001), with a trend post-infection (P=0.076). Treatment effects on ΔADG were significant pre- and post-infection (P<0.05) but not during infection, and the treatment×ΔADFI interaction showed a trend only post-infection (P=0.067). Slope comparisons revealed phase-dependent responses. In pre-infection, PROB had a steeper slope (b=0.859) than ATB (b=0.196, P<0.001) and PREB (b=0.384, P=0.026), with ATB showing no significant slope (P=0.138). During infection, ATB exhibited the strongest correlation (b=1.941), differing from NC (b=0.595, P=0.009) and PREB (b=0.789, P=0.024) while during post-infection, PREB slope became non-significant (b=-0.064, P=0.780) and differed from ATB (b=0.788, P=0.021) and PROB (b=0.660, P=0.013). These results indicate that feed additive effects on the intake–weight gain relationship vary with infection phase, with PREB potentially stabilizing intake post-infection and PROB improving intake and gain versus ATB, providing valuable insights for predictive models in broiler nutrition under sanitary stress.

**Keywords:** probiotics; prebiotics; broiler chickens; necrotic enteritis; antibiotic

### 51 Yeast-derived additives improve intestinal morphology in broilers under a necrotic enteritis challenge

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Modern poultry production continues to seek innovative feed additives that enhance bird health without compromising performance. Given the

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pivotal role of the gastrointestinal tract, strategies aimed at optimizing intestinal function have gained increasing attention. Among these approaches, yeast-derived additives have demonstrated significant potential in improving intestinal integrity and promoting mucosal development. Therefore, this study evaluated the effects of two yeast-derived additives on broiler intestinal morphology and integrity during a subclinical necrotic enteritis challenge. Day-old male broiler chicks (n = 600) were randomly assigned to four treatments (six pens/treatment): negative control (NC, basal diet), positive control (PC, NC + avilamycin), GlucanMos (GM, NC + GlucanMos), and GOLF/GlucanMos (GF/GM, NC + GOLF/GlucanMos). On d14, birds were orally gavaged with 2,000 sporulated oocysts of *Eimeria maxima*, followed by *Clostridium perfringens* (~$10^8$ CFU/mL) on d19 and d20. At days 14, 21, and 28, six birds per treatment were euthanized, and jejunal and ileal samples were collected, fixed in 10% buffered formalin, embedded, sectioned, and stained with Hematoxylin & Eosin (H&E). Intestinal integrity was evaluated by measuring villus height (VH), crypt depth (CD), and calculating the VH:CD ratio. Data were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS OnDemand), with the treatment group included as a fixed effect. Separate models were fitted for each tissue and sampling time. Pairwise comparisons were adjusted using Tukey and differences were considered when $P \leq 0.05$. At d14, both additives increased jejunal VH compared with NC and PC, and increased VH:CD compared with NC. In the ileum, both additives increased VH compared with PC, while GF/GM reduced CD relative to NC and increased VH:CD compared with NC and PC. At peack challenge (d21), GM birds had greater jejunal VH and VH:CD compared with those in NC and PC, while both GM and GF/GM had reduced ileal CD relative to NC. At d28, GM increased jejunal VH compared with NC and ileal VH compared with NC and PC. Overall, yeast-derived additives improved intestinal histomorphology in broilers, as evidenced by increased VH and VH:CD, along with reduced CD in specific segments and time points. These effects were more pronounced in the jejunum prior to and during the challenge, and in the ileum during the recovery phase (d28). The morphological modulation suggests enhanced intestinal integrity and absorptive capacity, as well as improved epithelial turnover dynamics of broilers under a necrotic enteritis challenge.

**Keywords:** Clostridium perfringens; necrotic enteritis; broiler; prebiotic; yeast

### 52 The effect of dietary supplementation of Organic Chaga Mushroom in broilers challenged with Necrotic Enteritis

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Necrotic enteritis(NE) is an enterotoxemia of poultry caused by *netB*-positive *Clostridium perfringens(*CP). Antibiotic growth promoters have been traditionally used to control NE in past decades. Since the ban on antibiotic use in the poultry industry, subclinical NE has reemerged as an economically significant poultry disease. Chaga Mushroom(CM: *Inonotus obliquus)* is a promising alternative to antibiotic growth promoters. The objective of the experiment was to investigate the effect of various levels of Chaga mushroom supplementation in broilers challenged with NE. A total of 420 one-day-old broiler chicks were randomly allocated into 5 treatments with 6 replicates, with 14 birds per replicate. Treatments included non-challenged control (NC), challenge control (CC), and CM1, CM2, and CM3, which were the groups challenged and fed 1%, 2%, 3% Chaga mushroom powder, respectively. On the 14$^{th}$ day, birds of the NC group were orally gavaged with 1ml of PBS on day 14, 18, and 19, and the challenged groups were orally gavaged with 10,000 sporulated oocysts of *Eimeria maxima* on day 14, followed by 1 × $10^9$ CFU of CP on d 18 and 19. Growth performance was recorded over 28 days. On d 21, one bird per pen was randomly selected and euthanised to collect jejunal and cecal contents for CP loads and cecal contents from one bird per pen for short-chain fatty acid (SCFA) profile. Body composition of birds was measured using Dual-energy X-ray absorptiometry (DEXA). The data were analyzed with one-way ANOVA, Wilcoxon/Kruskal-Wallis tests, and Tukey HSD, using JMP Pro 19 statistical software. On D28 (14dpi), the CC group had significantly lower BW and BWG compared to the NC group ($P$<0.05). Chaga mushroom supplemented groups had higher BW and BWG compared to the CC and were not significantly lower than the NC, particularly in the CM2 group. Chaga mushroom-supplemented groups showed an improved FI and FCR during 0-14 DPI compared to the CC group. NE challenge significantly increased jejunal digesta and cecal CP loads in all challenged groups. Among the challenged group, CM2 had a significant reduction in CP loads in jejunal digesta and cecal content compared to the CC group. The CM2 showed a lower jejunal NE lesion score compared to the CC and was not significantly different from the NC. The CM2 had significantly higher concentrations of total SCFA and butyrate compared to the NC. DEXA analysis revealed that the CM2 group showed increased lean muscle % and reduced Fat% compared to the other treatments. In conclusion, the results showed that dietary supplementation of 2% organic chaga mushroom powder is a potential mitigation strategy for NE in broilers.

**Keywords:** Necrotic enteritis; Mushroom; Feed additive; gut health

### 53 Evaluation of gallic acid supplementation on growth performance and gut health in broilers under Coccidiosis

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Coccidiosis remains a major concern that causes severe damage to gut integrity and impairs growth performance in chickens, leading to huge economic losses in the poultry industry. As the poultry production system has been facing the emergence of resistance to anticoccidials and vaccination gap, there is a growing demand for identifying effective natural alternatives. Gallic acid (**GA**), a natural polyphenol compound abundant in various fruits, plants, and nuts, exerts antimicrobial, antioxidant, and anti-inflammatory properties and may serve as a promising natural antimicrobial compound against *Eimeria spp.* in broilers. Therefore, a study was conducted to evaluate the effect of gallic acid supplementation on growth performance and gut health in *Eimeria*-challenged broilers. A total of 720 one-day-old male broilers were assigned to a 2 x 5 factorial arrangement having 6 replicates per treatment. The factors were *Eimeria* challenge and five treatments supplemented with graded levels of GA 0, 0.1, 0.2, 0.4, and 0.8 g/kg in the diet. On d14 (0 days post-inoculation: **DPI**), all birds from challenged groups were orally gavaged with an inoculum containing 10,000 *E. tenella*, 10,000 *E. maxima*, and 62,500 *E. acervulina* sporulated oocysts. Data were analyzed using two-way ANOVA and generalized linear model, followed by Tukey post-hoc, and the level of significance was set as $P < 0.05$. During the pre-challenged period (0-14d), GA significantly improved body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**) and feed conversion ratio (**FCR**) in a quadratic manner ($P < 0.05$). Following challenge (0-6 DPI), the birds showed reduced BW, BWG, and FI, and increased FCR ($P < 0.05$) when compared to the unchallenged group. *Eimeria* challenged groups had significantly higher serum FITC-d concentration compared with the unchallenged group, indicating destruction in gut barrier function**.** However, GA supplementation significantly improved body weight and reduced FITC-d level in serum, with moderate doses having greater efficacy. During 6-12 DPI, *Eimeria* challenge had a continued neg-

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ative impact on growth performance, whereas GA significantly improved the body weight ($P < 0.05$). During the overall challenge period (0-12 DPI), *Eimeria* challenge significantly reduced body weight, body weight gain, and feed intake and worsened feed utilization efficiency. No interaction effect between GA supplementation and challenged condition was observed ($P > 0.05$). In conclusion, *Eimeria* challenge had a profound effect on chicken health that reduced growth performance and increased gut permeability, while dietary inclusion of GA improved body weight and reduced intestinal permeability, with the inclusion of 0.2 g/kg being most effective.

**Keywords:** Gallic acid; Eimeria; broilers; natural antimicrobial compound; gut health

### 54 Safety Evaluation of Bacteriophage vB_SalS_KY05 Lysate via Drinking Water: Impacts on Health, Egg Production, and Intestinal Microbiota in Laying Hens

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The overuse of antibiotics and the emergence of antimicrobial resistance have become serious concerns for food safety and public health. In intensive egg production systems, bacteriophages (phages) have been considered potential alternatives due to their higher specificity and self-amplifying properties. Phages are typically propagated in lysate form, which is highly efficient and cost-effective for large-scale production. However, phage lysate may contain residual components of lysed bacteria, particularly endotoxin, which could induce inflammation and immune responses in animals. Bacteriophage vB_SalS_KY05 (KY05) is a natural phage isolated from local poultry farm. The objective was to evaluate the effects of KY05 lysate on the health status, laying performance, and intestinal health of laying hens. The lysate was propagated with a non-pathogenic *E. coli* K-12 strain and administered orally via drinking water for 56 days. 30 Lohmann Brown-Classic laying hens (53 weeks old) were randomly assigned to three groups (n=10): Control (without phage lysate), Low-phage ($1\times10^5$ PFU/mL) and High-phage ($1\times10^8$ PFU/mL). The average endotoxin level in phage lysate was $8.68\pm0.97\times10^4$ EU/mL. Data were analyzed with Kruskal-Wallis test when assumptions of normality or homogeneity of variance were not satisfied. The results demonstrated that phage lysate did not adversely affect water intake, body weight, egg production, and egg quality compared with the control group. Furthermore, no significant differences were observed among groups in plasma biochemical parameters, mRNA expression (*ZO-1*, *OCLN*, *CLDN1*, *IL6*, *TNF*A, *IL8*), cytokine (IL-1B, IL-6, TNF-A) levels, or intestinal morphology. Hens in phage-treated groups exhibited lower heterophil-to-lymphocyte ratios, suggesting that the animals were not under physiological stress. KY05 was re-isolated from the ceca at concentration of 5 log10 PFU/g on day 56, indicating the ability to colonize the intestinal tract. Additionally, the fecal *Lactobacillus* was not affected, while reduction of fecal *E. coli* showed in phage-treated groups compared to control group, although not statistically significant. In conclusion, our findings suggest that phage lysate is a safe strategy for use as a biocontrol agent in the poultry industry. Long-term administration did not induce adverse impacts on physiological or intestinal health. This approach may contribute to mitigating antimicrobial resistance while promoting food safety and sustainable poultry production. Further studies should investigate the long-term effects under field conditions, such as tap water systems or contaminated environments, to strengthen its practical potential for commercial application.

**Keywords:** bacteriophage; safety evaluation; laying hens; intestinal health; bacteriophage lysate

### 55 Jejunal transcriptomic responses to inorganic and amino acid complexed zinc and iron in ascites-challenged broilers

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Zinc (Zn) and iron (Fe) are essential trace elements involved in critical biological functions such gut integrity and immune function, as well as hemoglobin formation. Together, these minerals are a promising potential strategy to alleviate ascites. This study investigated the effects of different sources of Zn and Fe supplementation in broiler diets under an ascites-challenged model through transcriptomic analyses. In total, 1,320 male Ross 308 chicks were placed in a research facility (Neuro Livestock Research, South Africa) at ~1,180m above sea level. The birds were randomly allocated to one of two dietary treatments, being Zn and Fe supplementation as sulfate sources (Zn-S/Fe-S), which served as the industry-standard control group, or complexed to amino acids (Zn-AA/Fe-AA) (Zinpro Corporation, Eden Prairie, USA) at 60 and 30 ppm for each mineral, respectively. Each treatment was replicated 12 times with 55 birds placed per pen. Broilers were reared until 32 d of age and underwent an ascites cold stress challenge model in the first 2 d of life. At 24 d of age, 12 birds per treatment were euthanized and had jejunum samples collected. The RNA was extracted followed by mRNA sequencing (Illumina, 150 PE, poly-A, 20M reads per sample) and gene abundance calculated (Kallisto). Differentially expressed genes were tested among treatments (Deseq2), followed by a topology-based Quantitative Pathway Activation method (QPA, Biofractal, Portugal; adj$P<0.05$) that calculated standardized QPA scores indicating relative pathway inhibition (QPA<0) or activation (QPA>0). Pathway scores were tested by Kruskal-Wallis and adjusted for multiple testing using the Benjamini-Hochberg procedure. Supplementation of Zn-AA and Fe-AA downregulated jejunal pro-inflammatory cytokines such as IL-17 (QPA score = -0.97), IL-1 (QPA score = -1.44), and IFNΓ (QPA score = -1.49) signaling pathways in comparison to broilers fed Zn-S and Fe-S. Furthermore, an improvement in tight junction protein expression was reported in the broilers fed Zn-AA and Fe-AA, with improvements in tight junction regulation (QPA score = 1.98), scaffolding proteins (QPA score = 1.66), and transmembrane protein expression (QPA score = 0.77). Furthermore, 72.87% of mortalities in the control group were attributed to ascites, which was significantly reduced ($P<0.05$; ANOVA, student-t means separation in JMP) through Zn-AA/Fe-AA supplementation to 44.11%. These findings demonstrate that the supplementation of Zn-AA and Fe-AA enhanced the broiler immunocompetency through immunomodulation, reduced inflammatory signaling, and improved intestinal integrity as compared to inorganic Zn and Fe sources, all of which are expected to support improved gut health in broilers.

**Keywords:** broiler; iron; zinc; immunity; tight junctions

### 56 Cecal microbiota and short-chain fatty acids are associated with growth performance and organ attributes in pullets fed omega-3 fatty acids or yeast bioactives

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This study investigated the impact of yeast bioactives (YB) or co-extruded full-fatty acid as a source of omega-3 fatty acid (N-3 FA) on organ development, growth performance, their associations with cecal microbiota and short-chain fatty acids (SCFA) in Lohmann LSL pullets from placement to 16 weeks of age (woa) under different spacing allowances (SA). A total of 2,832 chicks were reared in an enriched cage: high (HSA,

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348 $cm^2$/bird) or low (LSA, 248 $cm^2$/bird) SA and fed control diet (C), C+3% N-3 FA, or C+0.05% YB. Growth performance, including body weight (BW), BW gain (BWG), BW uniformity (BWU), and feed intake (FI), as well as organs (small intestine, liver, spleen, and bursa), were measured at 4, 8 and 16 woa. Cecal digesta were sampled at 4, 8, and 16 woa for 16S rRNA microbiota analysis, and SCFA were quantified at 4 and 16 woa. Data were analyzed by a general linear mixed model with SA, diets, and sampling age as fixed factors, with cage as the experimental unit. Cecal microbiota and SCFA data were further used for exploratory Spearman correlation analyses to assess associations with growth performance and organ weight parameters. There were no dietary effects on BW, BWG, or FI compared with the control groups (*P > 0.05*). However, pullets reared in HSA had higher BW, FI, BWG, and BWU than those in LSA at all sampling ages (*P<0.001*), with a significant diet x SA interaction observed for BWU over 0-16 woa (*P<0.05*). No treatment effects on organ weights were observed; however, within LSA, intestinal weight was lower in N-3 FA- than in control-fed pullets at 4 woa (*P<0.05*). At 4 woa, N-3 FA-fed pullets showed a positive correlation between FI and *Bacillaceae* under HSA compared to LSA (*P<0.05*), while bursa weights correlated positively with *Rikenellaceae* (*P<0.01*). YB-fed pullets exhibited SA-specific correlations, including BWG with *Ruminococcaceae* under HSA (*P<0.05*). At 8 woa, N-3 FA had fewer significant correlations; liver weight positively correlated with *Bacteroidaceae* in LSA (*P<0.001*). By 16 woa, N-3 FA- and YB-fed pullets showed positive correlations between BW or BWG with *Lachnospiraceae* and *Bifidobacteriaceae* under HSA (*P<0.01*). In YB-fed pullets, FI was positively correlated with propionic acid under LSA at 16 woa (*P < 0.05*). Overall, dietary YB or N-3 FA and SA had limited effects on growth performance, whereas correlations revealed associations among microbiota, SCFA and performance traits, indicating a potential functional link.

**Keywords:** Pullets; Microbiota; Short-chain fatty acid ; omega-3 fatty acid; yeast bioactive

### 57 Natural Adsorbents Attenuate *Clostridium perfringens* Virulence via Toxin Sequestration and Modulation of Host Cellular Responses in Chicken Jejunal Epithelial Cells

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Virulence of *Clostridium perfringens* (CP) in necrotic enteritis (NE) is driven by toxin activity and results in host inflammatory responses, yet effective strategies targeting these mechanisms under antibiotic-free systems remain limited. The objective of this *in vitro* study was to evaluate the efficacy of natural adsorbents (NAs) in suppressing CP virulence across transcriptional, protein, and host cellular response levels. A panel of NAs including Kaolinite, Bentonite (4×), MX Wyoming clay, Sodium montmorillonite clay, Graphite, Wood biochar, and Agricultural waste biochar was tested across multiple concentrations (3.125-100 mg/mL). NetB toxin sequestration was quantified using ELISA following incubation of NAs with CP culture supernatant. Host responses were assessed using chicken jejunal epithelial cells derived from broiler, wound closure quantifying cell migration and proliferation (scratch assay) and relative gene expression (2^−ΔΔCt) of inflammatory cytokines (IL-1B, IL-6, TNF-A, CXCLi2, and IFN-Γ), inflammatory enzymes (iNOS), and toxin-associated markers (NetB and A-toxin). Results demonstrated a robust, dose-dependent reduction in NetB bioavailability across all adsorbents, with significantly lower NetB toxin levels compared to controls ($p < 0.05$). Functional assays revealed enhanced epithelial wound closure, indicating improved cellular recovery following NAs treatment ($p < 0.05$). At the transcriptional level, NAs significantly downregulated pro-inflammatory cytokines and toxin-associated gene expression in CP-challenged jejunal cells ($p < 0.0001$), suggesting attenuation of pathogen-induced host responses. Notably, clay-based adsorbents (Kaolinite, Bentonite, and Montmorillonite) and carbon-based materials (Graphite and Wood biochar) consistently demonstrated the highest multi-level efficacy, significantly reducing NetB toxin availability, suppressing inflammatory gene expression, and enhancing epithelial cell recovery. Collectively, these findings provide *in vitro* evidence that NAs mitigate CP virulence by simultaneously reducing toxin availability, dampening host inflammatory signaling, and supporting epithelial wound closure. This multi-modal mechanism highlights their potential as candidates for antibiotic-free feed additives for controlling necrotic enteritis and improving gut health in poultry production systems, warranting in vivo validation.

**Keywords:** Natural Adsorbents; Clostridium Perfringens; Necrotic Enteritis; NetB toxin; poultry gut health

## Student Competition: Metabolism and Nutrition - Enzymes

**206 (E-F) (MTCC)**

### 58 Effects of dietary B-Mannanase on growth performance, nutrient utilization, and intestinal integrity in broiler chickens

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It is well established that B-mannans, a class of non-starch polysaccharides found in corn-soybean meal diets, can increase intestinal viscosity and impair nutrient digestion by reducing the efficacy of endogenous digestive enzymes. Therefore, supplementation with an appropriate B-mannanase likely degrades these B-mannans, thereby potentially enhancing both nutrient utilization and metabolic efficiency in broilers. Thus, in this study, a total of 180 day-old AA male broilers were assigned to two treatments based on body weight with eight replicates of ten birds each, *i.e.*, a basal control diet without and with 150 mg/kg B-mannanase. Throughout the trial, birds were fed from 1 to 42 d under standard management conditions. Measurements included growth performance, intestinal integrity, and nutrient utilization parameters. Data were analyzed using One-way analysis of variance (**ANOVA**). Compared with the control group, dietary B-mannanase significantly reduced the ratio of feed intake to body weight gain from 1 to 21 d ($P < 0.05$), whereas it did not obviously alter body weight and feed intake ($P > 0.05$). At the age of 42 d, there were no significant alterations in carcass and meat quality among the three groups ($P > 0.05$), whereas B-mannanase intervention reduced lipid accumulation in liver and adipose tissue. Notably, B-mannanase manipulation significantly reduced intestinal digesta viscosity and improved digestibility of dry matter ($P < 0.05$), as well as upregulated the expression of nutrient transporters such as sodium-glucose co-transporter 1 (*SGLT-1*) and fatty acid-binding protein 6 (*FABP-6*). Moreover, enhanced intestinal barrier integrity was demonstrated by the upregulation of tight junction proteins, such as *Occludin* and zona occludens 1 (*ZO-1*), as well as elevated jejunal immunoglobulin A (**sIgA**) levels ($P < 0.05$). Furthermore, pro-inflammatory cytokines in ileum, including interleukin-1 beta (*IL-1B*) and tumor necrosis factor-alpha (*TNF-A*), were reduced by dietary B-mannanase supplementation. B-mannanase inclusion modified cecal microbial composition, showed by an increased relative abundance of Bacteroidetes and enrichment of beneficial genera such as *Alistipes* and *Lachnoclostridium*, along

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with a reduction in potentially unfavorable genera including *Negativibacillus* and *Paralachnospira*. In conclusion, dietary B-mannanase supplementation increases dry matter digestibility, reduced intestinal viscosity, potentially increased intestinal barrier function, and increased feed efficiency.

**Keywords:** B-mannanase; growth performance; nutrient digestibility; intestinal integrity; broiler chickens

### 59 Evaluation of metabolizable energy release from carbohydrase enzymes using restrict-fed broiler chickens and precision-fed roosters

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The objective of this study was to determine energy release from xylanases and a multi-carbohydrase blend using 2 bioassays. Bioavailable energy, apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn) were determined using restrict-fed broiler chickens in Experiment 1. The 9 dietary treatments were corn-soybean meal-based mashed diets, formulated to meet the nutritional recommendations for Cobb broiler chickens. Birds were fed a nutrient adequate starter diet ad libitum from 0 to 8 d-of-age. Experimental diets were restrict-fed to 70% of the expected ad libitum intake per breeder guidelines from 9 to 20 d-of-age. Diet 1 was adequate in all nutrients except energy. Diets 2 through 4 were used to generate a standard curve, where dextrose (glucose monohydrate) was added in increase the AMEn by increments of 70 kcal/kg. Diets 5 through 9 were same as diet 1, except the following enzyme treatments were added: diets 5 through 7 contained a 1x, 2x, and 4x of a multi-carbohydrase blend (BestaAg); diets 8 and 9 contained a 1x dose of xylanase from 2 sources (BestaAg or Huvepharma). Dextrose and enzymes were added in place of powdered cellulose. All diets included phytase (1,500 FTU/kg, BestaAg) and titanium dioxide was used as an indigestible marker. In Experiment 2, nitrogen-correct true metabolizable energy (TMEn) was determined using precision-fed roosters. Leghorn roosters with intact ceca were fasted for 26 h, precision-fed (crop intubated) 25 g of sample, and excreta were quantitatively collected for 48 h. Dietary treatments were the same as Experiment 1. Experiments contained 6 to 8 replicate pens per treatment. All data were analyzed using a 1-way ANOVA and BW gain was regressed on supplemental kcal/kg of ME from dextrose in Experiment 1. The significance value for all analyses was $P < 0.05$. In Experiment 1, body weight gain increased linearly with 0 to 140 kcal/kg from dextrose ($P < 0.05$, $R2 = 0.80$). All enzymes improved body weight gain, AME, and AMEn compared to diet 1 ($P < 0.05$). The estimated bioavailable energy release by enzymes based on growth performance ranged from 67 to 90 kcal/kg. In Experiment 2, TMEn did not differ among treatments ($P > 0.05$); albeit there was a numerical increase of approximately 50 to 60 kcal/kg by the higher multi-carbohydrase doses. In conclusion, enzymes evaluated were effective at releasing energy as shown by the improved growth performance and AMEn in restrict-fed broiler chickens. The restrict-fed bioassay demonstrated good sensitivity for carbohydrase evaluation; however, more work is needed to determine the sensitivity of the precision-fed rooster assay for evaluation of exogenous enzymes.

**Keywords:** carbohydrase; xylanase; dextrose; metabolizable energy; bioavailability

### 60 Evaluation of the effects of sodium bisulfate and exogenous phytase supplementation on phytic acid hydrolysis, mineral digestibility, P bioavailability, and gastrointestinal pH in precision-fed roosters and restrict-fed broiler chickens

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The objective of this study was to assess the effects of sodium bisulfate (AFG®; Jones-Hamilton Co.) and exogenous phytase on inositol phosphate (IP) degradation, mineral availability, and gastrointestinal pH. In Experiment 1, IP degradation was determined using precision-fed Leghorn roosters with intact ceca. Dietary treatments were as follows: diet 1 was 100% canola meal; diets 2 through 4 were the same as diet 1 except phytase was added at 500, 1,000, and 2,000 FTU/kg, respectively; diets 5 through 8 were the same as 1 through 4, except 0.3% AFG was added. Roosters were fasted for 26 h prior to being precision-fed (crop intubated) 25 g of sample and excreta were quantitatively collected for 48 h. In Experiment 2, IP degradation, mineral availability, and gastrointestinal pH were determined in restrict-fed broiler chickens. An adaptation phase was used from 0 to 6 d-of-age, where chickens were fed nutritionally adequate corn-soybean meal-based diets ad libitum that contained 0.0, 0.2, or 0.3% AFG. From 7 to 21 d-of-age, experimental diets were restrict-fed to 70% of the expected ad libitum feed intake. Diet 1 was a phosphorus (P)-deficient corn-soybean meal-based diet (0.20% non-phytate phosphorus). Diets 2 and 3 were the same as diet 1 except 0.11 and 0.22% P from KH2PO4 was added. The remaining dietary treatments were the same as diet 1 except: diet 4 contained 1,500 FTU/kg, diet 5 contained 0.2% AFG, diet 6 contained 0.3% AFG, diet 7 contained 1,500 FTU/kg + 0.2% AFG, and diet 8 contained 1,500 FTU/kg + 0.3% AFG. Titanium dioxide was used as an indigestible marker. Experiments contained 6 to 7 replicate pens (1 rooster or 5 broiler chickens). Data were analyzed using a 1 or 2-way ANOVA. Regression of tibia ash content against supplemental P from KH2PO4 was also performed in Experiment 2 to determine bioavailable P equivalency of phytase and AFG combinations using the standard curve method. In Experiment 1 with roosters, IP disappearance increased with phytase dose ($P < 0.05$). The AFG alone had no effect on IP disappearance ($P > 0.05$); however, degradation of IP6 (phytic acid) was improved by AFG + phytase compared with only phytase at 2,000 FTU/kg ($P < 0.05$). In Experiment 2 with broiler chickens, crop pH was reduced by AFG ($P < 0.05$). There was a phytase × AFG interaction for bioavailable P based on tibia ash, where a synergistic relationship was observed between AFG and phytase. Phytase and AFG improved IP disappearance and Ca and P digestibility ($P < 0.05$). In conclusion, the use of sodium bisulfate (AFG) can improve phytase efficacy through alteration to gastrointestinal pH, thereby improving nutrient availability for poultry

**Keywords:** sodium bisulfate; phytase; phytic acid; pH; mineral availability

### 61 Effects of dietary fiber and fiber-degrading enzymes on egg production, egg quality, and organ weights in Dekalb white laying hens during an extended laying cycle

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Extending the laying cycle is essential to the profitability and sustainability of the egg industry. However, keeping hens longer is challenged by higher risks of declining health and productivity. The objective of the study was to evaluate the effects of manipulating dietary energy using fibrous feedstuff and fiber-degrading enzymes (FDE) supplementation on egg production, egg quality, body, and visceral organs weight in aged laying hens. There were 3 experimental diets: 1) a corn-soybean-based positive control (PC) meeting specifications; 2) 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 than the PC; and 3) NC supplemented with 100 g/ton of FDE containing 5,600 xylanase units and 2,500 B-glucanase units/g. All

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diets included 800 FTU/kg of phytase and were prepared in mash form. A total of 120 Dekalb white hens at 76 weeks of age (woa) were placed in enriched cages (4 birds/cage) and allocated to 3 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 to 100 woa. Daily egg count was recorded, and feed intake (FI) was measured 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 5, 10, 15, 20, and 25. 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 were interactions between diet and period for Hen Day Egg Production (HDEP) (P = 0.0256) and Feed Conversion Ratio (FCR) (P = 0.0273). The PC and NC+FDE fed birds had higher HDEP than NC (87.12, 86.85 *vs.* 84.37 %; P = 0.0008) whereas FCR for PC and NC+XY birds was lower than for NC (1.892, 1.876 *vs.* 1.954; P = 0.0163). NC+FDE birds produced heavier eggs than NC (64.46 *vs.* 63.80 g; P=0.0229). SBS was greater in PC and NC+FDE hens (3.836, 3.857 *vs.* 3.682-kg*force; P = 0.0088) than NC. The EST was higher in NC+FDE birds than PC and NC (0.412 *vs.* 0.402, 0.401mm; P = 0.0128). Birds fed NC diet lost 11.5% of their BW (1828.63 *vs.*1616.67 g), NC+FDE birds 9.9% (1827.40 *vs.*1646.25 g), and PC birds 2.4% (1821.93 *vs.*1777.92 g; P = 0.0019). In conclusion, adding high fibrous feedstuff reduced egg productivity, quality, and BW; however, FDE supplementation restored performance and internal egg quality in NC diets. Further research is required to understand the FDE's role in nutrient digestibility of hens in extended lay.

**Keywords:** Extended lay cycle; Egg and egg quality; Fibrous feedstuff; Fiber-degrading enzymes; Dekalb white

## Student Competition: Genetics and Molecular Biology

**206 (E-F) (MTCC)**

### 62 Computational Identification of Natural Inhibitors Targeting Fiber Proteins of FAdV-1 and FAdV-4 Through Integrated Virtual Screening and Molecular Dynamics Simulations.

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Fowl adenoviruses (FAdVs) represent a major threat to poultry health, with serotypes FAdV-1 and FAdV-4 causing adenoviral gizzard erosion (AGE) and hepatitis-hydropericardium syndrome (HHS), respectively. A wide variety of afflicted birds, including chicken, pigeon, and psittacine species, have been reported to carry aviadenoviruses. The disease is highly contagious and spreads rapidly between flocks and farms through vertical and horizontal transmission. In this study, we implemented a multi-stage computational drug-discovery pipeline to identify natural inhibitors of the viral fiber proteins for both FAdV-1 and FAdV-4. A curated library of 7523 natural compounds from the African Natural Products Database (ANPDB) and the South African Natural Compounds Database (SANCDB) was subjected to pharmacophore-based filtering, molecular docking, ADMET prediction, and 500-ns molecular dynamics simulations against four structural targets: Fiber-1 and Fiber-2 of FAdV-4, and the Short and Long Fibers of FAdV-1. Three ligands ANPDB_6449 (-10.3 kcal/mol), ANPDB_2908 (-10.2 and -10.0 kcal/mol), and SANCDB_254 (-9.2 kcal/mol) consistently emerged as strong candidates across the entire computational workflow. While ANPDB_2908 demonstrated notable multi-target capability by binding to fiber proteins from both FAdV-1 and FAdV-4, ANPDB_6449 and SANCDB_254 exhibited strong serotype-specific potential, supported by stable interaction profiles and favorable drug-likeness characteristics. Together, these compounds highlight promising natural scaffolds for the development of targeted antiviral interventions against pathogenic FAdV serotypes. Runing Title: Natural Inhibitors of FAdV Fiber Proteins

**Keywords:** FAdV; Fiber protein ; ADMET; ANPDB; SANCDB

### 63 Population Structure of DNA Methylation and Its Relationship to Genetic Variation in Local Chicken Breeds

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Epigenetic variation plays a key role in phenotypic plasticity; however, epigenetic diversity remains poorly characterized. With the increasing availability of epigenomic data, there is a growing interest into understanding the relationship between DNA methylation and underlying genetic variation. This study leverages the diversity found within and among local breeds to characterize the population structure of DNA methylation and its relationship to the genome. Reduced representation bisulfite sequencing (RRBS) was performed on blood samples from 474 individuals across 16 breeds spanning seven countries, identifying a total of 832,897 CpG sites. However, CpG coverage varied across individuals, resulting in numerous missing calls which reflect both within and between breed CpG site presence variation. The mean and standard error of the number of missing sites per individual is 76,279 ± 5308. A total of 272,097 common CpGs were found across all breeds and were retained for across breed analyses. Missing values were imputed using breed-level mean values (i.e. conservative) and methylation levels were transformed to M-values. In addition to the methylation data, 61,500 SNPs were obtained from the RRBS data. Principal component analysis (PCA) revealed substantial variation within the methylation dataset, with the first three axes explaining 10% of the total variation. The relationship between genomic and methylation variation was assessed using the RV coefficient test, which indicated a significant association (RV = 0.47, $p$ = 0.001). Genetic differentiation was quantified using *FST*, while epigenetic differentiation was assessed using an *FST*-like measure (*PST*) based on methylation variation. In addition, the pairwise distances between environments were calculated based on Euclidean metrics. These metrics were used to test for isolation by distance (IBD) and isolation by environment (IBE) on the genomic/methylation variation using a mantel test. No significant IBD (r = 0.25, $p$ = 0.12) nor IBE (r = 0.15, $p$ = 0.23) was detected with the genomic data. However, both IBD (r = 0.40, $p < 0.05$) and IBE (r = 0.45, $p < 0.05$) were significant with the methylation data. This suggests that genomic variation is not strongly structured by geographic or climatic gradients, where methylation variation is. These findings highlight that DNA methylation reflects both genetic and environmental signals, proving its potentially adaptive role. This work contributes to our understanding of breed level epigenetic diversity and lays the ground work on the role of DNA methylation has on phenotypic plasticity within *Gallus gallus domesticus*. Funding: GEroNIMO (H2020 GA No 101000236)

**Keywords:** Epigenetics; RRBS; Diversity; Climate; Genomics

### 64 Integrated multi-omics analysis reveals breed-specific regulatory networks and hybrid vigor signatures underlying meat quality differentiation in ducks

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This study investigated breed-specific molecular regulatory mechanisms underlying meat quality differentiation in ducks by integrating transcriptomic and metabolomic profiling of pectoralis major muscle from three genetically distinct genotypes. A completely randomized design included eighteen male ducks comprising Cherry Valley duck (CVD), Muscovy duck (MD), and their F1 mule hybrid (MU) (n = 6 per genotype) raised under identical conditions. Breast muscle was analyzed using untargeted LC-MS/MS metabolomics and RNA-seq transcriptomics. Phenotypic traits were evaluated by one-way ANOVA with Tukey HSD post-hoc tests. Metabolomic variation was assessed using PCA and OPLS-DA, with differential metabolites defined by VIP > 1, fold-change thresholds, and $P < 0.05$. Differential gene expression was identified using DESeq2 ($|log2FC| \geq 2$, FDR < 0.05), followed by pathway enrichment through over-representation analysis with FDR correction. Multi-omics integration combined joint pathway analysis with correlation-based network analysis. Phenotypic evaluation showed that CVD had the highest dressing percentage and pectoralis major proportion ($P < 0.05$), whereas MD and MU exhibited larger body dimensions. MD muscle displayed significantly higher concentrations of glutamine, asparagine, serine, glycine, and lysine ($P < 0.05$) and favorable lipid indices including the lowest thrombogenic index and the highest hypocholesterolemic-to-hypercholesterolemic ratio. Integrated omics analysis revealed coordinated enrichment in MD of ECM-receptor interaction pathways involving 19 genes including LAMA5, LAMB1, and COL1A1, together with activation of mitophagy pathways (impact = 0.835, FDR = 8.17e-4; genes FUNDC1, BNIP3, ULK1) and strong downregulation of proteasome pathways (FDR = 5.17e-10). The mule hybrid displayed dual metabolic enrichment consistent with heterosis: enhanced carbohydrate metabolism relative to CVD involving PFKFB3 and GFPT1, and intensified lipid-mitochondrial flux relative to MD supported by HADHA, ACADSB, FABP2, and FABP6, converging on citrate cycle pathways (impact = 0.834). In contrast, CVD showed restricted pathway diversity consistent with yield specialization. Glycerophospholipid metabolism was significantly enriched across all breed comparisons. Overall, breed-associated meat quality differences arise from distinct molecular architectures: MD integrates ECM signaling with mitophagy and reduced proteasomal activity favoring protein preservation; MU shows metabolic flexibility through dual substrate utilization; and CVD exhibits a yield-focused but metabolically constrained profile. FABP2, FABP6, and PFKFB3 emerge as candidate genes for marker-assisted selection in duck breeding.

**Keywords:** Duck; meat quality; multi-omics integration; hybrid vigor; glycerophospholipid metabolism

### 65 Probiotic supplementation alters transcriptomic regulation of the yolk sac membrane in association with glycogen accumulation during broiler embryonic development

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**Background:**Glycogen stored in the yolk sac membrane (YSM) is a critical energy reserve during avian embryonic development, accumulating during mid-incubation and supporting energy supply prior to hatch. This reflects a developmental transition in YSM function from nutrient metabolism to energy storage and substrate translocation. Our previous study demonstrated that in ovo *Lacticaseibacillus paracasei* (LP) supplementation increased glycogen reserves in the yolk sac, supporting embryonic growth. However, the molecular mechanisms by which LP influences this functional transition in the YSM remain unclear. **Hypothesis:**Probiotic supplementation modifies developmental regulation of metabolic and cellular pathways in the YSM in association with glycogen accumulation and embryonic growth. **Materials and Methods:**Hatching eggs were sprayed with phosphate buffered saline (control) or LP (~9 log CFU/egg) and incubated under standard conditions. YSM tissues were collected on day 10, 14, and 18 (n = 6/time point) for RNA sequencing. Reads were aligned to the chicken reference genome using HISAT2, and gene expression was quantified using HTSeq-count. Differential expression was analyzed using DESeq2 (*Padj* < 0.05), and pathway analysis was conducted using Ingenuity Pathway Analysis. **Results:**In control embryos, differential expression analysis identified a significant transition between day 10 and day 14 characterized by downregulation of gluconeogenesis, carbohydrate metabolism, and nutrient transport pathways, with inhibition of AMPK signaling and activation of mTOR signaling, consistent with a shift toward energy storage. This state was maintained at day 18 and coincided with peak glycogen accumulation. Relative to this pattern, LP treatment resulted in significant differential expression relative to controls at all time points. At day 10, LP-treated embryos showed lower expression of genes related to metabolic activity and substrate transport (*FFAR4*, *GLRX5*, *SLC16A14*) and higher expression of regulatory genes. At day 14, LP-treated embryos showed greater suppression of metabolic and cell cycle regulatory pathways (*E2F/MYC/FOXM1*). By day 18, these differences were maintained. These transcriptional differences coincided with increased glycogen accumulation and greater embryonic growth in LP-treated embryos. **Conclusion:**Overall, LP treatment is associated with earlier and sustained differences in YSM gene expression, including reduced expression of metabolic and transport-related genes and increased regulatory gene expression. These changes coincide with increased glycogen deposition and improved embryonic growth, indicating that probiotic supplementation influences YSM function during embryonic development.

**Keywords:** Yolk sac membrane; Glycogen metabolism; Probiotics; Embryonic development; RNA sequencing

### 66 Construction of Probiotic *Lactobacillus*-based vaccines for turkeys against Clostridial Dermatitis using a stable recombinant-gene expression system via chromosomal integration

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Clostridial dermatitis (CD), caused by the toxin-producing *Clostridium septicum* bacteria, has re-surfaced in the turkey flocks in recent times causing necrotic dermatitis and high mortality, leading to huge economic losses. The growing ineffectiveness of antibiotics currently in practice coupled with a regulatory demand for growing poultry without antibiotics, has resulted in an urgent need for efficacious vaccines to prevent CD in turkeys. Our previous work identified a non-toxic domain in *C. septicum* alpha toxin, namely the non-toxic Alpha Toxin-Domain 2 (ntATX-D2) and showed that subcutaneous immunization of turkeys with ntATX-D2 subunit vaccine can induce robust protective immunity against CD. In the present work, we used two *Lactobacillus* isolates, namely *Limosilactobacillus reuteri* (Str. NCKL-22) and *Ligilactobacillus salivarius* (Str. NCKL-11), that were in-vitro characterized to possess superior immunomodulatory and anti-*C. septicum* properties to construct probiotic-based vaccines for turkeys. A double homologous recombination strategy was employed for the stable chromosomal integration and expression of ntATX-D2 antigen. Whole genomic sequencing of each strain facilitated the identification of the housekeeping phosphopyruvate hydratase (enolase) gene region and associated promoter to drive high-level constitutive expression of the ntATX-D2 antigen. To achieve site-specific integration following the enolase locus, but before the enolase promotor termination region, a gene

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fragment was designed that incorporated ~750 bp up- and downstream enolase homologous sequences (fragments A and B) flanking either side of the ntATX-D2 antigen. This gene fragment was cloned into a pUC-GW-Amp plasmid backbone, which was then used as a template to subsequently clone the gene fragment into the integration plasmid pTRK935 resulting in plasmid pTRK935+ntATX-D2. Transformation in *Lactobacillus* was executed using a dual-plasmid system involving the *RepA+* helper plasmid pTRK669 to facilitate pTRK935+ntATX-D2 replication and allow for the targeting of the enolase locus via flanking homologous recombination. Following single, and then double, homologous recombination, the ntATX-D2 antigen was permanently integrated into the *Lactobacillus* chromosome. Validation of ntATX-D2 antigen transcription and translation via PCR, Western Blot, and SDS-PAGE is underway, with the definitive goal to confirm the constructed oral vaccine as an efficacious, long-term alternative to antibiotics for the prevention of CD in turkeys.

**Keywords:** Clostridial Dermatitis; Clostridium septicum; Turkeys; Oral Vaccine; Probiotics

### 67 Erythrocytes in Chicken Lung Upregulate Innate Immune and Antiviral Genes through Development as Revealed by Single-Cell Transcriptomics

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Recurrent pathogen outbreaks in poultry can result in millions of bird deaths and billions of dollars in economic losses. The lack of effective countermeasures can in part be attributed to insufficient understanding of avian biology, such as the characteristics of nucleated erythrocytes. To address this knowledge gap, we aimed to investigate transcriptomic shifts underlying the development of major chicken lung cell types from *in ovo* to post-hatch conditions and to elucidate potential immune-associated characteristics of erythrocyte subtypes. Lung tissue from White Leghorn embryos on day 18 of incubation and seven-week-old pullets (n=3 each) were prepared for single-cell RNA sequencing. Cells were clustered based on transcriptomic similarity, and clusters were used to infer cell type and perform differential expression analysis (DEA). Briefly, libraries were prepared with the 10x Chromium Single Cell 3' kit and sequenced on the Illumina NovaSeq 6000. Reads were aligned to GRCg7b via CellRanger v7.0.1, and ambient RNA was removed with CellBender v0.2.0. Cells were then filtered by gene and mitochondrial/ribosomal thresholds. Downstream analysis was conducted in Scanpy v1.11.5, incorporating harmonypy, leidenalg, DESeq2, and LIANA+. All reported genes and interactions met a significance threshold of adjusted p-value (padj) < 0.05, calculated individually within each package. Applying these criteria, fourteen distinct clusters were generated from a total of 37,608 cells. Clusters were annotated using canonical cell type markers, identifying erythrocytes, fibroblasts, vascular cells, epithelial cells, leukocytes, T cells, and thrombocytes. DEA of erythrocytes revealed upregulation of multiple immune-associated genes in pullets, including interferon-induced *IRF9*, antiviral protein *MX1*, and antigen-presenting factors, *BF1* and *BF2*. Using DEA results for ligand-receptor inference, LIANA+ found upregulation of the antigen-presentation interaction B2M->CD1C along with pro-inflammatory IL15->IL2RA and C3->C3AR1. To determine potential subtypes contributing to these transcriptional shifts, erythrocytes were subclustered to generate seven distinct subclusters. Antigen presentation genes were found to be upregulated across all subclusters in pullets relative to embryos. Furthermore, a single subcluster of 237 erythrocytes uniquely expressed most of the interferon-inducible and antiviral genes detected from DEA, patterns largely absent in embryos. These findings expand on the underappreciated role of erythrocytes in immune defense and highlight developmental immune gene acquisition as a feature of erythrocyte maturation in chickens.

**Keywords:** single-cell; transcriptomics; virus; immune; genetics

## Student Competition: Immunology, Health and Disease - Immunology

**203 (B-D) (MTCC)**

### 68 Efficiency of *In-Ovo* eBeam-inactivated *Clostridium perfringens* vaccine Against Necrotic Enteritis in SPF layer chickens

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Necrotic Enteritis (NE), caused by *Clostridium perfringens* (CP), is a critical enteric disease in the poultry industry and leads to substantial economic losses worldwide. With no effective commercial vaccines currently available against CP, there is an urgent need to develop efficacious vaccination strategies to control CP colonization and reduce the incidence of NE in poultry. Electron beam (eBeam) technology inactivates pathogens by damaging their DNA while preserving antigenic epitopes, making it a promising approach for developing effective inactivated vaccines. The aim of this study was to develop a multivalent eBeam-inactivated CP vaccine and evaluate its efficacy in protecting chickens against NE. On day 18 of embryonation, a total of 265 fertilized specific-pathogen-free (SPF) eggs were allocated into three treatment groups: control, eBeam, and formalin. Eggs in each group received a 200 µL in ovo injection of phosphate-buffered saline (PBS), eBeam-inactivated CP vaccine, or formalin-inactivated CP vaccine, respectively. On the day of hatch, hatchability and chick quality parameters were assessed, and all chicks were subsequently housed in environmentally controlled floor pens. At 14 days of age, birds from all groups were orally vaccinated with a commercial live coccidia vaccine (COCCIVAC®-D2). Subsequently, on days 19 and 20, two-thirds of the birds in each group were orally challenged with $1 \times 10^8$ CFU/mL of CP to induce NE. Body weight gain was calculated from days 19 to 28 of age. On days 21 (1-day post infection, dpi) and 28 (8 dpi), cecal CP loads and gross intestinal lesion scores were evaluated in eight birds per group. Data were analyzed using one-way ANOVA or the Kruskal–Wallis test, followed by Tukey's HSD or Dunn's post hoc test, as appropriate. The eBeam group showed hatchability and chick quality parameters comparable to those of the control and formalin groups. Following the CP challenge, the eBeam group maintained body weight gains similar to the non-challenged control group and significantly higher ($P < 0.05$) than both the CP-infected control and formalin groups. Additionally, the eBeam group exhibited a significant reduction in gross intestinal lesion scores compared to the CP-infected control group ($P < 0.01$) but not compared to the formalin group at 1 dpi. No significant differences were observed in cecal CP counts among the groups. Together, these findings indicate that *in ovo* vaccination with eBeam-inactivated vaccine has no detrimental effects on newly hatched chicks and mitigates the adverse effects of NE in SPF chickens.

**Keywords:** Clostridium perfringens; eBeam; Vaccine; Necrotic Enteritis; Chicken

### 69 In ovo delivery of DOTAP-based mRNA vaccine elicits distinct innate immune activation profiles following TLR agonists co-administration in chicken embryos

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mRNA vaccines offer a rapidly deployable platform for avian disease control, though delivery efficiency and immunogenicity remain limiting, particularly for in ovo applications requiring stability and early immune activation. DOTAP (1,2-dioleoyl-3-trimethylammonium-propane), a cationic lipid, enhances mRNA condensation and cellular uptake, enabling efficient antigen expression, while concurrent Toll-like receptor (TLR) activation (CpG, Poly(I:C), R848) promotes early immune priming. This study aimed to evaluate an in vitro transcribed (IVT) mRNA vaccine encoding H9N2 hemagglutinin (HA), formulated in DOTAP-based lipid nanoparticles for in ovo delivery, and to assess the impact of TLR agonist co-administration on early innate immune responses. Specific pathogen free chicken eggs were vaccinated in ovo at embryonic day 18 using a DOTAP-based vaccine formulations. Six treatment groups were evaluated: (1) PBS control, (2) DOTAP-only control (LNP), (3) mRNA-LNP (5 µg/egg), (4) mRNA-LNP + CpG ODN (10 µg/egg), (5) mRNA-LNP + Poly(I:C) (15 µg/egg), and (6) mRNA-LNP + R848 (100 µg/egg). Each treatment included 8 embryos per time point, with sampling conducted at 24 and 48 hours post-vaccination. Quantitative Real-Time PCR was performed to assess the expression of key antiviral and innate immune genes in the spleen and bursa of Fabricius. Data were analyzed by one-way ANOVA (treatment effect), with Tukey's post hoc test for multiple comparisons when significant ($p < 0.05$); results are presented as mean ± SEM. Across time points, treatment effects were tissue- and gene-dependent. In the spleen, early responses (24 h) were characterized by significant modulation of interferon regulatory factor 7 (IRF7) ($p = 0.023$) and myxovirus resistance protein 1 (MX1) ($p = 0.0016$), with the strongest MX1 induction observed in the R848 group, while by 48 h only IFN-A remained significantly elevated ($p = 0.0079$), showing increased expression in mRNA and adjuvanted groups relative to DOTAP control. In the bursa of Fabricius, significant treatment effects were evident at 24 h for IFN-A ($p = 0.0167$), IRF7 ($p < 0.001$), MX1 ($p < 0.001$), and ISG12-like ($p = 0.0026$), with higher expression in control groups and reduced levels following mRNA and adjuvant treatments; while melanoma differentiation-associated protein 5 (MDA5) expression showed higher expression in vaccine with TLR agonists ($P < 0.001$). These findings demonstrate that mRNA-based vaccines can modulate innate immunity in chick embryos despite incomplete immune maturation, providing a mechanistic foundation for advancing DOTAP-based formulations to in vivo H9N2 challenge models to assess protective immunity, viral shedding, and durability of response.

**Keywords:** Avian influenza; mRNA vaccines; DOTAP-LNP; TLR agonists; Antiviral response

## 70 Enhanced proliferative capacity of liver ductal organoids from brown layer hens: a potential basis for spotty liver disease susceptibility

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Brown layer hens exhibit greater susceptibility to spotty liver disease (SLD) than white layers. The causative agent, *Campylobacter hepaticus*, primarily colonizes the biliary ducts; however, a lack of relevant *in vitro* models has hindered investigation into this breed-specific susceptibility. We hypothesized that the heightened SLD susceptibility in brown layers is linked to an intrinsically higher proliferative capacity of their biliary ductal cells. Therefore, the objective of this study was to establish a biologically relevant avian liver ductal organoid model to investigate these differences. Livers were harvested from embryonic day 18 Rhode Island Red (brown; n = 4) and White Leghorn (white; n = 4) embryos. Tissue was enzymatically dissociated using collagenase D and dispase II to isolate ductal fragments (> 40 µm). These fragments were embedded in Matrigel and cultured in an isolation medium for 4 days, then transferred to a liver expansion medium for long-term growth. Organoid growth and morphology were closely monitored using a Nikon Ti microscope, and images were collected on day 6. Quantitative measurements, including organoid counts, areas, and Feret diameters, were analyzed using ImageJ. Statistical analysis was performed using one-way ANOVA Tukey HSD test, with significance set at $P < 0.05$. We demonstrated the successful establishment of liver ductal organoids from both breeds, with the ability to undergo passaging and cryopreservation. Significant differences in organoid growth were observed between breeds, with brown liver ductal organoids exhibiting greater count, area, and Feret diameter compared to white liver ductal organoids ($P < 0.0001$ for all). These findings indicate enhanced growth potential in brown layer-derived liver organoids. Additionally, cryopreserved organoids were successfully revived and regrown, maintaining their growth characteristics, demonstrating the stability and reproducibility of this model for extended use. In conclusion, this study establishes an innovative avian liver ductal organoid model as a strong platform for long-term *in vitro* studies. The observed breed-associated differences in organoid growth suggest inherent biological variations that may contribute to increased susceptibility to SLD in brown layer chickens. Furthermore, the successful cryopreservation and regrowth of these organoids enable future RNA-sequencing and proteomic analyses, providing a powerful approach to investigate the molecular mechanisms underlying breed-specific differences in liver function and disease susceptibility. This model provides a valuable tool for future research into host-pathogen interactions and the underlying molecular mechanisms of SLD in poultry.

**Keywords:** Liver organoid; Layer hen; Spotty liver disease; Campylobacter hepaticus; Host-pathogen interaction

## 71 Effects of oviposition time and eggshell translucency on maternal antibody transfer in an aging broiler breeder flock

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Broiler breeder *Salmonella* vaccination programs induce *Salmonella*-specific maternal antibody transfer from hen to chick to reduce *Salmonella* incidence in progeny. Translucency and oviposition time may affect egg quality and hatchability, but information is limited regarding their impact on passive immunity. The objective was to evaluate how translucency, oviposition time, and flock age affect maternal IgY levels in eggs and chicks. Eggs were collected from the same commercial broiler breeder flock at 34, 44, and 53 wks-old at 2 oviposition times (A-9am and B-2pm) and sorted into 3 translucency scores (T1=low, T2=medium, T3=high), creating 6 experimental groups for each flock age: T1-A, T1-B, T2-A, T2-B, T3-A, T3-B. Egg yolks were collected (30 eggs/group/age), and remaining eggs were incubated under normal hatchery conditions. At hatch, residual yolk (Ryolk) and blood plasma were collected (n=10 chicks/group/age). Total IgY, *Salmonella*-specific (SAL) IgY, and proportion of SAL IgY in total IgY (%SAL IgY) in egg yolk, Ryolk, and plasma were quantified by ELISA. Data were analyzed by three-way ANOVA to assess the effects of age,

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oviposition time, translucency, and their interactions with significance at P≤0.05. Total IgY levels in yolk, Ryolk, and plasma increased with age (P<0.0001). SAL IgY levels in the egg yolk were similar from 34 to 44 wks but decreased from 44 to 53 wks (P= 0.002). There was an age by oviposition interaction for %SAL IgY in egg yolk (P=0.020). B had higher levels than A at 3 wks, and levels decreased by age with no differences by oviposition at 44 and 53 wks. There was also an oviposition by translucency interaction for % SAL IgY in egg yolk (P=0.039) where B-T1 had higher levels than all other groups regardless of flock age. SAL IgY levels in the Ryolk and plasma of chicks at hatch were significant by translucency (P= 0.012 and 0.031, respectively) where T2 had higher levels than T1 and T3 regardless of flock age. There was an age by oviposition interaction for SAL IgY and %SAL IgY in plasma (P=0.003 and P=0.002, respectively) where A eggs had the highest levels from 53 wk-old hens while B eggs had the highest levels from 44 wk-old hens, wherein B was only higher than A at 44 wks. %SAL IgY in Ryolk was significant by age (P=0.0002) where the proportion decreased from 34 to 44 wks but did not change from 44 to 53 wks. These results show that age has the greatest effect on *Salmonella*-specific maternal antibody transfer, but the effects of translucency and oviposition time require further evaluation. Future research will evaluate how these changes in maternal antibody transfer as a breeder flock ages affect chick susceptibility to *Salmonella* colonization.

**Keywords:** Translucency; Oviposition time; Passive Immunity; Antibodies; Yolk

### 72 Establishment and characterization of the first chicken thymic epithelial cell organoid platform

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Field observations suggest that brown layer chickens are more susceptible to spotty liver disease than white layers, indicating potential breed-related differences in immune function. The thymus is central to immunity, as its microenvironment, governed by thymic epithelial cells (TECs), orchestrates T cell development and establishes central tolerance. However, the underlying cellular mechanisms driving immune variations in avian species are poorly understood due to the lack of suitable *in vitro* models. We hypothesized that these differences in disease susceptibility stem from inherent, breed-specific variations in the cellular function and developmental capacity of thymic epithelial cells. Therefore, the objective of this study was to establish the first chicken TEC organoid model, providing a platform for investigating this breed-specific thymic biology. Thymic tissues from embryonic day 18 Brown Leghorn (N=3) and White Leghorn (N=4) embryos were dissociated and cultured in a Matrigel-based 3D system. Organoid growth and morphology (area, maximum diameter, and count) were captured and quantified using a Nikon Ti2 microscope. Maximum diameter, area, and organoid count were analyzed using one-way ANOVA with Tukey's HSD, while cystic proportion was analyzed using a chi-square test. Robust TEC organoids were generated from both chicken breeds, demonstrating capacity for serial passaging. Organoids were successfully cryopreserved and revived, confirming the model's stability. While organoids were morphologically similar at the initial passage, White Leghorns exhibited a larger average area (P=0.0014). By passage 2, more pronounced differences emerged, with the White Leghorn group developing a significantly higher proportion of cystic organoids (P<0.0001), total area (P<0.0001), and maximum diameter (P<0.0001) compared to the Brown Leghorn group, while organoid count did not differ between breeds (P>0.05). To investigate the molecular basis of these differences, organoids were collected for subsequent transcriptomic analysis. In conclusion, this first avian TEC organoid model reveals significant morphological differences between chicken breeds. The larger, more cystic organoids from White Leghorns suggest a higher proliferative capacity and a more functionally mature epithelial state. Conversely, the smaller phenotype in the disease-susceptible Brown Leghorns may indicate a less developed thymic epithelium, potentially compromising T cell maturation and contributing to reduced immune competence. This model provides a powerful new tool for dissecting the cellular basis of immune resilience, and the ongoing transcriptomic analysis is further elucidating the molecular pathways underlying these differences.

**Keywords:** Organoid; Thymus; Thymic Epithelial Cells; Immunology; Breed Differences

## Student Competition: Physiology and Reproduction I

**205 (A-D) (MTCC)**

### 73 Effects of chronic mild heat stress on reproductive performance, physiological status, and HPO hormones in broiler breeders

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This study aimed to evaluate effects of maternal chronic heat stress on egg production, physiological status, and egg quality of broiler breeders. A total of 160 broiler breeder hens (43 weeks of age) were randomly assigned to thermoneutral (20°C) or heat stress (32°C) conditions for 8 weeks. Body weight, water intake, organ weights, follicle number, egg production, and egg weight were recorded. All parameters were evaluated with a general linear mixed model (Proc Mixed). Results showed that the chronic heat stress significantly lowered water intake, numbers of large white follicles (LWF), uterus weight, egg production, and egg weight after 8 weeks of heat exposure ($p < 0.05$). Conversely, liver weight was significantly elevated in heat-stressed broiler breeders at week 8 ($p < 0.05$). Heat stress resulted in lower hypothalamus and serum estradiol and follicle-stimulating hormone and reduced luteinizing hormone in the serum. Furthermore, heat stress also decreased serum triiodothyronine and total cholesterol concentrations (all $p < 0.05$). In contrast, hepatic triglyceride deposition was significantly ($p < 0.05$) higher in the heat stressed breeders. Collectively, these findings demonstrate that chronic heat stress exerts significant adverse effects on the performance and physiological health of broiler breeders.

**Keywords:** Mild thermal stress; Broiler breeder; HPO axis hormone; Egg production; Follicle development

### 74 Do preventative supplementation with galacto-oligosaccharides and subsequent social stress alter aromatic amino acid profiles in low and high feather pecking laying hens?

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Severe feather pecking behavior negatively impacts the welfare and productivity of laying hens. Its causes are multifactorial and include stress, disruptions to the microbiota-gut-brain axis, and altered central serotonin metabolism. Tryptophan (Trp) is a precursor for central serotonin production but is also diverted into the kynurenine (Kyn) pathway, making the

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ratio of Kyn to Trp one indicator of Trp availability. Prebiotic galacto-oligosaccharides (GOS) may influence these pathways by feeding *Lactobacillus* species in the gut capable of producing Trp. To investigate how GOS supplementation followed by a social stress period influence peripheral Trp and related pathways, we used 144 White Leghorn laying hens from lines divergently selected for high (HFP) or low feather pecking (LFP) activity. Birds were housed in 12 floor pens with an equal proportion of both lines. During the supplementation period (19-22 weeks of age, woa), six pens received water with Nutrabiotic® GOS (74% GOS; Dairy Crest Limited, Davidstow, United Kingdom), while the other six received plain water (control). During the subsequent stress period (23-26 woa) only plain water was offered and half the previously supplemented and control pens experienced chronic social stress via repeated mixing of unfamiliar hens. The other pens remained undisturbed. Bodyweights and blood samples were collected at 17 (baseline), 22 (supplementation period), and 26 woa (stress period). Aromatic amino acid, Kyn concentrations, and their ratios in the plasma were analyzed within each period, using GLMMs (SAS v 9.04) with fixed effects of line, treatment, and their interactions. Bodyweight was included as a covariate, pen as a random effect; data were log-transformed as needed. Peripheral Trp differed only at baseline (LFP > HFP; $p < 0.0001$), suggesting inherent differences not evident after the treatments or onset of lay (19 woa). During supplementation, GOS did not alter Trp levels ($p = 0.4305$) but increased Kyn ($p = 0.0157$) and the Kyn to Trp ratio ($p = 0.0216$), indicating an acute shift in Trp metabolism toward the Kyn pathway in supplemented birds. Regardless of previous supplementation status, chronic social stress increased Kyn ($p = 0.0047$) with a trend for higher Kyn to Trp ratio in stressed hens ($p = 0.0553$). Our findings suggest GOS increased Kyn and Kyn to Trp ratio during active supplementation without buffering or worsening the response of birds to subsequent chronic social stress. This study provides evidence that GOS can acutely shift Trp metabolism toward the stress- and immune-associated Kyn pathway, which may influence central Trp availability.

**Keywords:** Feather pecking; Galacto-oligosaccharides; Kynurenine pathway; Social stress; Tryptophan metabolism

### 75 Melatonin Fun Times: Scotophase infusion of melatonin has no impact on geriatric laying hen production but decreases bone strength.

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Laying hens are one of the powerhouses of the animal industry, laying approximately 300 eggs per year. However, as they age, their production and bone health decline. However, research in other animals has found that melatonin (MEL) can be beneficial for bone health and production. The objective of our study was to investigate how MEL given during the scotophase impacts geriatric laying hen egg production and bone mechanical properties. For our study, we utilized 20 geriatric Lohmann Brown laying hens (85 WOA) and implanted infusion pumps. Once every hour of their scotophase for 18 days, the control group (CON; n=10) received 5 µL of saline (0.9% NaCl) and the MEL group (n = 10) received 5 µL of MEL solution (25.0 mg/mL). Egg production, egg weight, egg strength, thermal temperature, plasma MEL, brain MEL, and bone mechanical properties for the femur (F) and tibia (T) were measured. Brain areas measured were the mesencephalon (Mes), optic tectum (OT), diencephalon (Di), medial telencephalon (mTel), and lateral telencephalon (lTel). Bone mechanical properties measured were the second moment of inertia ($mm^4$), bone stiffness (N/mm), maximum bending stress (MPa), and Young's modulus of elasticity (MPa). Statistics for egg parameters, thermal temperature, and MEL levels were analyzed using a two-way ANOVA in SAS. Statistics for bone properties were analyzed using a generalized linear model in R. Data were considered significant when $p < 0.05$ and trending when $0.05 < p < 0.10$. There were no differences for egg production, egg weight, egg strength, or thermal temperature. There was an increase in MEL in the plasma of the MEL hens than the CON hens during the night each day measured (d7: $p = 0.0177$, d14: $p = 0.0321$, d18: $p = 0.0058$). There was also an increase in MEL in all MEL brain areas compared to the CON brains (Mes: $p = 0.051$, OT: $p = 0.0792$, Di: $p = 0.013$, mTel: $p = 0.0792$, lTel: $p = 0.0315$). Treatment (T: $p = 0.0009$; F: $p =0$ 0.0001), time of day (T: $p = 0.0389$; F: $p = 0.0067$), and their interaction (F: $p = 0.0144$) were significant where the tibiae and femurs taken from the CON group during scotophase had a higher breaking force than all other groups. Treatment (T: $p = 0.0336$; F: $p = 0.0012$) and time of day (T: $p = 0.0277$; F: $p = 0.0249$) were significant for maximum bending stress where tibiae and femurs taken during scotophase and those taken from the CON group were higher than those taken during the photophase and those taken from the MEL group. Our results suggest that exogenous MEL given during scotophase have no impact on production and have a negative effect on bone mechanical properties, suggesting there is an evolutionary cause why melatonin decreases as birds age.

**Keywords:** melatonin; geriatric; laying hen ; production; bone health

### 76 Effect of egg storage duration on broiler breeder embryonic gene expression

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Pre-incubation egg storage is a standard practice in commercial poultry production, yet extended storage durations are associated with declining hatchability and increased early embryonic mortality during the first three days of incubation. Previously, targeted multiple reaction monitoring -based lipidomic and metabolomic profiling identified storage- and mortality-associated yolk biomarkers. The objective of this study was to evaluate the transcriptional and metabolic responses associated with egg storage in broiler breeder embryos. Fertilized Cobb broiler breeder eggs were stored for either 2 or 10 days at 12 C and then incubated at 37.5 C. Yolk and embryo samples were collected on day 3 of incubation (n=8/storage duration). RNA expression data was analyzed using one-way ANOVA with storage duration as the main effect. Reverse transcription-quantitative PCR (RT-qPCR) was performed to evaluate mRNA levels of 15 genes associated with fatty acid metabolism, oxidative stress, cellular transport and signaling, and cellular differentiation between storage duration groups. Seven genes were significantly ($p<0.05$) up-regulated in the 10-day storage condition, implicating shifts in mitochondrial fatty acid oxidation (carnitine palmitoyltransferase 1A), oxidative stress (glutathione peroxidase 7), cellular transport (beta tubulin and clathrin heavy chain), and early developmental signaling (inhibitor of DNA binding 2 and polo-like kinase 1). These results indicate that some but not all of the genes analyzed for mitochondrial fatty acid metabolism, oxidative stress, cellular transport and signaling, and cellular differentiation were affected by egg storage. It remains to be determined whether any of the genes whose expression was affected by storage results in embryonic mortality. Future integration of metabolomic datasets may help provide biologically informed strategies for improved hatchability in commercial poultry production.

**Keywords:** Egg Storage; Gene Expression; Broiler Breeder; Yolk; Embryo

### 77 Effects of metabolizable energy intake and body weight restriction on reproductive physiology and metabolic cues of layer pullets

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This study examined the effects of rearing phase dietary metabolizable energy (Diet ME) and feed allocation (FA) on gene expression within the hypothalamus, pituitary, and liver of table egg pullets. The experiment utilized a 2 × 2 factorial arrangement of 2 FA levels: *ad libitum* or restricted to achieve the lower boundary of the breeder recommended target BW trajectory (Restricted); and 2 levels of Diet ME: Low (2,600 kcal/kg) and Standard (Std, 2,800 kcal/kg). Lohmann Brown-Lite pullets were randomly assigned to 8 floor pens (n = 26 birds per pen) from 0 to 30 weeks of age. Pullets were fed as a group and feed allocation for Restricted birds were adjusted weekly. All birds were photostimulated at 18 weeks of age. Data collection included hypothalamus, pituitary, and liver samples at 14, 18, 22, and 26 weeks of age for expression of 21 reproductive and metabolic genes using UV-visible spectroscopy via nCounter Nanostring technology. All counts were normalized to housekeeping genes and log2 transformed prior to analyses. The effects of FA, Diet ME, Age, and interactions were analyzed as a 3-way ANOVA. Tukey's multiple range test was used to separate means. There was no impact of Diet ME on the expression of genes involved in reproductive and metabolic signalling pathways as the pullets entered sexual maturity, while FA showed no effect on gene expression specifically in liver samples. At the time of photostimulation at 18 weeks of age, ad libitum birds had greater levels of follicle stimulating hormone subunit B (FSHB) (11.79 versus 10.88 relative log2 counts, P < 0.001) and cocaine and amphetamine regulated transcript (CART) (10.63 versus 9.86 relative log2 counts, P < 0.001) in the pituitary than Restricted birds. FSHB stimulates ovarian follicle growth, and CART regulates reproduction and energy balance, which suggested that *ad libitum* birds were more physiologically mature than Restricted birds. *Ad libitum* birds also came into lay earlier than Restricted birds. At 22 weeks of age, no difference in gene expression of FSHB and CART between *ad libitum* and Restricted birds were observed. Restricted birds reached the onset of lay later, which could lead to reduced total egg production over the laying period. Overall, Diet ME had no effect on reproductive of metabolic gene expression, but feed restriction delayed sexual maturity, suggesting that feeding pullets a Low Diet ME is sufficient to prepare them for sexual maturation, however, pullets should be fed ad libitum.

**Keywords:** metabolic physiology; gene expression; egg-strain pullet; hypothalamic-pituitary gonadal axis; feed restriction

### 78 Effects of Strains and Growth Trajectory on Body Composition and Entry into Lay in Layer Pullets

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Our previous studies showed that body composition during rearing may regulate physiological processes that influence sexual maturation independently of photostimulation. However, the exact parameters remain to be determined. This study evaluated the effects of dynamic changes in body composition on sexual maturation in two layer strains. A total of 72 pullets, consisting of Lohmann Brown-Lite (LB; n=36) and Lohmann Selected -Lite (LSL; n=36), were allocated to four treatment groups (n=18 per group) in a 2 x 2 factorial design of strain (LB vs. LSL) and divergent growth trajectories (ad libitum, AL vs. 20% feed restriction, R). Body composition traits (adipose tissue, lean mass, bone mineral content and density) were monitored using dual-energy X-ray absorptiometry (DEXA) with repeated measurements taken biweekly from 11–16 weeks of age (woa) and weekly from 17–24 woa. Egg production was recorded as an indicator of sexual maturation. Mixed-effects and spline regression models were used to characterize developmental trajectories across pre-pubertal (11–16 woa), peri-pubertal (17–20 woa), and post-pubertal (21–24 woa) rearing phases, while generalized linear mixed models were used to evaluate the association between body composition traits and age at first egg (AFE). Adipose deposition followed nonlinear patterns throughout development, with the steepest accumulation occurring during the peri-pubertal phase. Adipose deposition was greater in LB pullets (ß=2.270±0.446, P = 0.0001) and in birds fed AL compared to R birds (ß =1.645±0.446, P = 0.0004). Similarly, abdominal fat increased under AL than R feeding conditions (B = 0.1063 ± 0.0339, P = 0.0120). Lean mass increased steadily throughout the rearing period, with LB birds having higher lean mass than LSL (P < 0.0001). Bone mineral content and bone mineral density showed significant gains in both strains during early and mid growth regardless of treatment (P = 0.0001). Sexual maturation (AFE) was predicted by body composition, particularly adiposity-related traits such as abdominal fat, with stronger effects observed in LB than in LSL and under AL feeding (P = 0.0120). Overall, restricted feeding delayed AFE compared with AL in both strains, indicating a strain-dependent sensitivity to nutritional availability during reproductive maturation. The onset of lay appears to be influenced by adiposity, especially by the abdominal fat deposition.

**Keywords:** Body composition; layer pullets; adiposity; DEXA; sexual maturation

## Immunology, Health and Disease: Feed Additives I

**206 (A-D) (MTCC)**

### 79 Nutritional strategies to improve immune function, gut health and food safety

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Poultry production has advanced substantially since the mid 1900s through genetic selection, improved nutrition, and enhanced management of health, housing, and water quality. These innovations allow modern systems to provide consumers with affordable, high quality animal protein. Given the scale of the industry, even small gains in immune function, gut integrity, and food safety can yield meaningful improvements in overall performance. Subtherapeutic antibiotics once played a central role in supporting gut health and nutrient utilization, but concerns about antimicrobial resistance have driven their reduction or removal. This transition has accelerated the development of numerous antibiotic alternatives which, although generally less potent against susceptible bacteria, can beneficially modulate the gastrointestinal environment. Feed additives influence immunity, gut health, food safety, and economic outcomes through several interconnected mechanisms. Foundational to these outcomes is balanced nutrition, ensuring appropriate levels of energy, amino acids, vitamins, and minerals to support growth, maintenance, and immune activity. The intestinal microbiome of newly hatched birds—shaped by hatchery conditions, diet, and environment—can be guided through judicious ingredient selection and the use of antibiotic alternatives. Vaccination programs continue to face challenges due to short production cycles and interference from maternal antibodies, influencing how immune supportive additives are deployed. A variety of antibiotic alternatives, including botanicals, microbial fermentates, prebiotics, probiotics, organic acids, and targeted vaccines, offer strategies to enhance gut health and performance. Despite these advances, food safety remains difficult to improve because many zoonotic organisms do not trigger strong inflammatory or immune responses in poultry, underscoring the need for integrated nutritional and management approaches. This presentation will summarize current knowledge on how feed additives contribute to immune competence, gut integrity, food safety, and profitability in modern poultry production.

### 80 Modulation of gut microbiota by dietary inclusion of grape pomace in coccidia-challenged broilers (*Gallus gallus*)

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Gut microbiome plays an important role in the development and modulation of immune responses; however, enteric diseases, including coccidiosis, disrupt microbial homeostasis, thereby affecting health, performance, and recovery in chickens. Strategies to positively modulate the gut microbiome using antibiotic alternatives are being explored to improve disease resilience, intestinal function, and production. Grape pomace (**GP**), an agricultural byproduct rich in bioactive compounds and dietary fiber, has antioxidant, antimicrobial, and immunomodulatory properties and can modulate the gut microbiome. This study aimed to evaluate the efficacy of GP in positively modulating gut microbiome in broilers infected with coccidiosis. A total of 120 14-d-old male broiler chickens were randomly assigned to four treatments: i) non-challenged control + basal diet (**NCC**), ii) challenged control + basal diet (**CC**), iii) CC + 0.5% dietary inclusion of GP (**0.5GP**), and iv) CC + 0.75% dietary inclusion of GP (**0.75GP**). On d 14, broilers in CC, 0.5GP, and 0.75GP were orally inoculated with a 20× dose of commercial live coccidiosis vaccine to induce coccidiosis. On d 20 and d 26, DNA from cecal content was extracted, purified, amplified, and full-length 16S rRNA sequencing was performed using the PacBio (SMRT) sequencing platform. Downstream microbiome and statistical analyses were conducted in QIIME2 and R. At d20, challenged groups showed higher microbial richness than NCC, consistent with transient niche disruption after mucosal damage, while evenness was similar. By d26, richness normalized, but evenness was significantly lower ($P$<0.05), indicating community reorganization under selective pressure. Beta diversity analyses showed a distinct clustering between CC and NCC, whereas 0.75GP clustered closest to NCC by day 26, suggesting taxonomic and proportional community structure similarity ($P$<0.05). At d 20, *Lactobacillus,* especially *Lactobacillus crispatus,* was elevated (~15%) in CC and 0.5GP groups compared to NCC and 0.75GP (15%), whereas *Faecalibacterium,* a key butyrate-producing genus, was dominant in NCC and 0.75GP ($P$<0.05). At d 26, *Bacteroides fragilis,* an opportunistic bacterium, was predominant in CC and 0.5GP ($P$<0.05), suggesting maintenance of butyrate-mediated epithelial support. To conclude, coccidia challenge at d 14 disrupts the still maturing gut ecosystem, triggering a transient expansion of opportunistic colonizers. Dietary GP at 0.75% appears to buffer against both phases, maintaining butyrate-producing genera, limiting opportunistic pathobiont expansion, and steering the microbial community toward a non-challenged structure by day 26 - with a more consistent modulatory effect than 0.5% inclusion.

**Keywords:** Broilers; Coccidiosis; Gut microbiome; Grape pomace

### 81 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 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

### 82 Use of a Hydrolyzed Yeast Product for Prevention of Necrotic Enteritis in Broilers

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Control of both clinical and subclincal necrotic enteritis continues to be a priority for broiler veterinarians and nutritionists. It has been well established that *E. maxima* causes the initial damage to the jejunum resulting in the opportunity for *Clostridium perfringens* to proliferate, produce toxin and then damage the broilers' intestines. Prior to the antibiotic free or no human importance antibiotic programs, the continuous use of ionophores for coccidia control and antibiotics were routinely used by broiler companies. Today broiler producers are using coccidia vaccines, probiotics, essential oils and yeast based products in broiler feed to try to reduce the impact on bird health and performance of both the coccidia and the Clostridium. Two research studies were performed in broilers comparing a hydrolyzed yeast concentrated product, Ceretide B (200 ppm), a direct fed microbial (DFM-500 ppm), an antibiotic (Bacitracin-55 ppm), and an untreated challenge control. Both studies used Ross 708 day of age broilers that were vaccinated with 1 dose/chick by coarse spray of a commercial coccidia vaccine. Study 1 was 28 pens (7 replicates) with the challenge as re-used litter from a previous N.E. challenge study. Study 2 was 40 pens (10 replicates) of re-used litter with C.P. challenge on days 14, 15, and 21. Pelleted diets were starter crumble (day 0-14), grower (day 14-28), and finisher (day 28-42). All mortality during the studies were necropsied for cause of death. Statistical evaluation of data used Statistix using ANOVA with comparison of means using least significant difference (t-test) at $P < 0.05$. Study 1 had only 2 birds with N.E. mortality in the untreated control. The hydrolyzed yeast had numerically lowest overall mortality (2.86%[A]) versus the challenge control (3.43%[A]). The subclinical C.P. challenge was also mild with no significant differences

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in performance between any treatments. In the second study with the C.P. challenge, there was 1.20%$^{A}$ N.E. mortality in all treatments except the antibiotic (0.00%$^{A}$). Prior to the C.P. challenge, the antibiotic had significantly heavier 14 days body weight and lowest FCR. At the peak of the C.P. challenge on day 28, the DFM had the heaviest body weight (1.447kg$^{B}$) with the hydrolized yeast intermediate (1.421kg$^{AB}$). During the post challenge recovery phase (day 28 to 42), the antibiotic had the lowest FCR (1.719$^{B}$) followed by the hydrolyzed yeast product (1.738$^{AB}$). The hydrolyzed yeast had the lowest overall mortality (2.00%), at P=0.051 compared with that of the untreated control (4.4%) . Both the hydrolyzed yeast (Ceretide) and DFM lowered N.E. lesions which aided the birds in the early growth phase to improve performance over the challenge control.

**Keywords:** necrotic enteritis; hydrolyzed yeast; broilers; FCR; Clostridium perfringens

### 83 Positive effects of dietary marine sulfated polysaccharides derived from macroalgae in broilers chickens during a necrotic enteritis challenge

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Necrotic enteritis (NE) occurs from the colonization and proliferation of *Clostridium perfringens* in the small intestine, leading to the production of extracellular toxins. An experimental NE model involving a co-infection with *Eimeria maxima* (day [d] 14), and *Clostridium perfringens* (d 19) was used to assess the effectiveness of the product Algimun® (manufactured by Olmix SA, Brehan France), composed of marine sulfated polysaccharides extracted from macroalgae (green and red seaweeds, *Ulva spp* and *Solieria spp* respectively). This study employed an experimental NE model involving co-infection with *Eimeria maxima* and *Clostridium perfringens* to assess whether marine sulfate polysaccharides extracted from macroalgae could mitigate the adverse effects of NE in broilers. A total of 600 d-old Ross 708 male broilers were randomly assigned to one of four treatment groups: NC (negative control, fed a corn-soybean meal diet); PC (positive control, NC + 15 ppm Avilamycin and 125 ppm Amprolium); AGS (Algimun® Standard, NC + Algimun® added at 0.1 % of the diet); and AGH (Algimun® High, NC + Algimun® added at 0.2 % of the diet). Average daily feed intake and weight gain were calculated and adjusted for daily mortality on d 14, 21, 28, and 42. On d 21, four birds/pen were examined for intestinal NE lesions. On d 14, 21, and 42, serum and jejunum samples from one bird/pen were collected to measure the concentration of the biomarker calprotectin and mRNA abundance of cytokines and tight junction proteins including claudins (CLDN) and occludins (ZO). Data were analyzed using JMP and significance between treatments was identified by LSD ($P \leq 0.05$). NE lesion scores on d 21, and mortality and FCR during d 0 to 42 were lower in PC, AGS, and AGH compared to NC birds ($P \leq 0.05$). AGS birds had the lowest levels for serum calprotectin on d 21, while antibiotic growth promoter PC, and algae-based groups AGS and AGH had lower levels than the NC group on d 42 ($P \leq 0.05$). On d 14 and d 42, mRNA abundance of CLDN1, CLDN3, ZO1, IL1B, IFNΓ, IL10, and IL12B was greater in AGS and AGH birds compared to NC group ($P \leq 0.05$). Also, AGH had a greater abundance of TNFA on d 14 and d 42 compared to NC group ($P \leq 0.05$). Further, mRNA abundance of CLDN3, ZO1, ZO2, OCLDN, IL1B, IL10, IL12B, IFNΓ, and ANXA1 was greater in PC on d 21 compared to NC and AGS groups ($P \leq 0.05$). Based on these findings, dietary inclusion of the macroalgae-derived marine sulfated polysaccharides yielded a similar response to an antibiotic growth promoter under NE. Therefore, the algae-derived product can be used as a potential alternative for strengthening natural defenses hence leading to broilers more prepared to overcome sanitary challenges.

**Keywords:** macroalgae; immune modulation; disease resistance; Clostridium perfringens

### 84 Dietary guanidinoacetic acid supplementation improves resilience of broilers during a multifactorial necrotic enteritis challenge

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Necrotic enteritis (NE) remains a major challenge in broiler production, leading to reduced gut integrity and function, and facilitating bacterial translocation and systemic inflammation, which are energetically taxing. Guanidinoacetic acid (GAA), a precursor of creatine, has been shown to enhance broiler growth performance by improving cellular energy efficiency. This study aims to determine whether dietary GAA supplementation enhances broiler growth performance and intestinal recovery during a controlled NE challenge. The trial was conducted at PoulPharm research facility (Belgium). A total of 648 day-old Ross 308 male broilers were randomly allocated to 3 treatments (12 replicate pen/treatment; 18 birds/pen): IUC: infected untreated control; ITC: infected treated control, receiving amoxicillin at 20 mg/kg body weight (BW)/day via drinking water during days 17-21; and IG: infected receiving GAA at 1200 g per ton of feed during days 0-42. Birds were fed in 3 phases: Starter (days 0–16), Grower (days 16-23) and finisher (days 23–42). Diets were based on wheat, corn and soybean meal and formulated according to breeder recommendation, except the grower diet which contains high crude protein (29.38%) and 30% fishmeal and 5% rye as a part of the challenge. Birds received a 10 times overdose of Paracox 8 live *Eimeria* vaccination on days 14 and 16 via oral gavage, followed by daily oral inoculation with $10^{8}$–$10^{9}$ CFU *C. perfringens* Net B positive strain on days 18–21. BW, ADG, FCR, mortality, coccidiosis and NE lesion scores were measured. Statistical analyses used linear and mixed models, with significance at $P \leq 0.05$. Performance results are reported as means ± SE. During days 16–22, GAA supplementation significantly improved ADG (72.2 vs 63.6 ±2.77; $P<0.01$) and FCR (1.18 vs 1.31 ±0.03; $P<0.01$) compared with IUC group. During days 0–42, FCR (1.47 vs 1.53 ±0.03; $P<0.01$) and NE related mortality (1.85 vs 7.44%; $P<0.01$) were significantly lower in birds receiving GAA supplementation compared with those in the IUC group, and similar to the levels of ITC group. On day 21, GAA-supplemented birds showed significantly reduced coccidiosis lesion scores (0.60 vs 0.96; $P<0.01$) and E. maxima scores (0.52 vs 0.81; $P<0.01$) compared with those in IUC, indicating improved epithelial integrity. In addition, on day 22, GAA-supplemented birds exhibited reduced NE lesion scores compared with IUC birds (2.33 vs 3.25; $P<0.01$). In conclusion, GAA supplementation enhances broiler growth performance during the NE challenge, reducing coccidiosis and NE lesion scores. These findings highlight the potential of GAA as a nutritional strategy to support performance in production systems seeking to reduce antibiotic use.

**Keywords:** broiler; guanidinoacetic acid; necrotic enteritis; coccidiosis; resilience

### 85 Engineered probiotic for inhibition of *Clostridium perfringens* induced NE in broiler chickens

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Necrotic enteritis (NE), induced by the opportunistic bacterium *Clostridium perfringens* (*CP*) is an ongoing challenge for poultry producers. We are seeking strategies to control CP without antibiotic drug application. Bacteriophages able to specifically lyse *CP* have been isolated but resistant bacteria arise at high frequency. Lysis of the CP is achieved through

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expression of an endolysin that degrades the bacterial cell wall. We hypothesized that delivery of a phage-derived endolysin to the chicken gut by a probiotic could suppress *CP*-induced NE in broiler chickens. A *Saccharomyces boulardii* strain was engineered to produce and secrete a phage-derived endolysin with specificity for *CP*. The strain was tested *in vitro* for specificity of killing of *CP* and *in vivo* in a clinical infection model of NE that used 3 groups of 24 (n = 72) birds. A control group was provided with protective antibiotic (bacitracin at 500 mg/kg feed), a second group was treated with control yeast 5 x $10^6$ CFU/kg feed, a third group received the engineered yeast at 5 x $10^6$ CFU/kg feed. All birds were treated with a 15X dose of live coccidiosis vaccine (Coccivac B52) and $10^8$ CFU of *NetB*-positive *CP* by oral gavage on day 6 and a second dose of the *CP* on day 12. On day 18, animals were sacrificed, intestinal lesions counted and *CP* loads assayed. Data from *in vitro* tests were analyzed by one-way ANOVA and Tukey post-hoc test, significance set at p≤0.05, animal lesion data were analyzed by Kruskal-Wallis test with significance set at p≤0.05. *In vitro*, the secreted endolysin was effective in lysing *CP* but did not kill the native gut probiotic strains *Lactobacillus reuteri, L. buchneri* or *L. casei* (p<0.001). The engineered probiotic displayed effective killing of *CP* in intestine-like medium and reduced *CP in situ* in chicken intestinal samples from 7 x $10^5$ to 5 x $10^1$ CFU in 30 minutes. Results of the clinical infection trial showed that all animals displayed less severe lesions than anticipated (average score 1.0 for control animals and 0.5 for animals treated with engineered probiotic). Treatment effects on intestinal lesions were not detected (p = 0.65) , CP enumerated from cecum samples were not significantly reduced (p = 0.22) and less than 100 CFU of engineered yeast/g cecum were detected. In conclusion a probiotic producing *CP*-specific endolysin is extremely effective in killing CP *in vitro* but at the dosage employed we were unable to detect significant suppression of NE possibly due to the small animal number in the experiment or insufficient dosage of engineered probiotic.

**Keywords:** Clostridium perfringens; endolysin; necrotic enteritis; probiotic; yeast

## Student Competition: Animal Well-Being and Behavior

**206 (E-F) (MTCC)**

### 86 Quantification of the effect of dietary crude protein in broiler chickens on footpad dermatitis by meta-analysis

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Footpad dermatitis (FPD) is a major welfare concern in broiler production. Reduced crude protein (CP) diets have been proposed as an effective nutritional strategy to mitigate FPD through their effects on nitrogen excretion, potassium intake, water consumption, and litter moisture. However, the quantitative relationship between dietary CP reduction and FPD severity has not been clearly defined. This study aimed to quantify the effect of reduced CP diets on footpad score (FPS) in broiler chickens using a meta-analytical approach. Published studies from 2007 to 2025 reporting FPD outcomes in relation to dietary CP were compiled, generating a database of 13 trials from 12 publications, including 40 treatments and 59 observations conducted in USA, Belgium, France, Germany, Netherlands, Romania, and South Korea. Because dietary CP levels differed across feeding phases, a weighted average crude protein (WACP) value was calculated to standardize overall protein supply taking into account dietary CP and feed intake per feeding phase. FPD scoring systems were harmonized, when possible, into three categories (0 = no lesions, 1 = moderate lesions, 2 = severe lesions), and a unified FPS was recalculated using the formula: FPS = 100 × [(0.5 × number of birds scored 1) + (2 × number of birds scored 2)] / total birds scored, resulting in a scale ranging from 0 to 200. A linear mixed-effects model (Minitab 22) was used to evaluate the effect of WACP on FPS, with trial included as a random effect and significance declared at P < 0.05. Across the dataset, control diets averaged 20.6±0.89 % WACP, whereas reduced WACP diets averaged 18.5±1.14 %, representing a mean reduction of 2.1±1.13 %-points. Footpad scoring was conducted at a mean bird age of 35.86 ± 9.13 days, with assessment ages ranging from 20 to 57 days. Age was not included in the model, which should be considered when interpreting the findings. Mean FPS was 51.6±42 in control treatments and 33.5±32.2 in reduced CP treatments. Dietary WACP was positively and linearly associated with FPS (P = 0.001), and each one %-point reduction in WACP decreased FPS by 8.7 units. The trial effect was significant (P = 0.005), and the final model explained 87.8% of the variation in FPS. In conclusion, dietary CP level is a significant predictor of FPS. Moderate reductions in CP are consistently associated with meaningful improvements in footpad condition, supporting reduced CP diets as an effective nutritional strategy to enhance broiler welfare.

**Keywords:** Broiler welfare; Footpad dermatitis; Litter quality; Meta-analysis; Reduced crude protein

### 87 Effects of dietary protein level and classical conditioning on stimulus preference in broiler chickens

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The ability of broiler chickens to adjust feeding behavior according to their nutritional needs remains a key question in poultry science, particularly under dietary protein restriction. Classical conditioning may allow animals to associate sensory cues with nutrient sources and guide feeding decisions. This study evaluated whether broiler chickens develop conditioned preferences for nutrient-associated stimuli under different dietary protein levels. A total of 128 one-day-old male Ross 308 broilers were assigned to a 2 × 2 factorial design with two dietary treatments: a standard protein diet (SP), meeting all nutritional requirements, and a low-protein diet (LP), formulated at 30% below optimal crude protein; and two conditioning treatments (conditioned or non-conditioned), resulting in four treatments with eight replicate pens each. Birds were reared for 42 days. Conditioning (training phase) was conducted in two cycles starting on days 10 and 30, each consisting of 8 consecutive days with one daily 4-h session using colored drinkers (yellow or green) associated with either a carbohydrate solution (3% sucrose) or a protein-based solution (3% FMR), with associations counterbalanced across pens. Preference tests (evaluation phase) were conducted using the same birds after each cycle and consisted of 4 consecutive days (4 h/day) using a double-choice paradigm. In conditioned groups, drinkers contained water to assess learned preference, whereas in non-conditioned groups sucrose and protein solutions were used. Intake was normalized by metabolic body weight, and preference for the protein-associated stimulus was evaluated relative to 50%. Data were analyzed using two-way ANOVA with diet and conditioning as fixed effects, considering pen as the experimental unit. Preference data were analyzed separately for each evaluation phase, and one-sample tests were used to compare values against 50%. No significant preference for the protein-associated stimulus was observed in SP birds (P> 0.05). In contrast, LP birds showed a significant deviation from the neutral value indicating preference for the carbohydrate-associated stimulus during the first evaluation phase (P= 0.0076), with no preference in the second phase (P> 0.05). In non-conditioned groups, no consistent preference pattern was detected. Standard-protein birds showed no preference overall, although a significant avoidance of the protein-associated stimulus was observed in the second phase (P= 0.0048), whereas LP birds showed a trend towards protein selection (P= 0.0908). A two-way ANOVA revealed no significant effect of diet (P> 0.05), whereas conditioning influenced stim-

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ulus selection (P < 0.05), with no diet × conditioning interaction (P> 0.05).

**Keywords:** Broiler chicken; Classical conditioning; Preference test; Associative learning; Protein restriction

### 88 Social Rank Influences Resource Use and Finishing Growth Performance in Broilers

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As environmental enrichment is increasingly adopted by commercial broiler producers, it is important to understand whether social rank influences access to resources and performance outcomes. In group-housed poultry, agonistic interactions establish social hierarchies that may affect individual behavior and resource use, potentially influencing growth and well-being. The objective of this pilot study was to determine the effects of social rank on behavior and performance in growing broilers. Broilers (n = 30) were randomly assigned to enrichment or no enrichment floor pens (n = 5 birds/pen) on d0 and reared for 45 days. Enrichment pens contained one wooden platform (30.5 cm length × 11.4 cm width × 13.9 cm height) and one dust bath (36.6 cm diameter × 11.4 cm depth). Aggressive interactions observed were recorded on d1-5, 12, 19, 26, 33, and 40 using 5-minute videos every 2 h between 04:00 h through 23:00 h, and broiler time budget was analyzed using 5-minute videos every 6 h during the same days. Social rank was then determined using Aggressive Rank Index (ARI), calculated as the proportion of wins divided by total interactions. ARI was used to classify birds as low (0-0.32), medium (0.33-0.67), or high (0.68-1.00) rank. Performance outcomes were measured on d 0, 7, 14, 21, 28, 35, 42, and 45. Body weight gain and FCR were calculated weekly. Data were analyzed using RStudio (R version 2025.09.1+401). All variables were analyzed using ANOVA with social rank as the fixed effect. Body weight did not differ among social ranks from d0-35 (P>0.05); however, on d45, high rank birds were 49 g heavier than medium rank and 67 g heavier than low rank birds (P=0.04). Social rank did not affect gain nor feed conversion during wk 1-5, but during wk 6 high rank birds had 0.52 reduced FCR compared to medium rank birds and 0.50 reduced FCR compared to low rank birds (P=0.05). In wk 7, high rank birds had 0.35 lower FCR than medium rank birds and 0.68 lower FCR than low rank birds (P=0.04). During the first week of life, dust bath use differed by rank within enrichment pens (P=0.03), with high rank birds spending more time in the dust bath than low and medium rank birds. Across wk 1-6 in both enrichment and no enrichment pens, high rank birds spent more time at the feeder than medium and low rank birds (P=0.051; P=0.043, respectively). These data suggest that in small groups of broilers, higher-ranking birds may have increased access to resources and a growth performance advantage at market weight. Future work with larger group sizes is needed to characterize how social rank contributes to enrichment use and to validate growth-performance outcomes.

**Keywords:** Aggressive Rank Index; Behavior; Dominance; Performance; Social Status

### 89 Fun and fear: Effect of using platform perches on stimulated play and neophobia in broiler chickens

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Platform perches allow broiler chickens to perform natural behaviors. This may improve animal welfare, including affect. We aimed to determine the effect of platform perches on play and neophobia (fear) in commercial broiler chickens. Two commercial farms with two houses of 41,400 (Farm A) or 27,200 (Farm B) Ross 708 broiler chickens were used, with 6 flocks per treatment (total 12 flocks). The treatment house contained gridded plastic platform perches providing ~12% additional space (including platforms: final stocking density 31.7 kg/m2). The control house had standard commercial conditions (36 kg/m2). Stocking density and platform access were confounded in the design. At 2 weeks old, 4 transect walks were conducted per house to stimulate play behavior. Broilers are known to run and frolick into newly accessible empty spaces. Two-min videos were recorded at 4 locations per transect. Play bouts (frolicking, sparring, running, and jumping) were counted (192 videos) and assessed using GLMMs (Fixed: negative binomial, treatment and farm. Random: location within transect). Neophobia (fear) was tested via novel object test (NOT) at 6 weeks old. We recorded latencies to approach, contact, and number of birds within 0.5m every 20s for 5min at 4 locations (48 NOTs; n=720 scans). Latencies were analyzed with Cox proportional hazard regression (Fixed: treatment, farm, and interaction; by date). Scans were analyzed using GLMM (negative binomial) with treatment, farm, and interaction as fixed effects and scan time and observation as random effects. Farm was included as a fixed effect in both models since there were only two levels (excluding the option to use it as a random factor). More play bouts were observed in the control than in platform flocks (mean±SD: 137±10 vs 99±7 bouts; p<0.001). Latencies to approach and contact the NO were longer (more fear) in the control than the platform flocks at Farm A (median approach: 94 vs 24s; p<0.001; median contact: 132 vs 267s; p=0.006). Latency to approach did not differ at Farm B (8 vs 10s; p=0.12), but latency to contact was shorter (less fear) in control flocks (64 vs 104s; p=0.002). Fewer control birds approached the NO (more fear) than platform birds at Farm A (1±0.1 vs 4±0.3 birds; p<0.001), with no differences in Farm B (6±0.4 birds; p=0.91). Interpretation of stimulated play behavior can be complex. While play is typically considered positive, sensitivity to play stimulation may indicate a rebound from behavioral deprivation in control flocks. Still, stimulated play was frequent in platform flocks too. Platform access reduced fear at Farm A but not B, where fear was low in control houses. Thus, platforms may only reduce fear when it is relatively high.

**Keywords:** Broiler; behavior; welfare; enrichment; commercial

### 90 Growth performance and preference of broiler chickens under two light treatments

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Lighting is one of the most essential environmental factors in broiler production, affecting bird behavior, performance, and overall welfare. This study aimed to evaluate the preference of broilers for two lighting treatments: a full-spectrum LED (Full) and a control LED (Control) and assess preference patterns and feed intake. The full-spectrum LED light provides a broader wavelength distribution across the visible spectrum simulating the natural light. Both lights have peak spectral output at 450nm (blue), but Full maintains relative higher outputs (0.7-0.9) between 470-675nm (green-red) compared to Control (0.45-0.75). A total of 300 day-of-hatch male Cobb 500 chicks were placed into 12 pens, each divided into two freely accessible sections, with one light treatment per side, creating a paired-choice design in which all pens received both treatments. Pens were separated with black plastic to contain the light treatments. Light intensity was standardized for gallilux (CLUX), and irradiance ($W/m^2$) was measured using a spectrometer on day 0 (D0) and D14. From D0 to D14, light intensity ranged from 15-25 CLUX beneath the light at bird level, then decreasing to 5-6 CLUX for the rest of the study. Body weight (g), feed intake (g/bird/day), and FCR were recorded on D14, D26, and D42. Preference observations were conducted from video recordings on D0-1 (first

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20 hours), D2-3, and for 1 day per week in weeks 2-6. This preference was determined as the percentage of birds located under each light treatment during the scan observations. Light measures, body weight and FCR are reported descriptively, and feed intake and behavior data were analyzed using a one-way ANOVA in JMP. The average irradiance at both ages was 0.036 ($W/m^2$) for Control and 0.04 ($W/m^2$) for Full. Body weight increased throughout the experiment, reaching an average of 4084 g by day 42. Feed conversion ratio remained uniform during the starter and grower phases (1.37) and increased during the finisher phase (1.53), with a cumulative FCR of 1.46. During the grower phase, feed intake was greater ($p = 0.02$) under Full (79.6 g/bird/day) compared with Control (59.6 g/bird/day), with no differences during the starter or finisher phases. On D0, a greater proportion of chicks were on the Control side (56.4 ± 1.76 %) compared to the Full side (42.9 ± 1.76 %, $p < 0.0001$). This trend continued on D3 with 52.3 ± 1.17 % on the Control side compared to 47.7 ± 1.17 % on the Full side ($p = 0.005$). In conclusion, while feed intake was not strongly influenced, preliminary results suggest that broilers showed an early preference for the Control compared to the Full treatment. Future work will evaluate if this preference is maintained at older ages.

**Keywords:** lighting; preference; growth performance; behavior

### 91 PoultryLeX-Net: Dual-stream transformer architecture for large-scale poultry sentiment analysis

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The global poultry industry is expanding rapidly as demand for affordable animal protein increases. This growth has led to greater public attention on poultry production practices, animal welfare, and supply chain transparency. Social media platforms such as X (formerly Twitter), Facebook, YouTube, TikTok, and Instagram generate large volumes of text reflecting the opinions and perceptions ("sentiment") of stakeholders, including consumers, producers, and advocacy groups. In this study, sentiment refers to whether these opinions are expressed as positive, negative, or neutral (e.g., positive views on animal welfare improvements or negative concerns about housing conditions). However, analyzing sentiment in this domain is challenging due to complex language, unclear context, and the limitations of general-purpose language models. The objective of this study was to develop and evaluate a domain-specific model for more accurate sentiment analysis of poultry-related social media data. We introduce PoultryLeX-Net, a dual-stream transformer model designed to improve sentiment analysis in poultry-related texts. One stream focuses on poultry-specific terms (e.g., welfare, housing, antibiotics), while the other captures broader linguistic context. A cross-attention mechanism integrates both streams to improve interpretation. Latent Dirichlet Allocation (LDA) was used to identify key topics, such as production management and animal welfare, to support the interpretation of sentiment trends. Model performance was compared with baseline approaches, including a convolutional neural network (CNN) and pre-trained transformer models (DistilBERT and RoBERTa). Performance was evaluated using accuracy (overall correct predictions), F1-score (balance between precision and recall), and AUC-ROC (Area Under the Receiver Operating Characteristic Curve). Statistical comparisons were conducted using ANOVA with Tukey's HSD test ($A = 0.05$). PoultryLeX-Net achieved an accuracy of 97.35%, an F1-score of 96.67%, and an AUC-ROC of 99.56%, representing improvements of approximately 3–6% over baseline models. These results indicate that incorporating domain-specific knowledge and dual-stream attention improves sentiment classification compared to general models. This approach provides a scalable way to monitor public perceptions of poultry production and animal welfare. While not a direct decision-making tool, it can support researchers and policymakers by identifying emerging concerns and public opinion trends.

**Keywords:** Deep learning; Infoveillance; Natural language processing; Text mining; Poultry

### 92 Phenotype-based discrimination in laying hen pullets assessed through feather pecking and other non-aggressive interactions

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Laying hen social dynamics are influenced by phenotypic appearance, but it is unclear how recognition of social reference models (archetypes) occurs. This study aimed to determine whether archetype recognition is fixed during imprinting (1 wk post-hatch), or if a longer socialization period is needed, and to what extent flock phenotypic diversity affects archetype flexibility. Day-old ISA Brown chicks (n=720) were randomized in 30 groups of 24 birds. Two early social exposure treatments: 1 wk (imprinting) or 6 wk (imprinting plus early socialization), were combined with 5 phenotypic diversity treatments: 100% non-altered (NA), 100% altered (A), 84% A, 84% NA or 50% A (3 replicates/combination). Phenotype alteration consisted of a black mark on the nape feathers. After 1 or 6 wks social exposure, groups were halved, forming 36 groups of 12 phenotypically homogeneous individuals, with 6 phenotypic backgrounds: 100A, 100NA, 84A, 84NA, 50A and 50NA. Remaining birds from 100A and 100NA were used as test stimuli. At 1, 6, 10 and 14 wks, 2 familiar birds/pen were tested (1x1 m open field; 348 tests) for 10 min with 2 unknown stimuli birds, one with the same phenotype alteration as the familiars (Si) and another with the opposite alteration (Sd). Open field tests were recorded with a HD camera. From videos, non-aggressive interactions (gentle and severe feather peck (GE, SE), beak peck (BE), toe peck (TO), tag peck (TA)) given and received by tested birds were extracted. Data were analysed with mixed model ANOVAs. Most TA and TO occurred during wk 1 and 6 ($p<0.001$ both), most SE during wk 10 and 14 ($p<0.01$), and the least GE during wk 14 ($p<0.001$). Familiars delivered most GE and TA ($p<0.001$ both), Sd received most GE ($p<0.001$), and Sd and Si received most TA ($p<0.001$). Mark conspicuity was also noted, with Si receiving more GE and SE than Sd when Si was A ($p<0.001$ and $p<0.05$, respectively). Similarly, Sd received more GE and SE when Sd was A than NA ($p<0.001$ and $p<0.01$, respectively). In 84% and 100% treatments more GE were received when A than NA ($p<0.01$ both), with no differences in 50%. No effect of early social exposure was detected. The lack of directionality and variability across age of BE suggests that it was not associated with social recognition in the test environment. Contrarily, the early directionality of GE and TA towards unfamiliar birds suggests that familiarity recognition was already present at 1 wk. Directionality of GE and SE towards altered unfamiliar birds suggests that both have a strong social component and could be in line with the social cost of carrying the mark. **Acknowledgements**: A.H. & project funded by Spanish Ministry of Sci. & Inno. (PREP2022-000466 & PID2022-142406OR-100).

**Keywords:** Pullets; Phenotypic appearance; Feather pecking; Social recognition; Imprinting

### 93 Limited evidence for locomotor imitation in pullets using a ramp vs. jump two-action procedure

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Although chickens use social cues during foraging and resource exploration, it remains unclear whether they copy precise locomotor actions demonstrated by conspecifics. To test for such locomotor imitation, we employed a two-action task in which pullets observed either a ramp or a jump ascent demonstrated by same-age birds when both options provide access to a reward. This design was intended to assess action matching while limiting alternative mechanisms (e.g., social facilitation, local/stimulus enhancement). Observer pullets were assigned to one of three treatment contexts: 1. Trained demonstrator (trained to ascend via ramp-left, ramp-right, jump-left, or jump-right), 2. Untrained demonstrator (arena acclimated but untrained, free to choose ramp or jump), and 3. No demonstrator. Day-old white-feathered female chicks (n=48) were housed in four floor pens (12/pen) with a 15 cm high, 150cm long perch and water lines positioned at bird eye-height, but no other elevated structures. Birds were allowed to explore the test arena prior to testing. At 7 weeks, birds observed their assigned demonstration over 5 days in an arena with a start box, a 40 cm high platform, and a 15 cm wide, 65 cm long ramp set to 50°, making ramp use and jumping comparable in potential energy gain and likely to involve wing use. Before each session, observers were offered a high-value food reward (sweet corn, raisin, mealworm mix) and to join conspecifics in a crate, to ensure motivation. On day 5, observers were tested individually: after reward and social exposure, they were given 5 minutes to reach the platform via ramp or jump, with food and conspecifics present on the platform. Data were analyzed using a generalized linear mixed model (SAS), with treatment as a fixed effect and pen as a random effect. Fisher's exact test was used for sparse subgroups. Only 19 of the 48 pullets (39.6%) reached the platform. Of these birds (n=19), 45.7% had no demonstrator, 37.5% a trained demonstrator, and 37.5% an untrained demonstrator; treatment had no effect on platform reaching ($p = 0.8843$). Thus, most pullets did not reach the platform, and demonstrator exposure had no overall effect. Few birds jumped to reach the platform (2/19; 10.5%). Among birds observing a trained demonstrator (n = 12), ramp demonstration increased matching of ramp use ($p = 0.0101$). However, this effect was limited: only 5/48 (10.4%) both reached the platform and matched the demonstrated method, showing minimal locomotor imitation under the present conditions. Pullets relied mainly on individual learning when tasks were readily discoverable, showing copying only for the lower-risk ramp action, likely requiring less power than jumping, as they navigated 3-D space.

**Keywords:** Social Learning; locomotor; imitation

## 94 Building maneuvering skills of laying hens through training – no effect on daily energy expenditure

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Controlled flapping descent (CFD) refers to the use of wing beats to regulate a bird's downward movement from an elevated surface. CFD training has been proposed to build flight muscles and improve a bird's control during aerial movements, which may help mitigate future keel bone fractures (KBF). Understanding whether such training influences daily energy use is essential to ensure developing pullets retain enough metabolic capacity for growth, and later egg production. Doubly labeled water (DLW) is a method used to quantify daily energy expenditure (DEE, kJ/day) in freely moving birds. Since chicken flight muscles are anaerobic, supporting short, explosive flights, we hypothesized that regular CFD training during rearing would not elevate DEE once completed. A total of 88 pullets (42 white– and 46 brown–feathered) were distributed across 12 mixed–strain floor pens. Half of the birds were trained to complete CFDs, while the rest remained untrained. CFD training occurred from 6 days of age to 10 weeks of age (WOA). Birds trained 5 day/week, with two non-training days scheduled per week to prevent fatigue accumulation. Over the training period, the number of CFDs increased and rest between successive CFDs decreased. Pens were equipped with low perches (15cm) during CFD training. Interactions between strain and training were not the focus of the study, therefore, DEE was measured only in white-feathered pullets using DLW. At the completion of CFD training (10 WOA) and at 19 WOA, pullets received a dose of stable, non–radioactive isotopes deuterium ($^{2}H$) and oxygen–18 ($^{18}O$) via intramuscular injection. At one and 48 hours post–injection, blood was collected. Elimination rates of $^{2}H$ and $^{18}O$ were compared to estimate CO2 production, which allowed calculation of DEE for each individual bird. DEE was modeled using a repeated-measures generalized linear mixed model (SAS), with body mass and training status as predictors. From this model, age specific least–squares means of DEE were extracted, only results from 10 WOA are presented. Body mass had a significant positive relationship with DEE ($p < 0.0001$), indicating heavier birds expended more energy per day. DEE at 10 WOA did not differ between trained and untrained birds ($p = 0.6791$), therefore, CFD trained birds did not have any additional post–training energetic costs. This supports an approach in which CFD activity during rearing could be used to build maneuvering skill, without compromising energy for growth or other vital activities that require energy.

**Keywords:** Energy expenditure; Training ; Laying hen; Keel bone; Aerial locomotion

## 95 Add It, Take It Away: The Consequences of Enrichment Change in Pullets and Hens on Feather Cover and Mortality

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Feather Pecking (FP) in laying hens is when one pecks and destroys the feather cover of conspecifics. This remains a major welfare concern because it can escalate to skin injury, cannibalism, and death. Providing edible enrichments, like pecking blocks (PBs), is one approach to redirect the pecking away from conspecifics. Currently, enrichment practices differ widely, meaning that flocks may receive enrichment during the rearing, laying, throughout both periods, or not at all. Research from other species suggests that altering enrichment availability can be stressful, raising questions about how such changes affect feather damage and mortality. We conducted a longitudinal field study involving 29 pens across 6 rearing farms and 13 pens across six laying farms in Ontario (farm sizes: 5,000–15,000 birds; pens: 2,000–3,000 birds). Birds were assigned to one of 4 PBs provision strategies: PBs only 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. Farmers collected daily mortality. At the end of the rearing period (18 WOA) feather cover was uniformly good (all score=0) regardless of PBs. During lay, feather cover and mortality was analyzed via multinomial generalized linear

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mixed model with fixed effects of age, treatment, and strain and a random effect of pen within farm. A significant interaction between strain- treatment-age emerged (*p<0.0001*). At 65 WOA, hens receiving PBs throughout both phases (YY) showed the highest probability of retaining intact feather cover (22.7%), whereas birds that lost enrichment after rearing (YN) were least likely to do so (7.6%), even worse than hens that never received Pbs (NN,11.5%). These findings indicate that withdrawing enrichment my intensify FP, although mortality rose with age (*p=0.0005*), PB enrichment itself did not influence mortality (*p=0.5912*). Mortality was lowest from 19-30 WOA (0.4 ± 0.068%) and higher in later production intervals 30-50 WOA (1.4 ± 0.068%) and 50-65 WOA (1.5 ± 0.068%) w(*P=0.0114* and *P=0.0093* respectively). Consistent Pbs provision across both rearing and laying did not affect mortality but yielded best feather cover outcomes, while any changes, addition or removal, appeared to increase feather damage.

**Keywords:** Feather Pecking; Enrichment; Pecking Blocks ; Feather Cover; Mortality

### 96 Does location matter? The impact of pecking block placement on feather condition in laying hens

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Feather pecking (FP) in laying hens involves pecking and/or pulling at the feathers of conspecifics, often leading to feather damage (FD) and reduced welfare. FP is widely considered a redirected form of foraging behaviour, as under natural conditions, hens forage at ground level and peck upwards at foliage, insects, or berries located in plants above. In contrast, commercial environments typically provide feed at ground level and maintain high stocking densities, which may increase the likelihood that upward pecking is directed towards the feathers of other birds. Introducing elevated pecking blocks (PB) as edible enrichment has the potential to redirect FP away from conspecifics and encourage pecking towards the blocks. This study aimed to investigate whether elevating PB could redirect attention upwards and influence the onset and progression of FP behaviour, feather cover scores, and PB usage. A total of 364 1-day-old chicks from two commercial strains—Lohmann LSL (White) and Bovans Brown—were assigned to one of three treatments: access to hanging PB, ground PB, or no PB (n = 4 pens/treatment; 29–31 birds of mixed strain/pen; floor substrate: wood shavings). Feather condition was scored across four body regions (head/neck, back, tail, and belly), and the worst score per bird was used for analysis (0 = no damage, 1 = minimal damage, 2 = severe damage). A multinomial model with odds ratios (SAS V9.5) was used to analyze the effect of treatment on FD, where an OR > 1 indicates higher odds of worse FD. All birds exhibited intact feather cover at 17 weeks of age (WOA). At 30 WOA, 73.1% of birds retained intact feather cover, while 18.6% of birds had at least one body area with a score of 1 (minimal damage), and 8.3% of birds were scored in category 2 (severe damage). At 45 WOA, hens provided with ground PB had significantly greater odds of receiving the worst feather damage score compared to no PB (OR = 1.69, 95% CI: 1.06–2.70, p = 0.027), indicating a higher likelihood of severe feather damage in the ground PB treatment. Ultimately, feather cover declined with age across all treatments, underlining a general welfare concern; however, differences among enrichment placements were limited under the conditions of this study. These findings will be discussed in relation to scoring methodology, environmental context, and the challenges of disentangling causes of feather loss in enriched research settings. Ongoing analyses will evaluate whether individual pecking block usage and behavioural observations, such as FP behaviour, correspond with FD outcomes and whether they were influenced by PB placement.

**Keywords:** Feather pecking; Laying hens; Edible enrichment; Feather damage; Animal welfare

### 97 Turkey behavioral activity levels are influenced by age and walking ability, and minimally by environmental enrichment

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Leg health problems affecting mobility and access to feed and water are among the foremost welfare issues affecting tom turkeys. Broiler chicken research suggests that providing environmental enrichments (EE) may increase activity levels, which could improve bone development and leg health. This study investigated the impact of EE on activity-related behaviors (sitting/lying, standing, walking, and environmental pecking) in toms classified as having good (GG) or compromised (CG) gait approaching slaughter age. Toms (n=432) were randomly allotted to 6 treatment groups at 5 weeks of age (woa) with 4 replicates of 18 birds per group. Treatment groups included pecking block (PK), robot (RT), platform (PM), tunnel (TL), straw bale (SE), and control (CL). We chose 2 focal birds (1 tom with GG: gait score 0 or 1; and 1 tom with CG: gait score 2 or 3) per pen (n=24) for observation. In pens having no toms with CG, we observed 4 toms with GG. Video observations of focal birds were completed at 12 and 16 woa, at 3 time points [3h (morning); 8h (afternoon); and 13h (evening) after lights-on] for 15 mins. We assessed the effects of treatment, gait (GG vs CG), age (weeks), and time of day (timepoints) on the duration of behaviors using PROC GLIMMIX (SAS 9.4). Standing duration increased with age (12 woa: 115.71±10.30 s, 16 woa: 210±10.30 s; P<0.001). The birds stood more in the morning (256±11.57 s) than afternoon (118.73±11.57 s; P<0.001) and evening (114.64±11.57 s; P<0.001). Similar to standing, toms spent more time walking at 16 woa (80.50±11.84 s) than at 12 woa (49.34±11.84 s; P<0.001). Time of day influenced walking behavior such that toms walked more in the morning (95.14±12.15 s) than afternoon (49.80± 12.15 s, P<0.001) and evening (49.82±12.15 s, P<0.001). Treatment (P=0.68) did not influence walking duration. The duration of time birds spent engaged in environmental pecking (EP) declined with age (12 woa: 86.45±9.18 s, 16 woa: 47.33±10.33 s; P<0.01). Toms spent more time engaged in EP in the evening (83.40±11.22 s) than afternoon (47.93±11.06 s; P=0.02) but not morning (69.34±10.67 s; P=0.48). There was a tendency for gait to influence sitting/lying duration such that toms with GG spent less time sitting/lying (372.37±26.28 s) compared to toms with CG (433.59±29.81 s; P=0.06). Toms spent more time sitting/lying in the evening (485.23±29.26 s) than afternoon (424.75±29.26 s; P<0.001) and morning (298.95±29.26 s; P<0.001). Our results suggest a limited impact of EE on toms' activity levels at 12 and 16 woa; however, increased inactivity in toms with CG by 16 woa suggests that change in behavioral time budgets warrant further investigation as early indicators of compromised walking ability.

**Keywords:** Turkey; Walking ability; Gait; Behavior; Environmental enrichment

## Student Competition: Microbiology and Food Safety II

**202 (A-D) (MTCC)**

### 98 Survivability of *Campylobacter jejuni* and *Campylobacter coli* in broiler litter

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*Campylobacter* is one of the leading food-borne illness causing pathogens. Poultry is considered as the major reservoir of *Campylobacter,* and poul-

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try litter serves as a key reservoir that facilitates transmission between flocks. The objective of this study was to investigate the survivability and persistence of *Campylobacter* in litter. A completely randomized design was utilized with three treatments: T1. control, T2. *C. jejuni*, and T3. *C. coli,* each treatment had six replicates. Each replicate consists of 600 g of *Campylobacter* negative litter (initial moisture 7.5%) in an autoclaved container. T1 was inoculated with sterile water to adjust the moisture level to 20%, simulating farm conditions. Litter was inoculated at 5 log CFU/g of *C. jejuni* and *C. coli* in T2 and T3, respectively, and litter samples were incubated at 25$^{o}$C aerobically. Litter samples were collected immediately after the inoculation in (d0), and on d3, d7, and d14 post-inoculation. Thus, collected litter samples were utilized to enumerate *C. jejuni* and *C. coli,* and enrichment culture was done to determine the survivability of *Campylobacter* in litter. Additionally, pH, moisture, Aerobic Plate Count (APC), and Enterobacteriaceae (EB) counts of litter samples were measured. Data was analyzed using ANOVA and post-hoc Tukey test with *p*-value set at <0.05. On d0, enumeration of *C. jejuni* and *C. coli* showed the recovery of *Campylobacter* ranging between 3.18-4.55 log CFU/g for T2 and 2.70-4.65 log CFU/g for T3. Similarly, the enrichment culture showed 100% *Campylobacter*-like colonies in T2 and T3 on d0. However, enumeration culture did not show any *Campylobacter* at d3, d7 and d14. Enrichment, however, detected *Campylobacter*-like colonies in 66.7% for both T2 and T3 at d7, whereas the survivability for *C. jejuni* (T2) declined to 16% and *C. coli* (T3) declined to 50% on d14. On d0, the mean count of EB and APC between the treatments ranged between 6.0 to 7.0 log Cfu/g and 9.7 to 10 log Cfu/g respectively. Both pH ($p$ = 0.007) and moisture ($p$< 0.00001) were significantly impacted by the *Campylobacter* inoculation. Both T2 (20.8%) and T3 (20%) had significantly higher moisture than T1(10.1%), while only T3 had significantly higher pH compared to T2 on d14. In conclusion, aerobic incubation of litter at 25 °C significantly reduced the survival of *C. jejuni* and *C. coli* in the litter. These results indicate that *Campylobacter* can persist in viable form in litter up to d14, but at a very low prevalence. The reduced survivability might be due to a switch to a viable but non-culturable (VBNC) state in response to continuous aerobic stress.

**Keywords:** Broiler Litter; Campylobacter jejuni; Campylobacter coli

### 99 Virulence Determinants and Antimicrobial Resistance in Avian Pathogenic *Escherichia coli* Isolated from Poultry in Malaysia

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**Abstract** Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in poultry, resulting in substantial economic losses and raising public health concerns about antimicrobial resistance (AMR). This study examined the prevalence, virulence-associated gene (VAG) profiles, and AMR profile of APEC isolated from commercial broiler and village chickens in Malaysia. A total of 63 cloacal swab samples were collected, including 30 from clinically affected broilers and 33 from apparently healthy village chickens. Presumptive *E. coli* isolates were identified using standard biochemical techniques, and APEC was confirmed by multiplex PCR targeting eight VAGs, including *astA*, *iss*, *irp2*, *papC*, *iucD*, *tsh*, *vat*, and *cva/cvi*. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method, according to CLSI guidelines. APEC was confirmed in 43.3% of broiler isolates and 6% of village chicken isolates, indicating a significantly higher prevalence in intensive production systems. Broiler APEC isolates exhibited significantly higher frequencies of *iss*, *irp2*, and *iucD* genes (P < 0.001), indicating an enhanced virulence potential related to iron acquisition and serum survival. AMR was widespread in both production systems. All APEC isolates exhibited resistance to tetracycline, while broiler and village isolates showed 100% resistance to ampicillin and erythromycin, respectively. Multidrug resistance (MDR) was observed in both groups, with a higher MDR rate detected in village chicken isolates. In summary, commercial broiler chickens exhibited a greater prevalence of APEC with more diverse virulence profiles, while village chickens had fewer APEC isolates but a higher rate of multidrug resistance. These results highlight the influence of production systems, environmental exposure, and antimicrobial usage on APEC epidemiology and antimicrobial resistance, underscoring the importance of enhanced antimicrobial stewardship and ongoing monitoring in both commercial and village poultry sectors.

**Keywords:** Avian pathogenic Escherichia coli; Antimicrobial resistance; Broiler; Virulence genes

### 100 Pre-harvest heat stress: disruption of microbial ecology and subsequent shelf-life of raw poultry products

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Broiler heat stress (HS) continues to negatively impact the broiler industry by reducing broiler growth rate and welfare. Extensive research determining molecular mechanisms of heat stress in broilers, as well as behavioral changes and the physiological stress it causes, exists. However, there is little information connecting HS impact on the post-harvest poultry product shelf-life and microbiome. Heat stress in broilers may lead to systemic infection by weakening the intestinal barrier integrity allowing bacteria to spread throughout the body, which may impact the post-harvest shelf-life of poultry products. Therefore, a 42-d heat stress trial was conducted to determine the effects of heat stress on the subsequent post-harvest chicken breast fillets through a 20-d shelf-life study. Male Cobb 500 chickens were raised at the University of Arkansas Division of Agriculture Broiler Research Farm (Fayetteville, AR). On 29-d, treatments were implemented: Control: thermoneutral, TN (23°C), Cyclic HS (HS): cyclic high ambient temperature of 35°C for 8h/d, Pair-fed (PF): thermoneutral conditions of 23°C, but pair-fed to the HS group. On d 42, chickens were harvested and fabricated. Boneless, skinless chicken breasts were aerobically packaged in overwrapped foam tray with absorbent pads and transported on ice back to the University of Wyoming to be placed under a simulated retail display for a 20-d shelf-life study at 4°C. On d1, 5, 9, 13, and 20 chicken breasts were sampled to collect microbiome samples. Samples were extracted and library preparation occurred according to Earth Microbiome Project targeting V4 16S rRNA gene and sequenced on MiSeq i100 (Illumina). Microbial DNA was analyzed using QIIME2 to evaluate the alpha and beta diversity metrics as well as the rate of change from d1 to 5, 9, 13, and 20. There was an interaction of time and treatment on microbial ecology impacting the shelf-life of the chicken breasts (P<0.05). The richness of TN, PF, and HS differed from d1-5 but did not differ from d1-9 (P<0.05). From d1-13, TN differed from PF in richness and there were no differences from d1-20 (Q<0.05). The evenness differed between HS and PF from d1-5 (Q<0.05), and PF differed from TN and HS from d1-9 (Q<0.05). By days 13 and 20, there were no differences in evenness (P>0.05;Q>0.05). There were no differences in beta diversity except PF and HS treated samples differed from d1-9 (P<0.05). Towards the end of shelf-life, HS treated samples enriched *Streptococcus* and *Pseudomonas* (P<0.001), which may explain the reduced evenness and richness. Ultimately, HS-treated samples exhibited reduced evenness and richness resulting in the end of shelf-life, suggesting HS may negatively influence shelf-life.

**Keywords:** microbiome; shelf-life; heat-stress

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**101 The Effect of a Live-modified *Salmonella* Typhimurium Vaccine on the Performance Parameters of Broilers Challenged with *Salmonella* Enteritidis**

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*Salmonella* is a leading cause of foodborne diseases worldwide, with poultry serving as a major reservoir for human infection. Vaccination is considered a key preharvest intervention strategy to mitigate *Salmonella* contamination in broiler production; however, variation in booster delivery route may influence bird performance and remains insufficiently understood. This study evaluated the effect of a live modified *Salmonella* Typhimurium vaccine administered via two booster routes (spray or drinking water) on the growth performance of broilers experimentally challenged with *Salmonella* Enteritidis. A total of 800 day of hatch YPM × Ross 708 male broiler chicks were randomly assigned to four treatments: T1: standard vaccine protocol (vaccine spray + water booster); T2: alternative vaccine application (vaccine spray + spray booster); T3: positive control (PC; sham spray + sham booster [spray + water]); and T4, negative control (NC; sham spray + sham spray booster). Birds in T1, T2, and T3 were orally challenged with $10^6$ CFU/bird of *Salmonella* Enteritidis on day 10, whereas T4 remained unchallenged. Birds were fed phase specific corn soybean meal based diets across three phases: starter (day 0-14), grower (day 14-28), and finisher (day 28-35). Performance parameters, including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were measured at the pen level and adjusted for mortality prior to analysis. All statistical analyses were conducted in R with statistical significance set at $P \leq 0.05$. Treatment effects on BWG, FI, and FCR were evaluated using one way ANOVA, while mortality was analyzed using the Kruskal-Wallis test due to its non-normal distribution. Across all production phases, BWG, FI, and FCR were not affected by treatment ($P > 0.05$), with no differences between vaccinated (T1, T2) and unvaccinated birds (PC) under challenge, or between challenged (PC) and unchallenged birds (NC). Mortality did not differ among treatments over the experimental period ($P = 0.358$). Under the conditions of this study, neither vaccination route (spray or drinking water) nor *Salmonella* Enteritidis challenge affected broiler growth performance or mortality, indicating that *Salmonella* vaccination could be used in commercial vaccination programs without impacting performance.

**Keywords:** vaccine; live-modified; Salmonella; broiler; food safety

**102 *Salmonella* Typhimurium persistence on antimicrobial-infused polyurethane surfaces**

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*Salmonella* contamination is an important food safety issue in the poultry industry, and surface contamination in processing is one source of transmission. Integrating antimicrobials into processing surfaces could lower persistence while reducing the costs associated with routine sanitization. The objective of this study was to investigate *Salmonella*persistence on three polyurethanes infused with different antimicrobials, copper nonanoate, curcumin, and combination of the two after a short exposure time of 4 h. Polyurethane surfaces were prepared by combining toluene diisocyanate, polyethylene glycol, with each antimicrobial which was cured in glass petri dishes. Each surface type (empty control petri dish, copper, curcumin, mix) was inoculated with 2 mL of *Salmonella* Typhimurium at 5 log CFU/mL, then sampled at 0h and 4h. Sampling was performed by rinsing the surface with phosphate-buffered saline. The rinsates were plated onto XLD agar and incubated at 37 °C for 18 hours for *Salmonella* recovery. Each treatment had three replicates. Data were analyzed using a three way factorial design evaluating the effects of surface treatment (control, copper, curcumin, mix), exposure time (0 or 4 h), and *Salmonella* storage age (0, 24, 72 h) on *Salmonella* recovery (log CFU/mL). A fixed effect three-way ANOVA model (PROC GLM, SAS) was used. Least square means were compared using Tukey's adjustment to control for multiple testing. Significance level was set at 0.05. There were no differences ($p>0.05$) in *Salmonella* recovery at the 0 h timepoint regardless of how long *Salmonella* had been stored refrigerated. When surfaces were inoculated with freshly prepared *Salmonella* and exposed to treatment surfaces for 4 h, copper significantly reduced ($p<0.05$) *Salmonella* to below detection limits (<1 log CFU/mL), with an average reduction of 5.46 log CFU/mL. After *Salmonella* inoculum was refrigerated for 24 h, both copper and mix significantly reduced *Salmonella* (5.5 and 3.6 log CFU). After the *Salmonella* inoculum was refrigerated for 72 h, both copper and mix significantly reduced *Salmonella* to below detectable levels (5.13 and 4.76 log CFU). This research demonstrated that antimicrobial-infused plastics have the potential to be effective for reducing *Salmonella* persistence and should be further investigated. In addition, the persistence of *Salmonella* was impacted by storage of the *Salmonella* inoculum under refrigerated conditions.

**Keywords:** Salmonella; polyurethane; copper; curcumin; antimicrobial

## Student Competition: Physiology and Reproduction II

**205 (A-D) (MTCC)**

**103 Corticosterone increases but returns to baseline in both sexes during chronic heat stress, however met-enkephalin levels increased until recovery in Pekin ducks.**

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Sustained high temperatures are particularly damaging for Pekin ducks as their down feathers exacerbate the effects of heat stress (HS). We have previously shown that heat stress in breeder stock reduces fertility, decreases egg count, and increases glucocorticoid deposition into eggs. A grandparent Pekin duck stock (3 rooms per treatment) was subjected to a 3-week cyclical HS period (35°C/29.5°C daily) followed by a week at 22°C (recovery (REC)). Blood was collected at 4, 24, and 48hr, and at 1, 2, and 3 wk after the onset of HS and at the end of REC. Corticosterone (CORT) and met-enkephalin (MET) were quantified via ELISA (n=10/sex/treatment). Hormone data were log transformed and all analyzed by ANOVA with the duck being the statistical unit. At day 21 of HS thermal images were obtained using a Fluke thermal imaging camera and analyzed by targeting the bill and eye as thermal zones. Significant increases ($p<0.05$) in CORT were observed at 4hrs, 24hrs, and 2 weeks compared to control for hens but only at 24hrs for drakes; at REC, CORT was back to baseline for both sexes. Circulating MET remained at baseline in both sexes until 1 wk in hens or 24 hr in drakes at which time it was significantly ($p<0.01$) higher compared to controls and remained so until REC. MET was increased in both sexes at times when CORT returned to baseline. Increased temps in the bill ($p<0.0001$) and eye ($p=0.002$) regions were observed compared to controls. Our data suggest further neuroendocrine systems are active during chronic heat stress, and that MET may be an important player in regulating the hormonal stress axis.

**Keywords:** Welfare; SHPA Axis; Affective State; Thermoregulation; Chronic

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### 104 Differences in testicular morphology associated with sperm mobility phenotypes in broiler breeder roosters

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The objective of this study was to quantify testicular morphological traits in roosters with high and low sperm mobility and to determine if high oleic soybean meal could alter sperm dynamics. Cobb broiler breeder roosters were randomly allocated to the standard broiler breeder commercial diet or high oleic soybean diet (n=28) experimental groups at 21 weeks of age. Fresh semen was collected from each rooster twice a week and assessed for sperm concentration and mobility. Testes samples were collected from high and low sperm mobility roosters at 35 weeks of age from each diet (n=5). The right testes were weighed, fixed in 10% formalin, and sectioned for histological analysis of the seminiferous tubules (ST) (ST area, ST diameter, lumen width, and epithelial thickness). A one-way ANOVA was used to identify the impact of diet on semen concentration and sperm mobility. The birds fed a high oleic soybean diet had higher sperm mobility (0.571 ± 0.0167 AU) than the birds fed a standard soybean diet (0.519 ± 0.0144 AU). A two-way ANOVA was used to identify the impacts of diet and mobility phenotype on testes weight and testis diameter. The weight of the left testes was less in birds with the low mobility phenotype (13.169 ± 0.899 g) than the high mobility phenotype (18.639 ± 1.386 g). A three-way ANOVA was used to identify the impact of diet, sperm mobility phenotype, and ST location (internal vs. external) on ST parameters. The ST area was greater in birds with the high mobility phenotype (0.0809 ± 0.00419 $mm^2$) than the low mobility phenotype (0.0691 ± 0.00104 $mm^2$). The ST epithelium thickness was greater in birds with the high mobility phenotype (95.678 ± 1.287 Mm) than the low mobility phenotype (90.766 ± 1.329 Mm) and it was also greater in the internal testes region (96.552 ± 1.170 Mm) than the external testes region (89.905 ± 1.336 Mm). The ST diameter was greater in the external testes region (322.747 ± 4.400 Mm) than the internal testes region (305.475 ± 4.070 Mm). The lumen width was greater in birds fed a high oleic diet (120.05 ± 2.825 Mm) than the birds fed a standard soybean diet (110.589 ± 2.522 Mm) and it was also greater in the external testes region (127.028 ± 2.924 Mm) than the internal testes region (102.841 ± 2.145 Mm). Dietary inclusion of high oleic soybean meal improved sperm mobility and increased ST lumen width. The low mobility phenotype was associated with substantially lower left testes weight, smaller ST area, and smaller ST epithelial thickness. ST location influenced ST diameter, lumen width, and epithelial thickness. Future directions include analysis of differences in genetic or hormonal regulation of testicular growth and function.

**Keywords:** high oleic; semen; seminiferous tubules; mobility; broiler breeder

### 105 Embryos in motion: the influence of strain and sex on prenatal motility in commercial layer chickens

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Wild bird species differ markedly in their embryonic activity, with brood-parasitic cuckoos showing elevated movement that likely functions as pre-hatch conditioning for strenuous early behaviors such as ejecting host eggs. As embryonic activity contributes to musculoskeletal development in precocial birds, it is plausible that pre-hatch physiological priming may also occur in domestic chickens. Thus, we hypothesize that genetic strain, and sex, may influence embryonic movement, given the distinct lineages of white-feathered (White Leghorn-derived) and brown-feathered (Rhode Island Red-derived) birds. We incubated 408 eggs (200 white-feathered, 208 brown-feathered) in a NatureForm I-series unit at 37.5°C and 55% humidity with hourly turning. On embryonic day 17, eggs were removed in trays of 30, placed on a 37.2 - 37.8°C heated blanket, and covered with a 63-L container. Each egg was then positioned in a validated Egg Buddy monitor (Avitronics, UK), which detects internal movement via reflected light sensors. A GoPro mounted 20 cm above the device recorded 120 seconds per egg, 60 seconds for habituation followed by 60 seconds for movement recording. Videos were later scored to calculate embryonic movement rate (movements/min). Three hundred and thirty eggs hatched into 172 females (92 brown, 80 white) and 158 males (73 brown, 85 white). We used a generalized linear mixed model in SAS to assess how sex and strain, and their interaction, influenced embryonic movement rate (EMR). Overall white and brown strains did not differ significantly in EMR (p=0.3178), though males moved more than females (p=0.039). This effect was largely driven by a strain by sex specific interaction wherein brown males moved more than brown females (p=0.0316). However, this same effect was not observed within the white strain where EMR was similar between both sexes, despite white males having the highest numerical EMR. To our knowledge, this study provides the first evidence of sex differences in EMR in domestic brown-feathered chickens. The absence of a strain effect on day 17 suggests that baseline prenatal motor activity is similar between these genetically distinct commercial lines at this stage. Strain-related motor differences may instead arise at other developmental times or become clearer after hatch under varying motivational or environmental conditions. Ongoing analyses of earlier embryonic days and post hatch behavior will address these possibilities and will be presented at the conference.

**Keywords:** Embryonic activity; Avian development; Physiology; Laying hen; Genetic strain differences

### 106 Embryonic Movement Influences Postnatal Bone Mineral Density and Strength in Chickens

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**Introduction:** Fetal movements generate mechanical stresses necessary for skeletal development. While human data and paralysis models (chick/mouse) demonstrate that restricted or absent movement impairs bone structure, the specific effects of normal variation in embryonic movement on bone structure and strength have not been examined. This study aims for the first time, to quantify embryonic movements *in ovo* and test

for morphological and physiological effects upon the leg after hatch in chicks. **Method:** A total of 73 fertile eggs from White and Brown -feathered hens were incubated at the University of Guelph. Embryonic movement (EM) was recorded via digital monitor (Egg Buddy, Avitronics) for a duration of one minute on days 15 (EM15) and 18 (EM18). After hatch (day 21), female and male chicks (n=18-19 chicks/genetic strain/sex) were euthanized to assess cortical bone mineral density (Ct.BMD) and microstructure using microCT (left tibiotarsus), bone strength via three-point bending (left femur), serum procollagen type I N-propeptide (P1NP) via ELISA, and whole leg muscle weights. Linear regression analysis was used to assess the influence of the number of movements per min at day 15 and 18, sex, and strain on bone and muscle outcomes. **Results**: The mean±SD number of movements in EM15 and EM18 for the whole cohort were 91±41 and 104±34, respectively. Ct.BMD was 440±32 mg HA/ccm, and multivariable regression showed a significant interaction between EM15 and genetic strain (*p=0.002*) such that EM15 was not associated with Ct.BMD in White birds (*p=0.23*) but was significantly associated in Brown birds (*p=0.0004*). Sex and EM18 were not associated with Ct.BMD. Bone strength was 6.90±1.04 N, and EM18 showed a positive association in White males (*p=0.035*). Compared to White chicks, Brown chicks possessed significantly higher total cross-sectional area (0.40 $mm^2$; *p <0.0001*) including cortical area (0.13 $mm^2$; *p=0.0002*) and marrow area (0.28 $mm^2$; *p <0.0001*). Marrow area in Females was significantly lower (0.06 $mm^2$; *p=0.045*) than in males. No significant differences were found in P1NP levels or leg muscle mass across sex, genetic strain, or movement. **Conclusions:** Our results show that embryonic movement influenced bone mineral density and strength in a genetic strain and sex dependent manner. Since bone fractures in chickens are a major animal welfare concern, improving understanding of developmental mechanisms that promote bone strength will require further studies, which should feature longer measurement durations of embryos as well as studies to determine how long the effect of embryonic movement persists into the juvenile period.

**Keywords:** Embryonic Movement; Bone Mineral Density; Bone Strength; Mechanobiology; Chickens

### 107 Gene expression associated with the hypothalamic-pituitary-gonadal axis in response to synchronizing body weight and photostimulation in cage-free pullets

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The transition to cage-free housing has altered reproductive performance in laying hen flocks. This study evaluated the effects of synchronizing body weight (BW) and photostimulation (PS) on the activation of the hypothalamic-pituitary-gonadal (HPG) axis in cage-free laying hens. At 12 weeks of age (woa), 600 Lohmann LSL Lite pullets were placed among 4 rooms, each with 6 aviary-style pens (n=25/pen) reared under 9L:15D. Pens were assigned feeding regimens (FR) at 13 woa (N=3 pens/room): *ad libitum* (AL) and 20% feed restriction (R). PS (14L:10D, N=2 rooms/FR) was imposed at 18 woa (PS18) or 20 woa (PS20) upon reaching 1.45 kg. By 21 woa, all birds were fed AL. The hypothalamus, pituitary, and small white follicles (SWFs) were collected at 14 and 16 woa, weekly from 18 to 22 woa, and at 26 woa (n=6/trt/timepoint) to quantify gene expression of key HPG axis components by qPCR. Expression was normalized to the geometric mean of GAPDH and B-actin and calculated using the $2^{-\Delta\Delta Ct}$ method. Statistical analyses were performed using the GLIMMIX procedure in SAS to evaluate the effects of age, FR, PS, laying status, and abdominal fat accumulation. As hens reached sexual maturity, clear age-dependent changes occurred across the HPG axis. GnIH expression decreased after 19 woa (*P*<0.001), while GnRH-I expression increased between 16 and 21 woa (*P*<0.001). GnRH-RIII expression rose between 19 and 20 woa (*P*<0.001) while GnIH-R concurrently declined up to 19 woa (*P*<0.001). Within SWFs, FSH-R expression increased from 19 to 21 woa (*P*<0.001). PS age modified the magnitude of the pituitary response without altering the timing of activation. GnIH-R expression was greater in PS20 hens than in PS18 hens overall (*P*<0.05), while this PS effect was not observed with GnRH-RIII levels. Downstream, levels of FSH-B rose from 18 to 20 woa in the PS18 hens and between 19 and 20 woa in PS20 hens (*P*<0.05). Furthermore, PS20 had greater LH-B expression than PS18 at 22 woa (*P*<0.05). This suggests that PS age affects the pituitary response magnitude, whereas timing differences are confined to the SWF. FR did not alter HPG-axis activation but impacted follicular receptor dynamics, as LH-R expression in SWFs declined from 19 to 20 woa in R hens and from 20 to 21 woa in AL hens (*P*<0.01). Expression of GnRH-RIII and FSH-B was greater in hens that had entered lay and exceeded the fat threshold (*P*<0.001). Collectively, the activation of the HPG axis is primarily age-dependent, with FR and PS modulating pituitary responsiveness. Thus, the pituitary appears to be a critical integrator for metabolic signals that permit reproductive activation.

**Keywords:** Body composition; Cage-free; Gene expression; HPG axis; Photostimulation

### 108 Effects of genetic selection on thyrotropic axis gene expression in yolk sac and somatic tissues during broiler embryogenesis

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In the broiler industry, genetic selection for increased carcass yields and improved feed efficiency has reduced the grow-out period, making embryonic development roughly one-third of the time it takes to produce a market weight broiler. Despite improvements in growth performance, hatchability has declined in recent years. A shorter grow-out period combined with hatchability loss highlights the importance of understanding factors controlling embryonic growth and development. This study sought to elucidate impacts of genetic selection on developmental expression patterns within the thyrotropic axis that mediates growth and metabolism through the actions of thyroid hormones (THs). Embryos from modern, fast growing (Cobb) and legacy, slow-growing (Athens-Canadian Random Bred [ACRB]) lines were sampled on embryonic days (E) 3, 6, 9, 12, 15, 18, and day of hatch (DOH). The entire yolk sac tissue (YST) was collected at each age, embryo body was collected on E3 and E6, and liver and breast muscle were collected for the remaining ages. Male embryos were analyzed by RT-qPCR (n=4/line/age) to determine relative mRNA expression of genes mediating transport and bioavailability of THs. Data were analyzed by a two-way ANOVA followed by Fisher's LSD test. Transthyretin *(TTR)*, a TH transporter, was greatest in the YST compared to other tissues and decreased at E18 and DOH to low levels, with ACRB YST exhibiting greater expression at both ages (P<0.05). Upon entering the target cell, THs can be activated or inactivated by deiodinase (DIO) 2 or 3, respectively. While there were no differences between the lines for *DIO2*, hepatic expression levels were higher than other tissues after an increase from E18 to DOH (P<0.05). In muscle and YST, *DIO2* declined throughout development, but YST levels increased to intermediate levels between E18 to DOH (P<0.05). For *DIO3*, YST expression decreased after E9 to low levels in both lines and remained diminished through hatch (P<0.05). Levels of *DIO3* increased in ACRB YST on E18 and both lines on DOH, with ACRB exhibiting greater expression at both ages (P<0.05). The relatively few differences between lines were observed close to the time of hatch when embryo growth is nearly complete, suggesting that genetic selection has not greatly impacted thyrotropic axis activity in the developing embryo within the tissues examined. However, the dynamic expression patterns in modern and legacy line broiler embryos emphasize the role that the thyrotropic axis plays in regulating embryonic growth and metabolism

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and highlight it as a target for strategies that can be used to offset hatchability loss while continuing to improve post-hatch growth performance.

**Keywords:** thyroid hormones; embryonic development; hatchability; yolk sac tissue; deiodinase

### 109 Antibiotic growth promoters restore performance, acid-base balance, and kidney function during heat stress in modern broilers

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Rising global temperatures and high metabolic rates due to rapid growth have left broilers susceptible to heat stress (HS). Prior to their removal, antibiotic growth promoters (AGPs) were used to improve growth performance, especially during suboptimal conditions such as high ambient temperature, through poorly understood mechanisms. The kidney is vital in responding to HS by regulating gluconeogenesis, glucose reabsorption, and blood acid-base balance. We therefore sought to understand AGP effects on renal function during HS in order to develop novel antibiotic-free (ABF) strategies. Day (D) of hatch male Ross 708s were randomly assigned to four groups in a 2 x 2 factorial arrangement, with two diets (bacitracin methylene disalicylate [BMD; 50mg/kg] or ABF) fed for the entire study and two temperature treatments (thermoneutral [TN;72°F] or HS [95°F for 8h/day]) administered from D32-38 (n=12 pens/group). Standard growth performance parameters were determined [body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR)]. On D32 (acute) and D38 (chronic), whole blood and kidney were collected from one bird/pen and analyzed with i-STAT and VS2 or qRT-PCR to assess blood chemistry and gene expression related to kidney function, gluconeogenesis, and glucose reabsorption, respectively. Data were analyzed using one- and two-way ANOVA and Fisher's LSD test. From D32-D38, ADFI and ADG were lower in HS than TN ($P \leq 0.05$), and FCR was higher during HS ($P \leq 0.05$). Final BW was increased with BMD and decreased during HS ($P \leq 0.05$). Blood pCO2 and HCO3 were decreased, and pH was increased during acute and chronic HS, with pH being increased to a lesser extent in birds fed BMD during chronic HS ($P \leq 0.05$). Blood glucose was higher during acute and chronic HS, but to a lesser extent in birds fed BMD during chronic HS ($P \leq 0.05$). During acute HS, evidence of muscle breakdown and renal dysfunction was present, as indicated by increased aspartate aminotransferase, total protein, and albumin, and decreased phosphorus ($P \leq 0.05$). Higher expression of sodium-glucose cotransporter 1 was observed during acute HS only in ABF-fed birds, suggesting greater glucose reuptake ($P \leq 0.05$). During HS, levels of phosphoenolpyruvate carboxykinase 1, a gluconeogenic enzyme, were increased ($P \leq 0.05$). Expression of claudin 2, a tight-junction protein, was decreased during acute HS in both diets, while birds fed dietary BMD during chronic HS did not have lower expression ($P \leq 0.05$). Results indicate that while HS decreased performance and disrupted renal glucose metabolism and barrier function, dietary BMD mitigated some of these negative effects, indicating potential targets for AGP alternatives.

**Keywords:** Blood glucose; acid-base balance; gluconeogenesis; kidney function; pH

### 115 Identification of cells responsible for polyamine synthesis in the kidney of broiler chickens

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Polyamines (putrescine, spermidine, and spermine) are essential compounds for animal growth and development. Polyamines are synthesized from ornithine via ornithine decarboxylase (ODC), and ornithine are produced from arginine and proline. In mammals, polyamine synthesis occurs in various organs throughout the body, but in broiler chickens, the kidney is the primary producing organ. However, it remains unclear which renal cells are responsible for polyamine synthesis. This study aimed to identify the renal cell types involved in polyamine synthesis. The kidneys from 14 day-old of male broiler chickens (Ross) were fixed in formalin, and embedded in paraffin block. The kidneys collected from other broiler chickens were digested with collagenase, and the isolated cells were separated into four fractions by density gradient centrifugation. Among these fractions, the 2nd and 3rd fractions were collected as cell populations of renal tubules. A monoclonal antibody against chicken ODC was generated by conventional method. Serial kidney sections were analyzed by immunohistochemistry using the ODC monoclonal antibody, and by periodic acid-schiff (PAS) and hematoxylin and eosin staining. ODC and Aquaporin-1 (Aqp1; a marker of proximal tubule cells) expression levels in the cells were analyzed by Western blotting analysis and qPCR, respectively. The renal fractionated cells were incubated with $^{13}C6$, $^{15}N4$ arginine, and newly synthesized $^{13}C4$, $^{15}N2$ putrescine in both cells and culture medium were detected using LC-MS. Statistical analyses were performed using Student's t-test, with significance set at $p < 0.05$. Histological analysis showed that ODC-positive signals were observed in renal tubules. Serial section analysis indicated that these ODC-positive tubules also exhibited PAS-positive and strong eosinophilic cytoplasm staining. It is known that the proximal tubule has higher glycoprotein expression and higher levels of acidophilic substances than the distal tubule. These results indicate that proximal tubules are the important cells for avian polyamine synthesis. Among the 2nd and 3rd fractions, ODC expression was detected at only 2nd fraction. This 2nd fraction showed higher expression levels of Aqp1 than the 3rd fraction, which reconfirmed that the ODC-positive renal tubules are proximal tubules. Moreover, in tracer experiments, $^{13}C4$, $^{15}N2$ putrescine was detected at higher levels in both the cells and the culture medium of the 2nd fraction than the 3rd fraction. In conclusion, these results demonstrate that in broiler chickens, the renal proximal tubule cells are the main cells responsible for polyamine synthesis. This unique polyamine metabolism may contribute to the rapid growth of broiler chickens.

**Keywords:** Polyamines; Monoclonal antibody; LC-MS; Ornithine decarboxylase; Proximal tubules

### 110 Effects of fasting and semi-purified diets on hypothalamic mRNA expression in broiler chicks

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Provision of feed after hatching induces a metabolic switch from hepatic lipolysis during embryonic development to *de novo* lipogenesis post-hatch. Fasting or delayed feeding (DF) delays the metabolic switch and alters gene expression in the hypothalamus—a neuroendocrine organ regulating appetite, feed intake, and metabolism. Our lab has shown that DF increases hypothalamic mRNA for neuropeptide Y (*NPY*), which is responsible for driving feed intake. We previously reported that DF increased pituitary thyrotropin-releasing hormone receptor and growth hormone mRNA levels. While the effects of DF have been studied on the hypothalamus, the effects of specific dietary macronutrients have not been studied. The objectives of this study were to determine the effects of DF and specific dietary macronutrients on hypothalamic mRNA expression. Ross 708 broiler chicks were separated into 7 treatment groups (n=8/group): day of hatch (DOH), fully fed (FF), fasted (DF), and fed semi-purified (SP)-complete, SP-dextrin, SP-casein, SP-corn oil. FF chicks were fed *ad libitum*, while the SP diets were orally gavaged every 8 hr for 48 hr and consisted of 85% of its respective macronutrient on an isocaloric basis. Gene expression was measured using RT-qPCR and normalized to phosphoglycerate kinase 1 before statistical analysis by ANOVA (SAS Version 9.4). The following hypothalamic genes that regulate metabolism

and feed intake were analyzed: *NPY,* deiodinase 2 (*DIO2*), thyrotropin-releasing hormone (*TRH*), thyroid hormone receptor B (*TRb)*, thyroid hormone receptor A (*TRa*), growth hormone-releasing hormone (*GHRH*), and NPY receptor 5 (*NPY5R*). Genes downstream from hypothalamic *GHRH* and *TRH* expressed in the liver, such as growth hormone receptor (*GHR*) and deiodinases 1 and 3 (*DIO1* and *DIO3*) were measured and normalized to B-actin. No significant changes in hypothalamic *TRH, GHRH, NPY5R, TRa,* and *TRb* mRNA were found in DF chicks relative to FF. However, there was a significant increase (P<0.05, relative to FF) in hypothalamic *NPY* (DF=1.7±0.2; FF=0.7±0.1) and *DIO2* (DF=1.4±0.2; FF=0.7±0.1) mRNA in DF chicks. Liver expression of *GHR* showed no significant changes. However, *DIO1* (DF=0.2±0.1; FF=0.9±0.2) and *DIO3* (DF=4.9±1.5; FF=0.5±0.1) showed decreased and increased mRNA expression in the liver, respectively. We conclude that fasting newly hatched chicks alters thyroid hormone metabolism in the liver and hypothalamus and also induces hypothalamic stimulation of feed intake mechanisms. However, none of the SP-diets given by oral gavage mimicked the FF treatment group. Ongoing research is evaluating the effects of SP diets provided in a crumble form *ad libitum*.

**Keywords:** broiler; metabolic switch; hypothalamus; fasting; macronutrients

### 111 Pituitary thyrotropin responsiveness to thyrotropin-releasing hormone differs between low and high egg producing turkey hens

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Inefficiencies in egg production represent major economic losses in the turkey industry. Previous work has identified differences in the hypothalamus-pituitary-thyroid axis between hens with different levels of egg production, including increased thyrotropin releasing hormone (*TRH*) and thyroid-stimulating hormone beta subunit (*TSHB*) mRNA levels in low egg producing hens (LEPH) when compared to high egg producing hens (HEPH), as well as divergence in circulating thyroid hormone levels between LEPH and HEPH relative to the pre-ovulatory progesterone surge. We hypothesized that differences in pituitary responsiveness and/or sensitivity to TRH could be responsible for differences in thyrotropin production between LEPH and HEPH. To investigate this, primary turkey pituitary cells from LEPH and HEPH were cultured and treated with 0, 0.3 nM, 3 nM and 9 nM TRH. Circulating progesterone levels were measured via radioimmunoassay to determine pre-ovulatory surge status. Individual birds were sampled as biological replicates, but surge grouping resulted in an unbalanced design for each treatment condition with group sizes (n) as follows: Low-Basal = 3, Low-Surge = 6, High-Basal = 5, High-Surge = 4. Cells were lysed with QIAZOL, and RNA was extracted using RNeasy kits, followed by reverse transcription and quantitative PCR. We employed a 3-way ANOVA and mixed effects modeling to test effects of egg production x surge condition x treatment using base R and lme4 packages. To capture the non-linear response of cultured cells to TRH it was necessary to model the treatment effect as the natural cubic spline of the log of the dosage (2 degrees of freedom) in the mixed effects model using the base R splines package. *TSHB* mRNA levels showed a robust, nonlinear relationship to log of the dose (p<0.0001) and the shape of the dose-response curve differed by egg production level (LEPH vs. HEPH; p=0.004), with the maximum response in HEPH (4-fold) being greater than that of LEPH (2-fold). The 3-way Anova and the mixed effects model suggest a tendency for differences due to the pre-ovulatory surge (p=0.088 and p=0.062, respectively) in *TSHB* mRNA levels. TRH receptor (*TRHR*) mRNA differences were only due to treatment, with TRH depressing levels of *TRHR* mRNA comparably across all groups. These results indicate that thyrotropin responsiveness to TRH is greater in HEPH than in LEPH and that these responses may be affected by the pre-ovulatory surge. We conclude that the observed disparities in circulating thyroid hormones associated with egg production rates in turkey hens likely involve differences in pituitary TSH responses to hypothalamic TRH.

**Keywords:** thyroid; cell culture; turkey; pituitary; reproduction

### 112 Oviposition time on the early production stage of broiler breeder hens fed different feeding regimens

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Oviposition time represents the moment a hen lays a fully developed egg and is an important indicator for optimizing feeding schedules, egg collection efficiency, and egg quality. This study evaluated the effects of different feeding regimens on oviposition patterns of individually caged broiler breeder hens during early lay. A total of 103 Cobb 500 hens were monitored from 30 to 34 weeks of age (woa) under a 14L:10D photoperiod and a fixed 0700 h feeding time at lights on. Birds were randomly assigned to one of three feeding regimens: everyday control diet (ED), skip-a-day control diet (S), or everyday high-fiber diet (HF). These regimens were implemented beginning at 6 woa; S hens transitioned back to ED feeding at 18 woa, whereas HF hens remained on this diet throughout. Oviposition time was recorded using a photoelectronic sensor (PES) installed on each cage. Variables measured included oviposition time, egg formation time, clutch length, and pause days. Data were analyzed using a generalized linear mixed model to test the fixed effects of age and treatment (PROC GLIMMIX; SAS 9.4). Means separation was performed using Tukey-Kramer. Oviposition time was affected by age ($P < 0.001$), as hens at 30 woa laid eggs earlier in the day than hens at 31-34 woa. Feeding treatment did not significantly affect oviposition time; hens laid at 28.1, 28.0, and 28.8 h intervals in ED, S, and HF, respectively. Egg formation duration also differed by age ($P < 0.001$), as hens at 30 and 32 woa formed eggs faster than at 31,33 and 34 woa. Although feeding treatment did not affect egg formation duration, S hens formed eggs approximately one hour faster (26.4 h) than ED (27.2 h) and HF (27.3 h). Clutch length and pause days did not differ statistically among feeding treatments, although S hens averaged longer clutch lengths (16 days) compared to ED and HF (13 days). Across all treatments, hens averaged 5 clutches and 5 pause days over the 5-week period. Distribution of oviposition relative to lights on showed that 18% of eggs were laid two hours after lights on, 20% three hours after lights on, 18% four hours after lights on, and 15% five hours after lights on, indicating that most hens laid approximately three hours after feeding. Overall, hens laid and formed eggs more rapidly at peak production, with both oviposition and egg formation timing slowing as production begins its initial descent. Further studies are required to determine the long-term effects of feeding regimens on oviposition timing and egg formation, and to assess whether small shifts in these parameters translate into differences in eggshell quality.

**Keywords:** broiler breeder; clutch; oviposition; egg formation; sensors

### 113 Interactive Effects of Flock Age, Oviposition, and Eggshell Translucency on Egg Quality and Hatchability

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Eggshell quality variations, particularly translucency defects, are increasingly associated with reduced hatchability in broiler breeder operations. This study evaluated the effects of flock age, oviposition time, and eggshell translucency on hatchability in a commercial Cobb 500 flock (~11,000 birds). A total of 16,200 eggs were collected across three

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flock ages (34, 44, and 53 weeks; 5,400 eggs per age) during two periods (morning and afternoon) and classified into three translucency levels (low, medium, and high). The experimental design included 18 treatment combinations (3 translucency levels × 2 oviposition times × 3 flock ages) to assess their effects on hatchability and shell quality. Eggs were incubated at the MAFES Poultry Research Unit using a PS500 Jamesway incubator operated under standard industry conditions. For shell quality measurements, a subset of 540 eggs (30 per treatment) was analyzed. Eggshell thickness, shell strength, yolk color, and Haugh units were measured using an EggTester Ultimate instrument, and shell color (L* value) was determined with an electronic colorimeter. Data were analyzed using the GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means with significance established at $P \leq 0.05$. Results show a significant three-way interaction among flock age, oviposition time, and translucency level for shell thickness ($P = 0.0458$), L* value ($P = 0.0365$), and initial egg weight ($P = 0.0353$). The thickest shells (0.3704 mm) and highest L* values (70.17) occurred in high- and medium-translucency eggs collected in the afternoon at 44 weeks, whereas the thinnest shells (0.3317 mm) and lowest L* values (60.53) were observed in low-translucency eggs at 34 weeks. Initial egg weight was highest in low-translucency morning eggs at 53 weeks (68.8 g) and lowest in high-translucency morning eggs at 34 weeks (57.8 g). Hatchability was not affected by the interaction ($P = 0.0599$) but was independently influenced by translucency ($P = 0.0005$) and flock age ($P < 0.0001$). The lowest hatchability was observed at 53 weeks (86.14%), with no differences between 34 and 44 weeks, while low-translucency eggs showed the highest hatchability (91.86%), with no differences between medium and high translucency. These results emphasize that eggshell translucency is a key factor affecting hatchability, while its interaction with flock age and oviposition timing predominantly influences shell quality traits.

**Keywords:** Translucency; Oviposition; Age; Hatchability; Eggshell quality

### 114 Use of novel methodology to simulate external and internal keel tip fractures and assess keel tip strength

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Keel tip fractures in laying hens are a welfare concern that continues to plague the industry. The keel's unique shape and structure create challenges for direct strength assessments, so long bones, including humerus, have been used as proxies in studies aimed at improving layer bone quality to reduce keel damage. However, direct evaluation of keel integrity would greatly improve the chances of successfully mitigating keel damage. The project goal was to design methodology to test keel tip strength and simulate different fracture modalities. A rig was created that secured the proximal portion of the keel so that the distal 2 cm (keel tip) was freely extended and parallel to the floor. Keels could be oriented for loading on the external (ventral) or internal (dorsal) surface to simulate fractures occurring from external trauma or from repeated pressures of laying eggs. A texture analyzer was used to fracture the keel tip and measure fracture force and bone stiffness. Cleaned, undamaged keels from caged hens (n = 17) at pre-peak, peak, or post-peak production were fractured via external loading until fracture occurred. Fracture force ($P = 0.82$) and bone stiffness ($P = 0.18$) were not different between production phases (ANOVA). While we expected keel strength to decrease with age, the hens' humerus fracture force did not differ among production phases, corresponding to our keel findings. Keel strength parameters were moderately correlated with humerus strength parameters ($r = 0.50$-$0.68$; $P < 0.05$). Additionally, fracture via internal loading was conducted on six undamaged keels from cage-free hens in peak production. Half the keels were loaded on the internal surface until fracture occurred, while the other half underwent cyclic loading to 14.71 N for 100 cycles to simulate a repeated, lower internal pressure (such as egg laying). The cyclically-loaded (CL) keels were then internally loaded until fracture to determine if cyclic loading weakened the keel. Fracture force and bone stiffness between CL and non-CL keels were analyzed with a t-test, but no differences were observed ($P = 0.34$ and $P = 0.47$). However, two of the CL keels were observed to fracture at locations proximal to where the tip was supported, which could have resulted in a higher-than-expected fracture force. In future tests, keels loaded cyclically on the internal surface should then be loaded to fracture on the external surface to evaluate the theory that repeated egg-laying may weaken the keel and increase risk of damage from external trauma. This methodology was able to simulate multiple types of keel tip fractures and was correlated with humerus strength parameters, exhibiting promise as a new tool for advancing keel damage research.

**Keywords:** keel bone; breaking strength; keel fracture; laying hen ; biomechanics

## Student Competition: Processing and Products

**203 (B-D) (MTCC)**

### 116 Analyzing Egg Yolk Color Scores: Statistical and Colorimetric Evidence from the DSM YolkFan™

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Visual assessment of egg yolk color in poultry research commonly relies on the DSM YolkFan™, a 16–level scale in which darker yolks receive higher scores. In practice, these scores are frequently analyzed as numeric values, implicitly assuming equal spacing between adjacent fan levels. Whether this assumption is justified depends on the statistical methods applied and their underlying assumptions. Two previously collected experiments were analyzed: Study 1 (3 dietary treatments; n = 3,653 eggs) and Study 2 (2 dietary treatments; n = 628 eggs). In both studies, yolk color differences resulted from corn genetics, which determine the profile and concentration of xanthophyll carotenoids deposited into the yolk. Yellow corn (YC) non-selected for carotenoid genetics typically produces paler yolks, whereas orange corn (OC) diets contain higher carotenoid concentrations and produces darker yolks. In Study 1, a pigment blend was added to the YC diet and one OC treatment, whereas Study 2 diets contained no added pigments. To characterize the spacing of measurement scale underlying yolk scores, individual YolkFan™ levels were measured using a Nix colorimeter (D65 illuminant, CIE Lab*), and visual perceptual differences between adjacent levels were quantified using the CIEDE2000 color difference metric (ΔE00). Adjacent ΔE00 values ranged from 1.10 to 5.32 (median 2.51), indicating uneven perceptual spacing across the 16–level scale. Historical reflectance records for earlier yolk color fans show similar non-uniform changes in chromaticity and luminance. In parallel, statistical approaches treating the yolk color variable as ordinal, categorical, and continuous were compared. Study was included as a blocking factor so that treatment effects were assessed within each experiment. Bootstrap, permutation, and simulation procedures were used to evaluate null behavior, Type I error, and statistical power at n = 10, 30, and 60 eggs per group, with and without collapsing the 16–level scale. As predicted from the diets, treatment effects were strong in both experiments (Study 1 LR $\chi^2 = 515.09$, df = 2; Study 2 LR $\chi^2 = 304.46$, df = 1; both $P < 2.2 \times 10^{-16}$). YC eggs had substantially lower odds of higher YolkFan™ scores than OC eggs (OR ≈ 0.185 and 0.055). ANOVA produced similar directional

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conclusions but violated assumptions, including non-normal residuals and heteroscedasticity. Taken together, colorimetric evaluation of the scale and statistical outputs indicate that DSM YolkFan™ scores are best treated as ordinal data. Ordinal regression provides more appropriate and stable inference than interval-based methods, and modest category aggregation improves performance when extreme scores are sparse.

**Keywords:** Egg yolk color; Egg quality; Colorimetry; Ordinal Regression; DSM YolkFan™

### 117 Exterior Eggshell Characteristics: Rhode Island Red and Black Australorp vs. Single Comb White Leghorns

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Increased demand exists among backyard and small flock owners for reliable data to guide breed selection. To address this gap, we assessed exterior egg traits from two heritage breeds—Rhode Island Red (RIR) and Black Australorp (BA)—and compared them with eggs of the commercial Single Comb White Leghorn (SCWL). The heritage breeds, obtained from the same farm, were 30 weeks old, whereas SCWL eggs were obtained from a local store and flock age was unknown. One of the first things small flock owners consider when selecting a breed is eggshell color. A colorimeter was used to measure shell color uniformity for 20 eggs/breed, with 3 replications (reps). The shell color measures of the blunt, middle, and pointed were made, and L*a*b color scores were used to calculate the shell color index (SCI). Egg shape can influence grading and resistance to cracking, so length and width measures were used to calculate the egg shape index (ESI; 20 eggs/breed, with 3 reps). Physical shell traits were measured on 10 eggs/breed with 3 reps, and included whole egg weight, shell strength, shell weight, and shell thickness. General Linear Models procedure was used to analyze the L*a*b and SCI; length, width, and ESI; whole egg and shell weight; shell strength and thickness. Means were separated using Tukey's HSD with differences considered significant at P≤0.05. For shell color in all 3 regions, both heritage breeds grouped themselves as distinct from SCWL (P≤0.001), because the breeds lay brown eggs and white eggs, respectively. The SCI differed the most for the 3 breeds at the pointed end of the egg (P≤0.001). The egg width, ESI, whole egg weight, and shell weight for RIR and BA were not significantly different between the two heritage breeds, but the heritage breeds did differ (P≤0.001) from the SCWL. Interestingly, both the RIR and SCWL were statistically different from BA in egg length and shell thickness (P≤0.001). There was no significant difference between breeds for shell strength. Overall, for the majority of the measured traits, heritage breeds were similar to one another and very much unlike the commercial SCWL strain. This is likely due to the level of selection that has been made to the SCWL over the decades, whereas far less genetic selection has taken place with the RIR and BA breeds. However, further research may indicate that not all heritage breed eggs, from all hatcheries and breeders, behave in a distinctly different manner from that of the SCWL. By clarifying how heritage and commercial breeds differ in key exterior egg traits, this work offers small flock owners practical insight into breed performance and the suitability of eggs that they may wish to use at home or sell to customers.

**Keywords:** Heritage breed; Shell color; Egg shape index; Shell strength; Egg weight

### 118 Interior Egg Quality of Rhode Island Red and Black Australorp compared to Commercial Single Comb White Leghorn

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Due to a lack of data for heritage chicken breeds, this research was conducted to aid small flock owners in making decisions on which breed to select for eggs. Although heritage breeds are often broadly categorized as "noncommercial," our goal was to determine which interior characteristics from today's heritage breeds are the same as commercial grocery store eggs. Eggs from Rhode Island Red (RIR) and Black Australorp (BA) chickens were compared to the Single Comb White Leghorns (SCWL). The heritage breeds, obtained from the same farm, were 30 weeks old, whereas SCWL eggs were obtained from a local store and flock age was unknown. Ten eggs from each breed were tested in triplicate for all interior parameters. The Egg Tester Ultimate™ was used to find egg weight, Roche color, and Haugh Unit. A texture analyzer was used to find the vitelline membrane strength. Also, the yolk and albumen pH; yolk centering score; yolk index; and the individual weights of the shell, albumen, and yolk were collected and analyzed to provide foundational information on egg characteristics for these breeds. The General Linear Models procedure was used to analyze the pH levels of yolk and albumen; yolk centering score; Haugh unit; yolk index and Y: A ratio; weight of shell, albumen, and yolk per egg; vitelline membrane strength for the breeds. Means were separated using Tukey's HSD and differences were considered significant at P≤0.05. There was no difference in yolk pH; however, there was a significant difference between all 3 breeds for the albumen pH (P≤0.001) with RIR being the highest at 9.26. There was a difference (P≤0.001) in yolk height as RIR and SCWL differed from BA which was lowest at 15.34mm. Yolk width measures found that RIR and BA differed from SCWL (P≤0.001), which was the widest at 39.61mm. There was no significant difference for Haugh Unit, yolk center score, or Y: A ratio. The SCWL yolks had the darkest Roche color of 6.56, whereas BA and RIR were 5.96 and 5.26, respectively (P=0.007). SCWL had the highest vitelline membrane strength at 136.55g and RIR had the weakest at 76.64g; BA was in between at 82.36g (P≤0.001). Shell, yolk, and albumen weights most often grouped the SCWL as different from the heritage breeds with P-values of ≤0.001, ≤0.001, and 0.038. respectively, which stems from a larger whole egg weight. In conclusion, it was found that the RIR, BA, and SCWL did indeed differ from each other and the eggs from heritage breeds did not always group together. Further research on Roche color, yolk/shell/albumen weights would be interesting if breeds of the same age were raised together on the same diet to see how the breed may differ in how they allocate resources in forming eggs over time.

**Keywords:** Interior egg quality; Haugh unit; pH; Vitelline membrane; Roche color score

### 119 Functionality of Rhode Island Red and Black Australorp Eggs Compared to Single Comb White Leghorn Eggs

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The aim of this study was to provide backyard flock owners with information on which heritage chicken breeds to select based on the functionality of the egg. There is little information about how well heritage breed eggs whip, foam, and hold structure. Providing this information helps the average home cook, or flock owner, understand how eggs from different breeds might perform in everyday handling, cooking and baking. Eggs from Black Australorps **(BA)** and Rhode Island Reds **(RIR)** heritage breed chickens were compared to commercial Single Comb White Leghorns **(SCWL)**. The heritage breeds, obtained from the same farm, were 30 weeks old, whereas SCWL eggs were obtained from a local store and flock age was unknown. Yolk Foaming Ability **(YFA)**, Yolk Foam Stability **(YFS)**, Albumen Whipping Height **(AWH)**, and Albumen Foam Stability **(AFS)** were examined. Thick Albumen Stress **(TKAS)**, Thick Albumen Viscosity **(TKAV)**, Thin Albumen Stress **(TNAS)**, and Thin Albumen Viscosity **(TNAV)** were examined using rheometry. All eggs were stored at 4 C and allowed to come to room temperature prior to testing. YFA and YFS was performed with a 10,000 r/min homogenizer, used 6 eggs/breed, and had 6 replicates/breed. AWH used a single albumen separated from the yolk in a mixer, whipped on speed 10 for 150 seconds and foam height was

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measured at 3 locations. AWH had 10 replicates/breed. AFS used 130 ml of albumen per test and had 6 replications/breed. TKAS, TKAV, TNAS, and TNAV used eggs that had a pH of between 9.13, 9.22, and 9.26 for SCWL, BA, and RIR, respectively. These tests used 5 eggs/breed with 2 samples of thick albumen and 2 samples of thin albumen per egg. The General Linear Models procedure was used to analyze the YFA, YFS, AWH, AFS, TKAS, TKAV, TNAS, and TNAV; means were separated using Tukey's HSD and differences were considered significant at P≤0.05. There was no significant difference between the breeds for the YFA, YFS, AWH, TKAS, TKAV, TNAS, and TNAV. There was a significant difference in the AFS, or foam drainage, with a P≤0.001. The drainage from RIR (1.90 ± 1.12%) was significantly greater than both the SCWL (0.11 ± 0.10%) and the BA (0.15 ± 0.07%). Our research elucidated that there were no other differences in the functionality of the eggs from the different breeds, so it is suggested that the AFS research be repeated to determine what additional factors related to the RIR breed may be responsible for these differences. Continued research into heritage breed eggs may yield information on what other breeds produce eggs behave similarly to the RIR since baked goods from these breeds may have a less uniform structure or coarser crumb.

**Keywords:** Eggs; Heritage breeds; Albumen foam stability; Yolk foam; Rheometry

### 120 Angel Food Cake, Custard, and Mayonnaise Performance with Rhode Island Red and Black Australorp Eggs vs. Single Comb White Leghorn Eggs

Alyssa C. Rios*[1UG], Elizabeth F. Knight[1], Brigid McCrea[1] [1]*Alabama Cooperative Extension System, Alabama 4-H, Auburn, Alabama, United States*

Backyard flock owners often wonder which chicken breed will yield eggs that best meet their baking and cooking needs. To address this, we evaluated functional property differences among two heritage breeds, the Black Australorp (BA) and Rhode Island Red (RIR), and compared with the commercial Single Comb White Leghorn (SCWL). The heritage breeds, obtained from the same farm, were 30 weeks old, whereas SCWL eggs were obtained from a local store and flock age was unknown. Egg foaming and volume retention were assessed by measuring angel food cakes (n=9/breed) using the rapeseed displacement method. Custard hardness (n=18/breed) and mayonnaise firmness (n=3/breed) were evaluated with a texture analyzer. Custard weep (n=9/breed) was quantified using filter paper, mayonnaise color (L*a*b) with a colorimeter (n=18/breed), and custard stability by recording height at the edge and center (n=9/breed). The General Linear Models procedure analyzed cake rise; custard texture, height, and weep; and mayonnaise color and texture, with means separated using Tukey's HSD at P≤0.05. There were no significant differences between breeds for custard texture and weep; angel food cake volume; and mayonnaise firmness. There were differences with mayonnaise color for L*, a*, and b* with all 3 measures yielding a P-value of ≤0.001. RIR had the lightest yolks and differed from both BA and SCWL. The a* and b* values showed that all 3 breeds were distinct from each other with the SCWL having both the greenest and yellowest mayonnaise. There was no difference between the height of custards when measured at the edge (P=0.068), but there might to be a trend toward a difference which further research may reveal. There was a difference for the middle custard height (P=0.016). The SCWL had the lowest height and RIR had the highest with BA in between. This indicates that the eggs from the heritage breeds may lend themselves to a taller, more stable custard that is less likely to slump in the middle. It is interesting that the mayonnaise color differed so much for b* for the heritage breeds since they were at the same farm and had the same diet since diet plays a role in xanthophyll deposition in the yolk. It would be interesting to see what other heritage breeds vary in their mayonnaise L*a*b scores if fed the same diet. Overall, this work helps bridge the gap between breed information and kitchen performance, giving consumers clearer expectations about how different eggs behave in common recipes.

**Keywords:** Heritage breed; Eggs; Angel Food Cake; Custard; Mayonnaise

### 121 Untargeted Metabolomics to Understand the Impact of Controlled Atmosphere Stunning and Electrical Stunning on Metabolite Profiles of Early Postmortem Breast Fillet

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Pre-slaughter stunning influences postmortem muscle metabolism and may affect broiler breast meat quality and processing consistency. Although electrical stunning (ES) and controlled atmosphere stunning (CAS) are commonly used in commercial poultry production, the comparative evidence regarding their biochemical impacts during the early postmortem period remains limited. In our study, we utilized an untargeted metabolomics approach to identify metabolite profile alterations in broiler breast muscle following CAS and ES under commercial processing conditions. Early postmortem breast fillet samples from Ross 708 broilers stunned using either CAS or ES were analyzed using ultra-performance liquid chromatography mass spectrometry (UPLC-MS). Breast fillets (n = 6 birds per treatment) were collected approximately 30 minutes postmortem, with early pH measured at 15 minutes and instrumental color (*L**, *a**, *b**) recorded before snap freezing samples for metabolomics. Meat quality was analyzed using GLM in SAS 9.4, with stunning method as the main effect, Tukey's HSD for mean separation, and P < 0.05 for significance. CAS fillets exhibited a significantly lower muscle pH compared with ES fillets (P < 0.001), while instrumental color traits were non-significant. Metabolite normalization, statistical analysis, and pathway enrichment were conducted using MetaboAnalyst 6.0. We identified 511 known metabolites, of which metabolomic profiling indicated based on the t-test that there were five metabolites differing significantly between CAS and ES at P < 0.05 (uncorrected): methylglyoxal, gluconic acid, (R)-malate, aspartyl-leucine, and 1D-myo-inositol-3-phosphate. All significant identified metabolites were downregulated in CAS breast fillets compared with ES. Pathway analysis revealed significant effects on pyruvate metabolism and the pentose phosphate pathway, with additional changes in the TCA cycle, amino acid metabolism, and inositol phosphate signaling. Lower carbonyl stress metabolites and pentose phosphate intermediates in CAS fillets indicate reduced metabolic and oxidative stress, while TCA cycle and proteolysis-related differences suggest altered postmortem energy use. These findings show that stunning methods affect early postmortem metabolism, with CAS inducing fewer stress- and energy-related metabolic changes than ES. The untargeted findings are preliminary given the lack of false discovery rate correction, absence of targeted metabolite validation, and potential confounding by sex and body weight. These findings provide valuable guidance for optimizing stunning methods in commercial broiler production to enhance meat quality and consumer acceptability.

**Keywords:** stunning; metabolomics; animal welfare; meat quality

### 122 Effects of Endpoint Cooking Temperature on Broiler Breast Fillet Shear Energy and Yields

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USDA guidelines recommend poultry products be cooked to a minimum internal temperature of 73.9°C for food safety. However, most products exceed this temperature to ensure product safety. At higher temperatures,

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product quality may start to decrease due to further protein degradation and water evaporation. Product yield is important within industry, and excessive cooking can lead to decreased yield. As excessive heat causes proteins to denature, they contract to form a denser network of fibers and lead to a tougher product. Chicken breast fillets (n=16 per treatment over 4 replications) were cooked on wire racks in aluminum lined and covered pans in a convention oven at 177°C to an internal temperature of 73.9°C, 76.6°C, 79.4°C, 82.2°C, or 85.0°C. Internal fillet temperature was continuously monitored during cooking using wireless temperature probes. Fillets were weighed, cooked, and allowed to cool to room temperature for post-cook weight. Cook yields were then determined. Breast fillets were stored overnight at 4°C and sheared using a texture analyzer (Model TA-XT2Plus, Texture Technologies, Scarsdale, NY) and the Meullenet-Owens Razor Shear method measuring total energy (MORSE). Data was analyzed using ANOVA with rep, treatment, and rep x treatment as model effects (JMP, version 18.2.0). Means separated using Tukey's HSD test at P< 0.05. A multivariate pairwise correlation was used to determine relationships between endpoint temperature, energy, and yields. Cook yields decreased (P<0.05) with increasing endpoint temperatures (*r*=-0.74, P<0.0001). Fillets cooked to an internal temperature of 85°C and 82.2°C were lower yielding than all other temperatures (70% < 74-77%, P<0.05). Fillets cooked to 79.4°C were lower yielding than those cooked to 73.9°C (74% < 77%, P<0.05*)* with 76.6°C as an intermediate (76%). Fillets cooked to 85°C had higher MORSE, suggesting higher toughness, than those cooked to 73.9°C (182.2 N.mm >151.9 N.mm, P<0.05), and all other temperature treatments were intermediaries (165.6 to 154.4 N.mm, P>0.05). Cook yield and MORSE were negatively correlated (*r=-0.33*, P<0.01*)*, endpoint temperature and MORSE were positively correlated (*r*=0.34, P<0.01). The results of this study suggest that the greatest impact on yield was the highest cooking endpoint temperatures of 82.2°C and above whereas shear was mostly affected by the highest endpoint temperature of 85°C. Based on this study, it is recommended to use consistent endpoint temperatures, but target endpoint temperatures in the range of 73.9°C to 79.4°C to minimize changes in both yield and shear energy.

**Keywords:** MORS energy; Yield; Tenderness; Breast Fillets; Cooking Temperatures

### 123 Evaluation of the Prevalence of Wing Damage of Broiler Carcasses in a Commercial Processing Plant

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Wing damage during poultry processing is an ongoing product quality and animal welfare concern. The aim of this study was to evaluate the prevalence and types of wing damage across four processing stages - live haul, pre-scald, picking, and post-pick on a line with electrical stunning (ES) and a line with controlled atmosphere stunning (CAS). The ES birds were transported and tipped from modular coops with side doors and the CAS birds were transported and stunned in a tray-type system. The study was conducted using three groups of ES birds, and three groups of CAS birds (n= 2,450 ES and 4,032 CAS). Wing damage was recorded at each stage and classified by type (dislocated, broken, bruised, torn, and cut), then dislocated and broken wings were evaluated further by presence of protruding bones. SAS OnDemand for Academics was used to conduct Chi-square tests and Fisher's Exact Tests to compare the total wing damage prevalence as well as protruding bone occurrence by stunning method at each processing stage. PROC CATMOD and Cochran-Mantel-Haenszel tests were used to assess damage type distributions. When assessing total wing damage between ES and CAS, wing damage rates increased progressively through the stages, with ES birds having similar damage at live haul (0.90% vs 0.57%), picking (2.12% vs 1.54%), and post-pick (3.15% vs 2.53%). Only pre-scald was statistically significant (p=0.0179) with 1.63% for ES and 0.97% for CAS. When evaluating damage type distribution by stunning method and stage, both stunning method (p<0.0001) and processing stage (p=0.0007) significantly impacted damage type. Of all damaged wings, ES had more broken wings at 28.0% compared to CAS at 10.9%. CAS had more dislocations at 62.2% compared to ES at 48.2%. In dislocated wings, there was a significant difference at pre-scald (Fisher's p=0.0123), with ES having 18.75% of wings with protruding bones versus CAS at 0%. For broken wings, ES had significantly higher rates of protruding bone at picking (p=0.0036; 80.0% ES vs. 0% CAS) and post-pick (p=0.0038; 78.3% ES vs 25.0% CAS). Electrical stunning was associated with more severe defects, regardless of similar overall damage rates. These data suggest that the stunning method can impact the severity of wing defects in commercial processing.

**Keywords:** wing damage; broiler processing; carcass quality; stunning; dislocation

### 124 Wing Abduction Angle and Woody Breast Development in Broilers Fed Diets Differing in Nutrient Density and Probiotic Supplementation

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Woody breast myopathy is a meat quality concern in modern broiler production and is associated with rapid growth. Dietary nutrient density and probiotic supplementation may influence muscle development and metabolic stress, affecting the incidence and severity of woody breast. This study was conducted to assess woody breast development in broilers that were fed differing diet densities and probiotics. A total of 1,280 male Cobb 500 birds were assigned to dietary treatments (16 repliction pens/treatment; n=20 birds/pen/treatment) in a 2x2 factorial design with nutrient densities high (H) or low (L) and treatment control (C) or probiotic (P). Dietary treatments were corn and soybean meal-based diets. High density diets had 100 kcal/kg more than low density diets. The L diet contained 90% and H 108% of recommended amino acid (AA) levels resulting in an 18% greater AA in H throughout. Probiotic diets used "Argis" (Kent Nutrition), a *bacillus*-based probiotic. Five focal birds were subsampled per pen for body weight (BW, g), breast measurements (BM, cm), and wing abduction angles (WA, °) at D14, D42, and D56. BM were measured using a fabric tape-measure on the cranial region across the breast (wing to wing). WA were taken by gently raising wings until resistance, a photo was taken, and angles were measured using a digital protractor. WB was assessed at D42 and D56 after birds were processed. Data were analyzed as a linear effects model using density and probiotic treatment as fixed effects. Pearson correlations were run for BM, BW, and WA against WB scores at processing. There was no effect of diet on BW on D14 or D56 (P>0.05), but H tended to have higher BW at D46 than L (3604 vs 3470g, P=0.08). Breast measures were greater for H than L at D14 (11.2 vs 10.9cm, P=0.004), D42 (24.7 vs 24.2cm, P=0.02*)*, but not at D56 (27.2 vs 27.1, P=0.89). There was no effect of diet on WA on D14 (P>0.05), on D42 H had wider WA angles than L (83.9 vs 74.2°), and on

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D56 LC and HP had higher WA angles (101.5 and 99.9°) than LP and HC (88.3 and 87.7°). WB scores at processing were not correlated (P>0.05) with BW (*r*=0.06), BM (*r*=0.15) or WA (*r*=-0.08) from D14. WB was weakly correlated with BW (*r*=0.30) and BM (*r*=0.25) and strongly correlated with WA (*r*=0.63) on D42 (P<0.001). For D56, WB was moderately correlated with BW (*r*=0.41,P=0.02) and BM (*r*=0.4, P=0.05) and was strongly correlated with WA (*r*=0.62, P<0.001). Nutrient density influenced breast size and wing abduction angle but had limited effects on final body weight. Probiotics had minimal impact on woody breast development. Wing abduction angle at later ages was strongly associated with woody breast severity, suggesting it may serve as a useful indicator of woody breast.

**Keywords:** woody breast; wing abductions; broiler; breast measurments; nutrient density

### 125 Improving Functionality of Woody Breast-Affected Broiler Meat with Proteolytic Enzyme Marination: Implications for Yield

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Woody breast (WB), white striping (WS), and spaghetti meat (SM) are growth-related myopathies associated with rapid breast muscle development that reduce meat quality and economic value in commercial broiler production. The objective of this study was to evaluate the impact of papain marination on functional and textural properties of WB-affected broiler meat. Broiler breast samples (n = 12) representing WB scores 0–3 were subdivided into standardized nuggets (~15 g), yielding 48 samples assigned across four marination treatments (control, low, medium, high). The experiment was conducted across three independent runs. Samples were vacuum tumbled (−0.85 bar, 25 min, 4 °C) using marinades containing papain at increasing inclusion levels (0, 0.05, 0.11, and 0.22%) in a base solution of water, salt (1.63%), and sodium tripolyphosphate (0.33%). Marinade uptake, cook loss, compression force, and shear force were evaluated. Data were analyzed using PROC GLIMMIX (SAS v9.4) with WB score and treatment as fixed effects. Marinade uptake was not affected by enzyme inclusion (P > 0.05), but decreased significantly with increasing WB severity, declining from 8.01% in WB0 to 4.30% in WB3 (P = 0.01). Papain inclusion significantly increased cook loss, with control samples (5.32%) exhibiting lower losses compared to treated samples (7.08–7.22%) (P < 0.001). Compression force was significantly reduced by papain treatment (P < 0.001), indicating a softening effect, as control samples (37.98 N) exhibited higher values than low (28.76 N), medium (24.73 N), and high (27.20 N) treatments. Shear force was primarily influenced by WB severity (P < 0.05), while enzyme inclusion showed only a tendency to reduce shear energy (51.57 to 39.02 N*mm; P = 0.084). Overall, papain improved tenderness of WB-affected meat by up to ~30%, but this effect was accompanied by increased cook loss (~2 percentage points), indicating reduced water-holding capacity. WB severity contributed to variability in uptake and texture, but no significant treatment × WB interactions were observed, suggesting that enzymatic effects were consistent across severity levels. These results demonstrate that enzymatic tenderization is a promising strategy to improve the functionality and value of myopathy-affected broiler meat for further-processed applications, although optimization is required to balance tenderness and yield.

**Keywords:** Poultry processing; Compression Force ; Shear Force; Proteolytic enzymes ; • Water-holding capacity

### 126 Effect of poultry DAF skimmings' composition on hydrothermal carbonization yield and resource recovery

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Dissolved Air Flotation (DAF) is widely used in the poultry industry to separate waste materials from processing wastewater during pretreatment. However, the resulting DAF skimmings are difficult to dispose of in an environmentally conscious manner due to their high moisture content (~75%) and large volume. The dry component of these skimmings typically consists of 40–70% fats, oils, and grease (FOG), 15–40% protein, and 3–7% salts. Hydrothermal carbonization (HTC) provides a promising route for volume reduction and resource recovery of this waste. This study aimed to discover the effect of different amounts of protein, fat, and salts in the skimmings on the product yield and nutrient recovery. 60-minute HTC experiments were conducted at temperatures ranging from 180 to 250 °C, with feed compositions varying from 0–100% protein and fat, and with the addition of 5% FeCl3 or AlCl3. Products were assessed in terms of hydrochar production, process water composition, and volume reduction. Results show that HTC achieved substantial volume reduction, commonly more than 85% at higher temperatures, while producing carbon-enriched hydrochar, a coal-like material that can be used for energy. As temperatures increased, hydrochar yield decreased while carbon content rose. Analysis of the processing liquid was done using Hach water testing kits, including COD (chemical oxygen demand), TOC (total organic carbon), TKN (total kjeldahl nitrogen), and total ammonia tests. These revealed significant transfer of organic carbon and nitrogen into the liquid phase, with COD reaching $10^5$–$10^6$ ppm and nitrogen reaching $10^3$–$10^4$ ppm at higher temperatures. Feed composition strongly influenced nutrient recovery. Phosphorus concentrations in the filtrate increased from ~200 ppm in 100% fat skimmings to ~2100 ppm in 100% protein skimmings (a ~950% increase). Similarly, ammonium concentrations rose from ~0 ppm in fat-rich samples to ~6000 ppm in protein-rich samples. When metal chlorides (AlCl3 and FeCl3) were added, they increased volume reduction (~77% and ~20%, respectively) while decreasing hydrochar yield (~88% and ~34%, respectively), likely due to the catalyst promoting deamination and decarboxylation reactions that convert protein to gaseous products. Both additives also reduced nutrient recovery in the aqueous phase, with phosphorus decreasing by ~44% (AlCl3) and ~89% (FeCl3), and ammonium decreasing by ~19% and ~32%, respectively. Overall, HTC provides a tunable approach for reducing the volume of DAF skimmings while producing energy rich hydrochar and nutrient rich process water, though trade-offs exist between volume reduction and nutrient recovery depending on operating conditions and composition.

**Keywords:** Hydrothermal Carbonization; DAF skimmings; hydrochar; nutrient recovery; phosphorus

### 127 Effects of Sex, Defeathering Intensity, and Peracetic Acid Exposure on Broiler Meat Quality

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Aggressive defeathering and the use of antimicrobials, such as peracetic acid (PAA), in chiller water are common poultry processing practices that may adversely affect broiler meat quality; however, their impacts remain insufficiently characterized. This study evaluated the effects of sex (male, female), defeathering intensity (normal, aggressive: applied twice), and PAA exposure (no PAA, 200 ppm PAA) on the quality attributes of breast fillets with a 2 × 2 × 2 factorial design resulting in eight treatment combinations: T1 (male, normal, no PAA), T2 (male, normal,

200 ppm PAA), T3 (male, aggressive, no PAA), T4 (male, aggressive, 200 ppm PAA), T5 (female, normal, no PAA), T6 (female, normal, 200 ppm PAA), T7 (female, aggressive, no PAA), and T8 (female, aggressive, 200 ppm PAA). Three hundred birds (Ross 708) were raised to a target weight of 2.7 kg, and among them, 120 birds (60 males, 60 females) were body weight matched and processed. The processing line operated at 30 birds/min, with scalding at 128°F (53.3°C) for 2 min, followed by mechanical defeathering for 30 or 60 s. After 3 h of immersion chilling at 4 °C with or without PAA, carcasses were deboned. Breast fillet color and pH were measured, after which fillets were individually weighed, packaged in zip-top bags, and stored under refrigeration. After 24 h, breast fillets were reweighed to determine drip loss, and analyses of color, pH, shear force, lipid oxidation, and proximate composition (protein, moisture, fat, salt, and total collagen) were conducted. Data were analyzed using a General Linear Model (GLM) procedure in SAS On Demand for Academics. When significant effects were observed, means were separated using Tukey's HSD test, with significance declared at $P<0.05$. Aggressive defeathering (twice picking) and PAA exposure significantly reduced pH (5.70 and 5.70, respectively) compared to normal picking and no PAA (5.75; $P=0.0007$ and $P=0.0022$, respectively). Aggressive picking significantly increased drip loss (2.90% vs. 1.54%; $P<0.0001$) while reducing lipid oxidation (0.0857 vs. 0.4228; $P=0.0344$) compared to normal picking. Males exhibited higher lightness (L*=54.32 vs. 52.54; $P=0.0033$) and redness (a*=3.04 vs. 2.55; $P=0.0226$) than females. In contrast, females showed lower shear force values (6.30 vs. 7.08; $P=0.0019$), indicating softer meat. PAA treatment resulted in higher yellowness (b*=10.05 vs. 9.37; $P=0.0422$) and slightly lower protein content (22.59% vs. 22.90%; $P=0.0208$). These results indicate that aggressive defeathering significantly affects meat quality, specifically by compromising texture.

**Keywords:** processing ; peracetic acid; defeathering; meat quality; texture

### 128 Shelf-life dynamics of poultry parts following PAA and eBeam treatments during refrigerated storage

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Antimicrobials are widely used during poultry processing to reduce microbial load and extend shelf-life. However, their effectiveness is often evaluated based on immediate reductions rather than impact on microbial ecology during storage. While peracetic acid (PAA) is a common strategy, electron beam (eBeam) irradiation is an FDA-approved novel alternative with potential for extended food preservation. This study compared changes in microbial ecology of poultry parts treated with PAA and eBeam over 21-days refrigerated storage. Skin-on (wings) and skin-off (tenders) chicken parts were packed individually. Triplicate samples were exposed independently to five antimicrobial levels, sterile water (control), PAA at 250 and 500ppm for 10s, and eBeam at 2 and 3kGy. Samples were evaluated at 1, 3, 7, 14, and 21 days of refrigeration for microbial populations, including aerobic count (AC), Enterobacteriaceae (EB), *E. coli* (EC), coliforms (CC), and lactic acid bacteria (LAB) using Petrifilm™, while *Pseudomonas* was quantified with a selective agar. Common pathogens like *Salmonella*, *Campylobacter*, and *Enterococcus* were also monitored using standard culture methods. Log transformed microbial counts were analyzed using two-way ANOVA in R-Studio at $p<0.05$. Microbial ecology differed by treatment and storage time for both product types. On day 1, all treatments differed for all microbial populations, with eBeam having the best reduction. Precisely, 2 and 3kGy resulted in respective 5.3 and 6.0. log10 CFU/ml AC reduction, while PAA treatments had lower dose-dependent reductions of 1.2 and 2.6 log CFU/ml at 250 and 500ppm, respectively. Although microbial counts increased over storage, eBeam-treated samples steadily maintained lower counts than PAA-treated and control samples. Similar trends were observed for EB, EC, and CC, where eBeam resulted in near-complete reductions at early storage and remained low, compared to less effective reductions in PAA treatments. Overall, spoilage bacteria (LAB) increased gradually to 3.78 log CFU/mL over 21-days storage, but *Pseudomonas* exhibited a rapid growth reaching 6.13 log CFU/mL. For pathogens, *Enterococcus* was high (control = 100% wings, 93% tenders) and predominantly reduced at 3kGy to 0% wings and 7% tenders. *Salmonella* (33% wings, 60% tenders) was eliminated at 500ppm and both eBeam levels, while *Campylobacter* (7% wings, 20% tenders) reduced to zero across all treatments. Overall, product type influenced microbial dynamics, with wings supporting higher microbial persistence. Antimicrobial efficacy and shelf-life showed successive order of eBeam > PAA with the potential of low dose eBeam serving as a promising intervention to enhance the storage stability of chicken meat.

**Keywords:** poultry; shelf-life; refrigerated storage; eBeam irradiation; peracetic acid

## Student Competition: Metabolism and Nutrition - General Nutrition II

**201 (A-F) (MTCC)**

### 497 Influence of particle size, macromineral composition and physical properties of limestone on its solubility and acid-binding capacity

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The aim of this study was to evaluate the influence of the mean particle size (PS), macromineral composition, calcite content and porosity on acid-binding capacity (ABC) and limestone solubility. In total, 23 samples of commercial limestone were randomly collected from diverse geographical areas worldwide and analyzed. The PS distribution of the samples showed a geometric mean diameter (GMD) of 727 ± 568 µm, the mineral composition was of 38.5 ± 0.92% for Ca and 0.37 ± 0.31% for Mg, and the calcite content was of 95.7 ± 4.69%. The samples were sieved and separated into two distinct PS fraction subsamples: coarse fraction (CoF; GMD 1,000–1,400 µm) and fine fraction (FiF; GMD < 75 µm). However, 6 of them could not be analyzed for the CoF due to insufficient coarse material. The CoF and FiF subsamples were evaluated for ABC using HCl titration end-points at pH 4 and pH 3, and for limestone solubility after 5, 10, 15, and 30 min of incubation using two methodologies: 0.2 *N* HCl and a buffered solution at pH 3. In addition, porosity was measured by mercury intrusion porosimetry only in the CoF subsamples. Data were analyzed using ANOVA, with limestone PS fraction as main effect, and the relationships among the variables were studied by Pearson correlation analysis. ABC-4 (9,506 vs. 16,556 meq/kg) and ABC-3 (10,913 vs. 18,162 meq/kg) values were lower for the CoF than for the FiF ($P < 0.05$). Furthermore, limestone solubility was lower for the CoF than for the FiF across all incubation times, both with 0.2 *N* HCl (72, 81, 88, and 92% vs. 90, 92, 94, and 98%; $P < 0.05$) and at pH 3 (41, 54, 67, and 80% vs. 77, 81, 85, and 88%; $P < 0.05$). Independently of the PS, limestone solubility had a higher CV at 5, 10, 15 and 30 min of incubation for the pH3 method (35, 27, 19, and 11%, respectively) than for the 0.2 *N* HCl method (13, 8, 5, and 3%, respectively). Regarding relationships among variables, ABC-3 and ABC-4 were positively correlated with limestone solubility across both methodologies and all incubation times ($P < 0.05$), except for the 0.2 *N*

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HCl method at 30 min of incubation, where no significant correlation was detected. In addition, porosity was positively associated with limestone solubility for the pH 3 methodology after 5 min of incubation ($r$ = 0.707; P < 0.05). In conclusion, limestone sources showed marked differences in functional behaviour in spite of similar Ca content. Compared with the 0.2 *N* HCl method, the pH 3 method allowed better discrimination among samples due to its greater variability and lower solubility values. Overall, PS was identified as the primary determinant of limestone solubility and ABC values, with porosity also associated with variations in solubility.

**Keywords:** acid-binding capacity; limestone; particle size; porosity; solubility

### 129 Validation of a novel methodology to measure the internal particle size of pelleted feed and the degree of grinding during pelleting

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Accurate assessment of the particle size (PS) within pellet microstructure remains challenging due to particle agglomeration. This study aimed to validate a novel methodology to estimate internal pellet PS by disaggregating pellets and recovering particle structure, and to quantify PS reduction during pelleting across ingredient profiles. A 2 x 2 factorial arrangement was used with two ingredients (corn and soybean meal (SBM)) and two grinding levels (coarse and fine), resulting in four treatments: **T1** (coarse corn and coarse SBM), **T2** (coarse corn and fine SBM), **T3** (fine corn and coarse SBM), and **T4** (fine corn and fine SBM). Corn and SBM were ground using a two-pair roller mill to achieve PS of 1460 and 475 µm for corn and 1023 and 524 µm for SBM. Diets were mixed, conditioned at 80°C for 45 s, and pelleted through a 4.4 mm die. For validation, mash samples were analyzed either as dry mash or after hydration and dehydration. Hydration was conducted for 10 minutes using 130 g of sample and 350 g of water at 60°C, with 3 g of sodium hexametaphosphate to prevent particle re-agglomeration. Suspended fines were recovered by centrifugation, reincorporated into the sample, and dried using a TG 200 fluid bed dryer. Pellet samples were collected after cooling and processed using the same hydration-dehydration procedure prior to PS analysis using the ASABE S319.4 method. Data were analyzed as a completely randomized design using the GLIMMIX procedure in SAS, with means separated using Tukey's HSD test at P < 0.05. No differences (P > 0.05) in PS between dry mash and hydrated-dehydrated mash were observed for T2 (971 vs. 940 µm) and T4 (606 vs. 559 µm), whereas differences (P < 0.05) were detected for T1 (1294 vs. 1210 µm) and T3 (717 vs. 632 µm), indicating that methodology response depends on initial mash PS. Pelleting reduced (P < 0.05) PS in T1 (869 vs. 1210 µm), T2 (760 vs. 940 µm), and T3 (530 vs. 632 µm), but not in T4 (550 vs. 559 µm; P = 0.997), suggesting minimal additional size reduction when both ingredients were finely ground. The proposed methodology provides a practical approach to estimate internal pellet PS and quantify size reduction during pelleting driven by initial mash particle size.

**Keywords:** particle size; novel methodology; pelleting; grinding

### 130 Targeted Bacteroidales inoculation enhances early-life gut colonization in broilers

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Early-life microbial colonization plays a critical role in gut development and immune maturation in broiler chickens. Members of the order Bacteroidales, key contributors to carbohydrate metabolism, have limited diversity and abundance in intensively raised commercial chickens relative to extensively raised (outdoor) chickens, potentially limiting early-life microbial functionality. The consequence of this variability in the Bacteroidales order as well as determinants of their successful establishment in the broiler gut remain unclear. This study compared early-life colonization and competitive dynamics of Bacteroidales isolates obtained from broilers raised under extensive and intensive production systems. Day-old broiler chicks were randomly assigned to four oral gavage treatments: control (sterile PBS), intensively derived isolates (INT), extensively derived isolates (EXT), or a mixed community (INT+EXT) (n = 6). Birds were housed in microisolators, and euthanized on Day 5 post-inoculation. Cecal samples were analyzed using 16S rRNA gene amplicon sequencing to assess community composition, while Bacteroidales abundance was quantified by qPCR. Growth data were analyzed using one-way ANOVA, and microbial community differences were assessed using PERMANOVA (pairwise ADONIS). Bacteroidales successfully colonized all inoculated groups but with no detection in controls. Colonization of total Bacteroidales did not differ between treatment, however, EXT isolates were more abundant than the INT isolates when colonized in competition. All inoculated birds also showed increased body weight compared to controls (P < 0.01). These findings demonstrate that Bacteroidales can rapidly establish in the broiler gut and that colonization success is influenced by isolate origin. The competitive advantage of EXT isolates suggests that host-adapted strains are better suited for early-life establishment. Ongoing metabolomic analyses aim to further characterize the functional links between Bacteroidales colonization and host metabolic pathways. Together, these results highlight that intensively raised birds have very limited exposure to Bacteroidales and targeted inoculation strategies may enhance early colonization in the gut, contributing to better gut development and performance in broiler production.

**Keywords:** Bacteroidales; host adaptation; early-life colonization; production systems; gut microbiome

### 131 Effect of cyclic heat exposure on the expression of genes related to jejunal tight junction proteins and cecal microorganisms of broilers fed palm kernel meal

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The aim of this study was to evaluate the interaction between heat stress (HS) and the incorporation of palm kernel meal (PKM) in broiler chicken diets, as well as their effects on growth performance and metabolism. A total of 288 21-day-old male Arbor Acres chicks were used in a 21-day experiment. The birds were randomly assigned to two temperature conditions: thermoneutral (TN; 23°C) and HS (33°C for 9 hours per day). Each group received one of two diets (P0: 0% PKM; P1: 10% PKM) in a 2 × 2 factorial design, with environment (TN vs. HS) as the first factor and diet (P0 vs. P1) as the second. Each treatment included six replicates. On day 42, twelve birds from each temperature group were euthanised by bleeding. Approximately 2 cm of tissue was collected from the midpoint of each jejunum and transferred to individual tubes before being immediately flash-frozen in liquid nitrogen. RNA extraction and RT-PCR quantification were performed according to the method described in our previous work. Approximately 4 g of cecal digesta was collected from one bird per replicate after slaughter. The V3–V4 regions of the bacterial 16S rRNA gene were sequenced on the Illumina MiSeq platform by Personalbio Technology Co., Ltd. (Shanghai, China). The cecal microbiota data were analysed using QIIME2 (version 2024.5), R software (version 4.3.3) and the hilldiv package (version 1.5.3). A two-way analysis of variance was performed to analyse the relative mRNA expression of tight junction protein-related genes and bacterial species composition in

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the jejunum. Multiple comparisons among means were evaluated using the Šidák test, with statistical significance set at $p < 0.05$. All statistical analyses were carried out using GraphPad Prism software (version 8.0.2). Examination of tight junction proteins in the jejunum revealed no effects of temperature or diet ($p > 0.05$). The results showed no significant differences ($p > 0.05$) in A-diversity indices (Chao1 and Simpson) between the thermoneutral and heat stress groups. However, a significant difference ($p = 0.012$) was observed in the Shannon diversity index among these groups. PCoA analysis revealed significant differences in B-diversity ($p < 0.05$) between the TNP0, TNP1, HSP0 and HSP1 groups. Finally, analysis of the cecal microbiota revealed that temperature increased the relative abundance of several bacterial species ($p < 0.05$), whereas dietary treatment only affected the Phocaeicola species ($p < 0.05$). In summary, PKM supplementation did not mitigate the adverse effects of HS.

**Keywords:** Broiler; Cecal microorganisms; Cyclic heat exposure; Palm Kernel Meal; Tight junction proteins

### 132 Effect of apparent metabolizable energy, conditioning temperature, and retention time on crumble quality and pellet durability index of broiler diets

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Crumble quality (**CQ**) and pellet durability index (**PDI**) are key indicators of feed physical quality influencing feed intake, nutrient segregation, feed wastage, and broiler performance. These parameters are influenced by complex interactions between formulation and processing factors. However, limited research is available on the combined effects of apparent metabolizable energy (**AME**), conditioning temperature (**CT**), and retention time (**RT**). Therefore, this study assessed the effects of AME, CT, and RT on CQ and PDI of starter and grower diets using a 2 × 2 × 2 factorial design. Corn-soybean meal-based diets containing 2,850 or 2,975 kcal/kg AME were conditioned at 80 or 85°C for 45 or 90 s and pelleted through a 4.4-mm pellet die. Crumble quality was determined using a sieve stack with U.S numbers 6, 10, 18, and a pan on a Dura Tap shaker, which separated crumbles into four size groups: >3.35 mm, 3.35 to 2.00 mm, 2.00 to 1.00 mm, and <1.00 mm. Pellet durability index was measured by a Holmen NHP 100 for 30 and 60 s. Data were analyzed using a generalized linear model in JMP 19 (SAS, 2024), and means were separated using Tukey's HSD test with statistical significance accepted at $P \leq 0.05$. A significant AME × CT × RT interaction ($P < 0.05$) was observed for crumble size. Increasing CT and RT increased the proportion of large crumbles (>3.35 mm) particularly in the low AME diets, whereas intermediate crumble fractions (1.00 and 2.00 mm) were reduced under the same conditions. For PDI, a significant AME × CT × RT interaction ($P < 0.05$) was observed in the starter diet and for 30 s in grower diets. Increasing AME reduced PDI under mild processing conditions (low CT and short RT); but this reduction was partially mitigated by higher CT and longer RT. At low AME, PDI measured at 30 s remained consistent ($P > 0.05$) across processing conditions, whereas at high AME level, increasing CT and RT increased ($P < 0.05$) PDI. Overall, increasing AME negatively affected PDI but improved CQ under mild thermal processing conditions. In contrast, higher CT and longer RT improved PDI but increased the percentage of crumbles larger than 3.5 mm, highlighting the need to balance formulation and processing to optimize feed physical quality.

**Keywords:** pellet durability index; crumble quality; apparent metabolizable energy; conditioning temperature; retention time

### 133 potency of processed soy sauce meal as an alternative protein source for laying hens

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Agro-industrial by-product is potential resource to support the increasing demand of safe and sustainable protein source for poultry feed. The study aimed to assess the nutritional improvement of soy sauce meal (SSM) through acid extraction and enzymatic hydrolysis by using different enzyme types and dosages, and to evaluate its effects on the blood profile and intestinal histopathology of laying hens. SSM was subjected to acid solubilization (pH 4.5) and subsequently hydrolysed under submerged incubation conditions using papain and multi-enzyme complex at varying concentrations (0.1%–0.2% w/w). The processed SSM (PSSM) with the most favourable amino acid profile and gross energy (PSMM2) was selected for in vivo evaluation. PSSM retains considerable energy potential in addition to its protein content. Assesing gross energy is relevant for improving cost- effective feed formulation.A total of 500 Lohmann Brown laying hens (70 weeks old) were randomly allocated to five dietary treatments with 5 replicates (20 birds per replicate): control diet (T0), control diet formulated with 5% of SSM (T1), 5% of PSSM (T2), 10% of PSSM (T3), and 15% of PSSM (T4). All the experimental diets formulated to be iso-nutritional, differing only in ingredient composition, particularly in the inclusion levels of SSM, PSSM and soybean meal. The different Birds were fed experimental diets for 5 weeks. All data were tested for normality (Shapiro–Wilk test) and for homogeneity of variance (Levene's test). Data were subjected to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for multiple comparisons. Enzymatic hydrolysis with multi-enzyme treatments significantly ($P < 0.05$) improved several amino acid contents (Methionine, Threonine, Tryptophan, Cystine, M+C, Glycine, Serine), Gross energy and salt content of SSM. Hens fed diets containing 5% – 15% of PSSM showed no significant effects ($P > 0.05$) on Erythrose and Haemoglobin, indicating that PSSM did not impair erythropoiesis or induce anaemia. Blood protein and albumin also unchanged significantly ($P > 0.05$), indicate that hepatic protein synthesis and nutrient utilization were not disrupted. Hens receiving 5% -15% levels of PSSM showed normal leukocyte and lymphocyte counts ($P < 0.05$) compared to control group, provides insight that PSSM could help maintain immune status, physiological stress and other systemic effect. Intestinal histopathology showed normal villi structure across treatments, confirming gut integrity. In conclusion, PSSM can be safely included up to 15% in layer diets without negative effects on health parameters. These findings highlight the potential of PSSM as a sustainable alternative protein source in poultry feed.

**Keywords:** Sustainability; Soya sauce meal; Laying hen; blood; Intestinal

### 134 Evaluating the impact of palm oil Inclusion on growth performance, meat quality, and lipid deposition in turkeys

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The objective of this study is to investigate palm oils as potential oil sources for turkeys, with soybean oil (SO) serving as a benchmark. 300 one-day-old male turkeys were placed in floor pens with SO, crude palm oil (CPO), crude high oleic palm oil (HOPO), refined, bleached, and deodorized palm oil (RBDPO), and RBD palm olein (PALMOL) in corn-soy diets (isocaloric replacement of each oil source) for starter (d 0-28), grower I (d 29-42), and grower II (d 43 to 55) phases. Nitrogen-corrected true metabolizable energy (TMEn), and fatty acid (FA) profiles of the oils were de-

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termined before the diet formulation. Body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured at each feeding stage. On day 55, breast muscles were collected for meat quality evaluation (color, pH, and lipid profile), and blood serum was analyzed for lipid profile. The trial was repeated once to reach the target replicates (8 replicates/treatment). Data were analyzed using one-way ANOVA with treatment as a fixed effect and trials as a blocking factor. Means were separated using Tukey's HSD test ($P$< 0.050). Among all the palm oils, RBDPO showed lower BW at d 28 ($P$=0.001) and 42 ($P$<0.001), and lower BWG during the starter phase ($P$=0.001) and from 0-42 d ($P$<0.001). PALMOL also reduced early and overall growth performances ($P$<0.050). However, HOPO and CPO did not show a significant reduction in growth performance, and none of the palm oil sources affected cumulative FCR (0–42 or 0-55), with HOPO showing a numerically lowest FCR ($P$<0.050). Breast meat color ($P$>0.050) and pH ($P$=0.660) were not affected by dietary treatments. The FA profile of breast meat reflected the lipid composition of the oils. Higher PUFA were observed in the SO group compared with palm oil treatments, and HOPO showed a higher PUFA than PALMOL and RBDPO ($P$<0.001). MUFA were higher in palm oil treatments, where RBDPO exhibited a lower level than PALMO ($P$<0.001). Furthermore, HOPO had higher proportions of SFA than SO ($P$<0.001). At 28 days, cholesterol levels were higher in PALMOL than in HOPO. At 55 days, lower cholesterol levels were observed in birds fed HOPO than in those fed SO, RBDPO, or PALMOL ($P$=0.001). In conclusion, isocaloric replacement of soybean oil with palm oils did not affect overall growth performance in turkey poults up to 55 days, but turkey poults were sensitive to PALMOL and RBDPO during the early stages. HOPO showed the most comparable results to soybean oil. Additionally, HOPO exhibited a more balanced saturated-to-unsaturated fatty acid composition and higher MUFA levels than soybean oil.

**Keywords:** Palm oil; soybean oil; fatty acids; turkeys; meat quality

### 135 The effects of dietary crude protein level and poultry by-product meal or crystalline glycine inclusion on the growth performance of broilers challenged with necrotic enteritis

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Glycine (**Gly**) is a conditionally essential amino acid that is critical for intestinal health, though high dietary Gly concentrations can also support proliferation of *Clostridium perfringens* (**C**), the causative agent of necrotic enteritis (**NE**). Reduced crude protein (**CP**) diets inherently minimizes dietary Gly, whereas crystalline glycine (**CG**) and animal protein meals such as poultry by-product meal (**PBM**) can counterbalance this effect. The objective of this study was to determine if dietary CP concentration, PBM inclusion, or CG supplementation altered the responses of broilers to a NE challenge. Male Yield Plus x Ross 708 broiler chicks were allocated to 9 treatments (30 birds/pen; 8 pens/treatment) based on diet and challenge status. Treatments 1 and 2 were unchallenged and fed conventional CP (**CCP**) or reduced CP (**RCP**) diets, respectively. Treatments 3 - 9 were challenged with birds fed the same CCP (treatment 3) or RCP (treatment 4) diets, CCP diet with 3% PBM (treatment 5), a RCP diet with 3% or 6% PBM (treatments 6 and 7), or the RCP diet with low or high CG inclusion (treatments 8 and 9). Diets were maintained across starter (0-17 d), grower (17-26 d), and finisher (26-35 d) phases. A CP reduction of 2 percentage points was maintained between the CCP and RCP diets in all phases. Treatments 5 and 8 were formulated to have equal total Gly + Ser contents from either all intact proteins or CG. Other nutrients and energy levels were based on primary breeder recommendations. The challenge consisted of inoculation with 6,000 oocysts/mL of *Eimeria maxima* on d 14 and C ($10^8$ cfu) inoculation on d 18 and 19. Feed intake (**FI**), body weight gain (**BWG**), and feed conversion ratio (**FCR**) were determined at the end of each phase and on d 20. Mortality was recorded to adjust FCR. Data were analyzed with pre-planned orthogonal contrasts to test the effects of challenge, CP level, challenge × CP, PBM inclusion within challenge, CP × PBM within challenge, CG level within challenge, and Gly source (PBM vs CG) within challenge. The NE challenge reduced ($P$ < 0.05) FI (~3.5%) and BWG (~4%) and increased FCR (1.43 to 1.47) for the overall period. Reduction of CP reduced ($P$ < 0.05) BWG and increased ($P$ < 0.05) FCR, regardless of challenge status. Within challenged birds, increasing PBM inclusion from 3 to 6% reduced ($P$ < 0.05) FI and BWG and tended ($P$ < 0.10) to increase FCR, whereas CG inclusion did not affect ($P$ > 0.05) growth performance. In conclusion, these data indicate that CP reduction and PBM inclusion were generally detrimental to the performance of NE-challenged broilers. Future work will evaluate the impacts of these dietary factors on microbiota composition and function and other indicators of intestinal health.

**Keywords:** Glycine; Necrotic enteritis; Broiler performance; Poultry by-product meal; Clostridium perfringens

### 136 Effects of dietary palm oil inclusion on performance metrics and egg quality in laying hens

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This study evaluated the effects of palm oils (POs) on growth performance, laying performance, blood chemistry, and yolk fatty acid (FA) profiles in layers. A total of 320 Hy-Line W36 hens at 29 weeks were assigned to 5 treatments. Each treatment consisted of 8 replicates with 8 birds per individual cage. The replicate was considered the experimental unit. The diets were formulated for peak (29 to 37 weeks) and Layer II (38 to 45 weeks) phases, with soybean oil (SO) being replaced on a caloric basis with different POs: crude palm oil (CPO), crude high oleic palm oil (HOPO), refined, bleached, and deodorized palm oil (RBDPO), and RBD palm olein (PALMOL). Nitrogen-corrected true metabolizable energy (TMEn) and FA profiles for each oil were determined. Daily egg production and mortality were registered for hen-housed eggs (HHE) and average weekly egg production calculations. At 29, 33, 38, 41 and 45 weeks, body weight was obtained, and three eggs per replicate were evaluated for egg quality. Feed consumption was also measured to obtain feed conversation ratio (FCR) per dozens of eggs produced in each period. Also, at 33, 38, 41, and 45 weeks, the yolk FA profile for each treatment was determined. Fowl mite control using Ravap EC was required, which induced acute stress in the flock, as shown by a reduction in egg production from 34 to 38 wk. All data was analyzed using one-way ANOVAs with Tukey's HSD for mean separation, except for yolk color, which was used with non-parametric Kruskal–Wallis followed by Dunn's test. Differences were significant at P<0.05. No difference was found in growth performance and egg quality (P>0.05). Except for yolk color, which was greater in HOPO compared with SO. Similarly, CPO increased yolk color compared to SO at 33 and 38 weeks (P<0.005). At 37 weeks, egg production for the CPO was higher than for the SO (P=0.0277), and no difference was observed for the other periods (P>0.05). During the peak phase, the yolk FA profile showed a higher content of polyunsaturated FAs (PUFA) and a lower content of monounsaturated FAs (MUFA) in SO compared to the POs (P<0.05). For saturated FAs (SFA), HOPO had lower content in the yolk than in RBDPO and PALMOL, but no difference compared to SO and CPO (P<0.05). During the Layer II diet phase, total SFA concentration in the yolk did not differ between treatments (P>0.05). At 42 weeks, MUFA and PUFA followed the same trend as the peak phase. But at 45

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weeks total MUFA was higher in HOPO than in SO (P=0.0262). Serum ALT, AST, cholesterol, and triglyceride levels were not affected by the oils. The results showed the application of palm oil in laying hens and revealed that FA profiles in the yolk could be modified by dietary oil source.

**Keywords:** Palm oil; soybean oil; fatty acid; egg quality; laying performance

### 137 Effects of low-protein diets and nutrient conditioning on productive performance in broiler chickens

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Reducing crude protein in broiler diets is a common strategy to improve sustainability; however, it may impair performance under essential amino acid limitations. Nutrient conditioning, defined as the association between sensory cues and post-ingestive nutrient effects, may represent a mechanism to modulate feeding responses. The aim of this study was to evaluate whether nutrient conditioning modulates the effects of low-protein diets on productive performance in broiler chickens. A total of 128 one-day-old male Ross 308 broilers were assigned to a 2 × 2 factorial design. Birds were allocated to two dietary treatments: a standard protein diet (SP), formulated to meet nutritional requirements, and a low-protein diet (LP), formulated at 30% below optimal crude protein levels. Within each dietary treatment, birds were further assigned to either a conditioned or non-conditioned group, resulting in four treatments with eight replicate pens each (SP, SPC, LP, LPC). The conditioning protocol consisted of two phases (days 10–18 and 30–38), during which birds were exposed to colored drinkers (yellow or green) associated with either a carbohydrate solution (3% sucrose) or a protein-based solution (3% fish meal replacer); each session lasted 4 h following a short feed withdrawal period, and intake of both solutions was recorded. Birds were reared for 42 days under controlled conditions. Productive performance variables, including body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), were measured weekly throughout the experimental period and cumulatively at day 42. Data were analyzed using two-way ANOVA including diet, conditioning, and their interaction. Diet significantly affected all productive variables ($P < 0.001$), with LP birds showing lower BW, ADG, and ADFI compared with SP birds. Conditioning did not affect BW, ADG, or ADFI ($P > 0.05$). However, FCR was affected by diet ($P < 0.001$), conditioning ($P = 0.03$), and their interaction ($P = 0.003$). Specifically, conditioning improved FCR in LP birds compared with non-conditioned LP birds, whereas no differences were observed within SP treatments, indicating that the effect of conditioning depended on dietary protein level. These results indicate that, although nutrient conditioning did not directly affect growth performance, it improved feed efficiency under protein-restricted conditions, suggesting a role in enhancing adaptation to low-protein diets.

**Keywords:** Broiler chicken; low-protein diet; productive performance; feed conversion; classical conditioning

## Saturday, July 11, 2026

### Physiology and Reproduction

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### 138 Phenotyping Male Fertility Traits for Improving Broiler Breeder Fertility

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The modern broiler has undergone intense selection for rapid growth and feed efficiency, creating correlated declines in skeletal, cardiovascular, and reproductive performance that now threaten long-term fertility in broiler breeders. Broiler breeder fertility typically shows a pronounced decline between 40–50 weeks of age, and industry data indicate a sustained downward trend in hatchability, with projections suggesting fertility could fall toward 60% by 2050 if current trajectories continue. Traditional fertility endpoints are binary, noisy, and temporally distant from causal biological events, limiting their usefulness for genetic selection and mechanistic insight. To address these limitations, we developed longitudinal, male-centered phenotypes based on semen-quality traits measured throughout the production cycle in commercial broiler breeder males. Using computer-assisted sperm analysis (CASA), we quantified motility kinematics and morphology, including progressive motility, curvilinear and linear velocities, amplitude of lateral head displacement, and the proportion of morphologically normal sperm. For each male, non-linear models were fit to time-series trajectories of semen parameters, and derivatives were calculated to capture instantaneous rates of change and acceleration of decline, respectively. The age at which the second derivative crossed zero, corresponding to the point of maximal rate of decline in semen quality, was defined as the "Age of Peak Utility" (APU), a single-value phenotype representing the transition from stable function to accelerated reproductive aging. Our study estimated the APU as 9.4 months for modern breeders, which is substantially sooner than desirable, or what was standard only a decade ago. Derivative-based phenotypes revealed substantial among-male variation in both the timing and dynamics of reproductive decline, allowing classification of males as early, intermediate, or late decliners based on their APU values. Because the APU is derived from fitted models rather than individual time points, it is robust to noise, preserves information about the trajectory of change, and provides a biologically interpretable metric for genetic analyses. This framework enables the identification of males with resilient reproductive aging for targeted selection, and it can be generalized to other longitudinal traits in which the timing and rate of change are biologically important, including egg production curves and growth trajectories. These results support integrating derivative-based longitudinal phenotypes into broiler breeder breeding programs to improve fertility and sustain production efficiency.

**Keywords:** Breeder fertility; semen parameters; age of peak utility

### 139 Strain-Specific Variations in Oxidative stress and Immune Responses of Meat-Type Chickens Exposed to Cyclical Heat Stress

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Heat stress is a major constraint to poultry productivity, particularly in tropical environments, with genetic variation influencing adaptive capacity. This study assessed oxidative stress biomarkers and immune responses in different chicken strains under heat stress (HS). At 35 days of age, approximately 60 birds per strain were exposed to controlled short-term HS in a psychrometric chamber (34.5 ± 1°C; 75 ± 5% RH; THI > 90) for 6 h daily. Blood samples were collected on days 1, 3, 5, and 7. Peripheral blood mononuclear cells (PBMCs) were isolated, and gene expression analysis of heat shock proteins, oxidative stress, and immune-related genes was performed using quantitative real-time PCR. Cytokine levels (pro- and anti-inflammatory), total antioxidant capacity, and plasma lactate were quantified using ELISA and spectrophotometric assays. Data were analyzed using two-way analysis of variance (3×4) with strain (S) and heat stress duration (D) as main effects, followed by Tukey's post

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hoc test. Among reference genes, 18S rRNA was observed to be the most stable under HS conditions. Significant strain effects ($p<0.05$) were observed for IFN-Γ, HSP70, and HSP90, whereas GPx, NOX1, NOX4, SOD, and CAT were unaffected ($p>0.05$). Fast-growing CBD and CD strains showed higher IFN-Γ and HSP70 expression than COBB, indicating stronger activation of stress and immune pathways. HSP90 followed a similar but non-significant trend. Prolonged HS (D3–D7) significantly increased GPx, IFN-Γ, HSP70, HSP90, NOX1, SOD, and CAT, reflecting enhanced oxidative stress and antioxidant defense. GPx rose sharply from D1 to D3 and remained elevated. HSP70 and HSP90 peaked at D5–D7. NOX4 showed a fluctuating pattern, decreasing initially and increasing at D7, suggesting time-dependent ROS regulation. SOD and CAT increased up to D5, while SOD declined slightly at D7, indicating possible adaptive regulation. A significant S×D ($p<0.001$) was observed for all markers, demonstrating strain-specific responses. HS elevated plasma lactate levels ($p<0.001$), particularly in fast-growing birds, indicating greater metabolic stress. Slow-growing birds exhibited higher antioxidant capacity and lower lipid peroxidation ($p=0.001$), suggesting better oxidative resilience. Immune profiling showed higher IL-2, IL-10, and IFN-Γ levels ($p<0.05$) in slow-growing strains, with IL-10 increasing over time, indicating an adaptive anti-inflammatory response. In conclusion, slow-growing (CD) and medium-growing (CBD) strains demonstrated superior physiological and molecular adaptability to heat stress, highlighting their potential for sustainable poultry production under climate stress conditions.

**Keywords:** Meat type chicken; anti-oxidant; immunity; Heat stress

### 140 Hidden in plain sight: differences in color vision and visual acuity of the Pekin Duck compared to its wild progenitor the Mallard duck (Anas platyrhynchos).

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Much attention has been given to housing poultry under different colored lights, but little attention has been given to how these lights may affect their ability to perceive their environment. To accomplish this goal in Pekin ducks, we first had to assess the physiology of the retina (n = 13/sex), and compared that to wild-type Mallard ducks (n = 10/sex), the wild progenitor of the Pekin. We assessed retinal physiology by measuring eye size, eye shape, the transmittance of the ocular media, the absorbance properties of the photoreceptor visual pigments and oil droplets, and the density and distribution of photoreceptors within the retina, as well as comparing their overall photoreceptor sensitivity. We compared each of the traits measured between species using both general linear models and general linear mixed models. All models were run using R version 4.4.2 (2024-10-31 ucrt). All the codes for the statistical models and figures, along with the data, are available at: https://osf.io/qzh3p/overview?view_only=9e4d370736b64029a15884cf1ce69bb4. We found that Pekin ducks have larger eyes ($p < 0.003$) that are shaped in a way to enhance visual acuity, have over 1.3 million fewer ($p < 0.001$) photoreceptors in the retinas, but conversely, have an overall higher ($p = 0.0015$) visual acuity due to their larger size. Both the Pekin duck and Mallard duck forms possess a violet-sensitive color visual system with the same complement of cone and oil droplet classes as well as an oblique visual streak of high photoreceptor density. However, the Pekin duck possesses visual pigments shifted approximately 10 nm to longer wavelengths of light and does not have the P1-Type oil droplet typically present in avian double cone photoreceptors. These shifts in sensitivity and oil droplet absorbance selectively tune the sensitivity of the Pekin duck photoreceptors to simultaneously maintain color discrimination abilities and increase sensitivity to the longer wavelengths of light present in their housing environment. We also found that Pekin ducks have lower ($p < 0.001$) densities of the double cone, medium-wavelength sensitive, and long-wavelength sensitive photoreceptors, but differentially across the retina. Our results suggest that domestication has altered the Pekin ducks' ability to perceive their environment due to retinal differences compared to their wild progenitors. Whether this domestication effect is due to genetic drift, diet, or housing remains to be elucidated by further experiments.

**Keywords:** retinal anatomy; retinal physiology; visual acuity; visual perception; welfare

### 141 Did you see that? Impact of green, red or blue LED and white fluorescent vs incandescent lights on Pekin duck visualization of their environment and ducks actively avoid blue lights.

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Poultry scientists have studied the impacts of colored lights on production, behavior, and welfare on many poultry species, including ducks. However, no one has considered how these colored lights impact the birds' ability to visualize their environment. Our objectives were to first model how ducks visually perceive their environment, and second to understand duck preferences for colored lights. We first measured the spectral qualities of red (peak at 618-638 nm; total of 0.128Mmol photons/$m^2$/sec), green (peak at 534-566 nm; total of 0.133Mmol photons/$m^2$/sec), blue (peak at 457 nm; total of 0.143Mmol photons/$m^2$/sec), fluorescent white (peaks at 437, 489, 545, 588, and 613 nm; total of 0.163Mmol photons/$m^2$/sec) and incandescent lights (peak at 700 nm; total of 0.157Mmol photons/$m^2$/sec). We also measured the reflectance properties of all resources in the barn, the flooring and walls, and of several points on the bodies of the ducks. Adult ducks (~35 weeks) were housed at Purdue under 18:6 light cycle and under standard breeder conditions. All physiological and anatomical properties of retinas and eyes were assessed, respectively from adult ducks (n=14/sex). These visual system characteristics were then used to build a perceptual model of the duck through use of the receptor-noise limited model. Red spigot bases could not be resolved through chromatic or achromatic contrast under any lighting type due to their color and reflective properties. Similarly, it appears that the light peach-colored bill of Pekin ducks also cannot be resolved under any lighting types when viewed in front of walls or other ducks. The visual acuity model suggests that after 5 meters, object resolution is greatly diminished in Pekin ducks. In order to further understand ducks' preferences for light colors, we ran a preference test between the same red and blue lighting. When placed in the center of an empty room, pekin ducks (n=36/sex) preferred significantly the red (0.75) over the blue (0.25) lights (generalized linear model, $z = 3.65$, $P < 0.001$). This avoidance of the blue light was not affected significantly by sex ($X2 = 63.54$, $df = 55$, $P = 0.939$) or time of day ($X2 = 60.35$, $df = 53$, $P = 0.203$). In the room with a feeder at both ends, ducks again preferred significantly the red (0.74) over the blue (0.26) lights (generalized linear model, $z = 3.72$, $P < 0.001$). Sex ($X2 = 76.30$, $df = 66$, $P = 0.129$) or time of the day ($X2 = 71.91$, $df = 64$, $P = 0.111$) did not affect this preference pattern. Our results are beginning to suggest to us how barn resources should be recolored or how lighting systems can be redesigned based upon the visual properties of poultry.

**Keywords:** visual perception; object recognition; retinal physiology; lighting preferences

### 142 Significance of sex and season for the long-term effects of embryonic temperature training in broiler chickens

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During critical stages of development, body functions can be shaped by environmental factors, which has long-term effects on performance, adaptability and health. The aim of the study was to elucidate whether a short-term temperature training (STT) during the hatching phase effects neuronal molecular biomarkers related to developmental plasticity and energy metabolism as well as the stress hormone corticosterone (CORT) and the heterophil-to-lymphocyte ratio (HLR) in male and female Ross 308 broilers. Our hypothesis is that embryonal STT induces long-term physiological changes in neural and peripheral parameters which – depending on sex and ambient temperature (AT) – form the basis for improved adaptability to a stressful environment. A total of 126 eggs were used, which were incubated at 37.3°C; of these, 62 were subjected to STT on embryonic days 17–20 (+1°C for 2 hours per day). At 35 days of age the basal mRNA expression levels of Bdnf, Npy, and Pomc in the hypothalamic nucleus infundibular was investigated. Additional, CORT was detected in feathers using ELISA and HLR in blood smears. The experiments were carried out during a winter and a summer trial with consistently higher AT. Mean mRNA expression levels were descriptively higher for *Npy, Pomc,* and *Bdnf* in the STT group compared to the control group. The group difference for *Bdnf* was statistically significant when analyzed across both trials (N=72, padjust= 0.048). Multiple linear regression analyses revealed that this effect was mainly driven by differences in the summer trial. For all three target genes, we observed highest mean mRNA expression levels for the STT group in the winter trial and lowest expression levels for the control group in the summer trial, with the STT rescuing the dampening effect of the higher AT on the expression levels. For *Bdnf,* this difference was statistically significant. In both trials, no significant sex-specific differences were observed for any of the target genes. Compared with the control group, STT had no effect on CORT (Kruskal-Wallis test, p=0.14). When analysed by sex, a significant effect of STT on CORT was observed in male chickens (ANOVA, P=0.019). Taking the season into account, a trend towards different responses in summer and winter can be observed in female birds (higher summer values following stimulation, P=0.053). The HLR was numerically lower in the STT group in both the winter and summer trials (e.g. summer: control 0.76±0.46, STT 0.63±0.30). Our findings indicate that STT during a critical developmental period induces long-lasting changes in mRNA in avian hypothalamic neurons that are associated with developmental plasticity and improved stress resilience depending on gender and AT.

**Keywords:** thermoregulation; gene expression; energy metabolism; brain-derived neurotrophic factor; stress resilience

### 143 Mechanism of glutamate accumulation in chicken eggs

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Glutamate is a key umami component and a functional amino acid involved in energy metabolism, nitrogen utilization, and the synthesis of non-essential amino acids. Egg yolk contains a high level of glutamate, which may contribute to the characteristic umami taste of eggs. Although most egg yolk components originate from the hen's blood, the metabolic origin and regulatory mechanism underlying the marked enrichment of glutamate in yolk remain unclear. We hypothesized that glutamate in yolk is supplied from the ovarian tissue layer where the yolk is formed, and enzymatic and physiological approaches were employed to elucidate the metabolic mechanism responsible for supplying glutamate to the egg yolk. Plasma, liver, jejunum, granulosa cell layer (GC layer), theca cell layer, and yolk within the follicles were collected from laying hens (Lohman LSL lite, 30-50 weeks of age). The hens were fed a corn-soybean meal-based diet containing 17% CP and 2,800 kcal/kg ME. Amino acid concentrations and glutaminase activity, the enzyme converting glutamine into glutamate, were measured using LC–MS/MS. The activity of glutamate oxaloacetic transaminase (GOT), an enzyme involved in glutamate catabolism to A-ketoglutarate, was assessed using an NADH-based absorbance assay. Glutaminase expressions were analyzed by real-time PCR and Western blot. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Amino acid analysis showed that glutamine was the most abundant amino acid in plasma, whereas glutamate was present at low concentrations. In contrast, glutamate was the most abundant in the yolk of ovarian follicles, suggesting that its accumulation is not solely dependent on direct transport from circulating blood but may involve local synthesis within ovarian follicular tissues. Enzyme assay indicated that GC layer exhibited higher glutaminase activity compared to ovarian theca layer, liver and intestine, with particularly elevated activity in smaller follicles. Although GOT activity was also detected in the GC layer, its level was comparable to that in the intestine, an organ known to exhibit low GOT activity in chickens, indicating that glutamate catabolism is limited in the GC layer. Consistent with these findings, both mRNA and protein analyses confirmed high glutaminase expression in the GC layer, especially in smaller follicles. Collectively, these findings demonstrate that the GC layer actively converts glutamine into glutamate and supplies it to the egg yolk. This metabolic process appears most active in the early follicular stages. The GC layer thus represents a key metabolic tissue responsible for glutamate enrichment in egg yolk.

**Keywords:** glutamate; glutaminase; granulosa cell layer; LC-MS; umami

### 144 New insights into keel bone development: Keel tip integrity is unaffected by age at first egg in LSL-Lite pullets

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In laying hens, the keel bone's distal tip is not fully ossified by the time they come into lay. Our previous work has shown that the ossification progression appears driven by age, independent of age at first egg (**AFE**). However, it is unknown whether AFE affects the structural quality at the keel's tip. Bone strength has been positively correlated with bone mineral density (**BMD**) and trabecular bone connectivity, a topological indicator of structural integrity and porosity. We investigated indicators of keel bone strength in hens following experimental modification of AFE. In a split plot design, 600 Lohmann LSL Lite pullets were photostimulated (**PS**) at either 18 or 20 weeks of age (applied at the room level) and reared on either *ad libitum* (**AL**) feeding or a 20% feed restriction (R; applied at the pen level within rooms). As expected, AFE was earliest in AL pullets receiving 18-week PS, and latest in R pullets receiving 20-week PS. At 26 weeks, 96 hens from 24 pens (n= 6/ treatment combination) were euthanized and their keel bones collected. Using dual-energy X-ray absorptiometry imaging, the BMD (bone mineral content per $cm^2$) of the whole keel and the distal 2 cm of each keel were assessed. In addition, the keel tip (distal 2 cm) was imaged using micro-CT technology at a resolution of 40 microns. The micro-CT images were used to calculate connectivity density (**Conn.D**), an Euler connectivity analysis of porosity. Data were analyzed in R to predict the effects of feeding and age-at-PS treatments using linear mixed-effect models that accounted for the split-plot design. No treatment effects were found on BMD of the whole keel bone (age at PS: P= 0.91, feeding: P= 0.79) or distal tip (age at PS: P= 0.06, feeding: P= 0.71). No treatment effects were detected on Conn.D (age at PS: P= 0.69; feeding: P= 0.13). Conn. D showed substantial individual variability within each treatment group, with 2-3 times greater within-group

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variability than the group mean difference. Our data show no effect of AFE on the BMD or structural connectivity of laying hen keels, suggesting that the entry into egg production does not affect keel bone tip integrity. Whether the high variability in structural connectivity of the keel tip is related to individual risks of keel bone fractures remains to be investigated.

**Keywords:** Laying hen pullets; Keel bone ossification; Age at first egg; Bone mineral density; Bone connectivity density

### 145 Non-Destructive Assessment of Broiler Hatching Eggs Fertility Prior Incubation Via VOCs Profiling

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Technologies capable of accurately, and non-destructively determining egg fertility, viability, and sex during the early stages of incubation are highly desired for the modern poultry industry. Day 0 fertility assessment holds the most promise as it allows for evaluation prior to incubation, which could reduce costs, maximize incubator space utilization and improve welfare. Among various promising non-destructive methods at early stage of incubation (Day 3 or 4), collecting and analyzing the volatile organic compounds (VOCs) emitted from hatching eggs, using gas chromatography – mass spectrometry has demonstrated its discriminatory capabilities in classifying between unfertilized (UF) and fertilized (F) eggs on Day 0 for Athens-Canadian Random Bred (ACRB) in our previous research. The objective of this continued research is to determine and validate the egg fertilization assessment based on VOCs approach of commercial breed (Ross 708) hatching eggs on Day 0. Twenty-four pullets (24) and three roosters (3) were sourced from a local integrator and delivered to Southern Poultry Research Group. The breed is Ross 708 parent stock. At arrival, breeders were sorted by weight to ensure similar starting weights in each group. Breeders were assigned to two treatment groups: natural mating (1) vs. non-inseminated (2) with one replicate pen per treatment and twelve (12) pullets and three (3) roosters for treatment 1. The experimental facility was divided to accommodate each treatment within the same airspace. From 23 weeks for the next 3 weeks and from 28 weeks for the next 3 weeks, eggs were gathered and set by treatment. 45 eggs from both natural mating and non-inseminated groups were collected for four trials. VOCs were collected by magnetically attaching a Twister® to the inside of a lid and sealing the egg inside a glass jar for 1 hour. VOCs sampling was conducted on Days 0, 7 and 10 of incubation. VOCs were desorbed from the Twister® using a thermal desorption unit coupled to a cooled injection system and analyzed using two-dimensional gas chromatography- mass spectrometry in a non-targeted approach. LECO® ChromaTOF® software was used for data acquisition and analysis in conjunction with the NIST 2017 mass spectral database for compound identification. Principal Component Analysis and leave-one-out Partial Least Squares Discriminant Analysis were conducted separately for each day of VOCs collection to distinguish between “Fertile” and “Infertile” eggs. For data collected on Day 0, an F1 cross-validation score of 0.90 was achieved. The VOCs with the greatest influence on class separation were also identified.

**Keywords:** Broiler-hatching-eggs; Fertility; Non-destructive; Prior-incubation; VOCs

### 146 Tibiotarsus morphometry in broilers selected for water efficiency following short-term water deprivation

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Lameness remains a major welfare issue in broiler chickens, and bone morphometrics are essential for understanding factors that shape skeletal integrity. Although water deprivation can alter growth and behavior, its effects on bone development are poorly defined, and methods to measure skeletal integrity are archaic and limited and time consuming and there is a need for automated methods. This study evaluated whether short-term water deprivation alters tibiotarsus (tibia) morphology in broilers divergently selected for high (HWE) or low (LWE) water efficiency. Day-old chicks (N=382) were allocated by line (HWE or LWE) into 24 pens. On days 24-26 and 38-40, pens received either 0, 12, or 24h of water deprivation in a 2x3 factorial design in four staggered blocks. Across days 39-40, left and right tibiae were collected from 120 birds. Bones were autoclaved, 3D-scanned, landmark-aligned in a 3D Slicer software, and morphometric traits, including length, proximal (PH) and distal head (DH) widths, PH angle, cross-sectional widths at the 10, 25, 50, 75, and 95% length, epiphyseal and diaphyseal torsion, straightness, and robusticity, were quantified. Linear mixed models tested the effects of line, treatment, sex, and bone side, with body weight and tibia length as covariates. Water deprivation had minimal effects on bone morphometrics. Birds deprived of water for 24h had wider tibiae at 90% (12.4mm) compared to 0h (11.9mm), with 12h as intermediate (12.3mm, $p=0.01$), with a similar trend in PH width (0h=24.3mm, 12h=24.4mm, 24h=24.7mm, $p=0.08$). PH angle tended to differ between 12h (32.0°) and 24h (31.2°, $p=0.06$), with 0h as intermediate (31.5°). LWE broilers had longer tibiae (95.1 vs 93.5mm) and greater diaphyseal torsion (43.3 vs 37.0°), whereas HWE broilers had wider PH (24.8 vs 24.1mm), DH (18.7 vs 18.4mm), 50% (6.29 vs 6.07mm), 75% (7.64 vs 7.35mm), and 90% (12.4 vs 12.0mm), as well as greater robusticity (0.041 vs 0.040) and PH angle (32.1 vs 31.0°, $p<0.05$). Males had longer tibiae (96.4 vs 92.1 mm) that were also wider (at all measured widths) and had greater PH angle (32.3 vs 30.8°) and robusticity than females ($p<0.05$). However, females had greater diaphyseal torsion (45.4 vs 35.1°, $p=0.002$) and tended to have greater bone straightness (0.963 vs 0.959, $p=0.06$) than males. Body weight had a significant effect on bone width at all measured points, epiphyseal torsion, robustness, and straightness ($p<0.05$), while bone length only affected PH, DH, 10%, 75%, and 90% widths and robustness ($p<0.05$). Overall, short-term water deprivation had little effect on tibial morphology. However, substantial line and sex differences demonstrate the importance of genetics and body weight in shaping bone structure.

**Keywords:** broiler; water efficiency; water deprivation; bone morphometry; tibiotarsus

### 147 Effects of feeding level and feeding system on reproductive physiology of broiler breeders.

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A study was designed to analyze the effect of broiler breeder pullet BW and feeding system on gene expression within hypothalamus, pituitary, and ovary (stroma plus small and large white follicles) of broiler breeder females. Ninety-six day-old Ross 308 broiler breeder females were raised either in a precision feeding or a standard conventional feeding system until 60 wk of age. Birds were divided into three treatment groups: conventionally fed to the breeder-recommended target BW (CON or Control); precision fed to breeder-recommended target BW (PF Standard); and precision fed a meal at every visit (MEV, comparable to ad libitum feeding). Tissue collection was done in two birds per treatment group at 16 and 21

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wk of age (photostimulation age), and at the age of first egg (AFE). Expression of 46 genes was analyzed using the Nanostring nCounter-based® technology. Gene counts were normalized relative to positive and negative controls and based on housekeeping genes, and log2 transformed prior to analyses. Both PF Standard and MEV were contrasted against the Control group using the MIXED procedure in SAS 9.4. Tukey's multiple range test was used to separate means when significant at P < 0.05. PF pullets had lower levels of Neuropeptide Y (NPY) at 16 wk of age and greater levels of NPY at photostimulation age compared to CON pullets (P < 0.05). At AFE, MEV pullets had greater levels of gonadotropin-releasing hormone receptor I (GnRH-RI) in the pituitary compared to CON hens (P < 0.05). At AFE, PF Standard hens had lower levels of follicle-stimulating hormone receptor (FSH-R) and insulin-like growth factor receptor (IGF1-R) in small ovarian follicles compared to CON hens (P < 0.05). PF Standard breeders had multiple meals throughout the day compared to CON breeders (5 meals vs. 1 meal). The higher number of meals per day seemed to modulate the melanocortin system through NPY in PF Standard pullets. PF Standard breeders had lower egg production (15 fewer eggs) compared to CON breeders. Also, the higher number of meals likely decreased fat deposition in the PF Standard breeders, which might have explained the lower levels of FSH-R and IGF1-R in the ovary of PF Standard hens. Additionally, MEV broiler breeders came into lay before CON and PF Standard birds, and it might be the result of the greater levels of GnRH-RI in the pituitary. In conclusion, PF Standard breeders with several meals per day showed lower follicular development compared to CON breeders.

**Keywords:** precision livestock feeding; reproduction; feeding system; metabolic signaling; physiology

### 148 Target body weight and the length of the starter phase on fertility and hatchability of broiler breeders between 42 and 60 weeks of age

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Target body weight and the length of the starter phase affect feed allocation, cumulative nutrient intake, growth rate, metabolism and development, and consequently influence reproductive performance and hatchability. The objective of this experiment was to evaluate the effects of two target body weights (BW), low (L) and high (H), and the duration of the starter phase (4 or 6 weeks) in a factorial experiment. A total of 1,600 Cobb pullets were placed in a dark house with 16 pens (4 replicates/treatment) and fed starter, grower, and developer diets. Feed was allocated to achieve the target BW and was adjusted weekly based on actual values. Pullets were transferred to the laying house at 23 weeks with 72 hens and 7 Cobb MX roosters per pen. Eggs were collected three times a day, stored at 15 $^{o}$C for a maximum of 5 days, and 84 eggs per pen were incubated at 42, 44, 48, 52, 44, 57, 59 and 60 wk of age. Rooster spiking was conducted at 45 wk of age. Single-stage profiles were used and transferred to hatchers at 18 days. Fertility, hatchability, hatch of fertile (HOF), and embryo mortality were evaluated. Data was analyzed using a completely randomized design with a two-way ANOVA. Negative linear effects ($P$ < 0.001) of breeder age were detected in almost all variables evaluated. No interaction effect was detected ($P$ > 0.05), but fertility and hatchability were affected by target BW ($P$ < 0.05) and the length of the starter phase ($P$ < 0.001). Heavy hens had better fertility (93.2%) than the L hens (90.7%). Hens fed starter for 4 weeks had better average fertility (93.8 % *vs*. 90.1 %) and average hatchability (86.5% *vs*. 83.8%) during this late phase of egg production, than hens fed starter for 6 weeks. However, no effect of treatments ($P$ > 0.05) was detected on HOF. The length of starter feed also affected ($P$ < 0.05) embryo mortality between 1-3 and 15-18 embryo days (ED), and incidence of contaminated eggs. Hens fed starter feed for 6 wk had higher average 1-3 ED (3.1%) mortality, and contaminated eggs (1.24%) than hens offered starter feed for 4 weeks (2.3% and 0.82%), independent of target BW. The L hens fed starter for 4 wk had higher (1.3%) 15-18 ED mortality than L hens fed starter for 6 wk (0.46%), while no effect of starter length was observed in the H hens. No effects of treatments ($P$ > 0.05) were detected in other stages of ED, or in embryos mispositioned, or malformed. In conclusion, the length of the starter phase during rearing affected hen reproductive performance between 42 and 60 wk of age. Hens fed a starter diet for 6 weeks had lower fertility and hatchability, and higher early mortality and contaminated eggs, but lower mortality between 15-18 ED in L hens, independent of target BW.

**Keywords:** Fertility; hatchability; embryo mortality; target BW; starter phase

### 194 Dietary dual-strain *Bacillus*-based probiotic modulates the gut microbiota and improves productive performance, egg quality, blood parameters, intestinal morphology and bone mineralization in early-lay hens

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The point of lay imposes substantial stress marked by intensified mineral mobilization, shell gland maturation, microbiota shifts, and endocrine adjustments. The role of supplemental probiotics in modulating gastrointestinal ecology from onset of lay till 29 weeks of age was investigated on productive performance, egg quality, blood parameters, cecal microbiota, intestinal morphology, and tibia traits. A total of 324 Hy-Line Brown hens were housed in enriched cages (9 birds/cage) and randomly allocated to one of two diets including a corn-soybean meal basal diet or the basal diet supplemented with 1.3 × $10^6$ CFU/g of probiotic# (*Bacillus subtilis* 597 and 600 combined culture). Linear mixed model was used to evaluate the effects of treatment and age on body weight, productive performance, and egg quality that were measured periodically whereas other variables that were measured once at 29 weeks were analyzed by independent t-test using SPSS 29.0. Statistical significance was set at $P$ < 0.05 and a tendency at 0.05 ≤ $P$ < 0.10. Body weight up to 29 weeks was unaffected by treatment, whereas age affected body weight ($P$ < 0.001). Likewise, laying performance, egg loss rate, egg weight, egg mass, feed intake, and feed conversion ratio were affected by age ($P$ < 0.001) but not by treatment or treatment × age interaction ($P$ > 0.10). Probiotics led to improved shell color, thickness and breaking strength ($P$ < 0.05), but reduced yolk cholesterol ($P$ < 0.05). Age affected shell color, albumen height, Haugh unit, yolk color, shell thickness, yolk percentage, shell percentage, and yolk cholesterol ($P$ < 0.05). Treatment × age interactions were observed for yolk cholesterol and both shell thickness and percentage ($P$ < 0.05). Probiotics also increased tibia weight and calcium content ($P$ < 0.05), whereas tibia phosphorus and ash were marginally improved ($P$ < 0.10). Probiotics also increased villus height, villus height to crypt depth ratio, and villus absorptive surface area ($P$ < 0.001). As to cecal microbiota counts, probiotics increased *Lactobacillus* spp. and *Bifidobacterium* spp., but reduced *Clostridium* spp. ($P$ < 0.01). While triglycerides and very low-density lipoprotein ($P$ < 0.005) and low-density lipoprotein ($P$ < 0.10) were lowered with probiotics; high-density lipoprotein ($P$ = 0.008), serotonin, and alkaline phosphatase activity ($P$ < 0.001) were improved. Collectively, *Bacillus subtilis* 597 and 600 improved egg quality by enhancing gut microbial balance and ileal nutrient utilization capacity, without affecting laying performance. Modulatory effects on lipid metabolism, gut–brain signaling, and mineral metabolism were also observed. #Gallipro®Form (Novonesis, Copenhagen, Denmark)

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**Keywords:** Bacillus subtilis; cecal microbiota; early-phase laying hens; egg quality; tibia mineralization

### 195 Essential oils and sodium butyrate improve intestinal integrity in laying hens fed a low-crude protein diet

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This study investigated the effects of saponified essential oils (EOs) and sodium butyrate (SB) supplementation in an LCP diet on intestinal integrity in laying hens. A total of 150 ISA Brown laying hens at 58 weeks of age were randomly allocated to five dietary treatments consisted of SCP (standard crude protein, 18%), LCP (low crude protein, 16%), LCP + 750 mg/kg EOs, LCP + 750 mg/kg SB, and LCP + 750 mg/kg EOs + 750 mg/kg SB. Each treatment consisted of six replicates with five birds per replicate and reared 12 weeks feeding trial. At the end of the experimental period, jejunal samples were collected to determine the expression of genes related to tight junction integrity, inflammatory cytokines, and antioxidant enzymes using quantitative real-time PCR (qPCR). Data were analyzed by one-way ANOVA, and differences among treatment means were assessed using Tukey's post hoc test. Statistical significance was declared at P<0.05**.** The result show that LCP diet downregulated the expression of intestinal barrier markers (ZO-1 and OCLD-1) and antioxidant enzymes (SOD-1 and GPX-1), while upregulating pro-inflammatory cytokines (TNF-A and IL-8) (P<0.05). Supplementation with EOs and SB reversed these adverse effects, with the combined EOs + SB treatment showing the strongest response. This combination significantly increased the expression of tight junction-related and antioxidant genes, particularly CLDN-1 and SOD-1, while suppressing the expression of pro-inflammatory genes, including TNF-A and IL-8 (P<0.05). Overall, the combination of EOs and SB produced the most consistent improvement in intestinal barrier function and antioxidant status. These findings suggest that combined EOs and SB supplementation may serve as an effective nutritional strategy to mitigate the negative effects of LCP diets on gut health in laying hens.

**Keywords:** sustainable; low-crude protein diet; essential oils; sodium butyrate; intestinal integrity

### 196 Effects of an essential oil, a yeast-derived product, and an organic acid versus an antibiotic on production performance and egg quality of late-phase Lohmann laying hens

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This study evaluated the effects of feed additives based on an essential oil (EO), a yeast derivative (YP), and an organic acid (OA) relative to an antibiotic, bacitracin methylene disalicylate (BMD), on the performance and egg quality of laying hens from 52 to 73 weeks of age. A total of 240 Lohmann hens were housed in conventional cages and randomly assigned to one of five dietary treatments (basal diet-CON, BMD, EO, YP, and OA) with 8 replicates of 6 hens per cage. Diets were isocaloric and isonitrogenous, with the tested feed additives included at the levels of 0.30 g/kg EO, 0.75 g/kg YP, 0.60 g/kg OA, and 0.11 g/kg BMD. The experimental period included four periods (6 weeks each, except period 4 with 3 weeks). Body weight gain (BWG), average daily feed intake (ADFI), hen-day egg production (HDEP), egg weight (EW), and feed conversion ratio (FCR) were measured each period. Internal and external egg quality traits were evaluated using representative egg samples. Data were analyzed in SAS using mixed models and Tukey-adjusted comparisons with significance at $P < 0.05$ and tendencies at $0.05 < P < 0.10$. Hens fed OA showed higher BWG in period 3 compared with CON and BMD ($P < 0.05$) and tended to lose less BW over the entire experimental period ($P < 0.10$). BMD reduced ADFI relative to CON in periods 3 and 4 and overall ($P < 0.05$). All treatments showed improved FCR in period 4 compared with period 1 ($P < 0.05$), with a tendency for overall treatment effects ($P < 0.10$). The FCR was improved in BMD compared to CON, specifically in periods 3 and 4 ($P < 0.05$). HEDP and EW did not differ among treatments ($P > 0.05$), although EW increased across periods ($P < 0.001$) with a treatment × period tendency ($P < 0.10$). Specific gravity varied by period, with higher values in period 3 ($P < 0.001$). Haugh units were higher in EO than OA in period 3 ($P < 0.05$), and a treatment × period interaction was observed ($P < 0.05$). Breaking strength showed a significant treatment × period interaction ($P < 0.05$), although only marginal period-specific differences were detected ($P < 0.10$) and no main treatment effect was observed ($P > 0.05$). Yolk color was higher in OA, EO, and YP than in CON and BMD during periods 1–3 and overall ($P < 0.001$), with higher values in period 3. Eggshell thickness, albumen height, and shell weight did not differ among treatments ($P > 0.05$), though all varied across periods ($P < 0.001$). In conclusion, the evaluated feed additives maintained the body weight and improved the Haugh units and yolk color in older laying hens compared to the control and antibiotic groups, highlighting their potential as antibiotic alternatives.

**Keywords:** egg quality; essential oil; laying hens; organic acid; production performance

### 197 The effects of a *Bacillus*-based direct-fed microbial supplementation on Pekin ducks under heat stress challenge

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This study evaluated the effect of supplementing bacitracin methylene disalicylate (BMD), a *Bacillus-based* direct-fed microbial (DFM), or their combination to Pekin ducks on performance and welfare when exposed to heat stress. A complete randomized design was used to allocate 5 dietary treatments, each consisting of 11 replicate floor pens and 25 straight-run Pekin ducks per pen. The treatments were: Control (T1), a basal diet with no DFM, T1 + BMD at 55 ppm (T2), T1 + Novela® ECL (United Animal Health, USA) at $1.84x10^5$ cfu/g of feed (T3) or $3.68x10^5$ cfu/g of feed (T4), and T1 + BMD (55 ppm) + Novela ECL ($1.84x10^5$ cfu/g ) (T5). Feed and water were offered ad libitum. The feeding program consisted of 2 dietary phases (starter 0-14 d; grower 14-36 d). From d 21 to 36, temperatures were raised (32-35 °C for 12 h/d), and ventilation was reduced to induce heat stress on the ducks. Body weight (BW), feed consumption (FC), and mortality corrected feed conversion ratio (FCR) were calculated for d 14 and 35 of age. On d 35, blood was collected from 2 birds/pen for corticosterone (CORT), and heterophil/lymphocyte ratio (H/L) evaluation, and ileal mucosal swabs were collected and applied to FTA cards for nucleic acid preservation. Genomic DNA was extracted from the swabs and tested with a panel of qPCR assays for pathogen-specific markers and virulence genes. Gene data were log-transformed. On d 36, gait and paw scores were evaluated on all remaining ducks in each pen. Data were analyzed using a one-way ANOVA, and Kruskal-Wallis was used for gait and paw scores. Fisher's LSD was used for mean separation (P<0.05). On d 14, no differences were observed (P>0.05) in BW or FCR. After 35 d, T2 (3.38 kg), T4 (3.35 kg), and T5 (3.33 kg) had higher BW (P<0.05) than T1 (3.23 kg). Moreover, FCR was improved (P<0.05) during the grower phase (14-35 d) and cumulatively (0-35 d) for all treatments (T2-T5) when compared to the control (T1). There were no differences (P>0.05) in FC and mortality throughout the trial. CORT and H/L ratios decreased (P<0.05) in

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treatments T3, T4, and T5 compared with T1. Pathogen gene quantities, including avian pathogenic *E. coli* (*hlyF* P=0.07, *iroN* P=0.09 genes), were higher in T2 compared to the control (T1). *C. perfringens* alpha toxin (*cpa*) levels unexpectedly showed the highest numerical trend in the T2 group, despite the anticipated suppressive effect. There were no differences in paw and gait scores (P>0.05). In conclusion, these results indicate that the *Bacillus*-based DFM can improve Pekin duck performance and increase the welfare of the ducks experiencing heat stressors compared to the no DFM control and yielded similar results to the antibiotic BMD fed alone.

**Keywords:** Duck; Direct-fed microbial; Heat Stress; Performance; BMD

## Student Competition: Management and Production

### 203 (B-D) (MTCC)

### 149 Evaluation of EPA ammonia emission estimating method under contemporary broiler management

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Ammonia emissions from broiler production negatively affect bird health and productivity while contributing to environmental pollution. To standardize emission estimation, the U.S. Environmental Protection Agency (EPA) released ammonia emission estimating methods (EEM) for broilers in 2024 based on data from the National Air Emission Monitoring Study (NAEMS) collected between 2006 and 2009 at three commercial sites in California and Kentucky. However, these models may not fully reflect modern broiler production practices, including widespread litter amendment use, shorter grow-out periods, and contemporary genetic selection, which influence final bird weight, stocking density, litter nitrogen content, and ammonia emissions. Ammonia emissions were measured from two commercial broiler houses across 40 flocks raised between 2017 and 2025 in the northeastern United States under varying stocking densities and litter amendment programs. The objective of this study was to evaluate the accuracy of EPA ammonia EEM predictions by comparing model estimates with measured emissions under modern production conditions. Daily ammonia emission rates and cumulative emissions at the end of each production cycle were used for comparison. Measured emissions were compared with EPA-predicted values, and the effects of stocking density and litter amendment were analyzed using ANOVA. Emission rates at 49 days of age were evaluated across three stocking densities (0.065, 0.082, and 0.098 $m^2$/bird) and three litter amendment application rates (0, 500–1000 g/$m^2$, and >1380 g/$m^2$ sodium bisulfate equivalent). Stocking density did not significantly affect emission rates (P = 0.164), whereas litter amendment significantly influenced discrepancies between predicted and measured emissions (P = 0.0175). EPA estimates increasingly overpredicted emissions as amendment rates increased, with deviations of 34.9% at 500–1000 g/$m^2$ and 43.1% at 1380 g/$m^2$. In contrast, measured cumulative emissions showed significant effects of stocking density within amendment categories (P < 0.05), whereas density effects were not detected in EPA model predictions (P ≥ 0.165). These findings indicate that current EPA ammonia EEM may not adequately represent management-dependent variability in ammonia emissions under contemporary broiler production conditions.

**Keywords:** ammonia; emissions; method; broiler; comparison

### 150 Evaluation of *Miscanthusx giganteus* as an Alternative Bedding Material in Commercial Broiler Production

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*Miscanthus × giganteus*, a perennial bioenergy crop, has potential as an alternative bedding material for broiler production because of its favorable physical characteristics, including low initial moisture content, low bulk density, and high moisture-holding capacity. It can be produced on marginal land with relatively low irrigation and fertilizer requirements and can absorb excess nutrients, which may provide environmental benefits. In addition, the use of sterile *Miscanthus* hybrids reduces concerns about invasiveness. A commercial field study was conducted to compare chopped *Miscanthus × giganteus* bedding with conventional pine shavings litter in broiler production. Four commercial broiler houses (550 × 60 ft) were monitored over three consecutive flocks in a completely randomized design, with two houses assigned to pine shavings and two houses assigned to Miscanthus bedding. Each house was considered the experimental unit, and repeated measurements were collected across flocks. Ammonia concentration and emission were monitored continuously during each flock, and greenhouse gas emissions were measured biweekly using flux chamber methods. Broiler performance measurements included body weight, mortality, and footpad score. A two-week windrowing practice was applied between flocks. Data were analyzed using a mixed-model analysis of variance, with bedding type as a fixed effect and flock as a repeated measure; significance was declared at $P < 0.05$. No significant differences were observed between pine shavings and Miscanthus bedding for ammonia emissions (36.9 vs. 39.6 g/bird; $P = 0.54$), greenhouse gas emissions (25.2 vs. 23.3 g/bird; $P = 0.17$), or broiler performance variables ($P = 0.30$) during the first three flocks. These results suggest that *Miscanthus × giganteus* is a promising alternative bedding material for commercial broiler production without negatively affecting environmental emissions or bird performance. Continued monitoring for up to two years without total clean-out will provide additional information on its long-term performance under commercial conditions.

**Keywords:** Miscanthus; Bedding; Broier; Emission; Production

### 151 Laser environmental enrichment reduced incidence of woody breast and white striping without negatively impacting bone quality in Ross 708 broilers

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Genetic selection for rapid growth and increased breast yield has improved modern broiler bird efficiency, but it has also introduced myopathies affecting the pectoralis major muscle, such as woody breast (WB) and white striping (WS). Environmental enrichment strategies that encourage bird activity may support skeletal health and influence breast muscle quality. Previous research using 6-min laser enrichment exposures four times daily increased breast width and fillet weight and reduced the WS incidence in 42-day-old Ross 708 broilers. This study evaluated the effects of increasing laser enrichment to six 6-min exposures per day on broiler breast muscle yield, and meat and bone quality. Day-old Ross 708 chicks (n=1,360) were randomly assigned to pens in one of two treatment rooms: control (no enrichment) or laser enrichment provided from placement through a 7-wk grow-out period (20 pens/trt). Laser enrichment was deployed for 6-min laser periods, occurring six times daily for 49 days. On d49, 70 broilers were euthanized to assess breast weight (kg) and width (cm), WB and WS scores (normal, moderate, severe), and bone mineral content (g) and density (g/$cm^2$) using dual X-ray absorptiometry (DXA). Compression force using an Instron machine was measured on fresh, whole breast fillets 1d post-mortem. WB, WS and bone mineral content and density data were analyzed using the MIXED procedure (SAS 9.4),

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with significance denoted at P≤0.05. WB and WS severity were evaluated using the likelihood-ratio chi-square test and reported as percent distributions. There were no differences in breast width, compression force, bone mineral content, or bone mineral density (P≥0.05). Breast weight was 0.07 kg heavier in control fillets than fillets from laser-enriched birds on d49 (P=0.01). WB and WS prevalence were more severe in control than laser-enriched birds on d49 (P=0.006 and P=0.004, respectively), where 23.3% of control vs. 43.3% of laser enriched WB scores were normal, 36.7% of control vs. 50.0% of laser scored moderate, and 40.0% of control vs. 6.7% of laser WB scores were severe. For WS at d49, 26.7% of control vs. 51.4% of laser enriched scored normal, 40.0% of control vs. 45.7% of laser were moderate, and 33.3% of control vs. 2.9% of laser scored severe. Increasing the 6-min laser setting to 6 daily exposures did not impact bone quality measurements but significantly reduced WB and WS incidence at d49. Therefore, a 6-min laser setting with 6 daily exposures may be a practical management strategy to mitigate breast muscle myopathies in broiler production while maintaining product quality.

**Keywords:** broiler; environmental enrichment; meat quality; white striping; woody breast

### 152 Stepwise crude protein reduction with exogenous multicomponent protease supplementation improves broiler efficiency and environmental sustainability

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As concerns rise over the environmental impact of feed production, poultry nutrition has shifted toward strategies that balance performance with sustainability. This study assessed how stepwise crude protein (CP) reductions, combined with a bacterial protease (Jefo Nutrition Inc.), influenced broiler performance and environmental impact. A total of 720 one-day old male broiler chicks (Ross 308) were used in a completely randomized design and assigned to 1 of 5 treatments: a positive control (PC), two negative controls with 1.5 (NC1) and 3.0-point % (NC2) CP reduction, and both NC diets supplemented with protease. Each treatment had 12 replicates over a 42-day trial. Diets were formulated under a 3-phase feeding program: starter (d1-10), grower (d11–24) and finisher (d25–42), with initial CP levels of 23%, 21% and 19.5%, respectively. Growth performance responses, including, daily weight gain, daily feed intake, and feed conversion ratio (FCR), were collected and used as inputs into an attributional life cycle assessment (LCA) following the Livestock Environmental Assessment and Performance guidelines for feed additive and poultry supply chains. The LCA quantified greenhouse gas emissions (GHGEs) and eutrophication, using ReCiPe 2016 Midpoint (H) method. The results showed that protease supplementation effectively mitigated the negative effects of CP reduction by significantly improving growth performance compared with non-supplemented group ($p$<0.008), resulting in FCR values comparable to birds fed PC diets ($p$<0.05; FCR = 1.487 and 1.499 vs 1.501). One kg of PC diet per feeding phase was linked to 0.69–0.97 kg CO2-eq of GHGEs and 1.66–2.08 g $PO4^{3-}$-eq of eutrophication. However, a 1.5- and 3.0-point % reduction in CP, with protease supplementation, resulted in 10.4–16.9% and 8.4–20.3% decreases in GHGEs and eutrophication. Per kg of live weight, birds fed PC, NC1, and NC2 diets were associated with 2.03–2.18 kg CO2-eq and 2.92–3.28 g $PO4^{3-}$-eq, whereas birds fed with protease-supplemented NC diets resulted in 1.97–1.99 kg CO2-eq and 2.82–2.92 g $PO4^{3-}$-eq, representing 8.5–9.5% and 11.0–14.2% reductions, respectively. These decreases in environmental impact were largely driven by reductions in soybean meal (40–86 kg/t), soybean oil (4.3–10 kg/t), and lower farm emission intensities such as nitrogen and phosphorus excretion by up to 21.4%, relative to birds fed with PC or NC diets. An uncertainty analysis using 1,000 Monte Carlo simulations confirmed statistically significant difference across all impacts and treatments(p<0.05). Overall, the results demonstrate that dietary CP levels can be reduced without compromising performance when protease is included, supporting both productivity and sustainability.

**Keywords:** Crude Protein Reductions; Multicomponent Protease; Feed Conversion Ratio; Environmental life cycle assessment; Sustainable Production

### 153 IndigoLT altered commercial broiler litter microbiome structure over consecutive growouts and reduced *Enterococcus*-associated signatures

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Poultry litter is a major microbial reservoir in broiler production. However, the extent to which biological litter amendments reshape litter microbial ecology under commercial conditions remains unclear. This study evaluated the effects of IndigoLT on changes in the litter microbiome and physicochemical characteristics in a commercial broiler operation. Six houses were followed across consecutive growouts. Three received IndigoLT plus half-rate sodium bisulfate, and three control houses received full-rate sodium bisulfate. Litter was collected from five positions per house at flock removal during two consecutive growouts and analyzed by full-length 16S rRNA gene sequencing via Oxford Nanopore Technologies GridION. Barcode trimming and basecalling were performed on the GridION via MinKNOW and Dorado, with taxonomic classification performed via the Epi2Me Labs 16S workflow. All subsequent statistical analyses were based on a phyloseq object generated with the *phyloseq* R package in *RStudio*. IndigoLT-treated litter in growout 2 showed greater Faith's phylogenetic diversity and observed richness than controls (both p = 0.02). Beta-diversity analyses also showed strong growout effects on community structure (all $p = 1.0 \times 10^{-4}$). Treatment significantly affected the dissimilarity between microbial communities in the collective dataset (unweighted UniFrac, $p = 8.8 \times 10^{-3}$) and within growout 2 (Bray-Curtis, p = 0.006; unweighted UniFrac, p = 0.002). Differential abundance analysis identified 68 species and 22 genera associated with treatment (q < 0.05), including lowered *Enterococcus cecorum* in growout 2 (log2FC < -5), *E. hirae* in the collective dataset (log2FC < -3), and *Enterococcus* at the genus level in growout 2 (log2FC < -4.5). These results indicate that IndigoLT progressively reshaped litter microbial ecology over repeated growouts and was associated with reduced poultry-associated *Enterococcus* signatures under commercial broiler production conditions.

**Keywords:** Broiler Litter; Litter Amendment; Microbiome; Enterococcus; Commerical Poultry Production

### 154 Improving the Utilization of Dirty Eggs in Broiler Breeder Flocks Using Egg Washing

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The production of floor and dirty eggs is a common challenge in the poultry industry, as these eggs often carry higher bacterial loads and tend to have lower hatchability compared to clean nest eggs. While sanitization

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can help reduce contamination, there are concerns that washing may damage the eggshell cuticle, potentially affecting embryo development and chick quality. Additionally, limited information is available on the effects of automated egg washing on post-hatch performance. In broiler breeder production, declining fertility and a reduced supply of hatching eggs further increase the need to utilize all available eggs. Therefore, this study evaluated the effects of washing dirty eggs on hatchability and chick performance. A total of 1,620 eggs were collected from a commercial flock at 53 weeks of age and assigned to three groups: clean nest eggs (n = 540), dirty unwashed eggs (n = 540), and dirty washed eggs (n = 540). Washed eggs were processed using an automated washing system (Mach-C washing system - Mach-C Solutions, The Netherlands) with a chlorinated disinfectant for 8 minutes. Eggs were incubated under identical conditions. After hatch, chicks were reared in battery cages with 6 replicate pens per treatment (16 birds per pen) and evaluated for growth performance. Data were analyzed using JMP Pro 19.1, with significance declared at $P \leq 0.05$. Treatment significantly affected incubation outcomes. Hatchability was higher in nest eggs compared to washed and dirty eggs (56.30% vs. 49.63% and 47.04%, respectively; $P = 0.0009$), while contamination was lower in nest eggs (0.74%) than in washed and dirty eggs (10.93% and 12.22%; $P < 0.0001$). Early embryonic mortality was not affected ($P = 0.4634$), whereas late mortality tended to differ ($P = 0.0784$), with dirty eggs showing numerically higher late embryonic mortality compared to washed eggs. Although washed and dirty eggs did not differ statistically for some variables, washed eggs consistently showed numerically lower contamination and reduced late mortality. Broiler performance was not affected by treatment for body weight gain or feed intake ($P > 0.05$). However, birds from washed eggs exhibited numerically higher body weight gain across all periods, indicating that the washing process did not negatively affect post-hatch performance. In conclusion, washing dirty eggs improved hygienic quality and showed no negative effects on broiler performance, suggesting that egg washing may be a viable strategy to increase the utilization of floor eggs in broiler breeder operations.

**Keywords:** Egg-washing; Incubation; Hatchability; Dirty eggs

### 155 Producer-led evaluation of cricket and mealworm meals as alternative dietary protein sources in 6-week-old Ross 308 broiler chickens

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Volatility in feed ingredient costs, particularly soybean meal, poses a significant threat to the economic sustainability of poultry production systems. Identifying alternative, locally adaptable protein sources is critical for strengthening poultry systems and maintaining productivity under resource constraints. Our objective was to evaluate the inclusion of cricket meal (CM) and mealworm meal (MM) as sustainable alternative dietary protein sources and quantify effects on broiler growth and feed efficiency in a producer-run trial. Two independent experiments were conducted using Ross 308 broilers assigned to 5 diets: negative control fed on standard corn-soybean diet (NC; no insect meal), CM1 (1.5% CM), CM2 (2.5% CM), MM1 (1.5% MM), and MM2 (2.5% MM). The average external environmental temperature for Experiment 1 during April to June, 2025, was in the range 43.4-90.0°F, and that for Experiment 2 during July to September, 2025, was in the range 70.0-91.0°F. The birds were raised in a producer-run barn with appropriate conditions for bird care and management, with inputs sourced by the producer. Experiment 1 used 200 chicks (n = 40/diet), and Experiment 2 used 170 chicks (n = 34/diet). Birds were reared for 42 days with ad libitum feed and water; body weight (BW) and feed intake were recorded weekly to calculate body weight gain (BWG) and feed conversion ratio (FCR). Data on weekly BW, BWG, and FCR were analyzed by one-way ANOVA with post-hoc mean separation ($A = 0.05$). In Experiment 1, diet affected BW at weeks 1–2 and 5–6 ($P < 0.05$) but not at weeks 0 or 3–4; week 6 BW was 2.446 kg for NC and 2.330–2.596 kg for insect diets. Weekly FCR differed at weeks 5–6 ($P < 0.05$) but not at weeks 1–4; cumulative FCR differed among diets (1.73–1.91; $P < 0.05$). Experiment 2 showed alignment with the industry-standard performance for BW in 6-week-old broilers, indicating that weather conditions could influence producer-run experiments. In Experiment 2, BW differed at weeks 1–6 ($P < 0.05$) but not at week 0; week 6 BW was 3.100 kg for NC and 2.672–3.165 kg for insect diets. Weekly FCR differed at weeks 1–6 ($P < 0.05$); cumulative FCR differed among diets (1.481–1.71; $P < 0.05$). Overall, the producer-led experiments indicated that CM at 1.5% had BW and BWG comparable to NC, but not FCR, aligning with the results of our controlled nutrition experiments.

**Keywords:** broilers; cricket meal; mealworm meal; alternative feed ingredients; sustainability

### 156 Adjusting precision feeding targets reveals clever patterns of intake in broiler breeders

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Broiler breeder management involves substantial feed restriction to achieve the reproductive potential of rapidly growing, high-yielding meat-type chickens. Precision feeding (PF) systems have the potential to be implemented as a management tool in broiler breeder production through individualized feed delivery; however, no research has explored the effects of feeding to different targets. This study comprised 2 treatments to evaluate the effects of feeding to 2 types of targets: feed intake (TFI) and body weight (TBW) with precision-fed broiler breeder pullets between 7 and 154 days of age. The effects of the treatments were determined for body weight (BW), average daily feed intake (ADFI), feed conversion ratio (FCR), and behavioural outcomes: meal size, meal time, meal frequency, visit time, and visit frequency. Additionally, success rate was calculated as the frequency of visits per bird to a station, divided by the number of meals consumed. Day-old pullets (n = 65) were randomly assigned to the two treatments, distributed across 3 pens, and fed standard commercial diets, delivered via PF stations with 24-hour access. The TBW birds were fed according to a target BW updated hourly, while the TFI birds were fed the breeder-recommended feed intake, which was reset daily at midnight. Precision feeding stations recorded the radio frequency identification (RFID), body weight, feed intake, and the date and time of each visit, which allowed behaviour analysis. A two-way ANOVA was used to assess effects, with differences reported at $P \leq 0.05$. TBW and TFI birds did not differ significantly in ADFI ($P=0.53$); however, TFI BW was 1.02 times that of TBW at d 154 ($P < 0.001$). TBW birds visited the PF stations 2.73 times more frequently than the TFI birds ($P < 0.001$), with a lower success rate across the duration of the study (TFI: 17%, TBW: 37%; $P < 0.001$). The TFI birds developed a pattern of consistently consuming meals in the early hours of the day, between 12 am and 6 am, indicating an understanding of the availability of feed. In contrast, TBW birds exhibited a more even distribution of meal times throughout

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the day, which can be attributed to the potential for the birds to be rewarded with meals more irregularly under their feeding strategy. The TBW treatment yielded the highest BW uniformity at 22 weeks of age (CV = 1.1%). These results showed that feeding TFI affected the birds' temporal feeding patterns and caused them to learn when feed would be available and to adjust their behaviour accordingly, whereas the TBW birds did not modify their behaviour due to less predictable availability.

**Keywords:** broiler breeders; precision feeding; feeding strategy; feeding behaviour; smart agriculture

### 157 Canola meal or a pea-lentil mix as protein sources maintained laying performance and hatchability compared with soybean meal in broiler breeder diets

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Imported soybean meal (SBM) is the primary protein source in Australian poultry feed. Locally available protein sources, as alternatives to SBM such as canola meal, peas, or lentils, offer potential to enhance the sustainability of poultry production in SBM import-reliant regions. This study aimed to investigate the complete replacement of SBM with these protein sources in broiler breeder diets. We hypothesised that in well balanced diets, soybean meal could be fully replaced with either canola meal or a mix of peas and lentils without adversely affecting egg production performance and hatchability in broiler breeders. A total of 261 Ross 308 broiler breeders were assigned to four dietary treatments: Corn-Soybean (CS; 71.6% corn, 16.3% SBM), Wheat-Soybean (WS; 74.2% wheat, 9.1% SBM), Wheat-Canola (WC; 64.2% wheat, 11.3% canola meal), and Wheat-Pea-Lentil (WPL; 48.2% wheat, 11.6% pea, 12% lentils) from 30-37 weeks of age. Birds were allocated to 24 pens (nine/ten hens and one rooster per pen) in a randomised block design. Isocaloric (2800 kcal/kg ME), isonitrogenous (15% or 14.5% CP) and iso-essential amino acidic diets were formulated according to Ross 308 breeder guidelines. Egg production and egg weight were recorded daily and body weight weekly. At 35 weeks of age, 1200 eggs were incubated. At trial end, 12 hens per treatment were euthanised for organ weights, and blood was collected for gas and electrolytes analysis. Data were analysed using PROC GLM (SAS 9.4). The results showed that hen-day egg production (%) was 88.9±1.07 for CS, 87.9±1.51 for WS, 87.5±0.97 for WC, and 87.9±2.30 for WPL. Corresponding egg weights were 57.1± 0.25, 58.1±0.43, 58.1±0.40, and 57.5±0.38 g, while egg mass values were 50.8±0.69, 51.1± 0.76, 50.8±0.50, and 50.6±1.38 g/b/d, respectively. None of these parameters were significantly affected by dietary treatment (P>0.05). However, birds fed wheat-based diets (WS: 5.2±0.27; WC: 5.1±0.14; WPL: 5.1±0.15) had greater intestinal weight (%) than CS (4.4±0.11; P<0.01), possibly due to higher non-starch polysaccharide levels. Fertility (CS: 97.6±0.73; WS: 99.3±0.81; WC: 97.9±0.97; WPL: 97.4±1.05%) and hatchability (CS: 87.8±1.92; WS: 86.9±2.11; WC: 88.6±1.74; WPL: 92.4±1.42%) were also not influenced (P>0.05). Similarly, blood gas parameters (pH, pCO2, pO2, Na+, K+, Ca2+, Cl-, glucose, and lactate) were unaffected (P>0.05). In conclusion, canola meal or a pea-lentil mix can effectively replace SBM in broiler breeder diets without affecting egg production, fertility, or hatchability. This supports the use of local protein sources for sustainable Australian chicken meat production. Acknowledgements: The study was supported by the AgriFutures Chicken Meat Consortium (PRO-016111).

**Keywords:** Broiler breeder; alternative protein sources ; egg production; embryonic development; sustainability

### 158 Impact of housing type on broiler breeder body compositon and hen mortality

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Broiler breeder performance is vital to the success of the poultry industry. However, challenges with hen mortality (hMORT) and achieving hatching eggs per hen housed (HE/hh) have become a regular occurrence. Given the physiological stress from reproductive onset, loss of hens during early to peak production can heavily influence hMORT. Although, there has been a shift away from curtain-sided housing (CS) towards solid-sided walls (SS), CS housing still represents a large portion of the industry. Environmental and management differences between these housing types may be contributing to the hMORT and HE/hh. The aim of this study was to evaluate the impact of housing type on early hMORT and HE/hh in commercial flocks. Paired commercial flock data was obtained from a single integrator for both CS (n=12) and SS (n=12), containing hen: body weight (BW), body composition, environmental parameters, production, and blood plasma data from 19-45 wk. All data were analyzed in JMP 19, with $P \leq 0.05$ considered significant. For the statistical analysis, a linear mixed model was used with housing type, age, and their interaction as fixed effects with flock id included as a random effect. Lsmeans were compared with Tukey's HSD or polynomial contrasts. For BW, a significant interaction was observed (P = 0.004). At 19 wk, SS hen BW was numerically higher (+70 g) than CS hens (P>0.05). However, a linear trend in BW gain (P=0.028) was observed resulting in a higher BW for CS hens (+152 g) at 34 wk. For hMORT, no interaction was observed (P=0.623), but main effect differences were observed for housing type (P=0.001) and age (P<0.0001). The CS hens had an average increase in hMORT (+0.19%) over SS hens. No interactions were observed for HE/hh for 26-30, 31-35, 36-40, or 41-45 wk (P≥0.05). For body composition, an interaction for shank length was observed in which the 19 and 24 wk SS shanks were similar, but the CS shanks increased by 4.3mm on average (P=.0006). No differences were observed between housing type (P≥0.05) pelvic spread, abdominal girth, comb height, and feathers scoring, all of which were primarily driven by age main effect (P≤0.05). The study provides data suggesting environmental factors associated with curtain sided housing may likely impact hMORT in turn effect flock performance. While these results provide strong practical data, a future research facility study would provide robust statistical data to support these initial findings.

**Keywords:** Broiler Breeders; Mortality; Housing Type; Production; Body Composition

### 159 Deep neural networks for body weight category classification in 15-week-old pullets using depth images

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Monitoring the body weight of laying hens is essential to ensure egg production capacity. However, current monitoring methods have proven insufficient to meet the needs of the poultry industry, making alternatives necessary to automate and facilitate this process. This study aimed to develop a deep learning model for classifying depth images, using computer vision-based methods, aiming to estimate the body weight category of layer pullets. Two hundred 15-week-old Dekalb White layer-type pullets were

weighed and classified according to the standard weight of the strain, into 3 body weight categories: light, medium, and heavy. Eighteen pullets per category were selected for filming with a depth camera, covering the entire body of the bird, and the captures were subjected to computer vision image pre-processing techniques, applying 16-bit frame extraction, segmentation, and grayscale adjustment of the images. For machine learning classification, the captures were used directly as input data, totaling 19,176 images. These were divided into 3 groups: training, validation and test sets, with 60, 20 and 20% of the samples, respectively. The Convolutional Neural Network (CNN), Visual Geometry Group 16-layer Network (VGG16), Residual Network with 50 layers (ResNet50), and Convolutional Neural Network Next (ConvNeXt) models were tested for: accuracy, precision, recall, F-1 score, receiver operating characteristic area under the curve **(**ROC AUC), specificity, Matthews correlation coefficient (MCC), and Kappa score. The CNNs model showed better performance than other architectures used in accuracy (95.4%), precision (95.5%), recall (95.4%), F1 score (0.954), ROC AUC (0.96), specificity (96.4%), MCC (0.93), and Kappa score (0.93). In contrast, the ResNet50 architecture demonstrated the lowest performance among the evaluated models, with reduced accuracy (42.3%), F1 score (0.30), specificity (17.5%), MCC (0.131), Kappa score (0.074), and ROC AUC (0.534), indicating difficulties in learning discriminative patterns from depth images and correctly classifying into the classes. These results demonstrate that the CNNs were able to successfully estimate the body weight category of layer pullets at 15 weeks of age, using depth images, allowing the extraction of three-dimensional features and demonstrating great potential for practical applications.

**Keywords:** 3D images; artificial intelligence; computer vision; machine learning; segmentation

### 160 Optimizing the sample size for northern fowl mite population assessment in laying hens

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The northern fowl mite (NFM; *Ornithonyssus sylviarum*) is the most prevalent ectoparasite of North American laying hens, negatively affecting productivity and hen welfare. Monitoring NFM infestations currently involves manually checking laying hens for mites; however, the labor intensity required to accurately count mites makes monitoring difficult. The heterogeneity of NFM infestation creates large variation in the number of mites on hens within a flock, further complicating monitoring efforts. To streamline the process, we sought to quantify how many hens should be assessed to reflect the actualized NFM population within our experimental setup. Purebred white laying hens (replicate 1: n=393, replicate 2: n=600, total: n=993) were randomly assigned to floor pens (n=40 hens/pen, 5 pens/room). At week (wk) 0 of infestation (hens 24 wk old), all hens were infested with 50 NFM applied directly to the vent. At 2, 6, and 16-17 wk post-infestation (hens 26, 30, and 40-41 wk old, respectively), all hens were assessed for mites within a 4x6 cm area on the vent. Mite score was estimated using a published scale: 0 (0 mites), 1 (1-10), 2 (11-50), 3 (51-100), 4 (101-500), 5 (501-1,000), 6 (1,001-10,000), and 7 (>10,000; none observed in this study). Descriptive statistics were generated using PROC UNIVARIATE (SAS 9.4). The NLIN procedure was used to fit a broken line regression at 2 wk due to low variation in mite scores; at all other ages PROC NLMIXED was applied with random effects (replicate, room, pen). Slope and breakpoint estimates were generated with PROC GLM. Random effect variances were estimated using PROC MIXED. Ultimately, broken line regression was used to find the optimal sample size. At 2 wk post-infestation, mite scores ranged from 0-6 (median=1) with an optimal sample size of 5 hens/pen (12.5%; 95% CI: 2.6-6.6). During peak infestation (6 wk post-infestation), mite scores ranged from 1-6 (median=3) with optimal n=25 hens/pen (62.5%; 95% CI: 23.3-26.3). During declining infestation at 16-17 wk, mite scores ranged from 0-5 (median=1); optimal n=7 hens/pen (17.5%; 95% CI: 6.3-6.8). Across all ages, mite scores ranged from 0-6 (median=1); optimal n=8 hens/pen (20%; 95% CI: 4.0-12.8). When mite scores were most variable at 6 wk post-infestation, the model indicated that more hens needed to be assessed to capture the variation. When mite population was more uniform at 2 and 16-17 wk post-infestation, fewer hens were needed. To conclude, the variability of NFM infestation poses a challenge for NFM monitoring and broken line regression can provide a starting point for NFM monitoring protocols, though the optimal number of hens assessed is heavily dependent on how long the infestation has been present for.

**Keywords:** ectoparasite; northern fowl mite; laying hen ; sample size; monitoring

### 161 The effect of light wavelength on the behavior and health of Nicholas Select infrared beak-treated and sham-beak-treated turkey hens reared to 11 weeks

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The study evaluated the effects of light wavelength (L) and beak treatment (BT) on behavior, health, and welfare of Nicholas Select hens reared to 11 wk. Hens (n=2916 per trial; 2 trials) were housed in 9 rooms per trial (3 per L), each divided into 2 pens for infrared-treated (IR); or sham-treated (S) birds. Birds were reared under blue (B; 455 nm peak), green (G; 510 nm peak), or white (W) light and provided a commercial diet ad libitum. The 3×2 factorial experiment was analyzed as a RCBD (trial as block) using an ANOVA ($P \leq 0.05$). Data were checked for normality and log transformed when needed. Health responses varied with L. Gait score did not differ at 8 or 11 wk. A higher incidence of severe footpad lesions (>50% necrotic area) was observed under B at 8 wk ($P=0.04$). Feather cover (FC) was affected by L and BT. Poorer tail FC was observed under W ($P=0.04$) and poorer breast FC under B ($P<0.01$) at 8 wk. IR improved neck FC at 8 ($P=0.02$) and 11 ($P=0.01$) wk. Birds under B were dirtier overall ($P<0.01$), while IR birds were cleaner compared to S at 11 wk ($P=0.05$). Ocular measures differed with L, with heavier eyes ($P<0.01$) and larger mediolateral diameter ($P<0.01$) under B. Aggressive damage (AD; including culls) was absent under B from 0-11 wk. While B and G did not differ, W resulted in the highest AD ($P<0.01$). A BT x L interaction was found for wing damage (WD; $P<0.01$). Within BT, W was the highest with no differences between G and B. Under G or W, IR reduced WD, with no difference for B. Stress (heterophil-to-lymphocyte ratio) was lower under B than W, while G was intermediate at 11 wk ($P=0.02$). Behavioral time budgets were affected by L and BT at 3 and 8 wk. At 3 wk, perching ($P<0.01$) was higher under G than B or W, while aggressive pecking was higher under G than B, with G being intermediate ($P=0.02$). IR birds walked ($P=0.03$) and performed nutritive ($P<0.01$) behaviors more, whereas S birds rested ($P=0.05$) more and showed higher AD ($P=0.02$). At 8 wk, birds under B walked less compared to W, while G was intermediate ($P=0.03$) and rested more ($P<0.01$). Exploratory behavior was higher under G than B, with no differences to W ($P<0.01$). A novel abnormal behavior (cervical retroflexion) was observed only under B. Average litter quality was worse under B than W, while G was intermediate at 8 wk ($P=0.02$). Overall, B eliminated AD and reduced physiological stress compared to W but raised welfare concerns including increased footpad lesions, poorer feather cleanliness and FC, enlarged ocular measures, reduced exploration compared to G, and abnormal behavior

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emerged. W resulted in the greatest AD. IR improved neck FC and reduced aggression. L had notable and mixed effects on turkey hen welfare.

**Keywords:** Light wavelength; Beak treatment; Turkey hen welfare; Aggression and behavior; Physiological and health indicators

### 162 Characterizing Non-Infectious Finisher Mortality: Age of Onset and Patterns in Commercial Turkey Flocks

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This project was a continuation of a project examining brooder non-infectious mortality causes, onset, and patterns of mortality in which characterization of onset and patterns of non-infectious causes of mortality in the finisher were determined. To complete this objective, mortality data were used from 2 flocks of 3,072 and 1 flock of 2,196 male poults sourced from a single hatchery that did not experience high mortality (>5%) due to an infectious disease. Poults were placed in research pens at similar commercial stocking densities between 2 facilities. Flocks were reared using similar commercial standards and feed and water were provided *ad libitum*. The three flocks in this data set entered the finisher with 96.09%, 96.78%, and 90.03% livability, respectively. Daily mortality and cause of mortality was determined and recorded from 6 wk through 20 wk for each finisher flock. Ending livability was 87.92%, 85.68%, and 79.23%, respectively. Mortality was categorized as either: spontaneous cardiomyopathy (RH), enteric disease (ENT), leg issues (LEG, e.g. injured leg), infections (INFT), cannibalism (CAN), dehydration (DH), pendulous crop (PC), heart attack (HA), unknown lesions (NVL), and miscellaneous (MISC; e.g. accidental death). Data were analyzed for differences in causes of mortality in R for age of onset using the lmer package and frequency of onset using the chi.squared package. Age and cause of mortality were fit as fixed effects with flock fit as a random effect. Pairwise comparisons were determined using the emm package in R. Age of onset was different based on the cause of mortality ($P < 0.001$). Causes of mortality causes clustered into early average age of onset and late age of onset. Early mortality was associated with DH, RH, and PC ($P < 0.05$) with the average onset for DH at 9.72 wk, RH at 11.19 wk and PC 12.13 wk. Late mortality was associated with INFT and LEG ($P < 0.05$) with the average onset for INFT at 14.85 wk and LEG at 14.86 wk. The frequency of mortality was different by mortality category ($P < 0.01$). Of the causes of mortality, CAN and MISC had the highest mortality percent with 23.86% and 10.56% of the mortality percentage, respectively. PC, INFT, and LEG, ranged between 5.55% - 4.49% of the mortality percentage and all other mortality percentages were less than 3.0% of the mortality percentage. In conclusion, this research will allow growers assess their brooder flocks for abnormal in non-infectious causes of mortalities which will allow for ways to be identified to address these issues. However, additional work is needed to expand this data set to include more flocks and grower data.

**Keywords:** tom; non-infectious; finisher; mortality; grower

## Student Competition: Metabolism and Nutrition - Amino Acids II

**201 (A-F) (MTCC)**

### 163 Arginine silicate inositol and guanidinoacetic acid improve meat quality in market weight broilers

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Arginine silicate inositol (ASI) and guanidinoacetic acid (GAA) are vasodilatory compounds that may independently improve meat quality in broilers. Potential mechanisms include improved systemic blood flow and nitric oxide production through ASI, while GAA may support energy metabolism and meat yield through being a metabolic pathway precursor to creatinine. To determine the impact of these compounds on broiler meat quality through 49d of age, a phase-specific basal diet was formulated to be 99.85% complete, and three experimental diets were then produced: 1) control (CON) basal + 0.15% sand, 2) basal + 0.15% ASI, and 3) basal + 0.12% GAA + 0.03% sand. 1,560 one-day old Ross 708 broilers were randomly assigned to 39 4ft x 8ft floor pens containing 40 birds each. Pen weight and feed intake were measured at the start and end of each dietary phase (starter, grower, finisher 1, finisher 2) with mortality recorded daily and used to calculate average daily gain and feed conversion ratio. On d 49, twenty birds per treatment were euthanized for bone mineral content, bone mineral density, bone breaking strength, breast yield, woody breast, white striping, spaghetti meat, water holding capacity, marinade uptake, compression force, and pH. All data were analyzed with SAS 9.4 with significance set at $P \leq 0.05$; continuous data was analyzed with a one-way ANOVA and categorical data utilized Fisher's Exact Test. GAA significantly lowered starter phase FCR by 1.5 points compared to CON (P=0.0405). There were no other significant differences in performance throughout the study. The prevalence of spaghetti meat at d49 was significantly reduced in both ASI and GAA diets compared to CON (P = 0.0122), where CON had 50%, ASI had 25%, and GAA had 9.52% occurrence of spaghetti meat. Birds fed ASI diets had numerically lower woody breast compared to CON with 25 and 45%, respectively (P = 0.6162). Birds supplemented with ASI and GAA had no incidence of severe white striping compared to CON at 9% (P = 0.2031). There were no significant differences in other meat quality characteristics at d 49. Overall, ASI and GAA supplementation decreased the prevalence of breast muscle myopathies without negatively affecting performance.

**Keywords:** amino acid; muscle myopathy; vasodilator; woody breast

### 164 Practical assessment of dietary arginine needs of broilers

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Soybean meal (SBM) is the primary protein source in USA poultry diets and provides a substantial portion of the arginine (Arg) required for optimal broiler growth and metabolism. Consequently, titrating Arg levels through SBM inclusion can help determine optimal dietary Arg supply and its impact on growth performance and nutrient utilization in broilers. Therefore, two experiments were conducted to assess broiler responses to graded levels of dietary Arg met using different Arg sources. Experiments (Exp) I and II each utilized 96 floor pens (12 treatments; 8 replicate pens) containing 11 and 10 male Ross 708 broilers, respectively. Experimental diets were offered from 14 to 35 d in Exp I and 28 to 44 d in Exp II following common feeding periods. Dietary treatments were allotted to pens following a 6 × 2 factorial arrangement within a randomized complete block in both experiments. Dietary treatments were created by supplementing either a combination of SBM and L-Arg or L-Arg to Arg deficient grower (Exp I; 3,075 kcal/kg AME, 1.15% digestible Lys) or finisher (Exp II; 3,100 kcal/kg AME, 1.05% digestible Lys) diets at the expense of an inert filler to meet digestible Arg levels of 1.05, 1.12, 1.19, 1.26, 1.33, and 1.40%. Data were subjected to a full factorial analysis and linear and quadratic regression using JMP Pro 18 software, and significance was considered at $P \leq 0.05$. In Exp1, BW gain and FCR were improved in birds fed diets that supplemented Arg via SBM in comparison to diets supplemented with Arg using L-Arg regardless of Arg level (P<0.05). Carcass characteristics were not evaluated in Exp1. In Exp2, FI and fat yield decreased, and leg yield increased linearly with additional Arg supplementation regardless of Arg source. Birds fed diets with supplemented feed grade L-Arg had improved wing yield over diets that supplemented Arg via SBM (P<0.05). Birds fed diets that supplemented Arg via L-Arg have increased wing yield compared to bird fed diets supplemented Arg via SBM regardless of Arg level (P<0.05). Leg yield increased linearly with the addition of Arg supplemen-

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tation. However, the magnitude of the response was greater in birds supplemented with Arg via L-Arg, indicating an interaction between Arg level and Arg source (P<0.05). In conclusion, exp1 demonstrated that younger birds showed improved live performance measurements when fed diets supplemented with Arg via SBM. Conversely, Exp2 demonstrated that older birds had improved processing characteristics with additional Arg supplementation regardless of source except for leg yield, in which L-Arg had marginal improvements in compared to supplementation via SBM.

**Keywords:** arginine; soybean meal; broiler; nutrition; amino acids

### 165 Interaction between low crude protein and raised without antibiotics programs on nutrient utilization, plasma biochemistry, body composition, cecal digesta SCFA, and litter quality in broiler chicken

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This study evaluated the interactions between dietary crude protein (CP) and the raised without antibiotics (RWA) program on apparent nutrient retention, nitrogen balance, body composition, plasma biochemistry, cecal short-chain fatty acids (SCFA), and litter quality in broiler chickens. A total of 1,200 Ross 708-day-old male broilers were placed in floor pens (25 birds/pen). A standard broiler CP (SCP) or low CP (LCP; 4% points CP reduction across phases) was fed without (RWA) or with antimicrobial growth promoters (AGP), creating a 2 × 2 factorial arrangement. Diets were formulated to be isocaloric and to maintain similar glycine equivalence, with feed-grade amino acids (Lys, Met, Thr, Val, Arg, Ile, Leu, Trp, and Gly) used to meet minimum specifications as CP was reduced. Feeding phases included starter (days 1-10), grower (days 11-21), and finisher (days 22-35). The AGP group received BMD and ionophore, whereas the RWA group received a blend of short- and medium-chain fatty acids, organic acids, and a polyphenol plant extract, along with a coccidia vaccine. The treatments were allocated in a completely randomized design (n=12/treatment). Apparent nutrient retention (AR), nitrogen balance, plasma biochemistry, cecal SCFA, litter characteristics, and whole body composition were evaluated on day 35. The data were analyzed using generalized linear mixed models with fixed factors for CP, gut health programs, and their interactions. Over the 35 days, reducing CP by 4% point resulted in declined BWG and increased FCR (P < 0.001) in both gut health programs. Additionally, reducing CP increased AR of CP by 13% and AME by 3% while increasing N retention efficiency by 17% in both gut health programs (P < 0.001). Plasma Thr levels increased by 43% in broilers fed LCP diets in both gut health programs (P < 0.001). LCP diets reduced plasma uric acid concentrations and litter ammonium N levels, indicating improved nitrogen utilization and reduced nitrogen excretion (P < 0.001). The RWA-based gut health program tended to increase acetic acid (P=0.065), propionic acid (P=0.059), and total SCFA (P=0.085) concentrations at both CP levels. However, feeding LCP diets increased whole body fat deposition by 14% and reduced lean tissue accretion by the same magnitude in both gut health programs (P < 0.001). These results demonstrate that a 4% points reduction in dietary CP enhances nutrient and nitrogen utilization and improves litter nitrogen outcomes in broilers, including those under the RWA program. The changes in body composition highlight the need for careful optimization of amino acids and energy when implementing low-protein feeding strategies in antibiotic-free production systems.

**Keywords:** Broilers; Low crude protein ; Raised without antibiotics; Nitrogen utilization; Body composition

### 166 Early phase fortification of key amino acids supports broiler growth performance following later phase introduction of low soybean meal diets

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This study investigated whether early fortification of key amino acids (AA) in starter diets can provide a nutritional foundation that supports broiler performance following the introduction of low soybean meal (LSBM) diets in subsequent feeding phases. The LSBM diets were formulated by replacing SBM with alternative protein sources such as canola meal, canola seed, field peas and non-bound AA. A total of 336 day-old, off-sex male (Ross 308) broilers were randomly allocated to seven dietary treatments (8 replicates of 6 birds each) and fed the starter (0-14 d), grower (14-24 d), and finisher (24-35 d) diets. The control (CON) diets were wheat-SBM based and formulated to meet Aviagen nutrient specifications (2022). For starters phase, three diets were included: 1) CON, 2) CON+ 10% higher Lys, Met, and Thr (LMT), 3) CON + 10% higher Lys, TSAA, Thr, Val, Ile, Arg, Trp, and Leu (EAA). For grower phase, two diets were used either CON or LSBM diet with 50% reduction in SBM. For finisher phase, birds were fed either CON or LSBM diet with 100% SBM replacement. Based on these diets, seven feeding regimes for starter-grower-finisher were set as: T1 (CON-CON-CON); T2 (LMT-CON-CON); T3 (CON-LSBM-LSBM); T4 (LMT-CON-LSBM); T5(LMT-LSBM-LSBM); T6 (EAA-CON-LSBM); T7 (EAA-LSBM-LSBM). Growth performance and apparent metabolizable energy (AME; 32-34 d) were determined. Data were analyzed using one-way ANOVA with post-hoc pairwise comparisons adjusted for false discovery rate. During the starter phase, LMT and EAA diets significantly improved FCR by 2.7 and 3.9 points, respectively, compared to the CON (1.111 and 1.099 vs. 1.138, $P < 0.001$). Over the grower phase birds fed LMT or EAA starter diets followed by the LSBM grower diet exhibited improved FCR relative to birds fed the CON starter and grower diets (1.207 and 1.208 vs.1.235, $P = 0.002$). Over the entire production period, birds fed T5 showed lower FCR than those in T1 and T6 (1.339 vs. 1.389 and 1.392, $P = 0.005$). No significant difference in final BW was detected among treatments ($P = 0.365$). The highest AME (13.52 MJ/kg; $P = 0.003$) and AME: gross energy ratio (0.81; $P < 0.001$) was recorded for the birds fed the T5 diets. Both T5 and T7 achieved the highest nitrogen-corrected AME values (11.36–11.39 MJ/kg; $P = 0.014$). In conclusion, compared to birds fed a conventional diet throughout all phases, early fortification of Lys, Met and Thr provided a stronger nutritional foundation that helped sustain growth performance and improve energy utilization when LSBM diets were introduced in the later phases. Acknowledgements: The study was supported by the AgriFutures Chicken Meat Consortium (PRO-016111).

**Keywords:** Amino acid density; soybean meal reduction; broiler nutrition; growth performance; energy utilization

### 167 Interactive effects of yeast supplementation and amino acid density in the finisher phase on growth performance and organ weight in broiler chickens

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Optimization of amino acid density (AAd) and functional feed additives, such as yeast, can improve growth performance and carcass yield in broiler chickens. This study evaluated the effects of dietary yeast supplementation and finisher AAd on growth performance and organ weights in broilers. A total of 800 Ross 708 male broilers were allocated to 32

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pens (25 birds/pen; 8 replicate pens per treatment). Diets were formulated with two yeast supplementation strategies: no yeast or 0.2% yeast throughout all feeding phases. Starter (d 0–10) and grower (d 11–24) diets were formulated to meet recommended AAd specifications. For the finisher phase (d 25–39), birds from each yeast strategy were equally assigned to either a standard AAd diet (19.5% CP; SID Lys 1.04%) or a high AAd diet (21.5% CP; SID Lys 1.16%), resulting in a 2 × 2 factorial arrangement of treatments. All indispensable amino acids were maintained in fixed ratios relative to SID Lys; Birds had *ad libitum* access to feed and water. Body weight (BW), feed intake (FI), and mortality were recorded to calculate body weight gain (BWG) and feed conversion ratio (FCR). At d 39, organ weights were evaluated on 2 birds per pen. Data were analyzed using PROC GLIMMIX (SAS 9.4) with yeast, AAd, and their interactions as fixed effects and pen as the experimental unit. Initial body weight at d 25 (IBW) was included as a fixed covariate for BWG, FI, and FCR in the finisher phase. During the starter and grower phases, yeast supplementation improved BWG (1,502 vs. 1,448 g/b; P = 0.018) and FCR (1.285 vs. 1.321; P = 0.009) compared with the control diet, with no effect on FI (P > 0.05). During the finisher phase, yeast supplementation did not influence growth performance variables (P > 0.05), and no interactions between AAd level and yeast dose were observed. In contrast, birds fed the high AAd diet exhibited reduced FCR compared with those fed the regular AAd diet (1.597 vs. 1.694; P < 0.001), whereas BWG, FI, and body weights were not affected (P > 0.05). Yeast supplementation had no main effects on carcass traits or organ weights (P > 0.05), and no AAd × yeast interactions were detected. Additionally, dietary AAd level did not affect breast, thigh, or liver weights (P > 0.05), but birds fed the high AAd diet had lower abdominal fat compared with those fed the regular AAd diet (0.916 vs. 1.141%; P = 0.002). In conclusion, dietary yeast supplementation improved growth performance during the combined starter-grower phase but did not affect finisher performance, carcass traits, or organ weights. Increasing AAd in the finisher diet improved feed conversion ratio and reduced abdominal fat deposition without affecting body weight, breast yield, or thigh yield.

**Keywords:** Yeast supplementation; Broiler chickens; Reduced amino acid density; Reduced crude protein; Growth performance

### 168 Soybean meal inclusion rate and arginine supplementation on growth performance, body composition and carcass characteristics in broilers under cyclic heat stress

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A study was conducted to evaluate the effect of soybean meal (SBM) inclusion rates and arginine (Arg) supplementation on growth performance, body composition and carcass traits in broilers under cyclic heat stress. A total of 1,920 one-day-old male Cobb500 broiler chicks were randomly allocated to 96 pens and were fed a common starter diet from d0 to d14. On d14, birds were re-allocated to two rooms, with 6 treatments and 8 replicates each: Thermoneutral (TN) at 75°F and cyclic heat stress (HS) at 93°F for 8 h daily from d14 to d42. Treatments followed a completely randomised design with a 3x2x2 factorial arrangement with three levels of crude protein (CP)/SBM: low (17/16%), medium (20/19%), and high (23/22%); Two Arg levels (100% and 130% of recommended levels); and two environmental temperatures (TN and HS). Amino acid (AA) density was adjusted for up to 9 AAs relative to medium CP levels with a constant dArg:Lys ratio of 108%. The birds were fed experimental diets in two phases: grower (14-28d) and finisher (28-42d). Growth performance was measured weekly from d14 to d42. Body composition was measured using Dual Energy X-ray Absorptiometry (DEXA) on d21, d28 and d42. The birds were slaughtered, and carcass traits were evaluated on d43. The data were analysed using a three-way full factorial ANOVA, and the means were separated by Tukey's HSD (A = 0.05). Growth performance results showed no interaction between the three factors. Increasing CP levels significantly improved body weight gain (BWG) ($P < 0.05$) and better feed efficiency ($P < 0.05$) across all growth phases (d14-d42). High CP diets had significantly lower feed intake (FI) than low CP diets ($P < 0.05$). Arg supplementation at 130% significantly increased BWG during the finisher phase compared to the 100% levels. HS reduced BWG and FI compared to TN ($P < 0.05$). Body composition results showed that on d28, groups fed high CP diets had significantly lower tissue fat percentages than those fed low CP diets ($P < 0.05$). Arg supplementation at 130% significantly increased total lean tissue on d28 and d42 ($P < 0.05$). On d21, a significant Arg × Environment interaction was observed, in which HS birds fed 130% Arg had a higher tissue fat percentage than those fed 100% Arg ($P < 0.05$). Carcass results on d43 revealed that high CP diets increased hot carcass weight and breast muscle yield ($P < 0.05$) but also increased woody breast scores ($P < 0.05$). HS significantly reduced preslaughter weights and increased the relative abdominal fat pad percentage ($P < 0.05$). In conclusion, the combination of high-quality protein from SBM and Arg supplementation can serve as a nutritional strategy to mitigate the physiological challenges associated with heat stress

**Keywords:** Heat stress; Arginine; Soybean meal; Crude protein; Carcass study

### 169 Interactive effects of dietary energy and feed grade amino acid inclusion on the growth performance, processing characteristics, and body composition of broilers

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This study evaluated the influence of dietary protein reduction with feed grade amino acids (AA) on broiler responses to dietary energy from 14 to 35 d of age. A total of 2,592 Ross 708 male broilers were randomly distributed among 96 floor pens (27 birds/pen) and fed a common starter from 1 to 14 d of age. On d 14, all pens were standardized to 25 birds/pen with uniform body weights and assigned to 12 treatments (8 pens/treatment). Treatments consisted of 2 CP levels (22 and 20%) that were titrated with 6 ME (2,950, 2990, 3030, 3070, 3110, and 3150 kcal/kg) levels. The high CP diet contained feed-grade Met, Lys, and Thr, whereas the low CP diet contained Met, Lys, Thr, Val, Ile, Arg, and Gly to meet minimum specifications for these AA. The energy titration was obtained by increasing the inclusion of poultry fat at the expense of a purified fiber. Feed intake (FI), body weight (BW), and feed conversion ratio (FCR) were determined weekly to 35 d of age. On d 36, birds were processed to determine carcass and fat pad weight and yield. The comparative slaughter technique was used to determine body composition (BC) and energy retention of feather-free whole carcasses during the experimental period. The baseline BC was determined on 10 birds selected randomly and the final BC was determined on 1 bird/pen at d 37. Data were analyzed as a 2 x 6 (CP x ME) factorial arrangement using the MIXED procedure of SAS. Linear and quadratic contrasts were performed for energy and for the CP x ME interaction. Day 14 BW was used as a covariate for performance variables and statistical significance was set at $P < 0.05$. From 14 to 35 d, BWG increased ($P < 0.05$) and FI and FCR decreased ($P < 0.05$) as the dietary energy level increased. Broilers fed the high CP diet had a higher BWG and FI ($P < 0.05$) compared to broilers fed the low CP diet from 14 to 35 d. No differences were observed ($P > 0.05$) for carcass weight and yield between CP and energy levels. However, broilers fed the low CP diet had a higher ($P < 0.05$) fat pad weight and yield compared to broilers fed the high CP diet. On a DM basis, carcass protein was lower ($P < 0.05$) and

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carcass fat higher ($P < 0.05$) for broilers fed the low CP diet compared to broilers fed the high CP diet. The linear contrast for the CP x ME interaction was significant ($P < 0.05$) for carcass protein and fat and for energy retention during the experimental period. These results suggest that reducing dietary CP with feed grade amino acids influences the slope response to dietary energy for the energy retaining components of the carcass (i.e., protein and fat) but not for broiler performance parameters.

**Keywords:** crude protein; amino acids; energy; broiler performance; body composition

## Immunology, Health and Disease: Feed Additives II

### 206 (E-F) (MTCC)

### 170 Mechanistic programming of the gut by a novel health enzyme

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Even under ideal conditions, GIT is continuously exposed to dietary and environmental stimuli that impose persistent, low-grade stress on the animal. Though subtle, it triggers molecular and cellular mechanisms aimed at maintaining homeostasis and protecting the gut. For modern broilers with rapid growth rates, this continuous metabolic burden becomes even more significant, diverting valuable nutrients away from growth and performance. The objective of this study was to evaluate the effect of a novel health enzyme (NHE) on broiler performance and health. A health enzyme can be defined as an enzyme that does not directly improve nutrient digestion or absorption. Instead, its value lies in its indirect ability to reduce or prevent low-grade stress in the GIT, allowing nutrients and energy to be used more efficiently for growth and productivity. A trial was conducted as a completely randomized design with 1,008-d-old male broiler chickens. Birds were allocated to 2 treatments with 36 pens of 14 birds each: 1) Control group: fed a basal diet formulated to meet Ross nutrient recommendations; 2) NHE: fed a basal diet supplemented with the NHE. Performance data were collected for d 0-35. On d 35, one bird was selected from 20 of 36 pens per treatment (n=20), and mid-jejunum was collected. Total proteins were extracted, and concentrations of interferon (IFN)-y, interleukin (IL)-6, IL-10 and chemokine C-C motif ligand (CCL)-5 were measured via Luminex 200. Performance and protein expression data were analyzed using R, and Tukey's test was used for post-hoc analysis ($P \leq 0.05$). Furthermore, total RNA was isolated and used for next-generation sequencing on a NextSeq 2000 platform. After mapping, differential gene expression along with GO term enrichment analyses were performed in R. Over the experimental period (d 0-35), FCR was significantly improved in birds fed the NHE diet. NHE supplementation significantly reduced IL-6 concentration in the jejunum, while only numerically reduced IFN-y and CCL-5 concentrations. Lower concentration of proinflammatory cytokines in NHE group are indicative of alleviated inflammatory responses at the gut level on d 35. Transcriptome data indicated that NHE enhanced pathways supporting epithelial renewal and membrane-junction stability, suggesting a more resilient gut barrier, while reducing oxidative and metabolic stress pathways which reflects lower cellular strain within the jejunum. Overall, NHE improved broiler performance, likely through improved GIT barrier function and reduced proinflammatory responses. To improve gut health and sustainability, future research is required to evaluate the effect of NHE on the microbiome as well as identifying its impact during enteric disease.

**Keywords:** Health enzyme; Gut health; Immune response; Gut barrier; Broiler chickens

### 171 Methionine Hydroxy Analogue Bis–Chelated Trace Minerals **Reduce** *Enterococcus cecorum* **Translocation in Broilers**

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*Enterococcus cecorum* is an emerging broiler pathogen associated with septicemia, mortality, and lameness caused by colonization of skeletal sites such as the femoral head (FH), tibial head (TH), and free thoracic vertebra (FTV). Previous studies suggest that methionine hydroxy-analogue bis-chelated trace minerals (MMHAC; MINTREX®) may improve bone formation and barrier function, potentially limiting bacterial translocation. This study evaluated whether replacing inorganic trace minerals (ITM) with lower levels of MMHAC reduces *E. cecorum* translocation and skeletal lesion development in broilers. A total of 672 Cobb 500 broilers were randomly assigned to four treatments: negative control (NC; ITM Zn:Cu:Mn = 100:16:100 mg/kg), positive control (PC; ITM Zn:Cu:Mn = 100:16:100, mg/kg), MMHAC10 (Zn:Cu:Mn = 40:10:40 mg/kg), and MMHAC30 (Zn:Cu:Mn = 60:30:40 mg/kg), in a randomized complete block design (12 pens per treatment; 14 birds per pen). The starter diets (d 0 to 21) were provided in crumbles, and the finisher diets (d 21 to 42) in pellets. Except for NC, all birds were orally challenged with $1 \times 10^4$ CFU of *E. cecorum* on d 1 and 14. Performance, latency to lie (LTL), spleen *Enterococcus* recovery, and lesion scores (FH, TH) were evaluated from d 14 to 42. Samples of FTV were collected for bacterial recovery on d 42. Data were analyzed in JMP Pro 18 using one-way ANOVA, and means were separated using Tukey's HSD test ($p \leq 0.05$). From d 21 to 28, MMHAC30 significantly improved body weight ($p < 0.01$), weight gain ($p < 0.01$), and feed conversion ratio ($p < 0.01$) compared with other groups, particularly PC. On d 28 and 42, MMHAC30 also showed the lowest ($p < 0.01$) *Enterococcus* recovery from spleens, indicating reduced extraintestinal translocation. Lesion severity did not differ among treatments; however, FH lesions were consistently more severe than TH lesions. At d 42, LTL was greater in NC (63 ± 15.7 s) than PC (26 ± 5.4 s), with MMHAC groups showing intermediate values. Mortality was highest in PC (20) and lowest in NC (12) and MMHAC30 (12), a 40% reduction relative to PC ($p > 0.05$). In conclusion, MMHAC supplementation reduced bacterial translocation to the spleen compared to the inorganic trace mineral control groups. Early spleen detection of *E. cecorum* may indicate subsequent lameness risk, and total replacement of ITM with lower levels of MMHAC shows potential to mitigate translocation and enhance resilience to *E. cecorum* infection in broilers.

**Keywords:** Enterococcus cecorum; translocation; copper; zinc; manganese

### 172 Effect of dietary omega-3 polyunsaturated fatty acids (PUFA) on performance, yolk fatty acid composition, and antibody response to avian encephalomyelitis (AE) vaccination in pullets and layers

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Dietary omega-3 polyunsaturated fatty acids (PUFA) are widely used in laying hen nutrition to improve bird health, including modulation of immune responses, and to produce enriched eggs. Avian encephalomyelitis (AE) vaccination presents a relevant immunological challenge in poultry;

however, it remains unclear whether different omega-3 PUFA sources differentially influence antibody responses to vaccination and fatty acid deposition in egg yolk during long-term feeding. Therefore, this 25-week study evaluated the effects of diets enriched with distinct omega-3 PUFA sources on humoral immune responses in pullets and layers following AE vaccination, as well as on PUFA deposition in eggs. A total of 320 one-day-old Dekalb White pullets were randomly assigned to one of four diets: a corn–soybean meal-based control; a diet supplemented with 0.4% A-linolenic acid (ALA) from flaxseed oil (FSO); a diet with 0.4% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil (FO); and a diet with 0.4% algal DHA. Birds were fed starter, grower, finisher, and layer diets formulated to meet nutrient requirements. Each treatment included 16 cages with 5 birds per cage. At week 11, all birds were vaccinated with a live AE virus via intracutaneous wing-web injection. Blood samples were collected at week 9 (baseline) and weekly from weeks 12 to 25 ($n$ = 5 birds/treatment/week). Feed intake and body weight were recorded weekly. At week 25, four egg yolks per treatment were collected for PUFA analysis. Data were analyzed in R using repeated-measures models for performance and antibody data, and one-way ANOVA for yolk PUFA. Diet affected pullet performance mainly in the starter phase. The 0.4% algal DHA diet resulted in the highest body weight gain, while both the control and 0.4% algal DHA diets achieved the best overall feed conversion ratio. No dietary effect was observed on layer performance. Antibody titres were negative prior to vaccination and became positive from week 14 onward, with sample-to-positive ratios increasing significantly over time ($P<0.05$). Dietary sources of omega-3 PUFA altered yolk fatty acid profiles, with the FO diet increasing EPA, DPA, DHA, and total omega-3 PUFA, while lowering omega-6 PUFA and the omega-6 to omega-3 ratio ($P<0.001$). The FSO diet resulted in the highest ALA concentration in yolk ($P<0.001$). While AE vaccination triggered a humoral immune response, dietary treatments did not affect antibody responses. Consecutive studies will evaluate cellular immune responses, including T- and B-cell phenotypes and cytokine expression, to further elucidate the immunomodulatory effects of different PUFA sources.

**Keywords:** Omega-3 PUFA; Vaccination; Immune challenge; Antibody; Layers

### 173 Circulating immune cell populations and energy phenotype of broilers based on lipid source and pigmentation

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Lipids play dual biological roles in diets: 1) as an energetically dense feedstuff, but also 2) serve to modulate immune functions. Lipid feedstuffs and their components affect immunocompetence through many mechanisms. Degree of saturation and omega 3 to 6 ratios are relevant to the production of paracrine hormones such as thromboxane and prostaglandins: therefore, inclusion of various oil sources with differing fatty acid profiles may provide essential substrates for immune function. To investigate the cellular and metabolic response of immune cells to different lipid sources, 56 straight-run Ross 308 broilers were raised in floor pens on a common diet until d21, when they were placed in battery cages (33 x 51 cm) for individual feeding with two identical time replicates. Birds were fed experimental diets with 7.5% oil source (corn oil, palm oil, or soybean oil) ± natural pigmentation from d31-41. On d41, 3 ml of blood was collected into heparinized tubes, and peripheral blood mononuclear cells (PBMCs) were isolated through Ficoll-based centrifugal density separation. Fresh cells were utilized in an XF Cell Energy Phenotype Test to determine baseline and stressed oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). The remaining PBMC subsamples were stained for flow cytometry quantification of Bu-1, Monocyte/Macrophage, CD3, CD4, and CD8A cell markers. PROC GLM was used to analyze all data for the effect of oil source, pigmentation, and their interaction with significance set at $P \leq 0.05$. There was no significant effect of oil source or pigmentation on OCR or ECAR. The average OCR for all treatments was 62 pmol/min at baseline and 79 pmol/min under stressed conditions, resulting in a 122% metabolic potential shift. The average ECAR for all treatments was 16 mpH/min at baseline and 25 mpH/min under stressed conditions resulting in a 134% change in metabolic potential. There was no significant effect of oil source or pigmentation on circulating immune cell populations. Live cell populations ranged from 19-25% Bu-1$^+$, 3-8% Monocyte/Macrophage$^+$, and 18-24% CD3$^+$. The proportion of CD3$^+$ cells ranged from 42-55% CD4$^+$ and 12-19% CD8$^+$ cells. Overall, the oil source and pigmentation did not affect the immunometabolism or circulating populations of PBMCs in broilers. Despite differences in fatty acid profiles, the lipid sources used may have provided similar substrates for key immunological pathways, resulting in functionally similar outcomes. The lack of response may reflect homeostatic regulation of immune cell metabolism, with PBMCs maintaining functional stability across a range of dietary lipid profiles to preserve immunocompetence in the absence of challenge conditions.

**Keywords:** broiler; energy phenotype; flow cytometry; oil characteristics

### 174 Effects of Monosilicic Acid Supplementation on Lymphocyte Proliferation and Cytokine Expression in *Salmonella*-Challenged Broiler Chickens

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Silicon, in its bioavailable form as monosilicic acid (MSA), enhances phosphorylation pathways in immune cells and improves T- and B-cell proliferation in mammals. The objective of this study was to evaluate the *in vitro* and *in vivo* effects of monosilicic acid supplementation on immune cell activity in broiler chickens. Chicken peripheral blood mononuclear cells (PBMC) were cultured with 0, 0.01, 0.1, and 1.0% v/v MSA (Improx™, Addix Biotech LLC, Florida, USA) in the presence of 15 ng/mL lipopolysaccharide. On day 6, PBMC cultured with 0.1% MSA exhibited significantly higher proliferation compared to the control group and showed upregulated gene expression of genes involved in immune system development, antigenic response, and myeloid lineage differentiation. In the *in vivo* study, a total of 560 birds were randomly allocated to two treatment groups: Control and MSA (250 g/ton feed), with 10 replicate pens per treatment. All birds were vaccinated at hatch against infectious bronchitis, Marek's disease, infectious bursal disease, and Newcastle disease. On day 3 of age, 14 birds per pen were orally challenged with 1 X $10^7$ CFU of *Salmonella enterica* serovar Enteritidis. Data was analyzed by ANOVA and means separated by Student's t test ($P < 0.05$). On day 42, no significant differences ($P > 0.05$) were observed in BWG, FC, or FCR between treatment groups. On days 15 and 21, birds in the MSA-supplemented group showed significantly ($P < 0.05$) higher Concanavalin A-induced lymphocyte proliferation compared to the control group, indicating enhanced T-cell proliferative capacity. However, Salmonella antigen-specific lymphocyte proliferation was lower ($P < 0.05$) in the MSA-supplemented group on days 15 and 21 compared to the control group. On days 15, 21, and 28, birds in the MSA-supplemented group exhibited significantly higher ($P < 0.05$) IL-1 and IL-6 mRNA expression compared to the control group. It can be concluded that MSA supplementation improves lymphocyte proliferation and competence in poultry during a *Salmonella* challenge.

**Keywords:** Silicon; T cells; B cells; ConA; LPS

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### 175 A mixture of lauric glycerides yields stronger antibacterial effects *in vitro* than pure A-monolaurin

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Interest of investigations in Medium-chain fatty acids (MCFA) and their glyceride derivatives (MCFAG) as antimicrobial alternatives in animal production is growing rapidly. Previous research has demonstrated that caprylic (C8), capric (C10) and lauric (C12) acids exhibit *in vitro* antimicrobial properties, especially against Gram-positive (G+) bacteria, and that C8C10- and C12-glycerides (C8C10G, C12G) exhibit even stronger antibacterial activity. While these effects have been attributed to bacterial membrane-destabilizing effects of the A-monoglyceride component of MCFAG, some research has suggested that also 1,3-diglycerides can play a role in the antibacterial activity of MCFAG. We hypothesized that C12G, a mixture of mainly A-monoglycerides of lauric acid (monolaurin, glycerol monolaurate, GML) and 1,3-diglycerides of lauric acid (glycerol dilaurate, GDL) has equal or stronger antimicrobial effects against G+ pathogens *in vitro*, as compared to C8C10(G) or to GML alone. Minimal inhibitory concentrations (MIC) of C8C10, C8C10G, C12, C12G, GML, and GDL were determined against G- *Salmonella* Enteritidis, *Escherichia coli* ETEC F4 and G+ *Enterococcus cecorum*, *Clostridium perfringens*, and *Streptococcus suis* strains. Glyceride forms consistently exhibited stronger antibacterial activity than their corresponding free fatty acids. C8C10G showed broad-spectrum activity, inhibiting both G- and G+ bacteria at markedly lower MICs than C8C10. C12G demonstrated the most potent activity against G+ bacteria, with MIC values <20–40 ppm for *E. cecorum*, *C. perfringens*, and *S. suis*. Interestingly, despite containing 2.2 times less monolaurin than the purified GML product, C12G displayed lower MIC values for *S. suis*, and the same MIC values for the other bacteria, suggesting that the combined presence of GML and GDL enhances antibacterial efficacy. GDL itself, however, showed no or limited antimicrobial activity *in vitro*. C12G possess stronger antibacterial effects against the tested G+ pathogens compared to C8C10, C8C10G, C12 and GML. The superiority of C12G (mainly composed of GML and GDL) over GML cannot be attributed to the direct antimicrobial activity of GDL. Rather, the C12G product appears to benefit from synergistic actions of GML and GDL. These findings highlight the importance of glyceride composition, rather than A-monoglyceride content alone, when assessing MCFA-based antimicrobial products. Further research is needed to elucidate the mechanism by which GDL contributes to the synergy between GML and GDL.

**Keywords:** Glycerides of medium-chain fatty acids; monolaurin; GML

### 176 Anticoccidial drug resistance patterns using Anticoccidial Sensitivity Tests

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Coccidiosis is controlled with anticoccidial drugs and vaccines. Anticoccidial drugs are classified as either chemical or ionophores. Extensive use of anticoccidial drugs has resulted in selection of coccidia that is less sensitive to drug's action. Each anticoccidial drug is unique in mode of action and selection for decreased sensitivity. The degree of drug sensitivity is also varied when considering coccidia vaccine strains. To determine the most effective anticoccidial drug to use, a drug sensitivity profile for the coccidia from a poultry complex needs to be established. One tool to establish this profile is Anticoccidial Sensitivity Tests (ASTs). AST is a battery cage test that examines drug control of coccidia isolated from the field. The objective of three ASTs was to determine if differences in sensitivity exist between coccidiosis control programs. Each AST consisted of 52 battery cages with 8, 12d old chicks. The 13 treatments were replicated in 4 randomized blocks. Coccidia were isolated from farms using only chemicals (CHEM), only coccidia vaccination (VAC), or an ionophore only program (IONO). Treatments were: No Additive unchallenged (NMU) and challenged (NMI), (all drugs were used at commercial use level) Zoalene 125 ppm, Amprolium 125 ppm, Nicarbazine 125 ppm, Robenidine 33 ppm, Clopidol 125 ppm, Diclazuril 1 ppm, Decoquinate 30 ppm, Monensin 99 ppm, Salinomycin 66 ppm, Narasin 79 ppm, and Lasalocid 75 ppm. Birds and feed were weighed by cage on DO (12 days of age) and D8 (6d post challenge). On D2, birds were orally challenged with one of the 3 isolates. Each isolate contained *E. acervulina*, *E. maxima*, and *E. tenella*. On D8, all birds were humanely euthanized and coccidia lesion (LES) scored (0-4). Statistical analysis employed the General Linear Models procedure with a probability level of P <0.05 to determine significance. Each challenge significantly reduced performance and produced significant LES from all species (NMU AVG FCR 1.423 and LES 0.0; NMI AVG FCR 2.829 and LES 2.7). The CHEM AST, the avg. ionophores control was acceptable (FCR 1.639 and LES 1.0) and was significantly better than avg. chemical (moderate control) (FCR 2.077 and LES 1.8). The VAC AST, both the avg. ionophores control (FCR 1.606 and LES 1.2) and the avg. chemical control (FCR 1.681 and LES 1.7) were in the acceptable range. The IONO AST, the avg. ionophores control was moderate (FCR 1.867 and LES 2.1) and significantly better was the avg. chemical (acceptable control) (FCR 1.701 and LES 1.5). These results show anticoccidial control program, drug (chemical or ionophore) or vaccination vary in sensitivity levels. This information is critical to produce a long-term coccidiosis control program.

**Keywords:** Cocccidia; anticoccidial; Chemical; ionophore; anticoccidial sensitivity test

## Student Competition: Metabolism and Nutrition - Vitamins and Minerals

**205 (A-D) (MTCC)**

### 177 Broilers response to dietary calcium and digestible phosphorus from 0 to 10 days of age

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Calcium (Ca) and phosphorus (P) nutrition in broilers are critical for skeletal development and numerous physiological functions. These minerals are closely related, and imbalances can impair both absorption and post-absorptive utilisation. In recent decades, P nutrition has received considerable attention, as it is the third most expensive nutrient. However, the starter phase (from 0 to 10 d of age) has not received the same attention in research in comparison to grower and finisher. To refine dietary recommendations during early growth, 2 studies were conducted from 0 to 10 d of age using wheat-corn-soybean meal-based diets in micro-pellet form (2.5 Ø mm). In Experiment 1, 2,016 one-day-old males Ross 308 were assigned to 9 combinations of Ca (range: 0.35 and 0.90%) and digestible P (dP; range: 0.15 and 0.51%) using a central composite design (4 factorial points, 4 axial points and 1 central point). In Experiment 2, 3,200 one-day-old male Ross-308 were assigned to 4 levels of Ca (0.53, 0.70, 0.88, 0.11%) and 2 levels of dP (0.40 and 0.54%) in a 4 × 2 factorial scheme. After 10 days, birds received a common diet covering nutritional requirements. Growth performance, bone mineralization and serum parameters were evaluated in both trials. A combined database was created using data from both experiments. No exogenous phytase was included in the diets. Response equations were developed using $Ca^2$, $dP^2$, Ca, dP, Ca and Ca × dP as explanatory variables. Digestible P stands out as the most influential nutritional factor ($P < 0.001$), particularly for body weight gain (BWG) and feed intake (FI). Excess Ca had a greater negative impact when dP levels were low; increasing Ca from

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0.60 to 1.00% at dP = 0.30% reduced BWG by 10%, compared with 4% when dP = 0.40% ($R^2$=74.2; Ca × dP < 0.001). Maximum bone mineralization was observed at higher levels of both minerals. Like BWG, tibia ash was strongly reduced when Ca increased under low dP (Ca × dP < 0.001). Broilers fed low dP diets with normal or high Ca exhibited higher serum Ca (Ca × dP = 0.002). Serum P tended to be impacted by Ca and dP levels, being lower at high Ca and low dP (Ca × dP = 0.079). Regarding the 0 to 35 days period, broilers receiving Ca to dP ratio highly imbalanced feed in starter, were unable to fully recover BWG and FI (*P* < 0.001 for both experiments). In summary, when dP was set at 0.40%, the Ca needed to maximize BWG was 0.69% while that for bone mineralization was 0.82%. Using a minimum level of 0.82% Ca and 0.40% dP would result in a 1.3% reduction in BWG but ensure tibia mineralisation from 0 to 10 d of age.

**Keywords:** Calcium; digestible phosphorus; starter phase; broiler performance; bone mineralization

### 178 Effects of dietary calcium and phosphorus and phytase supplementation on phytate degradation and mineral digestibility in broilers

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Although dietary calcium (Ca), non-phytate phosphorus (NPP), and exogenous phytase (exPhy) individually influence phytate degradation (PPdisap) and mineral digestibility in broilers, their combined effects remain insufficiently characterized. This study evaluated the effects of dietary Ca and P and exPhy supplementation on PPdisap and on the apparent ileal digestibility (AID) of P and Ca in broilers from 1 to 21 days of age. A 2×2 factorial design was used, with two mineral contexts : High Ca/Low NPP (Ca 0.8%, NPP 0.2%) and Low Ca/High NPP (Ca 0.55%, NPP 0.4%) with (500 FTU/kg) or without exPhy supplementation, resulting in four treatments (n = 8 replicates of 8 birds). Ileal digesta were pooled to obtain eight composite samples per diet. Data were analyzed using a two-way ANOVA with mineral and phytase as fixed effects. In the absence of exPhy, PPdisap was markedly lower in the Low Ca/High NPP context (35.5%, P < 0.001) compared to High Ca/Low NPP (55.4%), suggesting that elevated NPP suppresses endogenous PP hydrolysis, possibly through P-dependent feedback on endogenous phytase activity. Despite this difference in PPdisap, AID P only tended to differ between mineral contexts without exPhy (49.3% vs 53.9%; P = 0.09), likely because dietary NPP itself provided additional absorbable P in the Low Ca/High NPP context, thus partially compensating for lower PP hydrolysis. The exPhy supplementation substantially increased PPdisap and AID P in both mineral contexts (P < 0.001), with mean increases of approximately 26 percentage points in PPdisap and 18 percentage points in AID P, regardless of mineral context. Importantly, AID P reached similar levels across mineral contexts with exPhy (68.1% vs 66.9%), indicating that exPhy effectively compensates for low NPP supply and equalizes P availability across contrasting mineral conditions. In contrast, AID Ca was not significantly affected by phytase supplementation (P = 0.12) but was strongly modified by mineral context (P < 0.001), with High Ca/Low NPP diets showing markedly higher AID Ca than Low Ca/High NPP diets (67.8% vs 51.8%, respectively). The higher AID Ca observed in the High Ca/Low NPP context may reflect a combination of factors, including greater Ca availability from soluble sources, reduced Ca-P precipitation, and potential upregulation of intestinal Ca absorption under low dietary P conditions. These findings demonstrate that exPhy and mineral context exert distinct and partially independent effects on PP degradation, AID P, and AID Ca. While exPhy consistently improves PPdisap and P availability regardless of mineral context, optimizing Ca utilization requires careful consideration of the luminal P environment shaped by dietary NPP levels.

**Keywords:** phytase; phosphorus; calcium; phytate

### 179 Effects of phytase level and trace mineral source on broiler performance, processing yield, and myopathies

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This study evaluated phytase (PHY) inclusion level and trace mineral (TM) source on growth performance, processing yield, and breast muscle myopathies in broilers subjected to heat stress at placement. The study was a 2×4 factorial with 2 PHY levels and 4 TM sources. A total of 1,760 day-old YPM × Ross 708 male broilers were assigned to 8 dietary treatments (0.076 $m^2$/bird; 10 replicates/treatment) consisting of PHY at 500 or 2,000 FTU/kg and TM sources of inorganic (INO) Fe (40 ppm) + Zn (100 ppm), organic Fe (OFe; 40 ppm), organic Zn (OZn; 60 ppm), or OFe (40 ppm) + OZn (60 ppm). Birds received a 1X dose of Coccivac®-B52 and were exposed to heat stress (35°C) for 1 hour prior to placement. A corn-soybean meal diet was fed for starter (d0-20), grower (d21-35), finisher (d36-42), and withdrawal (d43-49) phases. Body weight (BW), BW gain (BWG), feed intake (FI), mortality corrected feed conversion ratio (FCR), and mortality were recorded by phase and cumulatively. On d35, fecal samples from 2 pens were pooled within treatment for mineral content. On d50, six birds/pen were processed to evaluate yield and the incidence of woody breast (WB) and white striping (WS). Data were analyzed using two-way ANOVA in PROC GLM of SAS, and means were separated using Tukey's HSD test with significance at P≤0.05. In the finisher phase, a PHY level × TM source interaction for BWG (P=0.04) resulted in birds fed 500 FTU/kg PHY having increased BWG compared to birds fed 2,000 FTU/kg PHY within the OZn treatment, while no PHY-related differences were observed within other TM sources (P<0.05). A PHY × TM interaction for d49 BW and d0-49 BWG (P<0.05), showed birds fed OZn diets with 500 FTU/kg performed better than those fed OZn diets with 2,000 FTU/kg PHY. Fecal mineral analysis at d35 showed a PHY × TM interaction; birds fed INO Fe+Zn with 2,000 FTU/kg PHY had the highest S (P=0.03). Birds fed 500 FTU/kg PHY had lower fecal Na than those fed 2,000 FTU/kg (P=0.0007). Birds fed INO Fe+Zn had higher fecal Cu and Ca (P<0.05) compared to OFe. Birds fed INO Fe+Zn had higher fecal Zn and Na (P<0.05) compared to OFe or OFe+OZn. Wing yield was increased in birds fed 2,000 FTU/kg PHY compared to 500 FTU/kg (P=0.0108), while birds fed OFe had higher breast, tender, and boneless yields, but lower leg yield compared to INO Fe+Zn (P<0.05). Birds fed OFe+OZn with 500 FTU/kg exhibited reduced incidence of WB compared to birds fed OFe with 500 FTU/kg (P=0.03). Growth performance responses to PHY were dependent on TM source. OFe+OZn supplementation reduced WB incidence, indicating that strategic combinations of PHY level and TM source may improve broiler performance and mitigate breast muscle myopathies following early heat stress conditions.

**Keywords:** phytase; trace minerals; broilers; performance; myopathies

### 180 Interaction of adequate and reduced dietary macro- and micro minerals and phytase on performance and skeletal development of Hy-Line W-36 pullets

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Skeletal development during the pullet phase depends on adequate macro- and trace minerals and optimizing mineral source and inclusion level may improve nutrient utilization beyond conventional safety margins used to maintain performance and bone integrity. The objective of this study was to determine the effects of reducing dietary Ca, P, and trace mineral (**TM**) levels, in combination with different phytase doses, on mineral utilization efficiency and skeletal development during pullet growth. A total of 1,200 Hy-Line W-36 pullets were assigned from 0 to 15 wk of age to a randomized complete block design with a 3 × 2 × 2 factorial arrangement of TM source (100% inorganic, 50% inorganic, or 50% organic TM), where the TMs of interest were Cu, Zn, and Mn, with the organic TM supplied as metal amino acid complexes; Ca and P level (100% or 85% of breeder recommendation); and phytase dose (500 or 2,000 FTU/kg), resulting in 12 dietary treatments (10 replicate cages per treatment; 10 birds per cage). Body weight (**BW**) and feed intake (**FI**) were recorded throughout the pullet phase. At 15 wk, one bird per cage (10 replicates per treatment; n = 120) was euthanized for dual-energy X-ray absorptiometry to determine whole-body and right tibia bone mineral density (**BMD**) and bone mineral content (**BMC**). Right tibias were evaluated for 3-point bending strength and subsequently ashed for mineral concentration (1 bird per cage; n = 120). Data were analyzed using a three-way factorial ANOVA in SAS, and significance was declared at $P \leq 0.05$, with trends discussed at $0.05 < P \leq 0.10$. During the starter phase (0–6 wk), TM source showed a trend toward influencing BW ($P = 0.063$) and average daily gain ($P = 0.084$), with pullets fed 50% organic TM showing greater values than those fed 100% inorganic TM. Phytase dose also showed a tendency toward influencing FCR ($P = 0.061$), with birds fed 2,000 FTU/kg exhibiting improved feed efficiency. No treatment effects were detected for BW gain ($P = 0.646$), FI ($P = 0.903$) or FCR ($P = 0.860$) during any phase or overall. Also, dietary treatment did not change BMD ($P = 0.999$), BMC ($P = 0.519$), tibia ash percentage ($P = 0.201$), 3-point bend breaking force ($P = 0.370$), or maximum force ($P = 0.658$). In conclusion, pullets fed diets containing 85% of breeder-recommended Ca and P maintained growth performance, BW uniformity, and skeletal mineralization comparable to birds fed standard mineral levels regardless of TM source or phytase inclusion rate. These results indicate that moderate reductions in macro- and trace mineral inclusion, when supported by phytase supplementation, can sustain skeletal development while improving mineral utilization efficiency during pullet rearing.

**Keywords:** mineral efficiency; phytase super-dosing; pullets; trace minerals

## 181 Optimal intake of zinc amino acid complex in commercial laying hens from 30 to 48 weeks of age based on productive performance, egg quality, and bone densitometry

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Microelements are essential nutrients for laying hens, playing key roles in health, skeletal integrity, and productive performance. Zinc (Zn) participates in enzymatic reactions related to protein synthesis, mineral metabolism, antioxidant defense, eggshell formation, and bone turnover. Organic sources, such as zinc complexed to amino acids (ZnAA), have higher bioavailability than inorganic forms, potentially improving metabolic efficiency. This study aimed to estimate the optimal intake of ZnAA and evaluate its effects on productive performance, egg quality, and tibial bone characteristics in commercial laying hens. A total of 420 Dekalb White hens (30–48 weeks) were assigned to a completely randomized design with seven ZnAA levels (0, 5, 15, 25, 40, 60, and 80 mg $kg^{-1}$), six replicates, and ten birds per replicate. Other microminerals were kept constant. Performance variables, egg quality traits, and tibial characteristics, including bone mineral density (BMD) assessed by quantitative computed tomography, were evaluated. Data were subjected to analysis of variance (ANOVA), and when significant, regression models were applied. Nonlinear responses were evaluated using the Broken-Line model to estimate optimal ZnAA intake, considering a 5% significance level. Among performance variables, only egg mass was affected ($P < 0.01$), with a breakpoint at 0.528 mg $bird^{-1}$ $day^{-1}$ (≈4.92 mg $kg^{-1}$), reaching a plateau of 60.37 g. Feed intake, egg production, egg weight, and feed conversion were not influenced ($P > 0.05$). For egg quality, shell breaking strength responded significantly ($P = 0.0404$), with a breakpoint at 4.38 mg $bird^{-1}day^{-1}$ (≈40.85 mg $kg^{-1}$) and a maximum of 4.84 kgf. Eggshell weight increased linearly with ZnAA supplementation ($P = 0.0324$), while thickness was not affected. Yolk color was the only internal parameter influenced ($P = 0.0286$), with a breakpoint at 1.62 mg $bird^{-1}$ $day^{-1}$ (≈15.09 mg $kg^{-1}$). Bone morphometry, mineral matter, and Seedor index were not affected ($P > 0.05$). However, proximal tibial BMD was influenced ($P = 0.0461$), with a breakpoint at 8.15 mg $bird^{-1}day^{-1}$ (≈75.9 mg $kg^{-1}$) and a maximum of 661.5 mg/$cm^3$. ZnAA showed dose-dependent effects, with lower levels (≤15 mg $kg^{-1}$) optimizing egg mass, intermediate levels (~40 mg $kg^{-1}$) maximizing shell strength, and higher levels (≥60 mg $kg^{-1}$) improving bone density. Dietary inclusion around 40 mg $kg^{-1}$ is sufficient to support productive performance and bone integrity in laying hens.

**Keywords:** bone strength; dose-response; mineral metabolism; computed tomography; shell resistance

## 182 Estimation of the optimal supplemental zinc intake in the form of metal amino acid for laying hens from 55 to 71 weeks of age

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Zinc (Zn) is an essential trace mineral for laying hens, typically supplemented in inorganic forms. Additionally, zinc can be supplied through organic sources, such as zinc amino acid complexes (ZnAA), which are efficiently utilized by the animal. This study aimed to estimate the optimal ZnAA supplementation levels for laying hens in the aged production phase. A total of 336 Dekalb White laying hens (55–71 weeks of age) were distributed in a completely randomized design with seven treatments and six replicates, each consisting of eight birds. The treatments consisted of different ZnAA supplementation levels: 0, 5, 15, 25, 40, 60, and 80 mg/kg. The supplementation of the other trace minerals (Mn, Cu, Fe, I, and Se) was the same across all treatments. Productive performance was evaluated daily, and egg quality was assessed every 28 days. At 71 weeks of age, blood samples (0.5 mL) were collected from the ulnar vein for hematological assessment, and one bird per experimental unit was euthanized for tibia collection to evaluate bone quality. After confirming homogeneity of variances and residual normality, regression analysis was performed to determine the optimal zinc intake. The Broken Line model best described the relationship between zinc intake and measured variables. Significant effects were observed for egg mass ($P = 0.01$) and total egg production ($P < 0.01$), with estimated Zn requirements of 25.6 and 60 mg/kg of the diet, respectively. Albumen height exhibited a plateau response up to 45.5 mg/kg of Zn ($P = 0.05$), followed by a decline at higher supplementation levels, suggesting no additional improvement beyond this point. This relationship indicates that ZnAA supplementation beyond the breakpoint did not confer additional benefits. Medial densitometry showed a significant response ($P = 0.03$), with a breakpoint at 17.07 mg/kg of Zn in the diet. Hematological variables also responded to supplementation, with optimal levels for hemoglobin ($P = 0.01$), mean corpuscular volume (*P*

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= 0.04), and mean corpuscular hemoglobin concentration (*P* = 0.05) at 32.2, 27.5, and 21.8 mg/kg of dietary Zn, respectively. Based on these findings, supplementation with 60 mg/kg of ZnAA in the diet is recommended to optimize productive performance. For the remaining variables, lower zinc levels were required to maintain hematological homeostasis and bone quality in commercial laying hens from 55 to 71 weeks of age.

**Keywords:** aged laying hens; bone density; hematology; productive performance; trace mineral

### 183 Reduction of copper through proteinate supplementation in laying hens at peak production

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Copper (Cu) is a trace mineral (TM) required as a cofactor for several enzymes involved in metabolic processes and also plays key roles in antioxidant and immune functions, which are associated with laying performance and egg quality. Inorganic copper sources have low bioavailability due to interactions with other minerals and ligands in the feed, whereas copper proteinate has been shown to be more bioavailable. This study was designed to evaluate the productive performance and egg quality of laying hens at peak production (34 to 46 weeks of age) as influenced by Cu source and supplementation strategy applied during the growing, early laying, and peak laying phases. The study was conducted in a 2 × 2 × 2 factorial arrangement, consisting of two Cu sources evaluated across three production phases: growing, early laying, and peak laying. In each phase, hens received either 100% of the recommended Cu level (Brazilian tables) from copper sulfate (CuS) or 50% of the requirement using CuP (Bioplex Copper, Alltech Inc.). Thus, the eight treatments represented all possible combinations of Cu sources across phases (e.g., CuS–CuS–CuS, CuS–CuS–CuP, …, CuP–CuP–CuP), allowing the evaluation of carryover effects throughout the production cycle. A total of 392 hens were randomly assigned to 8 experimental treatments with 7 hens per experimental unit. Diets were prepared according to requirements of 10.43 mg Cu/kg for peak laying phase analyzed. Feed intake (FI), feed conversion g/g (FC), egg production, egg mass and egg quality were measured from 34 to 46 weeks of age over three 28-day periods. The data were analyzed by ANOVA in a 2 × 2 × 2 factorial arrangement, and the means were compared by Tukey test using SAS v.9.0. No evidence of interactions or main effects were observed for egg production (97.63%), FI (105.85 g), and FC in g/g (1.733 g/g), or kg/dz (1.311 kg/dz) (p>0.05). Main effect of Cu source was observed on the egg mass during the early laying phase, where hens receiving CuS had marginally higher (P = 0.054) egg mass. There was no significant difference (p>0.05) in egg quality variables among groups. However, there was a higher (p=0.020) eggshell percentage when birds were supplemented with CuP during the peak laying phase. In conclusion, laying hens can be supplemented with Cu as CuP at 50% of the requirement during the growth, early laying, and peak laying phase (34 to 46 weeks) without negatively impacting productive performance or egg quality. Furthermore, eggshell percentage improvements were observed with of the use of CuP.

**Keywords:** eggshell; copper proteinate; copper sulfate; egg quality; performance

### 184 Supplementation of selenium-enriched yeast in broiler breeder diets and its effect on incubation parameters

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The use of selenium (Se) in poultry nutrition plays a critical role, as it can influence the productive and reproductive performance of broiler breeders. Selenoyeast, obtained by enriching Saccharomyces cerevisiae strains with selenium, is used as a highly bioavailable organic source capable of providing different selenoproteins to the animal organism, including selenomethionine and selenocysteine, with absorption via active transport and greater tissue deposition compared to inorganic sources. The present study aimed to evaluate the use of selenium-enriched yeast and sodium selenite in broiler breeder diets and their influence on incubation performance. The experiment was conducted at the experimental aviary of the School of Veterinary Medicine and Animal Science – EVZ/UFG, using a flock of 56 breeders and 8 roosters of the Cobb 500® genetic line. Mash diets were provided to the breeders meeting the nutritional requirements recommended for the line, differentiated by the supplemented selenium source (Treatment 1: selenium-enriched yeast; Treatment 2: sodium selenite). A total of 167 hatchable eggs were selected, collected over seven days, and stored for nine days at a mean temperature of 18°C. Egg weight at the time of incubation (EWI), egg weight at transfer (EWT) at 18 days of incubation, moisture loss (ML) and egg weight loss (EWL) during this interval were evaluated. The experiment was conducted in a completely randomized design (CRD) and data were analyzed by ANOVA using R software, with means compared by the t-test (5%). The results obtained were as follows: EWI – T1: 69.31 g / T2: 68.22 g; EWT – T1: 61.63 g / T2: 59.65 g; ML – T1: 11.27% / T2: 12.06%; EWL – T1: 7.81 g / T2: 8.19 g. Statistical differences between treatments were found for EWT and ML (P<0.05), with the selenoyeast treatment presenting greater egg weight at transfer and lower moisture loss. These results indicate better embryonic hydration conditions and greater energy reserves available for the hatching and pipping process of the T1 embryos. Thus, it can be concluded that, under the conditions of this study, the replacement of sodium selenite by selenium-enriched yeast in broiler breeder diets influenced the egg incubation parameters.

**Keywords:** Mineral; Antioxidant; Selenoprotein; Nutrition

### 185 Effects of Calcium and Phosphorus Feeding Programs on Egg Quality of Cage-free Laying Hens 34-46 Weeks of Age

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The global egg industry is undergoing a significant transition from conventional cage to cage-free production systems. This shift to cage-free systems increases the need for optimal skeletal support, as increased locomotor activity elevates physiological demands for calcium (Ca) and phosphorus (P). Egg quality, a key factor of marketability, relies on adequate Ca and P intake to form medullary bone reserves for eggshell calcification during laying. In nature, birds have intermittent access to elevated levels of Ca and P during foraging, which suggests avian species flexible in regulating mineral metabolism under variable dietary intake. The phenomenon provides a nutritional opportunity for mineral optimization that mimics this natural circadian rhythm. Some egg producers have adopted this concept as “calcium boosting” to enhance eggshell strength. The primary objective of this study was to evaluate whether this approach provides measurable benefits compared to traditional options. We hypothesize that intermittent access to high levels of calcium and phosphorus (boosting) will have an impact on egg quality from 34 to 46 weeks of age. In a completely randomized design, 1200 Lohmann SLS Lite birds were assigned to 24 pens (10ft x 8ft), with 50 birds per pen.

Each pen had a 10-hole nestbox, two feeders, and two-line nipple drinkers, each with 4 nipples. Treatments were 4 Ca:P feeding programs: 1. Breeder recommended (TRT1) 2. Calcium and phosphorus boosting (TRT2). 3. Old commercial (TRT3), 4. New commercial (TRT4) assigned to six replicate pens per treatment. Management practices were based on the breeder's recommendation. The data were analyzed using one-way ANOVA, and differences were considered significant at $p \leq 0.05$ and trends $p > 0.05$ to $p \leq 0.09$. Significant means were separated using Fisher's LSD test. There was a significant effect of dietary treatment ($p < 0.05$) on egg weight, albumen height, specific gravity and yolk color. At week 38 and 46, there was a significant effect of dietary treatment on Haugh unit values and egg weight ($p < 0.05$) respectively, with TRT4 exhibiting fresher albumen compared to the other treatments while TRT1 produced eggs with greater weight. Week 38 specific gravity was lower ($p < 0.05$) for TRT1 compared TRT3 and TRT4 but not TRT2. The yolk color b at week 44 indicating deeper yolk pigmentation ($p < 0.05$) in TRT2 compared to the other treatments. These results suggest that dietary Ca and P may influence key egg quality parameters, but this might be age dependent.

**Keywords:** egg quality; calcium; phosphorous ; boosting; cage-free

### 186 Phase-specific optimization of dietary calcium and available phosphorus in laying hens: Effects on production performance, eggshell quality, skeletal integrity, and mineral utilization

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This study evaluated the phase-specific dietary calcium (Ca) and available phosphorus (AvP) requirements of Hy-Line Brown laying hens by assessing production performance, eggshell quality, skeletal characteristics, and mineral utilization from 16 to 70 week of age. Two feeding phases were applied based on Korean feeding standards for poultry: Phase 1 (week 16 to 48; 3.80% Ca and 0.32% AvP) and Phase 2 (week 50 to 70; 3.90% Ca and 0.31% AvP). Within each phase, five dietary regimens providing 80, 90, 100, 110, or 120% of the recommended Ca and AvP levels were formulated and designated as CaP80, CaP90, CaP100, CaP110, and CaP120, respectively. A total of 180 pullets were allotted to a completely randomized design with six replicates of six hens per treatment. Growth performance, egg production, eggshell quality, tibial breaking strength, tibial mineral composition, and ileal digestibility of crude ash, Ca, and P were measured. Data were analyzed by one-way ANOVA, and optimal dietary Ca and AvP concentrations were estimated using linear-plateau, quadratic-plateau, and quadratic regression models, with significance declared at $P < 0.05$. Dietary Ca and AvP regimens did not affect growth performance or egg production throughout the experimental period ($P > 0.05$). At 70 week, shell thickness and shell strength were greatest in hens fed CaP120 ($P < 0.05$), although values in CaP90 and CaP100 were comparable ($P > 0.05$). Tibial breaking strength at 70 week and tibial Ca and P concentrations at both 48 and 70 week showed similar responses, with no differences among CaP90, CaP100, CaP110, and CaP120 ($P > 0.05$). In contrast, ileal digestibility of crude ash, Ca, and P was higher in hens fed CaP80 and CaP90 at both 48 and 70 week ($P < 0.05$). Regression analyses indicated that higher Ca-AvP regimens were required to maximize eggshell quality and skeletal mineralization, with estimated optima of 3.53 to 3.58% Ca and 0.30% AvP at 48 week and 3.64 to 4.05% Ca and 0.29 to 0.32% AvP at 70 week. In contrast, maximal mineral digestibility was achieved at lower dietary concentrations, with estimated optima of 3.05 to 3.20% Ca and 0.26 to 0.27% AvP at 48 week and 3.09 to 3.53% Ca and 0.25 to 0.28% AvP at 70 week. These findings indicate that optimal Ca and AvP levels in laying hens are response-dependent and phase-specific, supporting precision mineral feeding strategies to maintain performance and skeletal health while improving mineral utilization efficiency and reducing phosphorus oversupply in commercial layer production.

**Keywords:** Available phosphorus; calcium; eggshell quality; laying hens; mineral digestibility

### 187 Assessment of water-soluble vitamin levels on Cobb 800 male broiler performance

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Sparse work has been done investigating vitamin responses in modern broiler chickens, particularly regarding water-soluble vitamins. The new Cobb 800 broiler has a lower initial feed intake than the Cobb 500 broiler and assessing critical nutrient needs early in the chicks' life warrants attention. Therefore, the objective of this study was to identify water-soluble vitamin responses in the starter phase for male Cobb 800 broiler chickens. A nutrient adequate starter diet, devoid of unbound water-soluble vitamins, based on corn and soybean meal, was formulated to contain both a mineral and fat-soluble vitamin premix. Formulation space was created at one pound per short ton for water-soluble vitamin treatments (9 treatments replicated 10 times with each replicate containing 10 chicks). The 90 floor pens were blocked resulting in a randomized complete block design. Treatments 1 and 2 represented a high and low water-soluble vitamin levels, respectively, of: niacin, 133 and 44 mg/kg; pantothenic acid, 32 and 11 mg/kg; riboflavin, 25 and 8 mg/kg; pyridoxine, 10 and 3 mg/kg; thiamin, 5 and 2 mg/kg; cobalamin, 4 and 1 mg/kg; and folic acid, 3 and 1 mg/kg. Treatments 3 through 9 represented the singular addition of the former vitamins from the low level back to the high level. Data were analyzed using the General Linear Models procedure of SAS 9.4 (alpha equal to 0.05) and means were separated using Fisher's Least Significant Difference. From day 0 to 14 birds consuming high levels of pyridoxine had a significant increase in BW gain over those consuming all other treatments while remaining similar to birds consuming diets high in thiamin and riboflavin (P=0.0003). Mortality corrected feed:gain significantly decreased for birds fed high levels of pyridoxine and thiamin (P=0.0040) when compared to birds fed low levels of water-soluble vitamins, while also numerically reducing feed:gain by three points when compared to birds fed high levels of water-soluble vitamins. Feed intake on a kilogram per bird basis was increased in birds fed high levels of pyridoxine when compared to both high and low water-soluble vitamin level treatments (P=0.0071). Mortality during the starter phase was not found to be significant and remained less than 1% through day 14 (P>0.05). Overall, pyridoxine and thiamin significantly improved BW gain and feed:gain over both high and low water-soluble vitamin treatments (P=0.0003, P=0.0040, respectively). Birds consuming low water-soluble vitamin levels had the poorest feed conversion ratio. In conclusion, further research is warranted to assess water-soluble vitamin interactions in modern broilers, especially in diets with higher levels of pyridoxine and thiamin.

**Keywords:** broiler; starter phase; water-soluble vitamins; pyridoxine; thiamin

### 188 Effects of Varying Levels of Vitamin A in the Starter Phase Diet in Male Cobb 800 Broilers

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The Cobb 800 broiler is a newly available genetic line. While this bird is similar to the Cobb 500 broiler, it inevitably has differing nutritional requirements in order to obtain optimal performance in the field. This lends itself to a need to reassess vitamin levels being offered in the feed. An

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eight-level vitamin A titration was administered to 480 male Cobb 800 broilers from d 1 to 14 to determine the impact of vitamin E level on live performance at d 14, 28 and 45, as well as 46 d processing traits and meat quality measurements. The experiment was a random complete block design with each titration level having 6 pen replicates of 10 birds each. Experimental diets with added vitamin A levels of 1,000, 3,000, 5,000, 7,000, 9,000, 11,000, 13,000, or 15,000 IU/kg of diet were fed from placement to 14 d of age. Common grower and finisher diets with surfeit vitamin A were fed for the remaining duration of rearing (45 d of age). Four birds per pen were wing tagged and processed at 46 d of age to assess processing yields and meat quality characteristics. All data was analyzed using both linear and quadratic regression models, as well as GLMM, in JMP pro19 software. Increasing dietary vitamin A from chick placement to d 14 quadratically (P < 0.05) improved BW gain and feed intake but not feed:gain or livability. Both BW gain and feed intake resulted in an asymptote response that approximated 8,000 IU vitamin A/kg of diet or 7,600 IU vitamin A/kg of diet calculated as 95% of the asymptote.

**Keywords:** Vitamin A; Broiler; Nutrition

## Student Competition: Metabolism and Nutrition - Feed Additives

**202 (A-D) (MTCC)**

### 189 Dose-response of an essential oil blend on nutrient utilization and nitrogen balance in broiler chickens

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Optimizing energy and nitrogen (N) utilization is an important challenge in modern broiler production because of its implications for feed cost and environmental sustainability. The objectives of this study were to evaluate growth performance and nutrient utilization, with particular emphasis on N utilization and N balance in broiler chickens fed diets supplemented with an essential oil blend (EOB), and to determine the optimal dietary supplementation level of EOB. A total of 360 one-day-old Cobb 500 byproduct male broiler chicks were randomly assigned to 5 dietary treatments, with 12 replicate cages per treatment and 6 chicks per cage. Dietary treatments consisted of a corn-soybean meal-based basal diet or the basal diet supplemented with 100, 200, 400, or 600 mg/kg of a novel EOB (Alltech Inc., Nicholasville, KY). Birds were fed starter (d 0 to 9) and grower (d 9 to 28) diets *ad libitum* and all diets were formulated to meet or exceed nutrient requirement estimates. From d 27 to 28, excreta samples were collected to determine apparent utilization of N, AMEn, and N balance, using the index method. Data were analyzed using the MIXED procedure of SAS, with dietary treatment considered a fixed effect. Polynomial contrasts were used to evaluate linear and quadratic responses to dietary EOB levels. Quadratic broken-line and quadratic polynomial models based on the NLIN procedure of SAS were applied to estimate the optimal dietary supplementation level of EOB. The experimental unit was the cage and statistical significance was declared at $P < 0.05$. From d 0 to 28, BW gain and feed intake increased linearly ($P < 0.05$), whereas gain-to-feed ratio (G:F) increased quadratically ($P = 0.002$) with increasing dietary EOB levels. With increasing dietary EOB level, apparent utilization of N and AMEn (DM basis) increased quadratically ($P < 0.01$), whereas N excretion decreased quadratically ($P = 0.012$). In addition, N intake and N retention increased linearly ($P < 0.001$) with increasing EOB supplementation level. Quadratic broken-line models estimated optimal EOB levels of 284 mg/kg for G:F (SE = 89; $R^2 = 0.36$; $P < 0.001$) and 337 mg/kg for apparent utilization of N (SE = 78; $R^2 = 0.49$; $P < 0.001$), whereas quadratic polynomial models estimated an optimal level of 251 mg/kg for both AMEn (DM basis; SE = 24; $R^2 = 0.29$; $P < 0.001$) and N excretion (SE = 38; $R^2 = 0.14$; $P = 0.013$). In conclusion, dietary EOB improved growth performance, nutrient utilization, and N balance in broilers in a dose-dependent manner. Although the estimated optimal supplementation level varied depending on the response variable, supplementation above 340 mg/kg was associated with diminishing marginal improvements in this trial.

**Keywords:** broiler; essential oil blend; nitrogen balance; nutrient utilization; optimal level

### 190 Dose-dependent effects of dietary propolis on growth performance, oxidative status, intestinal integrity, and body composition in broilers

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In broiler production, interest has increased in natural products as potential feed additives to support growth performance and physiological stability. This study aimed to evaluate the dose-dependent effects of dietary propolis supplementation on growth performance, oxidative status, intestinal integrity, and body composition in broilers. A total of 546 one-day-old broilers (Cobb 500, male) were randomly allocated into 42 cages (6 treatments with 7 replications) for 21 d. Treatments included six corn-soybean meal-based diets supplemented with 0, 250, 500, 1,000, 2,000, or 4,000 mg propolis/kg. Growth performance was measured weekly. Hepatic oxidative markers, jejunal morphology, expression of barrier-related genes, and body composition by dual-energy X-ray absorptiometry were measured on d 21. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, and orthogonal polynomial contrasts were applied to test linear and quadratic responses to dietary propolis levels. From d 1 to 21, body weight gain was greater in the 250 mg/kg propolis group than in the 0, 1,000, 2,000, and 4,000 mg/kg groups ($P < 0.05$). Feed conversion ratio was higher in the 1,000, 2,000, and 4,000 mg/kg groups than in the 250 and 500 mg/kg groups ($P < 0.05$). Hepatic malondialdehyde increased in the 1,000, 2,000, and 4,000 mg/kg groups compared with the 0, 250, and 500 mg/kg groups, whereas superoxide dismutase activity decreased with increasing dietary propolis ($P < 0.05$). Increasing dietary propolis linearly reduced the villus-to-crypt ratio and increased goblet cell count ($P < 0.05$). Increasing dietary propolis also linearly decreased zonula occludens-1, -2, and junctional adhesion molecule-2 expression, but increased claudin-1 and mucin-2 expression ($P < 0.05$). In addition, increasing dietary propolis linearly reduced bone mineral content and density ($P < 0.05$). These results demonstrate that dietary propolis at 250–500 mg/kg partially improved performance, whereas inclusion at 1,000 mg/kg or higher negatively affected growth, intestinal integrity parameters, oxidative status, and bone-related body composition. Thus, the present results suggest that dietary propolis at 250–500 mg/kg may be beneficial for broilers during the first 21 d, whereas higher inclusion levels may not be recommended.

**Keywords:** Broilers; Propolis; Feed additive; Growth Performance; Gut health

### 191 Evaluation of the effect of a spore-forming oligosaccharide-degrading probiotic on metabolizable energy, oligosaccharide disappearance, and cecal volatile fatty acid concentrations using restrict-fed broiler chickens

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The objective of this experiment was to determine fiber degradation and energy release by a probiotic (spore-forming Bacillus). The Bacillus used was previously selected for its ability to grow when provided galacto-oligosaccharides and other fibrous substrates. From 0 to 9 d-of-age, chickens were provided 1 of 2 nutritionally complete adaptation diets ad libitum. Diet 1 was a mash corn-soybean meal-based starter diet and diet 2 was the same as diet 1, except it contained $1 \times 10^6$ CFU/g of the probiotic. On d10, birds were individually weighed and allotted to treatment groups to equalize BW. Birds that were fed the probiotic during the experimental phase were also fed the probiotic during the adaptation phase. From 10 to 22 d-of-age, birds were feed restricted to 70% of the expected ad libitum feed intake per breeder guidelines to equalize feed intake. Dietary treatments were energy-deficient corn-soybean meal-based diets. Diet 1 was formulated to contain a nitrogen-corrected apparent metabolizable energy (AMEn) content of 2,547 kcal/kg as-fed. Diets 2 through 4 were the same as diet 1, except the AMEn content was increased in increments of 100 kcal/kg by adding dextrose in place of Solka Floc. Diets 5 and 6 were the same as diet 1, except they contained $1\times10^5$ CFU/g and $1\times10^6$ CFU/g of the probiotic, respectfully. Ileal digesta and excreta were collected at 22 d-of-age. Each treatment had 10 replicate pens of 5 birds. All data were analyzed using 1-way ANOVA and linear and quadratic regressions were performed for dietary treatments 1 through 4 by regressing BW gain vs. supplemental kcal/kg from dextrose. Bioavailable energy release for the probiotic was then calculated using the standard curve method. The significance value for all analyses was $P < 0.05$. There was a quadratic increase ($P < 0.05$) in BW gain as supplemental kcal/kg were added from dextrose ($R2 = 0.76$). The BW gain was greater for both doses of the probiotic compared with diet 1 ($P < 0.05$) and bioavailable energy release by the probiotic ranged from 39 to 46 kcal/kg. The ileal digestible energy and AMEn was increased by the addition of 1×106 CFU/g but not the lower dose of the probiotic ($P < 0.05$). Ileal oligosaccharide disappearance was numerically greater but not significantly different for diets 5 and 6 compared with diet 1 ($P > 0.05$). Acetic acid concentration in the ceca was greater for diets with the probiotic compared with diet 1 ($P < 0.05$). In conclusion, supplementation of the spore-forming Bacillus improved growth performance, ileal digestible energy, AMEn, and cecal acetic acid production, demonstrating that this additive can be used in poultry diets to increase energy availability. Supported by USDA-NIFA SAS # 2019-69012-29905.

**Keywords:** Bacillus; Metabolizable Energy; Oligosaccharide; Probiotic; Bioavailable Energy

### 192 Dietary supplementation of a blend of essential oils improves growth performance and intestinal barrier integrity in broiler chickens under tropical conditions

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High ambient temperature in tropical open-house systems can impair broiler productivity and intestinal barrier function; therefore, this study evaluated whether dietary supplementation with a blend of essential oils (CEO) could mitigate these effects. A total of 240 1-d-old male New Lohmann Indian River (MB 202 Platinum) broilers were assigned to a 2 × 3 factorial arrangement with two housing systems, closed house (CH) and open house (OH), and three CEO levels (0, 150, and 300 mg/kg). Each treatment had 8 replicates of 5 birds. Birds were managed from d 1 to 35, and dietary treatments were provided from d 11 to 35. The CH temperature and relative humidity were adjusted to broiler comfort standards, ranging from 30 to 20°C and approximately 60 to 70% RH, whereas OH temperature fluctuated from 24 to 34°C under tropical exposure, with estimated relative humidity of approximately 70 to 90%. Body weight and feed intake were recorded on d 21 and 35 to calculate body weight gain, feed intake, and feed conversion ratio. At d 35, birds were euthanized by halal slaughter through decapitation and jugular vein cutting, and blood, gastrointestinal and immune organs, jejunal tissue, and jejunal mucosa were collected. Jejunal histomorphology was evaluated using H&E-stained sections by measuring villus height, crypt depth, villus width, and villus height:crypt depth ratio, while tight-junction and inflammation-related genes were quantified by RT-qPCR. Data were analyzed using 2-way ANOVA for a factorial design, followed by Tukey's test at $P < 0.05$. Broilers reared in OH showed lower productivity and poorer jejunal histomorphology than those in CH ($P < 0.05$). Dietary CEO supplementation improved growth performance and jejunal morphology ($P < 0.05$). Open-house rearing upregulated IL-18 and IL-13 and downregulated OCLN, CLDN-1, JAM-2, and ZO-1 ($P < 0.05$), indicating impaired intestinal barrier function. In contrast, CEO supplementation downregulated TNF-A, IL-18, IL-6, IL-13, and IL-10, and upregulated OCLN, CLDN-1, JAM-2, and ZO-1 ($P < 0.05$). Significant housing × CEO interactions were detected for productivity and selected intestinal barrier endpoints ($P < 0.05$), with CEO supplementation improving responses more clearly under OH. In conclusion, dietary CEO supplementation, particularly 300 mg/kg, alleviated the negative effects of tropical OH conditions by improving broiler performance, jejunal morphology, and intestinal barrier integrity.

**Keywords:** broiler; essential oils; heat stress; intestinal barrier; tropical conditions

### 193 Effects of high-carotenoid corn and canthaxanthin on egg quality and reproduction in late-phase broiler breeders

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Broiler breeders in the late production phase experience declines in egg quality and reproductive efficiency, partly due to oxidative stress and physiological aging. Carotenoids have been reported to enhance antioxidant status and reproductive performance during this period. This study evaluated the effects of a high-carotenoid corn and canthaxanthin (carophyll red) on performance, egg quality, fertility, and hatchability in late-phase broiler breeders. At 49 weeks of age, 189 Aviagen Ross 708 females and Hubbard M99 males were randomly assigned to one of three diets: a control diet formulated with yellow corn, the same basal diet with a high-carotenoid corn replacing yellow corn, or the control diet supplemented with 6mg/kg carophyll red. There were 7 replicates per treatment, consisting of 8 females:1 male per replicate. Performance was measured from 49 to 52 weeks, and egg quality, fertility, and hatchability were assessed at 50 and 52 weeks. Data was analyzed using one-way ANOVA. Dietary treatments did not affect production variables, fertility, or hatchability ($P > 0.05$). Significant differences in yolk color scores were observed ($P < 0.001$), with carophyll red producing the darkest yolks, followed by the high-carotenoid corn at both 50 and 52 weeks. At 52 weeks, hens fed the high-carotenoid corn had lower shell thickness ($P = 0.028$) and shell breaking strength ($P < 0.001$) compared with the control and carophyll red treatments. Shell weight did not differ among treatments ($P > 0.05$). Hatchability remained high across all diets at 52 weeks but was not significantly affected. Overall, these findings suggest that while certain carotenoid sources can enhance yolk color and influence some shell quality parameters, they may not improve late-cycle reproductive efficiency in broiler breeder hens.

**Keywords:** Broiler breeders; oxidative stress; egg quality; fertility; hatchability

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## Metabolism and Nutrition: General Nutrition I

### 201 (A-F) (MTCC)

### 198 Methods for assessment of energy availability in lipid-based feedstuffs

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Lipids provide many functional roles in diets for poultry where they are a dense source of energy, improve nutrient digestibility, and increase pellet quality. Determining the availability energy content in lipid-based feedstuffs, however, is highly complex due to their extra-caloric and extra-metabolic effects that lead to metabolizable energy values that often exceed the gross energy content of the feedstuff. Relative comparisons of lipid sources can be made by substituting 1 level of each test ingredient into a common diet. Precise calculation of metabolizable energy values by difference for formulation matrixes using this approach should be done with caution, however, as the values are often overestimated in this approach. This is due to the test lipids reducing the digesta passage rate, which subsequently increases the metabolizable energy content of the base diet. A classical method for precise determination of the available energy content of lipids in broiler chickens utilizes energy-deficient diets that are restrict-fed to equalize feed intake among treatments. Lipids are then added at 2 increasing levels in place of an inert filler. The relative bioavailable energy content of test lipids is determined by regressing body weight gain against lipid inclusion level. A standard lipid source must be fed and the available energy content of the test ingredients is calculated by dividing the slope obtained for the test ingredients by the slope obtained for the standard reference ingredient. Newer approaches have focused on adaptation of the classical precision-fed rooster assay for lipid evaluation. In this approach, roosters are fasted for 26 h to allow for clearance of feed from the gastrointestinal tract, after which the birds precision-fed (crop intubated) approximately 25 g of their respective dietary treatments. For evaluation of lipid-based feedstuffs, the metabolizable energy of lipids can potentially be determined through direct measurement of lipid digestibility in diets where the test ingredient is the sole source of lipids. A regression method may also be used, in which 2 increasing levels of the test ingredients are added to the common base diet, which is often composed of corn or corn plus an inert filler. The determined metabolizable energy content of the diet is then regressed against the supplement lipid level. The relative metabolizable energy is calculated by dividing the slope obtained for the test ingredients by the slope obtained for the standard reference source. Multiple methods for evaluation of lipid-based feedstuffs now exist, which all offer different advantages depending on the objective of the study.

### 199 Metabolic markers linked to growth performance in Ross 708 broilers fed varying crude protein

Doyun Goo*[1], Seshidhar R. Gudidoddi[1], Sai Kumar Reddy Ragyari[1], Woo K. Kim[1] [1]*University of Georgia, Poultry Science, Athens, Georgia, United States*

A study was conducted to investigate the effects of different dietary crude protein (**CP**) levels on growth performance and the plasma metabolome of Ross 708 broilers at d 42. A total of 960 zero-day-old male broilers were randomly assigned to three dietary treatments with 16 replicates ($n$ = 16) per treatment (20 chickens per replicate). The corn-soybean meal-based three-phase diets (starter: d 0 to 14, grower: d 15 to 28, and finisher: d 29 to 42) were formulated to meet or exceed the Ross 708 Broiler Nutrition Specifications for energy and indispensable amino acids. The Medium (**Med**) CP diet contained 22.5%, 20.5%, and 19% CP for each phase, while the Low and High CP diets were formulated by decreasing or increasing the CP levels by 1.5% compared to the Med CP diet. Plasma was analyzed via GC-TOF-MS, and data were processed using MetaboAnalyst 6.0. Growth data were compared using independent t-tests, and correlations were assessed using Pearson ($r$) or Spearman ($\rho$) coefficients. At d 42, the Med CP group showed significantly higher body weight compared to both the Low and High CP groups ($P < 0.01$). For the comparison between the Med CP and Low CP groups, volcano plot analysis revealed that the Med CP group displayed higher levels of linolenic acid, pseudouridine, and glycine (FDR < 0.05). These metabolites were positively correlated with body weight, specifically for linolenic acid ($r = 0.351$, $P < 0.05$), pseudouridine ($r = 0.436$, $P < 0.05$), and glycine ($r = 0.385$, $P < 0.05$). The reduction of these metabolites in the Low CP group indicates a limitation in total nitrogen availability and potentially reflects a downregulation of protein translation machinery, even though essential amino acid levels were met. Among the top 15 metabolites ranked by VIP scores for the Med CP and High CP groups, malonic acid, sucrose, caproic acid, and oleamide were negatively correlated with body weight, while pipecolinic acid showed a positive correlation. The accumulation of malonic acid ($\rho = -0.517$, $P < 0.01$), which exhibited the significant negative correlation with body weight, indicates a disruption in the TCA cycle. Furthermore, the elevated sucrose ($\rho = -0.614$, $P < 0.001$) and oleamide ($\rho = -0.450$, $P < 0.01$) levels potentially reflect inefficient energy utilization and metabolic stress under high protein conditions. In conclusion, the Med CP level (19% in the finisher phase) is the metabolic optimum for broilers, as it maximizes growth by ensuring sufficient nitrogen for protein synthesis while avoiding the metabolic burden and accumulation of inhibitory metabolites such as malonic acid.

**Keywords:** broilers; crude protein; malonic acid; metabolomics; nitrogen metabolism

### 200 Testing metabolizable energy-derived net energy systems against an outcome-based energy system for predicting lean growth in broilers

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Poultry feed formulation is traditionally based on apparent metabolizable energy (AME) and recent proposed improvements rely on estimating net energy (NE). However, their capacity to predict biologically relevant outputs, particularly protein accretion (PAC), remains unclear. This study examined whether refinements of the classical NE framework improve prediction of PAC and fat gain (FG), and compared them with a directly measured, outcome-based system defined as productive energy (PE). Five energy systems were evaluated: (1) NE-1, classical NE, where NE = AME − heat increment (HI) and HI is derived from total heat production minus a reference fasting heat production (FHP-R); (2) NE-2, similar to NE-1 but using a measured fasting heat production value (FHP-M); (3) NE-3, incorporating directly determined net energy for gain (NEg) with dual-energy X-ray absorptiometry (DEXA) while retaining FHP-R; (4) NE-4, estimated from AME efficiency relative to body weight gain; and (5) PE, calculated as NEg (DEXA) plus net energy for maintenance (NEm, determined via indirect calorimetry using FHP-M). Eight experiments were conducted in a 96-floor pen facility using a total of 1920 day-old male broiler chicks, allocated to groups of 12 pens per experiment and fed 12 experimental diets differing in digestible amino acids (or digestible crude protein), digestible starch, digestible fat, and non-starch polysaccharides. All experiments were initiated simultaneously, with successive 7-day feeding periods initiated one week apart. A companion digestibility trial provided AME and AMEn values. Measured variables included body weight gain (BWG), PAC (g/bird per day), FG (g/bird per day), NEg (kcal/kg; DEXA), FHP-M, AME efficiency per BWG, and all derived NE estimates. Data were analyzed using a mixed model with treatment as fixed effect, experiment as random ef-

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fect, and 8 replicates per treatment. PE showed a strong relationship with PAC (P = 0.006; adj. $R^2$ = 0.98) and FG (P < 0.001). In contrast, NE-1 and NE-2 did not predict PAC (P > 0.20 and P = 0.15, respectively), although NE-2 showed limited predictive capacity for FG (P = 0.031). Incorporation of measured NEg in NE-3 improved statistical sensitivity but remained non-significant for PAC (P > 0.05). NE-4 similarly failed to predict PAC. These results indicate that modifications within the AME-derived NE framework do not resolve its limitations for predicting protein accretion. Systems relying on metabolizable energy appear insufficient to capture variation in lean tissue deposition, whereas directly measured productive energy provides a robust descriptor of both protein and fat gain.

**Keywords:** Net energy; Productive energy; Protein accretion; Meat production; Broilers

### 201 Practical interest of variables affecting the physical characteristics of commercial soybean meals

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The aim of this study was to determine the influence of the particle size, color, Fe content, and protein quality indicators (PQI) of commercial soybean meals (SBM) by country of origin. Samples were collected at random in European feed mills from 2015 to 2025 from Argentina (ARG; n = 115), Brazil (BRA; n = 89), and USA (n = 112). The variables studied were geometric mean diameter (GMD), Minolta color coordinates [L* (lightness), a* (redness), and b* (yellowness)], Fe content, and PQI [protein dispersibility index (PDI), KOH protein solubility (KOH), and trypsin inhibitor activity (TIA)]. Data were analyzed as a completely randomized design using the MIXED procedure of SAS, with SBM origin as main effect and year crop as a random effect. In addition, separate GLM regression models were performed to study the associations between L* or a* values with GMD, Fe content and PQI. Origin-specific slopes were derived from the fitted interaction model as linear combinations of the regression coefficients. Differences were considered significant at $P < 0.05$. The BRA meals were darker (L* = 66.0 vs. 67.6 vs. 68.7; $P < 0.001$) and redder (a* = 9.26 vs. 8.19 vs. 7.82; $P < 0.001$) than the ARG and USA meals, but b* values were not affected by SBM origin. The GMD (1,287 vs. 1,146 vs. 1,209 Mm; $P < 0.001$) and Fe content (138 vs. 102 vs. 110 mg/kg; $P < 0.001$) were higher for the BRA than for the ARG meals with the USA meals being intermediate. Also, all PQI values were higher ($P < 0.001$) for the USA meals than for the South American meals. The association between L* values and Fe content varied with the SBM origin. Overall, L* values decreased as the Fe content of the SBM increased ($P < 0.001$) but the slope was steeper ($P < 0.05$ for the interaction) for the ARG (-0.044) than for the BRA (-0.019; $P < 0.05$) or USA (-0.009; $P < 0.01$) meals. Also, L* values increased ($P < 0.001$) with increases in PDI, KOH, or TIA values, but no association was detected with the GMD of the SBM. The associations between a* and KOH varied with SBM origin. In fact, the a* values decreased with increases ($P < 0.001$) in KOH, a decline that was greater ($P < 0.05$ for the interaction) for the USA (-0.173) than for the BRA (-0.091; $P < 0.05$) and ARG (-0.080; $P < 0.01$) meals. Also, a* values increased with increases in Fe content ($P < 0.01$) but decreased with PDI ($P < 0.001$) and TIA ($P < 0.01$) values. In conclusion, L* and a* values of SBM are strongly associated with PQI. SBM color, which is assumed to reflect the intensity of the heat processing, is also affected by Fe content and SBM origin, effects that should be considered when the color is used as an indicator of SBM quality.

**Keywords:** Fe content; particle size; protein quality indicators; soybean meal color; soybean meal origin

### 202 Nutritional significance of condensed tannin content in laying hen diets in the context of high dietary inclusion of faba bean (*Vicia faba*): Effects on hen performance and objective egg quality

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Faba bean (*Vicia faba*) has tremendous potential as an alternative feed ingredient for laying hens, however most contemporary varieties grown in Canada and worldwide contain measurable levels of condensed tannins (CT), which are principally found in the hulls. The binding affinity of CT for proteins exerts an anti-nutritional effect by reducing amino acid digestibility and negatively impacting palatability. An experiment was therefore devised to test the null hypothesis that increasing CT content in diets containing high dietary inclusion of faba bean (36%) would have no effect on laying hen productivity or objective egg quality. Two varieties of faba bean differing widely in CT content ('Navi', low CT: 0.04 mg/g CT; 'Florent', high CT: 0.38 mg/g CT) were dehulled using a two-step procedure to yield hulls and de-hulled cotyledons. The procedure removed > 90% of hulls, and analysis indicated hulls accounted for 13.5% of total low CT faba bean wt. Hulls from Low CT and High CT contained 0.13 mg/g and 4.32 mg/g CT, respectively. A single wheat-based diet was formulated to include the equivalent of 36% (i.e., 31.14% dehulled cotyledon, 4.86% hull) of the low CT faba bean, which was also low in vicine/convicine. The treatments compared in this study were comprised of 4 test diets that were identical except for the ratio of low CT:high CT hulls added back: 100:0, 66:33, 33:66 or 0:100, respectively. Lohmann LSL hens (n=80; 42-54 wks of age) housed in conventional battery cages received one of the 4 test diets for a 12-wk experimental period in a randomized complete block design with 5 replicate cages per treatment. Productivity and objective egg quality were measured for each test cage in each of three, 4-week periods. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure (SAS Institute; Cary, NC, USA). Models included main effect of low CT:high CT hull ratio and block as a random term. Pen was specified as the subject of repeated measures in instances where measurements were taken on individual birds, eggs or portions thereof. The significance level for all statistical analyses was set at 5% (A = 0.05). The gradual substitution of low CT for high CT hulls had no effect on ADFI (P = 0.82), average egg wt (P = 0.44), lay percent (P = 0.43), or daily egg mass production (P = 0.22). Likewise, eggshell thickness (P = 0.99), Haugh units (P = 0.28); and proportional wet weight of albumen (P = 0.32), shell (P = 0.11) and yolk (P = 0.38) were unaffected by dietary treatment. In conclusion, if reduced AA digestibility is taken into account during formulation, laying hen diets containing high dietary inclusion of faba bean CT should not adversely affect hen productivity or egg quality.

**Keywords:** faba bean; laying hen ; antinutritional factor; productivity; egg quality

### 203 Immunoassay test coupled with HPLC-MS/MS allows for rapid screening and confirmatory quantification of mycotoxins in corn and poultry feed.

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Mycotoxin screening is critical in the poultry feed industry because, when ingested, these toxins can be detrimental to animal production and health. Protectively, there are mechanisms put in place to periodically test for the presence of these toxins in feed and feed ingredients to ensure that the levels are acceptable for animal consumption. The most common testing methods include High Performance Liquid Chromatography–Mass Spectrometry (HPLC-MS/MS) and Immunoassay-based Rapid Test. HPLC-MS/MS is the most precise and accurate measure, offering higher sensitivity and the ability to detect multiple toxins per assay. However, the Immuno-

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assay-based Rapid Test has its own advantages. The immunoassay approach is rapid, low cost, and requires less technical expertise, though it is limited to a single toxin per test and is limited in accuracy and precision by the matrixes. This study compared the performance of HPLC-MS/MS with a rapid, antibody-based detection system that used lateral flow test kits to quantify mycotoxins in corn (n=45), broiler feed (n=46), and layer feed (n=50) samples. The corn samples were analyzed for fumonisins (FUM), deoxynivalenol (DON), aflatoxins (AFLA), and zearalenone (ZEA). The feed samples were analyzed for AFLA, DON and FUM. The samples were collected from various locations within the southeastern United States. Descriptive statistic analysis showed that the rapid methodology tended to overestimate FUM concentrations and underestimated ZEA concentrations compared HPLC-MS/MS. The absolute errors for the rapid method tended to be less than 0.05 ppm for AFLA, less than 1ppm for DON and less than 3ppm for FUM, and less than 1ppm for ZEA. Although errors for the rapid method were sometimes large, when expressed as a percentage relative to HPLC-MS/MS. HPLC-MS/MS detected AFLA in 58% of the corn samples versus 98% with the rapid method, FUM was 98% for both methods. The rapid method detected both AFLA and DON in 98% and 6.5% AFLA and 83% DON in broiler feed samples. Both testing methods detected 100% FUM in both broiler and layer feed samples. These results show that the rapid method consistently detected AFLA in samples that HPLC-MS/MS did not detect. This was true across all sample types, but the effect was more pronounced in feed samples. While rapid mycotoxin testing methods provide numerous economic and ease-of-use benefits for observing mycotoxin levels in the real world, HPLC-MS/MS remains the gold standard due to its analytical accuracy.

**Keywords:** mycotoxins; HPLC-MS/MS; rapid screening; corn; feed safety

### 204 Vertical transfer of mycotoxins compromises broiler performance: mitigation through breeder-targeted intervention

Arnau Vidal[1], Fernando Lima[1], Don Ritter*[1] [1]*Innovad, Antwerp, Belgium*

Mycotoxin risk in poultry production is typically assessed through feed analysis at the broiler level. However, this approach overlooks early-life exposure arising from vertical transfer from breeders to progeny, potentially underestimating its biological and economic impact. This study investigated the role of breeder exposure in shaping broiler performance and evaluated a targeted mitigation strategy. A total of 15,000 Ross 308 breeders were allocated to two groups under commercial farm conditions: a control group receiving a standard diet and a test group receiving a diet supplemented with a mycotoxin detoxifier (Escent®; 1 kg/T). Systemic exposure in breeders was assessed through blood biomonitoring using dried blood spots. Eggs were collected and incubated, and progeny were reared under controlled conditions. Early-life performance parameters, including body weight, weight gain, feed intake, and feed conversion ratio, were evaluated during the first 14 days. Data were analyzed using a one-way ANOVA, considering treatment as fixed effect (n = 20 birds per group). Although mycotoxins in feed remained within accepted limits, multiple mycotoxins and metabolites were consistently detected in breeder blood. However, breeders supplemented with the detoxifier showed lower systemic mycotoxin levels, which was associated with numerically lower mortality compared to the control group. Breeder exposure was vertically transferred to progeny, as demonstrated by the presence of mycotoxins in day-old chicks that had not consumed feed. These findings highlight a key limitation of conventional risk assessment based solely on feed analysis. Broilers originating from untreated breeders showed reduced early-life performance, confirming that prehatch exposure has measurable biological consequences. In contrast, mitigation at the breeder level significantly improved outcomes. Broilers derived from supplemented breeders exhibited a +7% increase in body weight ($p < 0.05$), accompanied by consistent improvements in growth during the starter phase. These results demonstrate that mycotoxins are not only a feed-related issue but a systemic and transgenerational challenge that begins before hatch. Early-life exposure represents a critical window of susceptibility where performance trajectories are established. Managing mycotoxin exposure at the breeder level therefore constitutes a high-impact intervention point. Biomonitoring enables detection of hidden exposure and provides a more accurate assessment of risk than feed analysis. Integrating biomonitoring strategies with targeted mitigation at the breeder level represents a promising approach to improve broiler performance and production consistency under commercial conditions.

**Keywords:** Mycotoxins; vertical transfer; Broiler breeders; Biomonitoring; Performance

### 205 Effect of increasing deoxynivalenol (DON) contaminated diets containing corn DDGS on growth performance and health of broilers

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Exposure to Deoxynivalenol (DON) can negatively affect broiler growth performance and health, and severity depends on DON concentration in the feed. This study investigated effects of increasing dietary concentrations of DON on broiler growth performance and intestinal health. A total of 1,088 one-day-old male chicks were allocated to 44 floor pens. From day 0 to 13, birds were fed a diet containing 7% 'clean' cDDGS (2.7 ppm DON analyzed) to acclimate before trial initiation. At day 14, pens were assigned to one of 4 dietary treatments with DON levels: Low (0.3 ppm), Mid-low (1.8 ppm), Mid-high (2.5 ppm), and High (3.2 ppm). All experimental diets included 15% cDDGS. 'Contaminated' cDDGS (18 ppm DON analyzed) was blended with clean cDDGS to achieve the respective DON concentrations. Birds and feed were weighed to assess growth performance and feed efficiency at 0, 3, 7, 14, 21, and 25 days after DON exposure (ADE). At day 7 ADE, one bird per pen was euthanized, the small intestine was weighed, and jejunum sample collected for histological evaluation. Data were analyzed as a completely randomized design using PROC GLIMMIX in SAS and orthogonal polynomial contrasts tested linear and quadratic effects. There was a quadratic response ($P < 0.05$) for FCR on day 3, where broilers fed High DON had the highest value (1.54, 1.66, 1.55, 2.21 ± 0.15). Between day 7 and 21 ADE, there were no differences in growth performance across treatments (BW 1452 ± 22.5 g; ADG 79 ± 2.39 g/d; ADFI 118.6 ± 3.4 g/d; FCR 1.53 ± 0.05). BW at day 25 was similar across treatments (2380.7 ± 31 g); however, there was a quadratic response ($P < 0.05$) on day 25 to DON level for ADFI (186, 162, 182, 172 ± 5.7 g/d) and for the overall study period (131, 125, 131, 126 ± 2.4 g/d). A similar quadratic response ($P < 0.05$) was observed for FCR at day 25 (2.64, 2.00, 2.48, 2.44 ± 0.12 g/d) and overall (1.76, 1.66, 1.75, 1.71 ± 0.03 g/d). There was a quadratic response ($P < 0.10$) for the percentage of small intestine weight relative to body weight at day 7 ADE, with Low and High having the lowest percentages, and Mid-low and Mid-high having the highest values (3.6, 4.1, 3.9, 3.7 ± 0.13%). Jejunal villus height (710, 746, 873, 826 ± 0.03 µm) and villus/crypt ratio (3.3, 3.7, 4.2, 4.1 ± 0.35) increased linearly ($P < 0.05$) with increasing DON levels, while crypt depth showed no difference (223, 204, 209, 206 ± 15.7 Mm). These findings suggest that dietary DON levels up to 3.2 ppm may impair broiler growth performance during the first three days of exposure, but birds can adapt to those DON levels with prolonged feeding. Dietary DON levels around 3.2 ppm may have a stimulatory effect on the small intestinal.

**Keywords:** intestinal health; mycotoxin; poultry; vomitoxin

### 206 Environmental stressors amplify aflatoxin B1 toxicity in broilers: implications for risk assessment and mitigation strategies

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**under commercial conditions.**

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Aflatoxin B1 (AFB1) exposure occurs under conditions where birds are simultaneously subjected to multiple environmental stressors, which may amplify its effects. However, most experimental studies are conducted under controlled research conditions, potentially underestimating the real impact observed under commercial production. This study evaluated the interaction between AFB1 exposure and environmental conditions by comparing broilers reared in a commercial farm versus a controlled research facility, and assessed the efficacy of a dietary detoxifier. A total of 2,160 one-day-old Ross 308 broilers were assigned to a 2×2 factorial design comparing clean and naturally contaminated diets (22–45 µg/kg AFB1) under two environments: controlled research facility and commercial farm conditions. An additional treatment assessed a detoxifier (Escent®, Innovad) in contaminated diets under commercial conditions. Birds were reared for 35 days, and body weight (BW), feed intake (FI), feed conversion ratio (FCR), antibody titers, and plasma biochemical parameters were measured. Data were analyzed using two-way ANOVA to evaluate effects and interactions, and one-way ANOVA for detoxifier effects. Significant differences were observed between environments, with broilers reared under commercial conditions showing lower BW, reduced FI, and poorer FCR compared to those in the research facility (P<0.01), confirming the presence of a higher cumulative challenge. This divergence indicates that controlled research conditions underestimate the impact of production stressors. Within this context, AFB1 exposure significantly impaired performance (P<0.05), with a pronounced interaction between mycotoxin exposure and environment. Under commercial conditions, AFB1 reduced final BW by up to 12%, whereas the same exposure under controlled conditions resulted in minimal impact (~2%). These findings demonstrate that environmental stressors are a key determinant of mycotoxin toxicity, likely through cumulative metabolic burden, immune activation, and increased susceptibility to secondary challenges. Inclusion of the detoxifier significantly improved performance compared to the contaminated diet (P<0.05), increasing final BW by 6.5% and restoring FCR and FI to levels comparable to clean feed. Besides, it mitigated increases in plasma uric acid and stabilized immune responses. These results demonstrate that conventional experimental models underestimate mycotoxin risk under field conditions. Incorporating environmental complexity into experimental design is essential for accurate risk assessment, and targeted detoxification strategies can effectively mitigate the combined effects of mycotoxins and production stressors in commercial systems.

**Keywords:** aflatoxin B1; Broilers; Envrionmental stress; Mycotoxins; Detoxification

## 207 Locally produced rice distillers dried grains with solubles (RDDGS) as a suitable cost-effective protein ingredient for layer chicken diets

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Rising production costs in Sri Lanka's poultry sector have intensified the need to identify economically viable, locally available alternative feed ingredients for layer hen nutrition. Hence, a 10-week feeding experiment was conducted to compare the productive performance and nutrient utilization of laying hens receiving diets containing either locally produced Rice Distiller's Dried Grains with Solubles (RDDGS) or imported Corn Distiller's Dried Grains with Solubles (CDDGS) in the absence of enzymatic supplementation. A total of 360 Hy-Line White hens, commencing at 30 weeks of age, were randomly assigned across six dietary treatments following a 2 × 3 completely randomized factorial design. Each treatment was replicated with groups of 12 floor-housed birds, totaling 60 birds per treatment. Both DDGS sources were incorporated at three progressive inclusion levels of 50, 75, and 100 g/kg, and all experimental diets were formulated to be iso-caloric and iso-nitrogenous. Results revealed that the RDDGS fraction contained 14.45% ± 0.63 (dry matter basis) non-protein nitrogen substances. Among the measured parameters, egg weight and yolk index were significantly elevated in hens consuming RDDGS-supplemented diets at all inclusion levels up to 100 g/kg when compared to their CDDGS-fed counterparts (P<0.05). Conversely, no significant treatment effects were detected for egg shape index, shell thickness, egg mass, yolk color, Haugh unit scores, or feed conversion ratio. Although dry matter digestibility was significantly lower (P<0.05) in hens fed RDDGS diets relative to CDDGS diets (0.68 vs. 0.77), escalating levels of RDDGS inclusion progressively lowered the feed cost per kilogram of egg mass and per dozen eggs. Overall, RDDGS demonstrated suitability as a dietary ingredient for layer hens at inclusion levels of up to 100 g/kg without compromising productive performance, while delivering a feed cost reduction by approximately 3.8%. Rice distillers dried grains with solubles can be included in layer diets at levels up to 100 g/kg without adverse effects on production performance or egg quality, while improving egg weight and yolk index compared to CDDGS. Despite a reduction in dry matter digestibility, RDDGS inclusion reduced feed cost per unit of egg output by approximately 3.8%, indicating its economic advantage as a local alternative feed ingredient. Looking ahead, the use of locally produced RDDGS offers a promising strategy to enhance feed self-sufficiency and reduce costs in Sri Lanka's poultry sector, with future research needed to optimize nutrient utilization and validate long-term performance and sustainability under commercial conditions.

**Keywords:** distillery by-product; rice DDGS; alternative feed ingredients; feed cost reduction; egg weight

## 208 Impact of soybean origin and harvest year on the chemical composition, amino acid profile, and protein quality indicators of soybean meals collected from 2006 to 2025

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The chemical composition and protein quality of raw soybeans are influenced by numerous factors, including area of production, soil type, agronomic practices, and weather conditions. As a result, the nutritional value of commercial soybean meals (SBM) fluctuates depending on crop year. The objective of this research was to evaluate the effects of the country of origin of the beans on the chemical composition, amino acid (AA) profile, and protein quality indicators of commercial SBM collected at random from 2006 to 2025. In total, 954 samples from Argentina (ARG; n = 307), Brazil (BRA; n = 320), and United States (USA; n = 327) were collected at crushing plants and feed mills at different locations of the European Union by specialized personnel. Samples were analyzed by wet chemistry, except for AA that were determined by NIRS. Data were analyzed using mixed linear models (MIXED procedure of SAS), with country of origin of the beans as the main effect and harvest year as a random effect. Variance components were estimated to assess the contribution of the annual variability to the total variance. On 88% DM bases, CP (47.1 vs. 45.9 and 46.2%), crude fiber (4.83 vs. 4.15 and 4.19%), neutral detergent fiber (10.1 vs. 8.81 and 8.95%), and raffinose (1.34 and 1.27 vs. 1.11%) were higher ($P$ < 0.001), and sucrose (5.74 vs. 7.12 and 7.11%) and stachyose (4.33 vs. 4.83 vs. 4.17%) contents lower ($P$ < 0.001) for the

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BRA meals than for the ARG and USA meals. The abundance and the AA profile of the SBM varied with the origin. Lys (6.11 vs. 6.15 vs. 6.07% CP) and TSAA (2.81 and 2.81 vs. 2.76% CP) were higher ($P < 0.001$) for the ARG and USA meals than for the BRA meals. Also, Ca, P, and K were lower and Mg and Fe contents higher ($P < 0.05$) for the BRA than for the USA and ARG meals. Protein dispersibility index (PDI), KOH solubility (KOH), and trypsin inhibitor activity (TIA) were higher for the USA meals than for the South American meals ($P < 0.01$) but no differences were detected for urease activity or reactive Lys. Harvest year explained a substantial proportion of the variability for most of the variables studied. In this respect, crop year justified 40.0, 34.2, and 33.7% of the variability detected for sucrose, stachyose, and raffinose, respectively. Similarly, most of the variability for AA profile of the SBM was explained by harvest year, accounting for 33.6% and 74.5% of the variability of Lys and TSAA profile. For PDI, KOH, and TIA, 38.9, 56.1, and 40.4% of the variability observed was attributed to harvest year effect. It is concluded that harvest year and country of origin of the beans are the most relevant factors affecting the chemical composition, AA profile, and protein quality of commercial SBM.

**Keywords:** amino acid profile; chemical composition; harvest year; protein quality indicators; soybean meal origin

## Extension and Instruction

### 203 (B-D) (MTCC)

### 209 Know Your Flock: Audience-Centric Strategies for Extension and Technical Services

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The cornerstone of effective poultry science extension is not the technical data itself, but the "science of producing effective messages". Success in extension is defined by persuading a specific audience to adopt new technologies and change behaviors—a goal that is fundamentally audience-centric. While traditional classroom teaching often focuses on knowledge transfer in controlled environments, extension audiences are often subject to restrictions on availability, modes of interaction, and potential conflicts of interest, all of which necessitate a deep understanding of how people learn as a prerequisite for success. This includes recognizing the three primary types of learners based on how they perceive information: visual, auditory, and kinesthetic. In the digital age, blended in-person training and E-learning systems provide the necessary flexibility to cater to these diverse cognitive styles. Research in the poultry industry has shown that when digital tools are designed with the audience in mind—using interactive, self-paced modules featuring validation questions, practical exercises, and real-world production scenarios—they can achieve an 80% completion rate, significantly outperforming passive video formats. As we integrate Artificial Intelligence (AI) into extension services, the challenge lies in designing tools that prompt active learning rather than passive consumption. AI can save significant time by automating part of technical assistance, but its implementation must be rooted in the specific needs and "perceived usefulness" of the audience to ensure it is a functional tool for field improvement rather than a mere novelty. Its adoption is driven by the Technology Acceptance Model (TAM), in which users prioritize systems they believe will directly improve their job performance. Ultimately, knowing your audience is the most critical variable in extension work. To maximize outcomes, projects must move away from "one-size-fits-all" models toward content tailored to targeted audiences. Success is not measured by the volume of information delivered, but by the resonance of the message and resulting real-world behavior change. This presentation discusses these principles through several cases of extension projects that leverage modern digital and AI tools to ensure scientific innovations are not just communicated but successfully implemented in the field.

### 210 AI-Assisted Innovation Projects Enhance Applied Problem-Solving in Poultry Science Students

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The objective of this study was to evaluate the effectiveness of an AI-collaborative innovation assignment (FABricate) designed to improve systems thinking, problem-solving, and critical evaluation of generative artificial intelligence among undergraduate poultry science students. Students in Introduction to Poultry Science (POUL 2020) at the University of Georgia completed the FABricate project, in which teams identified a real-world poultry industry challenge and developed a proposed solution using iterative collaboration with generative AI tools from Google's Gemini or OpenAI's ChatGPT. Student learning outcomes were evaluated using two complementary approaches. Students completed pre- and post-semester Likert surveys (1 = strongly disagree, 5 = strongly agree) measuring confidence in identifying industry problems, proposing solutions, evaluating constraints, and critically using AI. Additionally, students completed short written responses to a structured prompt before and after the project. Responses were scored using a rubric assessing problem identification, solution development, constraint recognition, AI integration, and systems thinking (0–5 scale per category). Pre- and post-project responses were analyzed using descriptive statistics and Welch's t-tests. Likert survey responses increased significantly across all competencies following the FABricate project ($P < 0.01$). The largest gains were observed in students' ability to identify constraints in poultry production systems, evaluate stakeholder perspectives, and refine AI-generated ideas using disciplinary knowledge. Rubric-scored written responses also improved significantly, with mean scores increasing from $4.57 \pm 2.45$ pre-project to $7.23 \pm 2.03$ post-project ($t = 4.60$; $P < 0.01$). Student reflections indicated that generative AI accelerated brainstorming and idea development while requiring human evaluation to ensure biological feasibility and industry relevance. AI-collaborative design projects can effectively strengthen applied reasoning, systems thinking, and critical AI literacy in poultry science education. Integrating generative AI within structured innovation assignments provides a promising framework for preparing students to evaluate emerging technologies and develop solutions to complex challenges in poultry production.

**Keywords:** generative AI; poultry education; undergraduate instruction

### 211 Development and implementation of an online poultry medicine course for practicing veterinarians

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There has been a growing interest in small/home poultry flocks around the USA and the rest of the world. As with any animal production enterprise, health problems occur, and this can be a food safety concern. There are very few veterinarians that specialize in poultry medicine, and most of those are employed by large poultry production companies. Very few practicing veterinarians are comfortable working with poultry. To address this shortfall, an online training program designed to educate general-practice veterinarians about poultry issues in small/backyard flocks was developed. The course was launched in September 2022. To date 259 people have registered for the course. Of those, 116 have completed the course and received CE credits from AAAP. The average time to completion was 117 days but varied from less than 1 day to 594 days. Practicing veterinarians are required to get CE credits to renew their licenses, but finding the time to take the courses has proven challenging for many. Regular reminders helped to increase the number completing the course.

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An intro survey was conducted to learn more about the participants. The course was designed for practicing veterinarians. Of the 174 who completed the intro survey, 62 were veterinarians in small animal practice, 14 in large animal practice, and 37 were in mixed animal practice. 18 worked for a government agency and 18 took the course to help with their own flocks. The remaining were taking the course for other reasons. 58 were om a rural area; 45 in an urban area; and 35 worked in a suburban area. Although the course was designed for American veterinarians, there were participants from Canada, Western Europe, Asia, Africa and Australia/New Zealand. As has been the trend with veterinarian students, most of the participants were female. Some of the participants were students while others had been in practice for more than 15 years. Only 26 of the respondents had no poultry medicine experience, with the majority having some experience. 88 of the respondents had poultry at the time they were taking the course. 116 of the respondents anticipated seeing poultry as a part of their practice. After completion of the course, 124 participants completed the exit survey. Most of the participants increased their understanding of the veterinary needs of small/home poultry as well as increased their interest in providing veterinary care for poultry. However, only 58 of those responding to the exit survey ranked their confidence in their ability to provide veterinary care to poultry as high or very high. More training is obviously needed to increase the number of veterinarians willing to provide veterinary care for small and home poultry flocks.

**Keywords:** Poultry medicine; Online Course; Small/home poultry; Veterinarians

### 212 Development of a Comprehensive Scientific Writing Course for Animal and Poultry Science Undergraduate and Graduate Students

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Scientific writing is an essential part of undergraduate and graduate education in animal and poultry sciences for developing skills for processing and interpreting literature in these fields. However, many students have limited structured opportunities to develop these skills. This is important, as the writing of project reports, research papers, and disserations requires a command of the scientific literature and the ability to integrate information from different perspectives. This challenge is particularly evident among graduate students, who often begin dissertation literature reviews only after much of their research has already been completed, rather than using the review process to strengthen the project rationale and guide study design. Therefore, we hypothesized that a course that emphasizes the stepwise approach to comprehensive writing would address this issue. In response, we developed "Comprehensive Writing in Agricultural Sciences," a hybrid 2-credit course designed to support discipline-specific scientific writing through a process-based framework. The inaugural course enrolled 13 students, including 31% undergraduates and 69% graduates. Two writing tracks were established. Undergraduates developed peer-reviewed research papers, and graduate students generated review papers intended to serve as foundations for dissertation literature reviews. The course emphasized scientific writing as an ongoing process rather than a single final product. A studio-style format was used, focusing on reading strategies, synthesis, drafting, revision, and peer review. Oral progress updates were a common part of the class, with input from the entire class and the instructors. Students worked in small groups composed of contrasting research areas, allowing them to receive responses from peers with different disciplinary perspectives. Outcomes from the course indicated that repeated drafting, structured peer feedback, and cross-disciplinary discussion strengthened manuscript development and broadened students' appreciation for alternative scientific viewpoints. This course model shows a practical approach for integrating writing instruction into animal and poultry science training and may provide a useful framework for improving scientific communication skills at both the undergraduate and graduate levels.

**Keywords:** scientific writing; graduate students; undergraduate students; studio-style instruction; peer review

### 213 Chickens online: enhancing accessibility and impact of poultry science to youth through virtual and hybrid programming

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Poultry Judging(PJ) events (4-H, FFA, collegiate) are a well-established tool providing youth hands-on Poultry Science learning while fostering critical thinking, analytical skills, and career readiness. Youth are scored on their evaluation accuracy of multiple classes of Market Poultry, Market Eggs, and Past Production Hens while being introduced to opportunities in the vertically integrated poultry industry, NC's leading Ag commodity. However, youth participation in PJ can be hindered by logistical constraints including transportation barriers, limited access to animals, and a growing disconnect between our population and animal agriculture by which fewer knowledgeable mentors are available for coaching. NC poultry youth programs were moved to virtual formats during Covid shutdowns, modelling newfound accessibility. In the post-covid era, virtual tutelage remains relevant, especially among youth. Therefore, to bridge the gap between PJ training and access, this study evaluates the efficacy of innovative virtual and hybrid PJ instructional models. Building on seven years of successful implementation, a PJ pilot program was expanded to 38 youth across 18 NC counties, bringing youth together from distances spanning 350 miles. The curriculum utilizes a multi-platform approach, including Zoom, online annotations, and digitized materials from nationwide resources. Only materials participants provide are eggs, whole bird, digital 4-H PJ manual, and internet/computer access for the respective coaching sessions. Participants receive synchronous virtual instruction with real-time feedback via private chat with assistant coaches. To compare pedagogical outcomes, half of the participants followed a strictly virtual path, while the other half engaged in a hybrid model featuring in-person sessions every fourth meeting. Program efficacy is measured through longitudinal data, including online quiz performance and competitive scores at state-level 4-H Poultry Judging events compared against non-virtually coached peers. Preliminary results and qualitative feedback from parents and participants indicate that the program effectively builds workforce skills and encourages enrollment in collegiate Animal Science programs. By leveraging the portable and adaptable nature of PJ, this model demonstrates that virtual platforms can democratize access to livestock education, providing a scalable solution for youth who lack the resources to raise animals but seek a pathway into the poultry industry.

**Keywords:** Poultry Judging; Youth Education; virtual youth programming; 4-H and Extension; Poultry Life Skills

### 214 Strengthening agricultural literacy through outreach and public engagement at the University of Manitoba's Glenlea Research Station

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Engaging the public to learn about agriculture and where their food comes from is increasingly important, as more people have become disconnected from rural life. A well-informed public will support not only farmers but also the broader food system, including industry and academic efforts to enhance environmental sustainability through research. The University of Manitoba's Faculty of Agricultural and Food Sciences (FAFS) operates the Glenlea Research Station (GRS), a research and teaching facility that supports training and public outreach initiatives. Within GRS, the Bruce D. Campbell Farm and Food Discovery Centre (FFDC) provides immersive learning experiences that enhance public awareness and understanding of modern, sustainable food production. Visitors explore key aspects of agriculture, including crop and livestock production, animal welfare, biosecurity, nutrition, and food safety. The Centre also serves as an outreach platform for provincial commodity groups and highlights ongoing research conducted at the National Centre for Livestock and the Environment and within FAFS. The Manitoba Egg Farmers Learning and Research Centre (MEFLRC) is a poultry research, education, training, and outreach facility at GRS, established through a partnership among the Manitoba Egg Farmers, the university, and federal and provincial governments. The Centre provides learning experiences in egg production, animal welfare, nutrition, farm management, and the industry's societal role. Its unique design includes a public viewing area with direct observation of aviary and enriched housing systems, as well as egg collection and storage facilities. Through interactive exhibits, guided tours, workshops, curriculum-linked field trips and special events, both the FFDC and MEFLRC offer engaging and accessible learning environments for visitors of all ages and backgrounds. In 2025, the FFDC and MEFLRC hosted 3,172 visitors at public outreach events, including Discover Agriculture on the Farm, Amazing Ag Adventure, and other public programs. An additional 5,189 individuals participated in curriculum-linked programs and structured educational activities, of whom 59% were from urban areas. Furthermore, 1,163 industry and government representatives, along with 1,706 students and faculty members, visited the facilities over the year. Together, the Bruce D. Campbell Farm and Food Discovery Centre and the Manitoba Egg Farmers Learning and Research Centre demonstrate how integrated, hands-on learning within an academic setting can effectively enhance agricultural literacy, strengthen public trust, and promote meaningful connections between consumers, industry, and science.

**Keywords:** Bruce D. Campbell Farm and Food Discovery Centre; Manitoba Egg Farmers Learning and Research Centre; education; outreach ; public engagement

### 215 Regional Broiler Chicken Mortality Trends in the last 15 years

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The United States, with its geographically diverse broiler production system, faces region-specific challenges that impact mortality, and these dynamics have evolved over the past 15 years. This study evaluated regional trends in total mortality, harvest age, and live weight using the Agri Stats database, encompassing approximately 150 billion broilers produced between January 2010 and June 2025. Data were analyzed across five major U.S. regions (Midwest, Deep South, Northeast + NC, South Central, and Southeast). Linear regression models were fitted for each variable using year as the predictor. Statistical analyses were conducted using JMP v19 (SAS Institute). Regional differences in production strategies were evident. Harvest weight increased ($P < 0.05$) disproportionately in the Northeast + NC and South Central regions, at approximately 51 and 43 grams/year, respectively. These increases were accompanied by significant increases in harvest age of 0.24 and 0.17 days/year. In contrast, the Deep South and Southeast regions showed smaller but significant increases in harvest weight (9 grams/year), while harvest age decreased by 0.22 and 0.12 days/year, respectively ($P < 0.05$). In the Midwest, harvest weight did not change significantly ($P > 0.10$), whereas harvest age decreased by 0.37 days/year ($P < 0.01$). Total mortality increased ($P < 0.01$) in all regions, with the greatest annual increases observed in the Northeast + NC (0.20%) and South Central (0.22%) regions, followed by the Deep South and Southeast (0.15%), and the Midwest (0.08%). Broiler mortality trends in the United States are strongly influenced by regional production strategies. Regions pursuing heavier birds at extended grow-out periods exhibited the greatest increases in mortality, whereas regions reducing target weights showed more moderate mortality trends.

**Keywords:** Metabolism; Health; Performance; Diseases; Outbreak

### 216 Subclinical coccidiosis and its impact on broiler performance in medicated flocks in Southern Ontario

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Subclinical coccidiosis is a common field challenge in commercial broiler production. Even under a routine anticoccidial medication program, oocyst cycling can persist, quietly affecting gut health and feed efficiency without clear clinical signs. Due to reliance on medication-based control, the true scenario of subclinical coccidiosis may be underestimated. Oocysts per gram of feces (OPG) is a practical, low-cost technique to measure field coccidiosis pressure and provide feedback to update nutrition and anticoccidial programs under commercial conditions. This field study evaluated the relationship between OPG and performance efficiency using data from commercial broiler barns under anticoccidial medication programs. Between 27 and 29 days of age, ≥60 fresh fecal samples per barn were collected from 26 broiler barns in Southern Ontario over 15 months. Body weight (BW) and cumulative mortality were recorded at sampling, while final BW, final mortality, and feed conversion ratio (FCR) were obtained at flock close–out. Anticoccidial medication programs were rotated seasonally and applied uniformly across all barns. OPG was determined by the McMaster flotation technique, and results were log–transformed as log10(OPG + 1) prior to statistical analysis. Associations were evaluated using linear mixed effects models with log–transformed oocysts per gram (OPG_log) as a fixed effect, medication program and season as covariates, and barn as a random effect. OPG values were widely distributed, with 7.02% ≤1,000, 12.28% between 1,001 and 10,000, 33.33% between 10,001 and 50,000, 28.07% between 50,001 and 150,000, and 19.30% >150,000 oocysts per gram. Mixed effects models showed no consistent association between OPG_log and BW or mortality. In contrast, OPG_log was positively associated with FCR (+0.054 per unit increase in log10(OPG + 1)), with marginal and conditional $R^{<span style="font-size:10.8333px">2</span>}$ of 0.268 and 0.642, respectively, indicating a consistent negative impact of higher OPG on FCR independent of season and medication program. Therefore, in commercial broiler barns using anticoccidials, OPG counts were not related to BW or mortality, but were consistently linked to poorer FCR. These results indicate that subclinical coccidiosis contributes to sub–optimal economic efficiency in broiler production and support routine OPG monitoring even in medicated flocks. OPG can therefore be used as a practical tool to detect hidden feed efficiency losses and guide timely adjustments in nutrition and anticoccidial programs in commercial broilers.

**Keywords:** coccidiosis; Oocysts counts; Broiler performance; Anticoccidial; feed conversion ratio

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### 217 Industry-led collaboration to address emerging challenges in egg production and farm management

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Emerging challenges in farm management practices and environmental conditions must be addressed to ensure the adoption of best practices, high standards of animal welfare, and economic and environmental sustainability. Manitoba Egg Farmers (MEF), representing 170 egg and pullet producers across the province, is actively engaged in collaborative research initiatives to address emerging issues in egg production and farm management. These efforts aim to provide producers with reliable information and practical, evidence-based strategies that can be readily implemented on-farm. The Manitoba Egg Farmers Learning and Research Centre (MEFLRC), a poultry research, education, training, and outreach facility located at the University of Manitoba Glenlea Research Station, serves as a hub for these activities. Established in 2023 through a partnership among MEF, the University of Manitoba, and federal and provincial governments, the Centre enables collaborative research and knowledge transfer. It offers dedicated research and training infrastructure, including a large meeting space and a wet laboratory suitable for hands-on training for farmers and industry professionals. In partnership with Manitoba Agriculture, the University of Manitoba, and Elite Services (a company specializing in poultry handling, vaccination and euthanasia), a demonstration trial on end-of-lay hen composting was conducted in 2024. The method was demonstrated within a permitted solid manure storage facility at the MEFLRC. A composting brochure, released in 2025, provides a comprehensive overview of the trial and outlines best practices for composting end-of-lay hens using the "lasagna" windrow method. In addition, MEF supported a collaborative project with Manitoba Agriculture and the University of Manitoba to assess LED lighting in commercial egg production systems. Since LED flicker may negatively affect avian behaviour and performance, in-barn assessments were conducted in 2025 to evaluate its presence across three housing systems: aviary, enriched, and conventional. Each participating farm received a detailed report including measurements of light intensity, flicker percentage, and flicker index, along with recommendations for system improvements. As part of this initiative, two factsheets were developed by the University of Manitoba and distributed in 2025 to all registered egg and pullet producers. These initiatives highlight the value of collaborative, industry-driven research in addressing practical challenges in egg production. By integrating research, training, and knowledge transfer, the MEFLRC contributes to the adoption of innovative, science-based practices.

**Keywords:** Manitoba Egg Farmers Learning and Research Centre; training; in-barn trial; education; factsheet

### 218 Research and Innovation Perspectives: Global Pathways for Poultry Welfare in 2050

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Poultry welfare is evolving globally as technological innovation, stakeholder expectations, and sustainability priorities increasingly shape production practices. The objective of this work was to synthesize global stakeholder perspectives on the future of poultry welfare and identify priority research, innovation, and implementation needs to guide welfare improvements toward 2050. A structured stakeholder engagement approach was used to collect qualitative input on poultry welfare challenges, opportunities, and future directions across regions and poultry sectors. The Research and Innovation Committee of the International Poultry Welfare Alliance (IPWA) conducted seven facilitated icebreaker sessions at major poultry industry and scientific conferences between January 2024 and April 2025. More than 200 participants representing academia, industry, allied services, students, animal welfare organizations, and government from Europe, Asia, Africa, Oceania, and the Americas participated. Participants engaged in short, guided discussions addressing the question: *"What does poultry welfare look like in 2050, and how do we get there?"* Discussions were organized around eight topic areas: housing and management, positive welfare, artificial intelligence (AI), cross-poultry species applications, end-of-production to processing, nutrition, robustness, and welfare–sustainability interactions. Written responses were compiled across events. Responses were summarized using descriptive frequency analysis, including identification of the most commonly reported themes within each topic area and visualization using word cloud analysis. Across regions and stakeholder groups, consistent themes emerged. Participants emphasized the need for data-driven, outcome-based welfare indicators; integration of AI and precision livestock technologies for real-time monitoring and decision support; improved environmental enrichment and housing design; enhanced training and education across the value chain; and alignment of welfare, robustness, productivity, and sustainability goals. Transparency, global collaboration, and cross-poultry species learning were identified as critical driving forces. Global stakeholder input indicates that achieving meaningful improvements in poultry welfare by 2050 will require coordinated investment in research, innovation, education, and the adoption of technologies. These findings emphasize the need for future research to convert stakeholder priorities into practical, validated tools, metrics, and management practices that can be readily adopted across commercial poultry production systems.

**Keywords:** poultry welfare; stakeholder engagement; innovation; sustainability; precision livestock farming

## Metabolism and Nutrition: Feed Additives I

**202 (A-D) (MTCC)**

### 219 Antibiotic-free poultry production for food security, food safety, and environmental stewardship

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The progressive use of antibiotic growth promoters (AGPs) in poultry production has raised major concerns for human, animal, and environmental health due to the development and spread of antimicrobial resistance. Residual antibiotics in poultry products compromise food safety, while the release of residues into the environment through manure contributes to ecological contamination and accelerates resistance in non-target organisms. These risks have triggered global efforts to restrict or ban AGPs, particularly in high-income countries, and have intensified calls for sustainable, antibiotic-free production systems. Antibiotic-free poultry production seeks to safeguard food security and safety while mitigating

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environmental risks, but it faces challenges, including reduced productivity, higher disease susceptibility, and economic constraints. Addressing these challenges requires a holistic approach that integrates improved management practices, biosecurity measures, vaccination, natural antimicrobial alternatives, and supportive policy frameworks. At the same time, gaps in research and adoption remain most evident in low- and middle-income countries, where misuse of antibiotics persists and the burden of multidrug-resistant pathogens is high. This presentation will 1) examine the mechanisms by which AGPs promote growth and contribute to antimicrobial resistance, 2) address the challenges and opportunities of transitioning to antibiotic-free poultry systems using prebiotics, probiotics, synbiotics, bacteriophages, phytobiotics, acidifiers, and enzymes among others, and 3) explore regulatory and sustainability considerations. The presentation will also emphasize that achieving sustainable antibiotic-free poultry production requires coordinated action by researchers, policymakers, industry stakeholders, farmers, and consumers to establish resilient systems that deliver safe, high-quality poultry products while promoting public health, food security, and environmental stewardship.

Keywords: Antibiotics, Environmental health, Natural additives, Health care systems, Poultry, Sustainable production

### 220 Partial replacement of synthetic vitamin E by olive pulp in broilers: effects on performance, oxidative status, and breast meat quality

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This study investigated whether a standardized olive pulp (OP; Innovad Group, Belgium), specified to contain ≥7.5% total polyphenols and ≥1% triterpenoids, could partially replace synthetic vitamin E (VE) in broiler diets while preserving growth performance, oxidative status, and meat quality. A total of 770 male Ross 308 broilers were reared for 42 days (14 birds/pen; 11 pens/treatment) and assigned to five dietary treatments: CON+ (80 mg/kg VE), CON− (40 mg/kg VE), OLIVE1 (40 mg/kg VE + 40 mg/kg OP), OLIVE2 (40 mg/kg VE + 60 mg/kg OP), and OLIVE3 (80 mg/kg VE + 80 mg/kg OP). Birds were reared under standard commercial conditions. Performance parameters (body weight; BW, feed intake; FI, feed conversion ratio; FCR) were recorded at days 0, 10, 21, 35 and 42. At slaughter, breast fillet weight, drip loss (24 h post-mortem), and breast muscle myopathies (Wooden Breast; WB, White Striping; WS; Spaghetti Meat; SM) were assessed in two randomly selected birds per pen. Antioxidant status was evaluated using the KRL biological test (KIRIAL/SPIRAL) at day 37 (2 birds/pen), measuring resistance to free radical–induced hemolysis in whole blood and erythrocytes (RBC). Performance and carcass traits were analyzed by one-way ANOVA. Drip loss data were analyzed following logit transformation for variance stabilization. Breast myopathies were analyzed using the non-parametric Kruskal–Wallis test followed by post-hoc comparisons when applicable. KRL data were analyzed by ANOVA, with exploratory correlation analyses performed between KRL values and growth performance indicators. No significant differences were observed among treatments for BW, FI, or FCR at any time point, indicating that partial replacement of VE did not impair zootechnical performance. Breast fillet weight after 24 h chilling was not affected by treatment. There was a weak indication that treatment affects drip loss ($P = 0.10$). Birds fed OLIVE2 yielded lower WB scores than those on CON− ($P = 0.02$). No treatment effects were detected for WS or SM. Half-hemolysis time values in whole blood and RBC were higher in CON+ than in CON- ($P < 0.05$). OLIVE treatments showed variable responses, with OLIVE3 reaching values comparable to CON+. Exploratory regression analyses suggested a weak negative association ($P = 0.12$) between RBC half-hemolysis time to and weight gain. Partial replacement of VE with OP maintained growth performance and FCR, while indication the potential to reducing WB severity and drip loss. These findings support the potential of OP as a sustainable VE-sparing strategy, improving meat quality without compromising performance. Further studies with higher numbers of breast fillets analysed are required to support these findings.

**Keywords:** Poultry; vitamin E replacement; olive pulp; antioxidant status; meat quality

### 221 Effects of mango seed kernel extract on the gut microbiota of broilers following oral gavage

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Mango seed kernel contains multiple bioactive compounds reported to act as antimicrobial, antioxidant, and anti-inflammatory agents. This experiment aimed to evaluate the effects of mango seed kernel extract (MSKE), obtained using an optimized green extraction method, on inhibiting pathogenic microbes in broiler chickens and to determine the dose rate that achieves the highest inhibition under non-challenged conditions, using oral gavage. The research trial was conducted in two experiments, A and B, each with four treatment groups: A1–A4 (A1 did not receive oral gavage, while A2, A3, and A4 groups received oral gavage with MSKE at dose rates of 1x, 3x, and 5x, respectively) and B1–B4 (B1 group received oral gavage with distilled water (0x). In contrast, B2, B3, and B4 groups were orally gavage with MSKE at 0.5x, 1x, and 1.5x, respectively) on days 7, 14, and 21 to three birds per treatment. One-day-old male broiler chicks for experiment A (n=240) and B (n=252) were equally divided into four treatment groups, with three replicates each, following a completely randomized design. Throughout both experimental trials, the birds were fed a basal diet without antibiotics. The birds were euthanized at 2, 4, 8, 12, 24, and 36 hours after oral gavage and then sampled for gut microbiota counts by collecting intestinal contents from ileum and caeca. Contents from the selected gut locations from each bird per treatment per time interval were pooled into sterile falcon tubes and processed for microbiota count. The resulting data were analyzed using one-way ANOVA with Tukey's Post Hoc test. As a result, on days 7, 14, and 21, the MSKE treatment groups showed lower *E. coli* and Clostridium *perfringens* colony counts compared to without oral gavage) and gavage with distilled water groups up to 8 hours. In experiment A, on days 7, 14, and 21, A2 (1x), A3 (3x), and A4 (5x) showed lower *E. coli* and Clostridium *perfringens* colony counts than A1 (without oral gavage) for up to 8 hours. In experiment B, on day 7, B2, B3, and B4 showed significantly lower *E. coli* colony counts than B1 (given oral gavage with distilled water) for up to 8 hours. On day 21, B2, B3, and B4 showed significantly lower Clostridium *perfringens* colony counts than B1 for up to 12 hours. Similar results were observed for different dose levels (0.5x, 1x and 1.5x). Hence, it can be concluded that oral gavage of MSKE shows antimicrobial activity against *E. coli* and Clostridium *perfringens* for up to 8 to 12 hours, even at a low dose of 0.5x. Therefore, it can be concluded that the oral gavage method is useful for studying the effects of supplementing various feed additives on gut modulation at different time points.

**Keywords:** mango seed kernel; extract; oral gavage; gut microbiota; antimicrobial

### 222 Exploring the modulatory effects of spotted pumpkin (*Lagenaria breviflora*) fruit aqueous extract on growth performance, behaviour and blood profile of broiler chickens

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There is increasing concern over the health risks and antibiotic resistance linked to the use of synthetic growth promoters in poultry production. This study investigated the use of spotted pumpkin (*Lagenaria breviflora*) fruit aqueous extract as a phytogenic alternative for improving growth performance, behaviour, and physiological status in broiler chickens through its bioactive and antioxidant properties. Two hundred forty day-old Ross 308 chicks were randomly allotted to six treatments in a 2 (300 and 500 g/L) × 3 (0, 3, and 4 times/week) factorial arrangement within a completely randomised design and administered the extract via drinking water from day 7 to 42. Growth indices (weight gain, feed intake, water intake, and feed conversion ratio) were measured over the experimental period. Behavioural activities as indicators of welfare and activity levels (feeding, drinking, walking, dust-bathing, and wing-flapping) were recorded using closed-circuit cameras and analysed using scan sampling during the starter (day 7–21) and finisher (day 22–42) phases. Blood samples were analysed using standard laboratory procedures, and data were subjected to two-way ANOVA. Extract concentration did not significantly affect body weight gain or feed conversion ratio; however, birds that received 300 g/L had higher feed and water intake than those on 500 g/L. Administration frequency significantly ($P < 0.05$) influenced growth performance, with the control group recording the highest final weight and weight gain. Behaviour did not differ during the starter phase; however, during the finisher phase, birds on 300 g/L showed greater feeding, drinking, walking, dust-bathing, and wing-flapping than those on 500 g/L, whereas birds treated four times/week were generally less active. Haematological indices were not affected by treatment. Serum biochemical parameters were significantly affected ($P < 0.05$), with birds receiving 500 g/L showing higher total protein and albumin levels and lower malondialdehyde levels. In conclusion, *Lagenaria breviflora* extract affected behavioural expression and serum biochemical indices without adverse health effects. The lower concentration (300 g/L) increased feed intake and activity, while the higher concentration (500 g/L) improved antioxidant-related indices, indicating a dose-dependent response.

**Keywords:** broiler chickens; phytogenic additive; antioxidant status; serum biochemistry; behaviour

### 223 Effects of *Astragalus* polysaccharides on growth performance, antioxidant capacity, gut microbiota, and cecal metabolome of yellow-feathered broilers under microplastics exposure

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Microplastics (MP) have become an emerging environmental threat to the poultry industry, causing oxidative stress and gut dysbiosis. *Astragalus* polysaccharides (APS), a bioactive natural polysaccharide, possess potent antioxidant properties. This study aimed to investigate the protective effects of dietary APS supplementation against MP-induced impairments in yellow-feathered broilers. A total of 240 one-day-old male broilers were randomly assigned to four treatments (6 replicates of 10 birds per replicate) for 54 days, including Control group (basal diet), MP group (basal diet + 200 mg/kg polystyrene MP), APS group (basal diet + 200 mg/kg APS), and APSMP group (basal diet + 200 mg/kg MP + 200 mg/kg APS). Data on growth performance, antioxidant capacity, and intestinal morphology were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Results indicated that MP exposure reduced the overall average daily gain (ADG) and body weight (BW). However, the APSMP group successfully ameliorated the MP-induced growth inhibition, restoring the final BW to a level comparable to the Control group (1763.8 vs. 1738.0 g; $P>0.05$), partly through a compensatory increase in feed intake. For serum antioxidant status at 54 d, MP exposure significantly increased the malondialdehyde (MDA) content compared to the Control (6.22 vs. 4.04 nmol/mL; $P<0.05$). Conversely, APS supplementation (APSMP group) effectively reversed this lipid peroxidation, significantly reducing the MDA level to 3.25 nmol/mL ($P<0.05$). Intestinal morphology analysis revealed that the APSMP group had a significantly increased jejunal villus height compared to both the Control and MP groups ($P<0.01$), expanding the absorptive surface area. Furthermore, 16S rDNA sequencing of cecal microbiota demonstrated that MP exposure drastically increased the relative abundance of the stress-related genus *Rikenellaceae_RC9_gut_group*, whereas the APSMP treatment significantly suppressed its overgrowth ($P<0.05$). Additionally, untargeted metabolomics of cecal contents revealed that the APSMP intervention significantly modulated 246 differential metabolites (114 in positive and 132 in negative ion modes) compared to the MP group alone. KEGG pathway enrichment analysis indicated that APS mitigated MP-induced metabolic shifts primarily by regulating the biosynthesis of amino acids and lipid metabolism pathways. In conclusion, dietary APS supplementation effectively alleviates MP-induced oxidative damage, improves jejunal morphology, and restores gut microbiota homeostasis and metabolic profiles, highlighting its potential as a promising nutritional intervention against environmental MP toxicity in broilers.

**Keywords:** Astragalus polysaccharides; broiler; microplastics; gut microbiota; metabolomics

### 224 The effects of a polyherbal formulation versus choline chloride 60% or betaine HCL on broiler growth performance, carcass cuts, and muscle myopathies

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Choline is an essential vitamin for optimal bird health and performance. It is traditionally supplied through dietary ingredients and choline chloride (CC). Betaine, a choline derivative, is used as a feed additive for methyl donation, heat stress mitigation, and potential gut health benefits. A proprietary polyherbal formulation (PHF; Kolin Plus) has demonstrated performance benefits in poultry and nursery pigs but has limited data regarding carcass yield. This experiment compared CC, betaine, and PHF on broiler performance and carcass characteristics from 0–57 days. A total of 2,250 Ross 708 chicks were assigned to three treatments in a completely randomized block design and fed diets containing 2.0 lbs/ton CC (60%), 1.1 lbs/ton betaine HCl (95%), or 0.3 lbs/ton PHF. Data were analyzed using ANOVA with the MIXED procedure of SAS, with significance set at $P \leq 0.05$. From day 0–16, PHF-fed birds showed reduced ($P \leq 0.01$) body weight (BW), feed intake (FI), and average daily gain (ADG), but both PHF and betaine treatments exhibited improved ($P \leq 0.01$) feed conversion ratio (FCR) compared to CC. From day 16–35, birds fed PHF or betaine had lower ($P < 0.05$) BW and FI than CC-fed birds. Betaine-fed birds had the highest ($P < 0.05$) FCR during the grower phase, which persisted throughout the study. During the finisher phase, PHF-fed birds performed similarly to CC-fed birds in ADG and FI ($P > 0.05$), while betaine-fed birds were lighter and consumed less feed ($P = 0.004$). Overall performance from day 0–57 showed no differences between CC and PHF in ADG or FCR. Betaine-fed birds had the lowest BW, lowest ADG, and highest FCR ($P < 0.05$). Carcass analysis showed a trend ($P = 0.064$) toward reduced boneless leg weight in betaine-fed birds, with no differences in wing, breast, or tender weights. Birds fed CC or PHF had fewer ($P < 0.05$) woody breast positives than those fed betaine. In conclusion, PHF reduced performance from days 0–35 but achieved comparable results to the CC control by day 57, while betaine fed birds were outperformed by PHF and CC fed birds over the entire experimental period. PHF and

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CC fed birds showed positive improvements in woody breast instances compared to betaine fed birds, showing that PHF can effectively replace CC and betaine while reducing woody breast incidence in broiler diets.

**Keywords:** choline chloride; betaine ; woody breast; choline alternative; feed conversion

### 225 Dietary guanidinoacetic acid supplementation favorably modulates energy dynamics and nitrogen balance in broilers

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The arginine-sparing potential of guanidinoacetic acid (GAA) has been well documented. However, information regarding its effects on energy dynamics and nitrogen balance in broilers is limited. It was hypothesized that dietary GAA enhances the efficiency of energy and nitrogen utilization and reduces energy losses as heat, thereby improving the feed efficiency of broilers. This study evaluated the effects of dietary GAA supplementation on heat increment, net energy, efficiency of energy utilization, and nitrogen and energy balance in broilers using closed-circuit calorimetry chambers. Day-old Ross 308 male broilers (n = 80) were fed common starter and grower diets until 18 d of age. From d 19, birds started to receive the grower diet supplemented with 0%, 0.03%, 0.06% or 0.09% GAA. Following a four-day acclimation period in chambers (d 21 to 25), indirect calorimetry measurements were conducted from d 25 to 28 using two birds per chamber. Each dietary treatment consisted of eight replicates, divided across two consecutive experimental runs, with four replicates per run. The efficacy of GAA on growth performance was evidenced by a linear improvement in feed conversion ratio (FCR, P = 0.022) as GAA levels increased, along with a tendency for quadratic responses in body weight gain and feed intake. Dietary GAA did not affect apparent metabolizable energy (AME) or nitrogen-corrected AME (AMEn) of the feed. However, dietary net energy (NE) tended to increase linearly with increasing GAA inclusion. The efficiency of AME utilization for NE, expressed as NE/AME and NE/AMEn, was affected by GAA supplementation, with a linear increase in NE/AMEn (P = 0.017) and a tendency for a linear increase in NE/AME. Dietary GAA linearly decreased (P = 0.031) AME intake per unit of body weight gain while it had no impact on NE intake. Increasing dietary GAA levels increased nitrogen (N) retention in a quadratic manner (P = 0.017) and linearly improved N utilization efficiency (P = 0.002). Heat production decreased linearly (P = 0.015) with increasing GAA concentration. There was no effect of GAA on total retained energy (RE) but the RE as protein increased quadratically (P = 0.022) and RE as fat tended to decrease linearly with increasing GAA levels. In conclusion, dietary GAA improved nitrogen utilization efficiency and the efficiency of AME utilization for NE, and favorably modulated body energy partitioning by enhancing energy retention as protein, thereby improving feed efficiency in broilers.

**Keywords:** Broiler; Energy metabolism; Guanidinoacetic acid; Net energy; Nitrogen balance

### 226 *The use of immunoglobulin enriched blood plasma in broiler chicken nutrition to improve growth performance*

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The use of feed enriched with immunoglobulin Y (equivalent to mammalian IgG) represents a form of passive immunization that may improve broiler chicken growth performance. The use of IgY-rich plasma, obtained by centrifugation of blood collected during poultry slaughter, offers a means of valorizing slaughter by-products within waste-recycling framework. The study aimed to produce IgY-enriched blood plasma preparations and evaluate their effects in broiler nutrition. Dried plasma with an activity of approximately 200 g IgY/kg protein (plasma 200) was used alongside preparations enriched to 400 and 600 g IgY/kg protein using plasma membrane separation (microfiltration) or salting-out of IgY-containing globulins in NaCl solutions. These preparations were incorporated into feed to achieve IgY levels of 1 or 2 g/kg. The feeding trial was conducted in two phases. The initial small-scale experiment involved 819 Ross 308 broilers housed on a floor system and allocated to seven groups: a control group and groups receiving plasma 200, 400, or 600 at two levels of IgY inclusion levels. A standard diet (energy value 2960-3150 kcal/kg) was used. In the second phase, a large-scale industrial trial was performed with 222,000 chickens divided into three groups: control and groups receiving plasma 200 or 400 at an IgY level of 1 g/kg, with three poultry houses per group. IgY activity was quantified by radial immunodiffusion, and specific activity against *Salmonella*, *Escherichia coli* and *Campylobacter* was determined using ELISA method. Growth performance parameters, including body weight, feed intake, and mortality, were recorded and analyzed using one- or two-way ANOVA (statistically significant differences were tested using the Tukey test). Enriching plasma with IgY increased activity against *Campylobacter*, whereas the effect against *Salmonella* was limited. The most favorable growth performance was observed with plasma 400 in feed at an inclusion level of 1g IgY/kg, resulting in a 4.73% increase in body weight relative to the control (control = 100%) and a 2.2% reduction in feed intake. In the large-scale trial, birds receiving plasma 400 achieved the highest body weight (2.86 kg vs. 2.67 kg in the control), corresponding to an increase of approximately 7%, although feed intake was not reduced. In conclusion, supplementation of broiler diets with IgY-enriched chicken plasma at a level corresponding to 1 g IgY/kg improved the growth performance under both experimental and industrial conditions. This research was co-financed by the National Center for Research and Development (Poland) from European Funds-project No. POIR.01.01.01-00-0849/18.

**Keywords:** blood plasma; immunoglobulin G; broiler; growth performance

### 227 Validating predictions for AME and digestible arginine sparing of guanidinoacetic acid in protein-reduced broiler diets

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Guanidinoacetic acid (GAA), a creatine precursor, can enhance cellular energy utilization and improve feed efficiency in broilers. Considering the metabolic pathway of creatine formation in vertebrates, supplementation of GAA can also spare dietary arginine and glycine. Based on these modes of action and several broiler trials, the GuanAMINO® Recommendation Tool was developed and patented (Evonik Operations GmbH, Germany). This tool estimates the sparing potential of GAA for metabolizable energy (AME) and standardized ileal digestible arginine (SID Arg) depending on dietary nutrient levels, sex, feeding phases and performance level of broilers. Therefore, a broiler feeding trial was conducted to evaluate the suggested AME- and Arg-sparing potentials estimated by the GuanAMINO® Recommendation tool at Klipkraal Broiler Research Institute, South Africa. It was hypothesized that performance can be maintained by applying AME and SID Arg reductions predicted by the GuanAMINO® Recommendation Tool when supplementing 600 g GAA per ton of feed, whereas omitting GAA supplementation would impair broiler performance. A total of 2,160 male day-old Ross 308 chicks were allocated to 48 floor pens (9 or 10 pens/treatment, 45 birds/pen) and fed

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starter (1 to 8 d), grower (9 to 18 d), and finisher (19 to 30 d) diets. Five treatments were: T1) Positive control diet formulated to meet the AA and energy requirements (PC); T2) T1 but with AME reductions (51/70/95 kcal AME per kg in starter/grower/finisher) + 600 g/t GAA (AME); T3) T1 but with Arg-sparing (0.035/0.055/0.057% SID Arg in starter/grower/finisher) + 600 g/t GAA (Arg); T4) AME & Arg-sparing as in T2 and T3 combined, respectively + 600 g/t GAA (AME&Arg); and T5) AME & Arg-sparing as T4 but no GAA (NC). Grower (19.9%) and finisher (18.0%) diet CP-levels were reduced versus local practice by approx. 1% point while starter diets contained 22% CP. Body weight and feed intake were measured weekly; FCR was corrected for mortality. Data were analyzed by one-way ANOVA (Tukey; P<0.05). Body weights did not differ among treatments throughout the trial. From 1 to 30 d, broilers fed the NC diet had the poorest FCR and greater than the PC diet (1.285 vs 1.255; P<0.05). From 1 to 21 d, broilers fed the NC diet had greater FCR than Arg (1.187 vs 1.153) and AME&Arg (1.144) treatments (P<0.05). From 1 to 7 d, the broilers fed the NC diet had a greater FCR than the broilers fed the AME diet (1.157 vs 0.958; P<0.05). In conclusion, applying the sparing for AME and SID Arg predicted with the GuanAMINO® Recommendation Tool at 600 g/t GAA maintained growth performance parameters throughout the trial while reducing overall feed cost up to 3.7% in a South African price scenario.

**Keywords:** broiler; guanidinoacetic acid; metabolizable energy; standardized ileal digestible arginine; feed conversion ratio

### 228 Muscadine Grape Pomace Alters Oxidative Stress and Cecal Microbiota Without Affecting Growth in Broilers

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The muscadine grape (*Vitis rotundifolia*) is native to the southeastern U.S. and widely used for wine, juice, and other products. The wine processing leaves behind skins and seeds, or grape pomace (GP), which contain polyphenolic compounds with antioxidant and immunogenic properties. This study evaluated the effects of dietary supplementation with 2.5% GP on broiler chicken performance, organ development and serum biomarkers. It was hypothesized that GP supplementation would enhance growth, organ development, and health metrics. Fifty-four Ross-708 broilers were assigned to either a control or GP-supplemented diet in a randomized complete block design with nine replicate cages per treatment and three birds per cage from days 25 to 35 post-hatch. At day 35, birds were euthanized via carbon dioxide asphyxiation for necropsy. Performance parameters, including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were measured alongside internal organ and breast muscle traits. Nutrient digestibility was measured with titanium dioxide as an indigestible marker. Serum was analyzed for cholesterol, corticosterone (CORT), and oxidative stress markers. Data were analyzed using a one-way ANOVA in the MIXED procedure of SAS (SAS Institute Inc.), with cage as the experimental unit, and significance declared at P < 0.05. No significant differences were observed in BW, BWG, FI, or FCR between control and GP-fed birds. Relative organ weights (g/kg) of the liver, heart, pancreas, and bursa, as well as breast muscle traits, were unaffected by GP supplementation, though a marginal reduction in relative spleen weight (P = 0.08) was noted in GP-fed birds. Serum LDL and HDL cholesterol, CORT, and glucose were unaffected by dietary treatments. However, serum hydrogen peroxide concentration increased (0.29 vs. 0.38 µg/mL), while total antioxidant capacity (TAC, µM) decreased in GP-fed birds compared to controls (P < 0.05). Alpha diversity in cecal digesta was reduced GP-fed birds (P < 0.05, Shannon index) while beta diversity indicated no major shifts in overall community structure. Nevertheless, several taxa differed in abundance between treatments (P < 0.05), suggesting selective microbial responses to GP constituents. While digestibility data are still being processed, current findings indicate that muscadine grape pomace exerts selective physiological and microbial effects without altering growth and underscores the need for further work clarifying its functional role in broiler nutrition and health.

**Keywords:** Grape pomace; Broiler; Oxidative stress; Polyphenols; microbiome

### 229 Assessing a Novel Phytogenic Formulation in Broilers Challenged with Subclinical Enteritis

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The study assessed the effect of a novel phytogenic formulation in broilers challenged with enteritis. Two hundred eighty male day-old chicks were distributed into 40 battery cages adapted to contain litter and assigned to four treatments: NC (negative control; unchallenged), PC (positive control; challenged), and NG1 and NG2, consisting of birds challenged and fed diets with 50 or 70 ppm of a proprietary phytogenic formulation (Nature Grow; CKM SAC; Peru), respectively. PC, NG1, and NG2 birds received inocula of *Clostridium perfringens* ($10^8$ CFU/bird per day) at 18, 19, and 20 d, with a 12-h fasting period prior to each inoculation. All birds were fed crumble (1-21 d) or pellet (22-42 d) wheat-soybean meal diets high in crude protein (1-21 d, 26% CP; 22-42 d, 21% CP), including a partially overcooked soybean meal, and were in contact with reused litter (100% used litter). At d 21, birds were transferred to 32 battery cages, maintaining the treatments. Performance, excreta quality, and body temperature were monitored weekly, along with necropsies. Watery droppings were determined using Whatman absorbent paper when the diameter of the moisture halo was at least twice that of the fecal droplet. Body surface temperature was recorded in each bird with a handheld infrared thermometer (non-contact) at five anatomical sites: periocular region, wing surface, shank, dorsal region, and inguinal region. Data were analyzed under a one-way ANOVA, and further analyses were performed using mixed models in JMP 16. The results showed a negative influence of the challenge (PC vs. NC) on performance (BW 21, 28 d; BW gain 15-21, 21-28 d; P<0.05) and excreta quality (watery, from 21 d onward; P<0.05). NG influenced bird performance (improved BW gain, attenuated the negative impact of the challenge on feed intake, P<0.05), and improved excreta quality (P<0.05). NG2 influenced the body temperature before and after the challenge (P<0.05). Results indicate that NG2 modulates energy metabolism, likely through bioactive compounds influencing gut function and metabolism, as evidenced by (1) reduced body temperature in non-challenged birds (d 7 and 14; -0.2 to -0.6 °C; P<0.02), suggesting decreased heat production, consistent with reduced basal metabolic rate and maintenance energy expenditure, and (2) increased body temperature during the acute phase response (d 21; +0.6 °C; P<0.01), consistent with an enhanced metabolic response. In conclusion, the phytogenic formulation mitigated the negative effects of enteric challenge, while NG2 promoted more favorable energy partitioning, characterized by reduced basal heat production and enhanced metabolic response during the acute phase.

**Keywords:** Phytogenic; Energy metabolism; Challenge; Antibiotic-free; Broilers

## Sunday, July 12, 2026

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## Metabolism and Nutrition: Vitamins and Minerals I

### 206 (A-D) (MTCC)

### 230 Advancing Robust Calcium and Phosphorus Nutrition in Modern Poultry

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Conventional nutrition systems rely on estimating feed ingredient values to formulate a ration at the lowest cost, meeting nutrient requirements and ingredient incorporation constraints. To be more robust considering the changing and various production objectives and optimization criteria among farms multi-criteria approaches are needed. This is particular to nutrients related to sustainability concerns such as phosphorus (P) for which both animal and plant production rely on a non-finite resource that is thus subject to price peak. Nevertheless, P is essential for both soft tissue and bone growth and its deficiency have a strong negative impact on feed intake and growth performance in poultry. Its deposition into bone necessitate calcium (Ca) has they are fixed together in the form of hydroxyapatite in a fixed ratio; Ca is thus tightly linked with P efficiency optimization. To make matters more complex, Ca can negatively affect P utilization by forming insoluble complexes in the gastrointestinal tract, thereby reducing its absorption. In addition, high dietary Ca can impair bird health and performance due to high acid-binding capacity of limestone, and the fact that excess Ca increased water consumption leading to wet litter and pododermatitis as well as a greater risk of necrotic enteritis. To handle the complexity of Ca and P interactions and optimize P use while ensuring growth potential and optimal bone mineralization, mechanistic modeling is particularly appropriate. Combining animal experiments and meta-analyses to generate empirical data is also essential for developing and validate robust multi-criteria models in both broilers and laying hens. To achieve this, a highly generic and context-adaptable approach (e.g., applicable to both fast- and slow-growing birds) is developed, with the additional final objective of enabling real-time simulations.

### 231 Digestible Calcium Based Diet Formulation Maintains Broiler Performance Across Limestone Sources with Varying Solubility

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Calcium and phosphorus balance is vital for poultry growth and skeletal health. Despite advances in digestible P systems, in practice diets remain over formulated for total Ca, reducing precision and efficiency. Improved understanding of limestone (LS) quality is driving adoption of digestible Ca systems. The study aimed to validate a newly developed tool which calculates the dig calcium coefficient of LS (dig Ca) using a database of 50 LS samples. The tool was used to generate dig Ca coefficients for 3 distinct LS which were subsequently used to formulate to both total and digestible Ca and available and digestible P systems. Both performance and bone mineralisation were evaluated. In total, 3060-day-old Ross 308 broilers were randomly assigned to 9 treatments (10 pens/treatment with 34 birds/pen). Three LS were: a coarse, slow solubility LS (Dig Ca 31.27%), a medium LS with intermediate solubility (Dig Ca 51.73%), and a very fine, highly soluble LS (Dig Ca 57.51%). Diets were formulated using three approaches: available P with total Ca, dig P with total Ca, and dig P with dig Ca. Dig Ca: dig P ratios were formulated by phase to meet dig Ca requirement detailed by literature. All diets contained 1,000 FTU/kg Axtra® PHY GOLD with the associated matrix contribution for Ca, P, ME, AA and Na recommended by supplier and fed *ad lib*. BWG, FI, FCR, livability, and European Production Efficiency Index (EPEI) were determined to day 35. Statistical analyses using ANOVA with Tukeys post-hoc (JMP 16). Across all LS profiles, diets formulated on a dig Ca: dig P basis supported performance, livability, and tibia ash values comparable to those obtained with tCa formulations. In diets utilising a fast soluble LS, typically detrimental to performance, the 35d BWG was (2662g vs 2596 and 2629g in dCa: dP vs tCa:AvP and tCa:dP respectively, P>0.05) and livability was 99.63% vs 98.84 and 99.71% (P>0.05). The economic return was maintained (EPEI 532.25 vs 518.21 and 531.45) and FCR was comparable (1.42 vs 1.40 and 1.41, P>0.05). In coarse LS containing diets, dig systems also maintained 35d BWG (2606 vs 2599 and 2604g in dig Ca:dig P vs tCa:AvP and tCa:dP), with comparable FCR 1.42 (vs 1.42 and 1.41) and livability (99.68% vs 97.77 and 99.58%). These results demonstrate that in the presence of a fast-acting phytase, it is possible to formulate using variable LS qualities to digestible systems, challenge historic minimal total Ca constraints (5.6g/kg in total Ca finisher diets vs 3.44-5.05g/kg in dig Ca diets) and utilise previously perceived detrimental LS qualities without compromising broiler performance or skeletal integrity.

**Keywords:** phytase; broilers; digestible calcium; formulation

### 232 Whole body composition in broiler chickens fed low available phosphorus corn and soybean meal-based diets supplemented with doses of monocalcium phosphate or *E. coli* phytase

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Phosphorus (**P**) nutrition in broilers is commonly evaluated using growth performance and bone traits, although the whole body is the ultimate sink for dietary nutrients. Building on a previous study that established non-phytate phosphorus equivalence values for a novel *Escherichia coli* phytase in corn-soybean meal diets, this study evaluated whole-body responses to graded available phosphorus (**AvP**) from monocalcium phosphate (**MCP**) or phytase in a low-AvP diet. A total of 2,060, 21-day-old male Ross 708 chicks were placed in floor pens (22 birds/pen) housed in three rooms and allocated to 10 diets (n=7). Birds were fed a common starter diet from d 0 to 7 and experimental diets in grower (d 8 to 21) and finisher (d 22 to 35). The AvP diets were formulated to contain (%) 0.22/0.18, 0.27/0.23, 0.32/0.28, 0.37/0.33, and 0.42/0.38 (grower/finisher) through MCP supplementation. Phytase diets were created by adding 250, 500, 1,000, 1,500, or 2,000 FTU of phytase/kg in 0.22/18 diet. Birds had free access to water and feed throughout the study. On d 35, one bird per pen was randomly selected, euthanized, and the whole body defeathered, weighed, ground, freeze-dried, and analyzed for dry matter (**DM**), crude protein (**CP**), fat, and ash contents. Data were expressed as g/kg defeathered BW on a DM basis. The data were analyzed using PROC GLIMMIX in SAS, with diet as a fixed effect and block (room) as a random effect. Orthogonal polynomial contrasts were used to test responses to AvP and phytase. The pen was the experimental unit. Increasing AvP had no effect on whole-body DM or fat content ($P \geq 0.203$) but increased defeathered BW linearly ($P = 0.041$). However, CP showed linear and non-linear ($P \leq 0.047$), whereas ash exhibited a quadratic response ($P = 0.018$) to AvP. In contrast, phytase linearly increased BW ($P = 0.011$), CP ($P = 0.005$), and ash ($P = 0.012$). Phytase increased fat content quartically ($P = 0.050$). Overall, 1,000 FTU/kg appeared to be the optimal inclusion level, producing the greatest defeathered BW, crude protein, fat, and ash values. These findings indicated that whole-body protein, fat, and mineral contents responded to the provision of AvP via MCP or to phytase via phy-

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tate degradation in broiler chickens. The results support the use of whole-body composition as an additional metric for evaluating phytase efficacy.

**Keywords:** Broiler; phytase; phosphorus; whole-body composition

### 233 1,25-Dihydroxycholecalciferol glycoside improves feed efficiency and intestinal immune response of broilers at 42 days of age

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The use of active vitamin D3 metabolites in broiler nutrition has received increasing attention due to their potential to enhance mineral utilization and growth performance. 1,25-Dihydroxycholecalciferol glycoside (1,25(OH)2D3-Gly), extracted from *Solanum glaucophyllum,* is a source of vitamin D3 that can be directly added to the diet in its biologically active form, thereby bypassing the need for metabolic activation in the liver and kidneys. Despite growing interest in this additive, information regarding its use in breeder diets and its subsequent effects on offspring broiler performance and gene expression remains limited. This study aimed to investigate the effects of 1,25(OH)2D3-Gly supplementation on the performance and gene expression of broilers hatched from breeders with or without supplementation of the same additive. A total of 1,152 one-day-old male Ross 308 AP chicks were distributed in a completely randomized design with a 2 × 3 factorial arrangement. The first factor was the inclusion (100 mg/kg) or absence (0.0 mg/kg) of 1,25(OH)2D3-Gly in the breeders' diets from 21 to 62 weeks of age. The second factor consisted of three levels (0.0, 50, and 100 mg/kg) of 1,25(OH)2D3-Gly in the broilers' diets from 1 to 42 days of age. Diets were based on corn and soybean meal, and birds had ad libitum access to feed and water throughout the experimental period. Data were tested for normality and homogeneity of variance and subsequently subjected to analysis of variance (ANOVA). When significant effects were detected, means were compared using Tukey's test at a 5% significance level. At 42 days of age, broilers supplemented with 50 and 100 mg/kg of 1,25(OH)2D3-Gly from breeders supplemented with 100 mg/kg of the same additive showed lower feed intake (4,759 and 4,733 g, respectively; P=0.047), a lower feed conversion ratio (1.513 and 1.518 g/g, respectively; P=0.015), and higher concentrations of calbindin D28K (0.436 and 0.478 A.U., respectively; P=0.027), interleukin-10 (2.307 and 2.403 A.U., respectively; P=0.001), and interleukin-1B (1.606 and 1.733 A.U., respectively; P=0.001) in the jejunum. These results indicate improved intestinal nutrient handling and modulation of the intestinal immune response in broilers at 42 days of age. Therefore, supplementation of 50 and 100 mg/kg of 1,25(OH)2D3-Gly in broiler diets may be considered a viable strategy to optimize nutrient utilization, intestinal absorption, and immune function when broilers originate from breeders supplemented with 100 mg/kg of the same additive.

**Keywords:** Performance

### 234 25 Hydroxyvitamin D3 in broiler nutrition: effects on performance, bone quality, and plasma vitamin D3 status

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The objective of this study was to evaluate increasing dietary levels of 25 hydroxyvitamin D3 fed to broiler chickens from 0 to 35 days of age on growth performance, bone parameters and plasma 25 hydroxyvitamin D3 levels. A total of 154 birds were allocated to 4 treatments, with 11 replicates of 14 birds each (male Ross 308). The treatments consisted of different inclusion levels of a nutritional additive based on 25 hydroxyvitamin D3 (Ultra 25-D, AB Vista, Marlborough, UK), as follows: 4 µg/kg of 25 hydroxyvitamin D3 (minimum level adopted in Europe for evaluation, corresponding to 175 IU/kg of feed; Treat 1); 32 µg/kg (corresponding to 1,410 IU/kg; Treat 2); 62.5 µg/kg (corresponding to 2,760 IU/kg; Treat 3); and 93 µg/kg (corresponding to 4,110 IU/kg; Treat 4). Body weight gain - BWG, feed conversion ratio - FCR, feed intake - FI, and mortality were evaluated at 21 and 35 days of age. In addition, tibia ash from 11 birds per treatment (expressed as % of dry matter), bone breaking strength - BBS (N), tibia weight (g, dry matter basis), and plasma 25-hydroxyvitamin D3 concentrations (nmol/L) were analyzed at 21 days of age. Statistical analyses were performed using JMP Pro 16.2 software. The results demonstrated that BWG, FCR, FI, and mortality from 0 to 35 days of age did not differ (P > 0.05) among the evaluated treatments. Similarly, tibia ash and BBS data were not significantly affected (P > 0.05). However, a significant linear response (P = 0.028) was observed for tibia weight at 21 days of age, with mean values of 2.81, 3.00, 3.09, and 3.14 g/tibia for treatments 1 to 4, respectively. Furthermore, analysis of plasma 25 hydroxyvitamin D3 concentrations at 21 days of age indicated a linear effect (P < 0.001) associated with increasing inclusion of the additive, resulting in plasma values of 20.1, 64.6, 121.0, and 181.6 nmol/L for Treatments 1 to 4, respectively. Overall, although the inclusion of 25 hydroxyvitamin D3 improved bone quality parameters and increased plasma levels of 25-hydroxyvitamin D3, no effects were observed on growth performance. This lack of response may be attributed to the fact that control diets already met vitamin D, calcium, and phosphorus requirements, as well as to the use of phytase (Quantum Force, 500 FTU/kg, Ca, avP, and Na matrix values applied) in all experimental diets. In conclusion, the use of the 25 hydroxyvitamin D3 feed additive increased tibia weight, and plasma 25 hydroxyvitamin D3 concentrations exhibited a linear dose–response effect.

**Keywords:** Dose–response; Tibia weight; Plasma; Nutritional feed additive; Bioavailability

### 235 Dietary 25Hydroxyvitamin D3 maintains Vitamin D activation pathways and improve organ resilience during an induced renal and hepatic stress in broiler chickens

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Vitamin D plays essential roles in immunity, bone mineralization, and muscle development in animals and humans. Different dietary sources—vitamin D3, 25 hydroxyvitamin D3 (25OHD3), and 1,25 dihydroxyvitamin D3(1,25(OH)2D3- vary in efficiency, particularly when kidney and liver functions, central to vitamin D metabolism, are impaired. This study evaluated the molecular effects of induced renal and hepatic stress and compared the metabolic impact of these three vitamin D sources in broiler chickens. A total of 256 male Ross 308 broilers (1 day old) were allocated to 32 pens (8 birds/pen) and fed one of four treatments for 35 days. The control group (T1) received vitamin D3 (4000 IU/kg). Three groups were challenged with 1000 ppb ochratoxin A (OTA), a potent nephro and hepatotoxic mycotoxin. Challenged birds received vitamin D3 (4000 IU/kg; T2), vitamin D3 (1240 IU/kg) + 25OHD3 (69 µg/kg; T3), or vitamin D3 (4000 IU/kg) + 1,25(OH)2D3 (1 µg/kg; T4). Blood, kidney, and liver samples were collected on days 21 and 35, and plasma vitamin D metabolites, renal qPCR, and hepatic RNAseq analyses were conducted. qPCR was analysed by two-way ANOVA, and RNAseq by edgeR with FDR correction (p < 0.05). On day 21, OTA challenged birds (T2–T4) showed a pronounced downregulation of CYP2R1 (p < 0.001), indicating impaired conversion of vitamin D3 to 25OHD3. CYP27C1 and CYP24A1 expression remained stable, but CYP27C1 was preserved in T3, suggesting that dietary 25OHD3 supports renal vitamin D activation under OTA stress. Plasma metabolite patterns aligned with transcriptional

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effects: 1,25(OH)2D3 levels decreased in T2 but were restored to control levels in T3, while T4 supplementation did not fully compensate. Liver transcriptomics at day 35 revealed substantial OTA induced alterations, with 71 differentially expressed genes (DEGs) versus T1, reflecting mitochondrial dysfunction, oxidative stress, and activation of inflammatory, immune, proteasomal, and cell cycle pathways. Supplementation with 25OHD3 (T3) markedly attenuated these effects, reducing DEGs to 25 and normalizing pathways related to lipid metabolism, immune regulation, proteostasis, and mitochondrial function. Birds receiving 1,25(OH)2D3 (T4) showed partial mitigation (38 DEGs). In conclusion, OTA challenge impaired expression of gene encoding renal first step hydroxylation of vitamin D3 and induced progressive hepatic transcriptomic stress. Supplementation with vitamin D metabolites, particularly 25OHD3, remains biologically active and appears to preserve key metabolic and regulatory pathways in both the kidney and liver, suggesting that higher circulating levels of 25OHD3 may contribute to the protection of these organs.

**Keywords:** Vitamin D metabolism; 25OHD3; hepato-renal stress; trancriptomics; organ protection

### 236 Aspartate-chelated copper maintains broiler growth performance at reduced copper levels under natural coccidial exposure

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Pharmacological copper supplementation is widely used in poultry production to promote growth and antimicrobial effects; however, excessive dietary copper increases mineral excretion and environmental burden. This study evaluated whether aspartate-chelated copper (Asp-Cu; a patented copper source, Insighter Inc., Guangzhou, China) could maintain broiler performance at reduced copper levels under commercial floor-rearing conditions with natural coccidial exposure. Two floor-pen trials were conducted using pen as the experimental unit. Body weight (BW) and average daily gain (ADG) were analyzed by one-way analysis of variance (ANOVA) with Tukey's test ($P < 0.05$), and blood feces counts were analyzed using repeated-measures models. In Experiment 1, 1,018 one-day-old Lingnan broilers were assigned to five treatments (four replicate pens; 50–51 birds per pen): copper sulfate (Sulf-Cu) at 16 or 110 mg/kg copper, and Asp-Cu at 16, 30, or 110 mg/kg copper for 56 days. During the starter phase (days 1–21), BW and ADG were greater in birds fed Sulf-Cu at 110 mg/kg and Asp-Cu at 16–110 mg/kg than in those fed Sulf-Cu at 16 mg/kg ($P < 0.01$). Asp-Cu at 16 or 30 mg/kg did not differ from Sulf-Cu at 110 mg/kg in BW or ADG ($P > 0.05$). Oocyst shedding did not differ among treatments ($P > 0.05$). Blood feces patterns indicated multiple infection waves, with treatment effects differing by production phase: Sulf-Cu at 110 mg/kg and Asp-Cu at 110 mg/kg reduced blood feces burden in the finisher phase ($P < 0.05$), whereas no differences were observed in earlier phases. In Experiment 2, 1,000 one-day-old Arbor Acres broilers were assigned to five treatments (four replicate pens) and fed copper sulfate at 125 mg/kg or Asp-Cu at 25, 50, 100, or 125 mg/kg for 42 days. Final BW did not differ between birds fed Asp-Cu at 25–125 mg/kg and those fed Sulf-Cu ($P > 0.05$). Oocyst shedding did not differ among treatments ($P > 0.05$) and exhibited clustered distribution among pens. Overall, Asp-Cu maintained broiler growth performance equivalent to pharmacological copper sulfate while enabling reduced dietary copper inclusion, indicating improved efficiency of dietary copper use and robust production performance under commercial rearing conditions with natural coccidial exposure.

**Keywords:** aspartate-chelated copper; broilers; dietary copper level; coccidiosis; growth performance

### 498 Transcriptomic Evidence of the Mechanism of Action of Bis-Chelated Copper in Broiler Chickens

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Three independent broiler trials with a total of 3,624 birds (Ross 708 or Cobb 500) under intestinal challenges evaluated the effects of copper (Cu) sources on performance and transcriptomic responses. Each trial compared methionine hydroxy analogue bis–chelated Cu (MMHAC Cu; MINTREX® Cu, 30 ppm) with tribasic copper chloride (TBCC, 100–125 ppm) and low–Cu ITM controls (10 ppm sulfate). Diets were formulated to be iso-methionine and meet broiler nutrient requirements. Birds from TBCC and MMHAC Cu treatments representing the average body weights were selected for gene expression analysis in the duodenum and liver (Trial 1), liver and breast muscle (Trial 2), and ileum (Trial 3). At 28d, tissue samples were collected and mRNA was sequenced (Illumina paired–end, 150 bp, poly(A)–enriched, 20 M reads/sample). For performance, data was analyzed by PROC MIXED with Cu source as a fixed effect and trial location and pen (nested within location) as random effects. Contrasts compared TBCC and MMHAC Cu treatments with low Cu and between TBCC and MMHAC Cu sources. For gene expression, transcript abundance was quantified using Kallisto, differential expression analyzed with DESeq2, and pathway activity determined using topology–based Quantitative Pathway Activation (QPA; adjP < 0.05) that calculated standardized QPA scores indicating relative pathway inhibition (QPA<0) or activation (QPA>0). Birds fed TBCC and MMHAC Cu diets had increased feed intake and body weight through 28 d ($P < 0.05$) compared with Low–Cu ITM controls, but differences were not maintained thereafter. In the intestine, MMHAC Cu inhibited inflammatory pathways, including NLRP3 inflammasome signaling in the duodenum (QPA =–0.82) and ileum (QPA=–1.40), while enhancing antigen presentation (MHC II complex) in the duodenum (QPA=+1.04) and ileum (QPA=+0.92) compared with TBCC. Cell turnover (WNT pathway) activity decreased in the duodenum (QPA=–1.72) and decreased in the ileum (QPA=-1.65) with MMHAC Cu. Transcriptomic profiles indicated greater mitochondrial biogenesis and lower glycolytic signaling in liver, muscle, and ileum in response to bis-chelated copper consistent with a shift toward oxidative phosphorylation. Amino acid metabolism pathways showed that MMHAC Cu increased methionine salvage and reduced catabolism of sulfur amino acids and lysine compared with TBCC. Collectively, MMHAC Cu supplementation consistently improved mitochondrial function, suppressed inflammatory signaling, and optimized amino acid metabolism compared with TBCC, providing mechanistic support for the performance effects.

**Keywords:** Chelated-Copper; tissue transcriptomic; broiler performance

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**206 (E-F) (MTCC)**

### 237 The Avian Organoid Biobank: A versatile platform for modeling infectious diseases in poultry

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To overcome the limitations of traditional animal models, our research addresses the critical need for advanced, physiologically relevant systems to combat infectious diseases in poultry. Our work has met this challenge by establishing a comprehensive avian organoid biobank, a platform that pioneers a new paradigm for studying host-pathogen interactions and developing novel therapeutics. Our avian biobank is a unique collection of long-term 3D organoid cultures derived from intestinal, hepatic, and tracheal tissues of broilers, layers, and turkeys, including the first-ever avian liver organoid models. The stability of this resource is demonstrated by the successful revival and passaging of intestinal organoids after 2.5 years of cryopreservation. Furthermore, characterization confirms the

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models' physiological integrity; for instance, late-passage tracheal organoids maintain key multipotent stem cell populations that are TP63-positive, ensuring their suitability for long-term studies. The platform is now being applied to address high-priority poultry health challenges, offering unprecedented insights into disease mechanisms. Current projects are leveraging our intestinal biobank to investigate host-parasite interactions with *Eimeria spp.,* while turkey enteroid and liver organoids are being used to dissect the pathogenesis of *Histomonas meleagridis*. Our newly established avian trachea organoids also provide an essential tool for generating vital insights into the viral-host dynamics of respiratory pathogens such as H5N1 avian influenza. This multi-tissue, multi-species organoid biobank represents a transformative resource for the scientific community. It provides a powerful platform for exploring disease mechanisms, evaluating nutrient supplementation, screening therapeutics, and accelerating vaccine development. By enabling detailed mechanistic studies, this work embodies the principles of the 3Rs (Replacement, Reduction, and Refinement), significantly reducing the reliance on animal testing while providing more physiologically relevant data. This research has profound implications for improving avian health and paves the way for a new era of sustainable, ethical poultry disease research.

### 238 Fumonisin B1-induced hepatotoxicity in broilers through gut microbiota-liver axis dysregulation-mediated ferroptosis: ameliorative effects of selenium nanoparticles

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Fumonisin B1(FB1), a prevalent *Fusarium*-produced mycotoxin, poses severe health hazards to animals and humans and can significantly damage the liver. However, the mechanism underlying FB1-induced hepatotoxicity, particularly in the gut microbiota-liver axis of broiler chickens, remains unclear. Thus, we hypothesized that FB1-induced liver damage is mediated by gut microbiota-liver axis dysregulation via ferroptosis signalling pathway activation, and that selenium nanoparticles differentially alleviated this toxicity by inhibiting ferroptosis. A total of 144 one-day-old Cobb 500 broiler chickens were divided into three groups (n = 8 replicates, 6 birds/cage): a control (CON) fed a basal diet, and two groups fed basal diets supplemented with 2.5 or 5.0 mg/kg FB1. On day 42, 8 birds per group were slaughtered to collect serum, liver (liver, kidney, brain, and lung for histomorphology), and cecal content samples for further analysis. Ferroptosis was validated in HepG2 cells (a liver toxicity model) treated with FB1 (200 mg/L) with or without Fer-1 (10 μM), SeNa, or SeNPs (0.5 μM). Data were analyzed by one-way ANOVA using R (v4.3.3) and GraphPad Prism (10.4.2). Spearman correlation was applied. Data are presented in the form of mean±SE with the level of significance at P < 0.05. Compared with CON (0.83±0.01), dietary FB1 at 2.5 (0.78±0.02) and 5.0 (0.77±0.02) mg/kg reduced weight gain ($P$ < 0.05). FB1 at both 2.5 and 5.0 mg/kg increased serum liver biochemistry ALT ($P$ < 0.05), whereas AST, TP, ALP, TBIL, and oxidative stress indicators (MDA, PC, and Fe) were elevated at 5.0 mg/kg ($P$ < 0.05). In contrast, 5.0 mg/kg reduced GLU and increased inflammation and necrosis ($P$ < 0.05). FB1 induced gut microbial dysbiosis, particularly by decreased relative abundance of *Bacteroides* and increased *Clostridia_vadinBB60_group* ($P$ < 0.05), which can dampen the anti-inflammatory response and promote inflammation and oxidative damage. FB1 activated oxidative stress and inflammation-related pathways, including oxidative phosphorylation, non-alcoholic fatty liver disease, and ferroptosis ($P$ < 0.05). Furthermore, integrative analysis of the microbiota-transcriptome revealed that the ferroptosis signaling pathway was associated with FB1-induced hepatic injury ($P$ < 0.05). The *in vitro* experiment showed that ferrostatin-1 (Fer-1) reduced FB1-induced oxidative damage ($P$ < 0.05), which confirmed the involvement of ferroptosis. Notably, selenium nanoparticles (SeNPs) decreased ROS and 4-HNE levels more effectively than sodium selenite (SeNa) ($P$ < 0.05). Collectively, FB1 induced hepatotoxicity by dysregulating the gut-liver axis, with ferroptosis as a key pathway, while SeNPs offer a promising strategy against FB1-induced toxicity.

**Keywords:** Ferroptosis; Fumonisin B1; Gut microbiota-liver axis; Hepatotoxicity; Selenium nanoparticles

### 239 One drop of blood is enough to perform indirect and competitive ELISA tests

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Collecting blood samples faced significant challenges during the COVID19 pandemic. We needed to find ways to collect samples while adhering to restrictions and improve biosecurity by reducing the necessity for technicians to visit for sample collection. Additionally, we sought to simplify the transportation of blood samples for remote areas. The classical method involves bleeding the bird and centrifuging the whole blood to obtain plasma. The innovative serum and blood transport cards provide a new solution for ELISA tests. After collecting a blood droplet by capillarity using a specific cannula and deposing it onto the card, the whole blood is automatically separated into plasma by migration along the strip. To investigate the effect of sample deposition on the blood transport card on subsequent ELISA analyses, ELISA tests were performed on samples before and after deposit on the transport cards, as described below. A total of 15 chickens were sampled at 1 day and 22 days of age. For each sample, serum obtained from whole blood was compared with the corresponding serum derived from the blood strip using four IDvet poultry indirect ELISA kits (FLU, NDV, IBV and IBD). The ELISA assays demonstrated that blood samples on cards or in tubes resulted in similar ELISA titers at flock level, with mean titers ranging between 90 and 14 674 depending on kit and correlation coefficients $R^2$ ranging from 0.9531 to 0.9896. The integration of transport cards optimises sample collection, transport, and storage, thereby improving the feasibility of serological monitoring. This method is a less invasive alternative for collecting blood, particularly in day-old chicks, which contributes to improved welfare. An example of a field application with the monitoring of Influenza H5 vaccination in duck population will be further presented.

**Keywords:** Serum Transport Card; Serological monitoring; Indirect ELISA; Blood sampling; Flock health monitoring

### 240 Zinc-Induced Molting Modulates Oxidative and Health Biomarkers in White Leghorn Hens

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Induced molting is a widely adopted management strategy to extend laying cycles and improve post-molt productivity. However, conventional molting methods, particularly feed withdrawal, are associated with physiological stress, oxidative imbalance, and welfare concerns. Alternative approaches such as zinc-induced molting have been proposed, yet their systemic effects on oxidative status and cellular integrity remain insufficiently characterized. This study evaluated the effects of zinc-induced

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molting on various oxidative stress biomarkers, antioxidant capacity, and DNA integrity in commercial White Leghorn layers (67-week-old). Following a three-week of acclimatization, birds were allocated to molted (ML; n = 12) and a non-molted control group (NML; n = 12). Three g ZnO/kg of diet was supplemented in ML feed and feed was offered at 35g/bird/day to both the groups for three consecutive weeks of induced molting. Total oxidant status (TOS) and total antioxidant capacity (TAC) were assessed in serum and tissues (liver, heart, kidney, and muscle). Serum Homocysteine level and the activity of ceruloplasmin, paraoxonase, and arylesterase enzymes were measured in the serum. The DNA damage was evaluated through comet assay in the lymphocytes isolated from blood run on SDS-Gel doc to see comet formation under florescent microscope. Data were analysed using independent samples t-tests, and results were considered statistically significant at $P < 0.05$ and $P < 0.01$. Zinc-induced molting significantly increased TOS levels in the liver ($P < 0.05$), heart ($P < 0.01$), and kidney ($P < 0.05$) compared with controls. TAC was elevated in serum ($P < 0.01$) but found significantly reduced in the heart ($P < 0.05$), kidney ($P < 0.01$), and muscle ($P < 0.01$) in ML birds. Serum homocysteine concentrations and lymphocyte DNA damage were significantly higher ($P < 0.01$) in the ML group, whereas paraoxonase and arylesterase activities were markedly decreased ($P < 0.01$). Although molting was associated with increased oxidative stress and compromising the DNA integrity in cells, zinc-induced molting appeared to mitigate these effects, as evidenced by an increase in circulating antioxidant capacity. This suggests that zinc supplementation may help alleviate the physiological stress associated with molting.

**Keywords:** Zinc-induced molting; Oxidative stress; Total antioxidant capacity; DNA damage; White Leghorn layers

### 241 Vaccination monitoring and DIVA strategy for Infectious Bursal Disease

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**INTRODUCTION** Infectious Bursal Disease (IBD) prevention relies on breeder vaccination to confer maternal antibodies (Ab) and on chick vaccination, where timing remains critical. New vaccines, including vectorized HVT-VP2 vaccines, were developed to overcome this constraint, creating a need for specific ELISA tools to monitor immune responses. The ID Screen® IBD VP2 Indirect kit (IBDVP2) measures Ab produced after HVT-VP2 vaccination. In contrast, ID Screen® IBD Indirect (IBDS) detects Ab against the whole IBDV antigen and allows a partial DIVA (Differentiating Infected from Vaccinated Animals). To improve differentiation, we developed ID Screen® IBD VP3 Indirect (IBDVP3), targeting Ab against VP3, a protein produced during natural infection. This kit shows high specificity and sensitivity, detecting infected animals without false positives in HVT-VP2 vaccinated poultry. Combined with IBDVP2, it enables an efficient DIVA.**MATERIALS AND METHODS**Vaccination monitoring5 broiler groups with different backgrounds were tested with the 3 kits:Groups 1–2: vaccinated with recombinant (rec) HVT-VP2 (manufacturer A (Mfr)); group 2 challenged (11 dpi)Groups 3–4: vaccinated with rec HVT-VP2 (Mfr B); group 4 challenged (11 dpi)Group 5: vaccinated with a live vaccine (Mfr A)Group 6: controlDIVA strategy24 broiler samples from 3 groups were collected at 4, 6 and 8w and tested with the 3 kits:Group 1: unvaccinatedGroup 2: vaccinated at 1d with HVT-VP2 (Mfr A)Group 3: vaccinated at 1d with HVT-VP2 (Mfr B)**RESULTS**Vaccination monitoringIBDVP2 detected Ab induced by HVT-VP2 vaccines from both manufacturers.IBDS, based on the whole virus, detected low titers in vaccinated non-infected birds and became positive after challenge.IBDVP3 clearly detected infection after challenge while remaining negative in vaccinated non-infected birds. All three kits detected responses to live vaccines, with IBDVP2 and IBDVP3 showing higher and similar sensitivity.DIVA strategy-IBDVP2 monitored rec vaccination from 4w with both vaccines. Positivity in unvaccinated birds at 4w was due to maternal Ab and disappeared by 6w.IBDS showed weak positivity with VP2 vaccines, making infection and vaccination difficult to distinguish. Because of the weak positivity measured, it is not a perfect DIVA.IBDVP3 remained negative in vaccinated birds since it does not detect VP2-induced Ab, making it a real DIVA.**CONCLUSION**IBDVP2 detects Ab induced by HVT rec vaccines.IBDVP3 shows high specificity and sensitivity to detect infection without false positives in HVT-VP2 vaccinated poultry. Combined use of IBDVP2 and IBDVP3 provides a comprehensive serological solution to monitor vaccination and implement an effective DIVA in poultry vaccinated with rec VP2 vaccines.

**Keywords:** serology; ELISA; Infectious Bursal Disease; Vaccination monitoring; DIVA strategy

### 242 Evaluation of Increasing Doses of an Attenuated *Eimeria* Vaccine, Evant®, on Broiler Growth Performance and Gut Health: Establishing an In Vivo Coccidiosis-Challenge Model

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This study evaluated the effects of increasing doses of the attenuated *Eimeria* vaccine Evant® on broiler growth performance and gut health from 0 to 42 days of age, with the aim of establishing an in vivo coccidiosis-challenge model. A total of 352 one-day-old broilers were randomly assigned to 32 pens (11 birds/pen; 8 pens/treatment) and allocated to four treatments based on the Evant® dose administered on day 14: non vaccinated control (C) or Evant® at 30×, 60×, or 90× the standard dose (T30, T60, T90). Birds received a corn–soybean meal diet formulated to Ross 308 requirements for the starter (0-14 d), grower (15-28 d), and finisher (29-42 d) phases. BW, ADG, FI, and FCR were assessed on days 7, 14, 21, 28, 35 and 42. On days 22, one bird per pen was sampled for histopathology of the duodenum, jejunum, ileum and ceca, and *Eimeria* lesion scoring. An ANOVA was conducted to evaluate performance data. Results were considered statistically significant at a threshold of $P < 0.05$. Significant treatment effects emerged after the vaccine challenge ($P<0.05$). At days 21 and 28, birds in T60 exhibited significantly lower BW than all other treatment groups (-12% and -9%; $P<0.01$), and this disadvantage persisted at days 35 and 42, where T60 birds remained lighter than birds in C and T90 (-6 % and -5 %; $P<0.05$). During the grower phase (14-28 d), vaccine dose significantly affected ADG and FCR ($P<0.05$), aligning with the typical peak of post-vaccination *Eimeria* cycling. T60 birds showed the lowest ADG (-13.5%; $P<0.001$) and the highest FCR (1.50 vs. 1.28; $P=0.005$). Overall (from 0d to 42d), FCR was also worsened in T60 ($P=0.0068$) by 7 points compared to all other treatments. At 8 days post-vaccination (day 22), T30 and T60 birds showed higher *E. acervulina* lesion scores than C and T90 ($P<0.0001$), while *E. maxima* and *E. tenella* lesions were similar between treatments ($P>0.05$). Control birds had the shortest crypt depths in the duodenum, jejunum and cecum (145±17µm; 129±17µm; 224±16µm, $P<0.05$) and the highest villus:crypt ratios in the duodenum and jejunum (12.2±0.8; 9.2±0.5, $P<0.001$). In contrast, T60 showed the greatest crypt depth (316±17µm; 259±17µm, 306±16) and reduced villus:crypt ratios (4.9±0.4; 3.6±0.5). In the ileum, control birds exhibited decreased crypt depth compared to T30 birds and T90 ($p=0.008$). In conclusion, administration of Evant® at 60× the standard dose produced a robust coccidiosis-like challenge, as evidenced by reduced growth performance, increased lesion severity and decreased villus:crypt ratios relative to the control, 30×, and 90× doses. These findings support the use of the 60× Evant® dose as a reliable in vivo coccidiosis-challenge model.

**Keywords:** coccidiosis; broiler; vaccine; model; performance

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### 243 Modeling Spotty Liver Disease and *Campylobacter hepaticus* infection in Leghorn Hens

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Spotty Liver Disease, caused by *Campylobacter hepaticus* (CH), has increased in field prevalence over the last several years along with the increasing cage-free and free-range egg rearing/laying systems. The disease causes increased mortality along with a drop in egg production making it an important welfare and economical issue for egg producers. One limitation for validating preventions or interventions for this bacterial infection is the lack of reproducible models. Our research group has been unable to consistently reproduce the disease utilizing techniques and guidance from those who developed an existing CH challenge model. The objective of the projects to be presented were to create an alternative repeatable Spotty Liver Disease model for screening of products. Both the unsuccessful and successful model outcomes utilizing different isolate and methodologies culminated in increased understanding of the disease. In the current model, oral and intravenous (IV) routes of *Campylobacter hepaticus* administration were compared. Hy-Line W-36 leghorn hens were orally or intravenously exposed to *C. hepaticus* (NCTC) (~$10^8$ CFU/bird). For each group, a subset of ten (10) hens were challenged directly and an additional twenty (20) hens remained as horizontally exposed (sentinels). Environmental stressors, including fasting and temperature swings, mirrored the model validated by the researchers at UGA. Bile samples were collected and liver lesion scores were evaluated on day 16 and 35 post challenge. On day 16, no liver lesions were observed; however, in the IV challenge, 1 of 3 direct (33%) and 1 of 6 indirect (17%) were CH positive. No birds were culture positive in the orally challenged group on day 16, and no statistical differences were observed between the challenge groups. By day 35, 100% of direct (5/5) and 100% of horizontal (11/11) birds in the intravenous group were culture positive for CH. One of ten (10%) birds in the oral challenged group was CH positive. The IV challenge had significantly greater culture positive prevalence in the horizontal birds on day 35 compared to the orally challenged horizontals ($P<0.05$). Lesions were observed in both groups. Further refinement of this model includes validation of liver lesion scores and culture results with or without environmental stressors. More evaluation is needed to better understand the epidemiology of this disease, but based on this model, intravenous challenge may create a more robust and quantifiable model for product evaluation. Cumulatively, these pilot studies indicate *C. hepaticus* prevalence in horizontally exposed hens may be used as an indicator of product or vaccine efficacy.

**Keywords:** Spotty Liver Disease; Laying Hens; Campylobacter hepaticus; Egg Production; Modeling

### 244 Electron Beam–Killed Bacterial Vaccine as an Innovative Approach to Prevent Bacterial Chondronecrosis with Osteomyelitis Lameness in Broiler Chickens

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Broiler chicken lameness caused by Bacterial Chondronecrosis with Osteomyelitis (BCO) poses a significant challenge to animal welfare and the economic viability of the poultry industry, with annual revenue losses estimated to exceed tens of millions of dollars. BCO lameness is associated with many opportunistic bacterial pathogens that inhabit the respiratory and gastrointestinal tracts. Subsequently, these pathogens, especially *Staphylococcus* species, cross mucosal barriers, enter the bloodstream, and eventually invade the growth plates of long bones, causing necrosis and lameness. Our objective was to develop a *Staphylococcus*-based vaccine to prevent lameness in broiler chickens. Electron beam (eBeam) technology irreversibly damages bacterial DNA, restricting replication while maintaining surface epitopes crucial for immune recognition. We hypothesized that an eBeam-treated *Staphylococcus* vaccine (2 strains) would elicit a targeted, strong immune response in broilers against BCO. A total of 210 day-old chicks, *in ovo* vaccinated with eBeam-inactivated *Staphylococcus*, formalin-inactivated *Staphylococcus* (same strains), or control (vehicle without bacteria) treatments, were placed in 6 litter-floor pens at 2 pens/treatment, 35 chicks/pen. The birds were challenged with *S. agnetis* on days 20 and 21. Blood was sampled on days 11, 33, and 56 to compare anti-*Staphylococcus* antibody profiles and leucocyte responses via ELISA and flow cytometry, respectively. Daily cumulative lameness was evaluated from days 22 through 59. Birds were prompted to walk, and those that were resistant to walking/standing were identified as clinically lame and humanely euthanized. The following analyses were conducted via GraphPad Prism software. Lameness incidence was compared using Kaplan–Meier survival analysis followed by Mantel–Cox post hoc tests. Differences in whole-blood cell populations between treatments were analyzed by two-way ANOVA, and differences in the antibody responses between treatments were analyzed by one-way ANOVA, both followed by Tukey's post hoc tests. $P<0.05$ was considered statistically significant. The results show a 50% decrease in lameness incidence among eBeam-vaccinated chickens compared with the control group, but no significant difference between the formalin and control groups. Additionally, the eBeam-vaccinated birds showed increased anti-*Staphylococcus* IgA titers, suggesting a robust and specific humoral immune response. These findings endorse the effectiveness of the eBeam-inactivated *Staphylococcus* vaccine as a promising approach to reduce BCO-related lameness in broiler production.

**Keywords:** Lameness, broiler; Electron beam; Staphylococcus ; Osteomyelitis

### 245 Reused litter shapes the early cecal microbiota succession and the immune cells distribution in layer chickens

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The development of gut microbiota and immune system during the early life of chicks are influenced by environmental exposure, with litter acting as a key source of microbial colonization. Reused litter may serve as a natural microbial inoculum shaping this process. We hypothesized that reused litter alters gut microbiota establishment and immune development compared to fresh litter and evaluated these effects in layer chicks. A total of 160 newly hatched female Hy-Line W80 layer were housed on fresh or used litter (n = 80/group). Body weight was recorded weekly throughout the study. At days 7 and 35, cecal samples (n = 5/group/timepoint) were collected for gut microbiome analysis, and ileum and spleen samples (n = 8/group/timepoint) were collected for intraepithelial lymphocyte (IEL) and lymphocyte analysis, respectively. Statistical analyses were performed using GraphPad Prism v10.0.03, with chickens as the experimental unit. Body weight was analyzed by independent sample t-test. Cecal microbiota (16S rRNA metagenome) was processed in QIIME (v1.9.1), with PERMANOVA for community differences and LEfSe for differential taxa. Flow cytometry data were analyzed using the Mann-Whitney U test. Results showed that birds reared on fresh litter exhibited higher body weight from day 14 to 35 ($P < 0.05$), with similar mortality between groups. Cecal microbiota showed significant differences at day 7 ($P < 0.05$), but no differences were observed by day 35, indicating microbial convergence over time. Bacillota remained

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the dominant phylum, with an age-related increase in Bacteroidota. No differences in IEL subsets were detected at day 7; however, by day 35, used litter significantly increased $TCR^-$, $TCR\Gamma\Delta^+$, $TCRAB^+$, $TCRAB^+CD8AB^+$, and $TCRAB^+CD8AA^+$ IEL subsets, as well as splenic $TCR\Gamma\Delta^+$ and $TCRAB^+CD8AB^+$ populations ($P < 0.05$). These findings highlight that reused litter modulate early cecal microbiota and enhanced IEL and splenic T-cell expansion in layer chicks, with microbial communities converging by day 35; future studies should evaluate reused litter impacts on laying performance and resistance to enteric pathogen challenge.

**Keywords:** Reused litter; Layer; Gut microbiota; Intraepithelial lymphocytes; 16s rRNA sequencing

### 246 The poultry-wildlife interface: opportunities for biosecurity breaches and interspecies pathogen transmission

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Uncontrolled direct or indirect interactions at the poultry–wildlife interface can facilitate the introduction of pathogens into poultry holdings. To counteract this risk, the consistent implementation and strict enforcement of biosecurity measures represent one of the most effective strategies for preventing disease transmission. The present study aimed to characterize the spatiotemporal patterns of wild birds and mammals visiting the surroundings of poultry houses. Eight camera traps were deployed over a one-year period on three commercial layer farms, including a free-range and two barn production systems, located in a densely populated poultry area in Northern Italy. Time-series analysis, correspondence analysis, and generalized linear model, were applied to investigate daily wildlife visitation patterns and identify influential variables. Overall, 5,978 videos featured wild birds, 567 featured wild mammals, and 1,866 featured pets (cats and dogs). Wildlife visits increased during the spring and winter months. Among mammals, coypus (*Myocastor coypus*) and domestic cats (*Felis catus*) were the species observed most frequently. Among birds, Eurasian magpies (*Pica pica*), ring-necked pheasants (*Phasianus colchicus*), and Eurasian collared doves (*Streptopelia decaocto*) were the most common visitors. Birds were predominantly engaged in foraging activities near poultry houses, highlighting the importance of reducing site attractiveness to wild animals. Effective mitigation measures may include regular inspections of farm premises, prompt removal of feed spills and eggs fallen from automated egg-transport systems, and the implementation of deterrence strategies to prevent wildlife habituation. Overall, these findings provide a foundation for the development of targeted monitoring and intervention strategies aimed at preventing disease transmission. Furthermore, visual evidence of wild animals approaching poultry houses may support veterinary and public health authorities in educating farmers and raising awareness about wildlife presence on farms and the associated risks of pathogen spillover.

**Keywords:** Wildlife-poultry interface; Camera-traps; Layer farms; Biosecurity

### 247 Environmental source tracking of H5N1 in Alberta: linking migratory waterfowl to commercial poultry outbreaks (2022–2025)

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This study aimed to characterize the molecular evolution and transmission dynamics of Highly Pathogenic Avian Influenza (HPAI) H5N1 within the Alberta poultry-wild bird interface. By integrating environmental sampling with structural bioinformatics, we sought to identify the genomic drivers behind the sequence identity observed between wild migratory birds and commercial outbreaks, while assessing the zoonotic risk associated with regional mammalian spillover. Whole-genome sequences were recovered from three Alberta commercial poultry outbreak sites and wild waterfowls. Sequence alignment and mutation mapping were performed using Geneious Prime. Phylogenetic reconstruction and temporal divergence were analyzed using BEAST v2.7 and associated molecular clock tools. To assess the "Glycan Shield" evolution, NetNGlyc 4.1 was utilized to predict N-glycosylation occupancy potentials, while PyMOL was employed for 3D structural modeling of the Hemagglutinin (HA) globular head. Site-specific analysis focused on the Multibasic Cleavage Site (MBCS), Antigenic Site A, and the Q226 residue within the Receptor Binding Domain (RBD). Phylogenetic analysis confirmed that Alberta isolates belong to the NA-Eurasian Clade 2.3.4.4b, specifically sub-clades B.3.10, B.3.13, and D.1.1. All sequences maintained a confirmed HPAI MBCS motif. Viral trafficking aligned strictly with the Central and Pacific Flyways, with identical genomic signatures shared between wild waterfowl and infected premises, confirming a direct environmental source of infection. While all isolates retained the avian-preferring Q226 residue, structural modeling revealed a significant evolutionary shift in the glycan shield. The Asn142 site showed an increased glycosylation potential (from 0.58 in 2022 to 0.71 in 2025), suggesting an "optimized" shield that masks Antigenic Site A, potentially facilitating poultry adaptation. Furthermore, phylogenetic analysis revealed that the H5N1 genotypes circulating in Alberta commercial barns and wild waterfowl had high degree of sequence identity with isolates of mammals (skunk and foxes) from Alberta and Saskatchewan. Our findings demonstrate that H5N1 in the Central Flyway has undergone significant structural optimization since its 2021 introduction. The high-potential glycan shielding at the HA head suggests a virus increasingly "fitted" within poultry environments. These results underscore the critical role of environmental sampling in tracking the source of infection and highlight the risk of mammalian spillover. Continuous monitoring of the poultry-wildlife-mammal interface in Alberta is essential for early detection and the development of effective bio-exclusion strategies for the commercial industry.

**Keywords:** H5N1 Clade 2.3.4.4b; Environmental Surveillance; Migratory Waterfowl; Mammalian Spillover; Structural Bioinformatics

## Management and Production I

**205 (A-D) (MTCC)**

### 248 Post Peak Adaptation of Lohmann LSL-LITE Hens to Delayed Nest Box Introduction

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The egg industry is undergoing a major shift as producers move away from conventional cages toward cage-free systems. This change is driven by growing consumer expectations, retailer commitments, and evolving regulations focused on animal welfare. However, this transition is not without its challenges. One of the most persistent issues is the occurrence of floor eggs, which require more labor for collection. These eggs can increase food safety risks, and they can compromise overall egg quality and marketability. Addressing this challenge requires a more deliberate approach to flock management. Early pullet training and optimal nest accessibility are all critical strategies to encourage proper nesting

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behavior and minimize floor egg production. In the current study, we hypothesized that after peak egg production, Lohmann LSL-Lite hens will not adapt their nest box utilization after experiencing delayed access to nest box early in the life of the hens. A total of 1200 Lohmann LSL-Lite pullets, 16 weeks of age, were randomly distributed to 24 (10ft x 8ft) floor pens with 50 birds per pen in a completely randomized design. Each pen had two feeders and two-line nipple drinkers. At 17 and 23 weeks of age, a 10-hole next box was introduced to 12 replicate pens to create each treatment group (On-Time) and (delayed), respectively. Floor eggs were recorded from 41 to 71 weeks of age. The weekly data were analyzed using one-way ANOVA, and the entire data set was subjected to a repeated-measure analysis with week as a factor. Differences were considered significant at ($p \leq 0.05$). Hens introduced to nest boxes on time consistently showed a higher percentage of eggs laid in nest boxes compared to delayed hens, particularly during the early laying period (weeks 41–48). Peak nest box utilization was observed around weeks 51–52, where on-time hens reached up to 75.21%, compared to 63.24% in delayed hens. In the later laying phase (weeks 56–70), no significant differences ($P > 0.05$) were observed between treatments. The main effects of nestbox placement (P=0.045) and weeks (P=0.00) were significant, but their interaction (0.618) was not. These findings suggest that early access to nest boxes significantly improved nest box use during early post-peak egg production, but this advantage diminished with age, resulting in similar performance between treatments later in the hen's life.

Keywords: Lohmann LSL-Lite, nest box, cage-free, floor eggs

### 249 Economic Viability and Nutritional Efficiency of Phytase Supplementation in Diets with Varying Phosphorus Levels for Commercial Laying Hens

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The objective of this study was to economically evaluate the effects of available phosphorus (aP) requirements, with and without the addition of the enzyme phytase, in the diets of light commercial laying hens, based on feed intake and egg production variables. Phosphorus is one of the most expensive components in poultry nutrition, and phytase is frequently utilized to release phytate-bound phosphorus from plant-based ingredients to reduce formulation costs and environmental impact. A total of 576 Hissex White layers were evaluated from 25 to 40 weeks of age using a completely randomized design with eight treatments, nine replicates, and eight birds per experimental unit. Diets were corn and soybean meal-based, isonutritive, and varied only in aP levels. Economic analysis was performed by calculating the gross marketing margin (GMM), total revenue (TR), and treatment cost (TC). Ingredient costs were based on 10-year historical average prices, deflated by the National Consumer Price Index (IPCA) for December 2022. Egg revenue was based on an average producer price of R$ 141.13 per box, according to CEPEA/ESALQ/USP data. Data were analyzed using JMP 14 software and subjected to analysis of variance (ANOVA) at a 5% significance level. Differences between means were determined by Tukey's test, and polynomial regression analysis was used to establish equations describing the behavior of GMM, TR, TC, and the TC/TR ratio. No dietary effects were observed on feed intake or egg production ($P > 0.05$), indicating that economic differences were due to ingredient costs. The TC per bird increased linearly as aP requirements increased, both with and without phytase ($P < 0.001$). Without phytase, the cost difference between the highest (0.42%) and lowest (0.18%) aP levels was R$ 0.99 per bird. With phytase inclusion, a linear reduction in the GMM was observed as phosphorus increased, indicating that the 0.12% level provided the highest profitability per bird ($P = 0.0037$). The TC/TR ratio showed a linear increase ($P < 0.001$) as phosphorus requirements grew, representing a linear reduction in profitability percentage. In conclusion, requirements of 0.18% aP without phytase, or 0.12% with phytase inclusion, can be utilized in commercial layers without affecting productive performance. These levels allow for reduced production costs and increased economic profitability.

**Keywords:** Nutrition; phytase; phosphorus; economy; layers

### 250 Can hen performance be affected by lighting uniformity and degree of nest enclosure?

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The transition of egg industry to cage-free systems poses new challenges to production and bird welfare. For instance, free transition in the system may lead to unintended use of resources and floor eggs. Mislaid eggs may originate from nests not being attractive, not enough nest space per bird, or litter areas being more attractive nest sites. Furthermore, a uniformly lit environment may help bird navigation and resource use. The present research explores the modulation of nest enclosure and light to increase nest attractiveness and reduce floor eggs at a low economic investment. The experiment was carried out in Big Dutchman's NATURA STEP aviaries which provide litter access on both aviary sides. At 16 weeks of age (WOA), floor-reared Hy-Line Brown pullets were moved into 4 aviary rooms further divided into 4 sections and stocked with 164 birds/section to ensure a minimum of 929 cm2 of floor space per bird. From 16 to 18 WOA, all pens were illuminated with ceiling lights (CL), and central nest flaps were removed to facilitate nest exploration prior to onset of egg laying. At 18 WOA (and until 32 WOA), light bars on the wall over the litter area (WL) were turned on in two rooms (randomization was not possible, as light could not be prevented from illuminating adjacent pens in a room). Moreover, every other nest flap was removed (partial enclosure, P), or nests were fully enclosed (F) (randomized design across pens of each room). Egg production and location (nest, system, floor), and mortality were monitored daily from 17 to 32 WOA. Hen body weight (BW) was recorded from 5% of hens per pen at 17, 20, 26, 31 WOA. Data were analyzed with a General Linear Model with WOA and treatments as fixed effects. Hen performance reflected guideline values and egg production was not affected by treatments. However, floor eggs were greater in WL than CL ($P<0.05$), and more eggs were laid in P nests compared to F nests while system eggs were higher in F compared to P. Mortality was significantly greater in CL compared to WL ($P<0.01$), and in P compared to F ($P<0.001$) from 23 until 32 WOA. Finally, less than 10% BW variation was observed in all groups, and BW was lower in F hens compared to P hens at 20 WOA ($P<0.05$). In conclusion, light uniformity and nest enclosure affected mortality, egg laying location, and body weight. Although the presence of two rooms per WL and CL, the room effect still represents a confounding factor and additional exploration with more replicates is needed. Further research is also necessary to 1) understand the relation between egg laying location and nest use, and 2) find a balance between litter provision and uniform lighting that supports litter use without increasing floor egg laying.

**Keywords:** poultry; layer; egg; aviary; cage-free

### 251 Effect of Heat Stress and biofortified Orange Corn during pullet rearing on early egg production

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Increasing high-heat days are intensifying heat-stress challenges for U.S. laying hens, impairing eggshell formation. The link is unclear if heat stress impairs ovarian maturation potentially impacting egg production making the pullet rearing a vulnerable period for producers. Dietary ingredients, such as biofortified orange corn, may mitigate heat stress disruptions. The objective of this study was to assess the impact of acute heat stress during pullet rearing on early egg production and if orange corn can mitigate effects. 1,600 LSL white pullets were reared in cages (10/cage) in three identical rooms. Pullets were fed isocaloric diets made with yellow corn or biofortified orange corn. At 9 and 14 weeks of age (woa), room temperatures in one room of pullets were increased for 4 days to achieve a temperature heat index (THI) of 82 for at least 10 hours with nighttime THI of at least 76, maintaining the heat stress through the night. Each heat challenge was conducted in a separate room, so pullets undergoing the heat challenge were naïve to heat stress. One room remained thermoneutral (TN) throughout. Facility limitations required that heat be applied at room level (n = 1); thus, we are unable to separate room and heat treatment effects in this study. At 17 woa all hens were moved into enrichable colony cages (20/cage) fed a common yellow corn diet following breeders' recommended photostimulation. At transfer TN hens were heavier than heat challenged hens ($P < 0.001$), all were in range of breeder recommendations. Three cages per diet × heat challenge group were monitored for daily egg production to 25 woa. From 20 to 25 woa a full day's production was evaluated weekly for fresh egg quality. Data were analyzed with two-factor ANOVA. Orange corn did not impact lay performance. Age of first egg was earlier for TN pullets compared to heated groups (18 vs 19 woa). Daily egg production was greatest for TN pullets until 24 woa and greater than all heated groups until 21 woa ($P \leq 0.04$). The 14 woa heated pullets had lower egg production than 9 woa heated pullets during 20, 21, 22 woa ($P \leq 0.001$). Pullet background did not impact egg size ($P \geq 0.11$). Fresh egg quality metrics only differed between groups at 25 woa, where Albumen height, and Haugh units were numerically increased in groups fed orange corn as pullets ($P = 0.06$). Despite the delay in first egg, heat stress as pullets did not decrease fresh egg quality during early lay. However, the delayed first egg could potentially be ameliorated by delaying photostimulation based on the lighter transfer weight.

**Keywords:** pullet; laying hen ; heat stress; orange corn; egg quality

### 252 Effects of Stocking Density on Welfare, Egg Quality, and Production of Cage-Free Laying Hens in Multi-Tiered Aviaries

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As U.S. egg production is shifting towards cage-free systems, producers must meet minimum legislative and retailer floor space requirements; however, the extent to which hens utilize available space in aviaries remains unclear. The aim of this study is to investigate how factoring colony nest space into the usable floor space equation can impact egg production, egg quality, and animal welfare. A total of 1,436 Tetra Brown laying hens were housed in cage-free aviaries at 0.09 $m^2$/hen of floor space under two stocking densities: high density (192 hens/pen), where nest space was included in the floor space calculation, and standard density (167 hens/pen), where nest space was excluded. Four room replicates were used with 1 pen/treatment in each room (n=4 pens/treatment). Egg count and location (nest, tier, floor) were recorded daily and averaged weekly from 18 to 37 weeks of age (WOA). Egg quality was assessed every 28-day period. Welfare indicators (comb damage, footpad dermatitis, feather condition) were evaluated at 28 WOA (25 hens/pen). Data were analyzed in R Software (4.5.1) with linear mixed effects and generalized linear mixed effects models. Density, or period, and their interaction were included as fixed effects and room as a random effect for egg production, egg location, and egg quality parameters. Density was included as a main effect and room as a random effect for welfare metrics. The high-density birds laid fewer nest eggs ($p<0.001$), fewer tier eggs ($p=0.002$), and more floor eggs ($p<0.001$) than standard density birds. Across treatments, nest and floor eggs increased from 18 to 24 WOA, then plateaued from 25 to 37 WOA ($p<0.001$ for both), whereas tier eggs increased from 18 to 19 WOA and plateaued after 20 WOA ($P < 0.001$). Stocking density did not impact egg quality parameters, including weight ($p=0.26$), yolk height ($p=0.72$), Haugh unit ($p=0.17$), vitelline membrane strength ($p=0.46$), vitelline membrane elasticity ($p=0.25$), shell strength ($p=0.36$), and shell elasticity ($p=0.45$). Stocking density had no effect on comb damage ($p=0.46$) nor footpad dermatitis prevalence ($p = 0.26$). Feather condition was excellent in both treatments, with only one hen showing plumage damage (high density). While stocking density did not affect egg quality or animal-based welfare indicators, the higher prevalence of floor eggs laid by hens in the high density treatment suggests that nest use is compromised at higher densities. Alongside lower prevalences of nest and tier eggs from high density hens, there may not be sufficient space for hens to utilize the aviary in high densities. Eggs laid outside of the nest also warrant more labor for egg collection and higher risks for egg loss for producers.

**Keywords:** cage-free; multi-tier aviary; stocking density; egg production; egg quality

### 253 A method for comparing mathematically modeled fertility and hatchability curves

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Within breeding flocks, serially measured (e.g. weekly) fertility rates comprise an early increase, a peak, and a subsequent age-related decline. Our hypothesis was that this relationship could be mathematically modeled using biologically relevant parameters. Further, we hypothesized that differences between curves describing unique scenarios, and the area under the curves (representing life of flock number of fertile eggs) could be compared statistically. We found that a double exponential model in the following form described the fertility curve in a robust manner: F=L(1/(1+exp(-k1(Age-a1))))x(1/(1+exp(k2(Age-a2)))), where F was fertility rate; L was a coefficient linked to peak fertility; a1 was the inflection point of the early rise in fertility (d) and k1 was the steepness or rate of rise; and a2 was the inflection point of the age-related fertility decline (d) and k2 was the steepness or rate of decline post-peak. Coefficients for a Ross 308 standard fertility curve (for North America) and for a single commercial broiler breeder flock where fertility was measured bi-weekly between 182 and 378 d of age were estimated with the NLIN procedure of SAS. The bootstrap method of the NLIN procedure was used to repeatedly resample the data, refit the nonlinear models, and generate a distribution of 5000 sets of coefficients for each scenario. This propagated parameter uncertainty to derived outcomes (e.g., the area under the curve; AUC). The AUC for each set of coefficients was determined using a trapezoid integration method over the period from 182 to 378 d of age, using steps of 0.1 d. The cumulative AUC was divided by the number of days, yielding lifetime fertility rate estimates. These estimates were analyzed as a one-way ANOVA using the MIXED procedure in SAS. Lifetime fertility rate during the 182 to 378 d period was 91.4% for the commercial flock, which was less than the 95.7% fertility for the Ross 308 standard curve during the same period ($P < 0.001$). This method provides a way to statistically compare serially measured fertility from 2 or more flocks. By

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extension, this approach could be used for hatchability curves as well.

**Keywords:** broiler breeders; mathematical biology; simulation modeling; statistics; fertility

### 254 Eggshell translucency, age related structural changes, and their combined impact on hatch of fertile eggs in commercial broiler breeder flocks

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Declining hatchability has become a significant challenge in modern broiler breeder production, yet the specific eggshell characteristics that may influence embryonic survival remain insufficiently defined. This study aimed to determine the relative contribution of eggshell quality traits and flock age on the hatch of fertile eggs (CHOF) and examined their correlations with CHOF, while controlling for the effect of flock age. Data were collected from 738 commercial Ross 708 broiler breeder flocks with 30 eggs each flock, representing 22,140 eggs. All eggs were individually assessed for breaking strength (BS), thickness (TH), lightness (LH), and translucency (TS; translucency score 1, 2, and 3). Translucency was completed with Zinpro® BlueBox™ using a 3 point score system: TS1 = none or few small translucent spots; TS2 = several small translucent spots; TS3 = many large translucent spots. Machine learning based algorithms LASSO penalized regression and random forest regression were used for identifying the most relevant eggshell quality parameter with flock age as a covariant and determining their relative contribution to CHOF. The partial correlation was calculated to isolate age adjusted associations between shell quality and CHOF. Significance value was set at 0.05. Age was identified as the most contributing factor to CHOF by both LASSO and random forest regression. Additionally, LASSO regression results showed eggshell TS emerged as the only trait providing independent explanatory power beyond age, while BS, TH, and LH were largely collinear with age driven structural changes. These results show that CHOF is fundamentally associated with age and TS, even though age showed a larger effect. Flock age exhibited a strong quadratic relationship with CHOF and significantly influenced every shell parameter measured, confirming age as the primary determinant of shell phenotype and hatchability. After controlling for age, higher TS, particularly a greater proportion of high translucency eggs (TS3), was correlated with reduced CHOF (r= - 0.30, P= 0.031. Conversely, TS1 was positively correlated with CHOF (r = 0.28, P = 0.042). None of the other eggshell quality parameters retained a meaningful correlation with CHOF once age was held constant. These findings identify TS as a biologically meaningful, non invasive indicator of functional eggshell quality and demonstrate its utility as a robust, field applicable predictor of hatchability independent of flock age. Incorporating TS scoring into breeder level monitoring programs could provide a quantitative framework to support shell quality assessment and enhance reproductive performance in aging flocks.

**Keywords:** Translucency; Egg-shell quality; hacth of fertile; breeder

## Metabolism and Nutrition: Feed Additives II

**202 (A-D) (MTCC)**

### 255 EFFECT OF EXOGENOUS ENZYMES SUPPLEMENTATION IN FERMENTED SOYBEAN MEAL BASED DIET ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND CARCASS CHARACTERISTICS OF BROILERS

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The study evaluated the impact of exogenous enzymes (protease and non-starch polysaccharides) supplementation in soybean meal (SBM) and 5 % fermented soybean meal (FSBM) based diet in broilers. A total of 800 newly hatched broiler chicks were randomly distributed among four treatment groups, each comprising 10 replicates (20 birds in each replicate). Treatments consisted of SBM based control diet (without enzymes), an SBM-based diet with exogenous enzymes (protease and non-starch polysaccharides), a 5% FSBM diet and a 5% FSBM diet with exogenous enzymes (protease and non-starch polysaccharides). L*actobacillus, Bacillus subtilis*, and yeast were utilized as fermentation inoculants. Data regarding growth parameters were recorded every week. On days 21 and 35, Celite® (an external marker) was used for nutrient digestibility and two birds were slaughtered from each pen for carcass characteristics, including thigh, breast, liver, heart, gizzard and abdominal fat percentage. The data were statistically evaluated under a Completely Randomized Design using the General Linear Model, with Tukey's test for mean comparison. Results demonstrated that body weight gain (BWG) and feed conversion ratio (FCR) were improved in broilers fed 5% FSBM supplemented with exogenous enzymes ($p$<0.05). Highest nutrient digestibility (crude protein and ether extract) was noticed in 5% FSBM based diet without exogenous enzymes on days 21 and 35 ($p$<0.05). However, no impact on feed intake and carcass characteristics ($p$>0.05) with the supplementation of exogenous enzymes in SBM and 5 % FSBM based diets was observed. In conclusion, dietary supplementation of exogenous enzymes in 5 % FSBM based diets effectively improved BWG, FCR and nutrient digestibility (crude protein and ether extract) as compared to non-supplemented diets.

**Keywords:** body weight gain; exogenous enzymes; feed conversion ratio; non-starch polysaccharides; protease

### 256 Reduction of liver injury and fibrosis induced by carbon-tetrachloride in chickens supplemented with artichoke extract, lemon essential oil and silymarin

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Phytogenic feed additives (PFA) enhance the growth parameters, especially in antibiotic-free poultry production. The poultry industry uses essential oil and extract of plants as an antioxidants and growth promoters that improve the productivity, meat quality and immunity. This study assessed whether supplementation with a mixture of artichoke extract, lemon essential oil, and silymarin can attenuate carbon tetrachloride (CCl4-) induced liver damage and fibrosis in broiler chickens, through modulation of oxidative stress and the inflammatory response. The experimental model of CCl4- induced liver injury was used to simulate oxidative stress and liver inflammation observed under production conditions such as

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mycotoxin exposure, heat stress, and intensive diets. Artichoke extract, lemon essential oil and silymarin are PFA that show antioxidant and liver protectant effect. However, the effective concentration and combinations of PFA against liver damage is still unclear. A total of 540 male chicks (one-day-old) were used in this experiment completely randomized, with three groups, six replicates and thirty birds per replicate. The groups were as follows: Treatment (CCL4- /basal feed + blend of 2.5% Artichoke extract + 0.5% lemon essential oil and 4% silymarin - 0.5 g/kg), Positive control (CCL4- /basal feed) and Negative control (basal feed). Birds of treatment and positive control group were inoculated with CCl4- in olive oil (2 ml/kg BW) at 14d, 17d, 21d and 24d of age Growth parameters, gross lessions, biochemical analysis, histology analysis and liver mRNA expressions of fibrogenesis were evaluated. The data were analyzed as one way- ANOVA, and the means showing significant differences were then compared using the Post-Hoc Bonferroni test (p≤0.05). The supplementation with artichoke extract, lemon essential oil and silymarin reduced feed conversion ratio (p = 0.027) compared with the groups without PFA. Furthermore, positive control group showed higher percent of fat drop accumulation and hydropic degeneration in liver histology compare with treatment and negative control group (p=0.029). About liver mRNA expressions of fibrogenesis (TGF-B1, COL1A, ACTA2), chickens reared with PFA underexpressed these compared with both control groups (p= 0.031). Artichoke extract, lemon essential oil and silymarin improved the productive performance and showed liver protective effect, reducing the injury and fibrosis in chickens with and without CCl4- induced liver damage.

**Keywords:** Chicken; Artichoke; Lemon; Silymarin; Liver

### 257 Dietary protein source and Salmonella modulate cecal monoamine oxidase activity in chickens

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**Objective:** Little is known regarding the interplay of feed type and *Salmonella* infection in chickens on intestinal function. Monoamine oxidase is an enzyme present in the chicken intestinal tract that fulfills a number of critical functions, including the breakdown of monoamine neurochemicals and detoxification of dietary amines. As intestinal neurochemicals, in part regulated by MAO activity, contribute to host susceptibility to *Salmonella* infection, we sought to determine the impact of feed type and *Salmonella* infection on monoamine oxidase activity in the chicken ceca. **Experimental design:** Male, day-old by-product breeder chicks were acquired from a local hatchery, randomly assigned into six treatments in a 2×3 (Uninfected vs *Salmonella* infected × 3 dietary treatments) factorial arrangement. Three dietary treatments were soybean meal (SBM) diet, black soldier fly larvae meal (BSFL) diet, and fish meal (FM) diet. Chicks were housed in floor pens and managed under standard, age-appropriate conditions. At 5 days post-hatch, the *Salmonella* Infantis group of chickens were orally infected (10^7 cfu/bird). All chickens received ad libitum access to feed and water throughout the entire study. **Materials and methods:** Chickens were euthanized at 2 or 4 weeks of age, cecal tissue collected and snap frozen until protein extraction. Tissue lysis buffer and a protease inhibitor cocktail were added to ceca tissue immediately before homogenization and protein extraction. Monoamine oxidase (MAO) activity in each sample was then performed using the Amplex Red monoamine oxidase assay kit according to manufacturer instructions, on a Cytation 5 multi-mode plate reader. **Statement of statistical analysis:** Cecal MAO activity (n=7 chickens per treatment) were analyzed using two-way ANOVA followed by Tukey post-hoc test to determine the impact of *Salmonella* infection and feed type on cecal MAO activity. Salmonella and feed type were the main factors. The age groups at 2 or 4 weeks of age were analyzed separately. Any comparison between age groups is descriptive and was not formally statistically tested. **Results:** Within the week 2 age group, chickens fed the BSFL diet in the *Salmonella* challenged group exhibited greater ($p < 0.05$) cecal MAO activity compared to BSFL-fed chickens in the control group. Within the week 4 group, cecal MAO activity was significantly ($p < 0.05$) higher in the control group than in the *Salmonella*-infected group, irrespective of dietary treatment. **Conclusions:** Cecal MAO activity in chickens was influenced by *Salmonella* infection and feed type. Future studies are warranted to understand how MAO activity may be related to neurophysiological and/or detoxification outcomes within the chicken intestinal tract.

**Keywords:** Gut; Salmonella; Neurophysiology; Neurochemical ; Feed

### 258 Cross-model chemical and cellular modulation of oxidative stress by olive pulp: comparison with vitamin E forms

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Vitamin E (VitE) is a key molecule in poultry nutrition. Beyond its nutritional role, VitE is primarily valued for its antioxidant function, raising interest in alternative natural antioxidant sources able to provide comparable redox protection. This study applied a cross-model approach combining in vitro and ex vivo assays to compare a standardized olive pulp (OP; Innovad Group, Belgium) specified to contain ≥7.5% total polyphenols and ≥1% triterpenoids with different VitE forms: natural VitE (d-A-tocopheryl acetate, 40.5%) and synthetic VitE (all-rac-A-tocopheryl acetate, 50%). Redox activity was assessed using $DPPH^{\cdot}$, $ABTS^{\cdot+}$, and $O2^{\cdot-}$ radical scavenging, Reducing Power, CUPRAC assay, plasmid DNA relaxation against $ROO^{\cdot}$, ORAC, and KRL test (KIRIAL/SPIRAL). Results were expressed as IC50, AU0.5 and TE/g and analyzed by one-way ANOVA. Cellular effects were assessed ex vivo in HepG2 cells using an H2O2-induced oxidative stress (OS) model (positive control, PC), which was compared against non-stressed control cells (negative control, NC). Intracellular reactive oxygen species (ROS), glutathione (GSH) and lipid peroxidation (TBARS) levels and NRF2 gene expression were determined. Non-cytotoxic concentrations (5 and 10 Mg/mL) were selected based on viability screening (XTT). Group comparisons were performed using unpaired t-tests with Welch's correction. OP exhibited strong and multi-modal redox activity, characterized by higher radical scavenging, electron-transfer capacity, protection against oxidative DNA damage and resistance to free radical induced hemolysis compared to both natural and synthetic VitE ($P<0.05$). In HepG2, PC displayed a pronounced OS phenotype compared to NC, evidenced by elevated ROS, TBARS, and NRF2 expression, alongside depleted GSH levels ($P \leq 0.045$). OP reduced ROS and TBARS, increased GSH levels and reduced NRF2 expression compared to PC ($P\leq0.009$)), indicating attenuation of OS and limitation of stress-response signalling. Synthetic VitE reduced ROS and NRF2 expression compared to PC ($P\leq0.003$) while effects on TBARS were inconsistent, remaining elevated at the lowest concentration ($P=0.005$) and partially reduced at the highest concentration ($P=0.001$) compared to PC. Natural VitE showed variable responses across endpoints. ROS levels were decreased, compared to PC, only at the highest concentration ($P<0.001$). NRF2 expression and TBARS levels were decreased ($P\leq0.01$) while GSH levels were increased ($P<0.02$), compared to PC, only at the lowest concentration. Overall, these in vitro data suggest that OP may contribute to antioxidant protection in poultry nutrition in a manner compatible with partial replacement of VitE. However, confirmation of this hypothesis requires in vivo validation.

**Keywords:** Poultry; vitamin E replacement; olive pulp; antioxidant status; HepG2

### 259 Influence of oregano-derived essential oil (Ecodiar®) supple-

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**mentation on production performance, egg quality, and egg income in late-phase laying hens**

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Extended-lay egg production systems require nutritional strategies that sustain persistency and economic efficiency beyond 90 wk of age. This study evaluated the effects of dietary supplementation with a Greek oregano–derived essential oil product (Ecodiar® Powder) on performance, egg quality, blood biochemistry, and economic returns of late-phase laying hens. A total of 630 Hy-Line Brown hens (50–102 wk) were assigned to 3 dietary treatments in a complete randomized design with 10 replicates of 21 hens each: control (0 lb/ton), 0.33 lb/ton, or 0.66 lb/ton oregano extract. Hens were housed in enriched colony cages and fed experimental diets for 52 wk. The production was divided into 4 phase 54-69 wk; 70-85 wk; 86-102 wk; and overall 54-102 wk. Data were analyzed using PROC GLIMMIX of SAS 9.4. Across the entire 50–102 wk production cycle, hens fed 0.66 lb/ton oregano extract had greater cumulative hen-day egg production (90.30%) compared with control (88.88%) and 0.33 lb/ton (88.58%) treatments (P < 0.01). Cumulative feed conversion (egg basis) was improved in hens receiving 0.66 lb/ton (1.933 vs. 1.964 and 1.966; P = 0.003). Income per hen over 50–102 wk was greater in the 0.66 lb/ton treatment ($57.01) compared with control ($55.61) and 0.33 lb/ton ($54.98; P = 0.018), while feed cost per henwere not different among treatments. Egg weight, feed intake, and most shell and internal egg quality parameters were unaffected. However, vitelline membrane strength at 102 wk was increased in oregano-supplemented hens (P = 0.002). Serum glucose concentrations were reduced at 102 wk in supplemented treatments (P < 0.001). Dietary inclusion of oregano-derived essential oil at 0.66 lb/ton improved production and economic returns in extended-lay hens without compromising egg quality. These findings support phytogenic supplementation as a viable nutritional strategy for late-cycle commercial egg production.

**Keywords:** antioxidants ; Laying hen; Oregano

**260 Astaxanthin Improves Systemic Stress Responses and Jejunal Barrier Integrity in Aged Laying Hens Under Summer Heat Stress**

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Heat stress during summer disrupts redox balance, immune regulation, and intestinal integrity in laying hens, with aged birds being particularly vulnerable. This study examined the effects of astaxanthin (ASTA) supplementation on systemic stress responses and jejunal barrier-related indices in aged laying hens exposed to heat challenge during summer period. A total of 180 aged laying hens (65 weeks old) were assigned to three dietary treatments: control (Basal diet), basal diet plus 40 mg ASTA, or 80 mg ASTA, with five replicates of 12 birds each, over a 6-week period. Birds were exposed to elevated ambient temperatures (average 31–34°c during peak periods), monitored using automated sensors. Serum and jejunal samples were collected at the end of the trial for biochemical, morphological, and gene expression analyses. Data were analyzed using SPSS, one-way ANOVA was done with Tukey's post hoc test at P < 0.05, and GraphPad Prism for graphs to visualise results. Astaxanthin supplementation reduced serum cortisol levels compared with the control, with the lowest values observed in the 40 mg group (P < 0.05). Serum GPX3 and IL-10 increased, while TNF-A decreased in ASTA-supplemented hens (P < 0.05). In the jejunum, 40 mg ASTA upregulated tight junction barrier-related genes TJP1 and CLDN1 (P < 0.05). Heat stress was associated with altered expression of Nrf2 and HMOXI in the control group, while ASTA supplementation moderated these changes (P < 0.05). Villus height and villus height-to-crypt depth ratio increased, and crypt depth decreased in ASTA-treated hens (P < 0.05). Our findings revealed that astaxanthin supplementation improved antioxidant status, reduced inflammatory responses, and enhanced intestinal integrity in aged laying hens under heat stress, with 40 mg showing greater consistency than 80 mg. Therefore, astaxanthin may be an effective nutritional strategy to improve resilience to heat stress in laying hens.

**Keywords:** Astaxanthin ; Aged Laying hens; Heat stress; Jejunal Integrity; Systemic Response

**261 Effects of natural feed additives and antibiotics on bone strength, organ weights, and gut morphology in late-phase laying hens**

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Late-phase laying hens (>50 weeks) exhibit unique physiological challenges compared to younger birds, making targeted feed additives particularly valuable. With age, hens tend to show weaker bones, shorter villi, deeper crypts, and impaired barrier function, reducing the absorptive area and nutrient uptake. This study investigated the effects of natural feed additives and antibiotics on tibia breaking strength, organ weights, and gut morphology in laying hens. 240 Lohmann Lite hens (52 wk) were assigned to 5 dietary treatments: control (CON), bacitracin methylene disalicylate (BMD; 110 mg/kg), essential oil (EO; 300 mg/kg), a yeast-derived product (YP; 750 mg/kg), and an organic acid product (OA; 600 mg/kg). Hens were housed in conventional cages (6 hens/cage; 8 replicates/treatment) and fed their respective diets for 21 wk. At the end of the trial, tibia bones, intestinal segments (duodenum, jejunum, ileum), spleen and liver were collected. Data were analyzed using one-way ANOVA. Tibia breaking strength, liver weight, and fatty liver score did not differ (P>0.05) among treatments. However, absolute spleen weight was >17% higher in hens fed OA and YP *vs*. CON (*P*<0.001), and relative spleen weight was greater in OA hens (*P*<0.05). Gut morphology responses varied by intestinal segment (*P*<0.001). Villus height (VH) increased in the duodenum and ileum of BMD-fed hens, whereas YP increased VH in the jejunum. Crypt depth (CD) was lower in the duodenum and jejunum of BMD hens but highest in OA and CON groups. In the ileum, CD was lowest in OA and highest in EO. Jejunal VH: CD was unaffected by treatment (*P*>0.05). However, duodenal VH: CD was highest in the CON group and lowest in OA, while ileal VH: CD peaked in YP and was lowest in OA. These findings suggest that the tested natural additives did not compromise bone or liver health, but YP and OA increased spleen weight. All additives improved intestinal histomorphology in at least one segment compared to the control, suggesting enhanced intestinal absorptive capacity in laying hens and indicating their potential as antibiotic alternatives.

**Keywords:** Antibiotics; Laying hens; Gut morphology; Bone strength; Natural feed additives

**262 Feed supplementation with a Triple-Strain *Bacillus*-based pro-**

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**biotic improves performance and leg skin condition in turkeys**

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Probiotic products containing *Bacillus*s spores appear to be suitable candidates in supporting performance and wellbeing of turkeys because they exhibit high potential in promoting the health status of birds and in contrast to antibiotics, they are not contributing to resistance mechanisms in bacteria. This study evaluated the performance and foot skin conditions (FPD) as a result of dietary *Bacillus*-probiotic supplementation. In the experiment, a total of 980 one-day-old male turkeys Hybrid Converter from the commercial hatchery were kept in pens with netting walls and divided into two treatment groups of 14 replications of 35 birds each. The control group was fed with a standard diet (CON) and the probiotic (PRO) group was fed with the same standard diet supplemented with a commercial probiotic containing three selected strains (*Bacillus subtilis* - 597, *Bacillus subtilis* - 600 and *Bacillus amyloliquefaciens* - 516 ) at the dose of $3.2x10^6$ CFU/g feed the first 4-week phase and $1.6x10^6$ CFU/g feed thereafter. The length of rearing period was 20 weeks, and the performance results, and leg skin condition was assessed at the end of the experiment. Data were analyzed using 1-way ANOVA with Duncan procedure. Significance (P<0.05) or tendency (0.05≤ P< 0.10) were considered. Average daily feed intake (ADFI) in the period days 1-28 was significantly higher in CON (P=0.006), whereas FCR was significantly better in PRO (P=0.005). The same was true for the period 29-56 and 1-56 days. Body weight (BW) at day 84 was significantly better in PRO (P=0.009) due to the higher average daily gain (ADG) for the period of 57-84 days (P=0.012) and of the one of 1 to 84 days (P=0.017). On the overall trial duration, ADFI was significantly higher in CON vs. PRO (P=0.035) and FCR was better in PRO group (P=0.001). FPD evaluation of birds at 140 days of age revealed the absence of birds with the score 0 (no lesion) and score 4 ( highest severity of FPD). 53.6% of birds in PRO received a score 1 (vs. 37.5% in CON).46.4% in PRO received score 2 (vs. 51.8% in CON) while10.7% in CON received score 3. The total index of FPD averaged 173.2 ± 45.4 for CON and 146.4 ± 29.1 for PRO (P=0.075). Furthermore, a beneficial shift in short-chain fatty acids profile was evidenced in PRO fed-birds. In conclusion, with regard to productivity, feeding a triple-Strain *Bacillus*-based probiotic significantly increased BW at 84 days, reduced feed intake in most feeding phases and for global period, maintained better FCR and tended to increase European production efficiency factor. Finally, legs skin condition of birds fed with the probiotic-supplemented diet was improved due to a lower severity of FPD.

**Keywords:** Bacillus subtilis; Bacillus amyloliquefaciens; performance; leg condition; turkey

**263 Study on the Effect and Related Mechanism of *Rosa Roxburghii Tratt* Extract in Alleviating Hyperuricemia in Magang Geese**

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Hyperuricemia (HUA) has recently become a common metabolic disease in poultry, posing a significant challenge to China's goose industry. This study established a HUA model in Magang geese via a high-calcium, high-protein diet to explore the alleviating effects of *Rosa roxburghii Tratt* (RRT) extract, aiming to provide nutritional strategies for this issue. Sixty healthy 1-day-old Magang geese with similar body weights were randomly divided into 5 groups (4 replicates of 3 geese each): CON (control), HUA (model), HUA + 750 mg/kg RRT (L-RRT), HUA + 1500 mg/kg RRT (M-RRT), and HUA + 3000 mg/kg RRT (H-RRT). The trial lasted 14 days. Per replicate, 2 or 3 geese near mean wt sampled for serum, tissues, joint fluid, gut, cecal feces. One-way ANOVA or t-test was performed using spss22.0/GraphPad Prism 9. The significance threshold is ($P$<0.05). Compared with CON, the HUA group showed significantly lower average daily feed intake (ADFI), higher heart, kidney, muscular gizzard, and pancreas indices, elevated serum uric acid (UA), creatinine (Cr), relative knee joint width, and tumor necrosis factor-A. Compared with HUA, RRT groups had reduced liver and muscular gizzard indices, narrower knee joints, lower kidney and pancreas indices, and decreased serum UA and Cr ($P$ < 0.05). Histopathology revealed increased hepatic and renal inflammatory cells and elevated hepatic xanthine dehydrogenase (XDH) and renal glucose transporter 9 (GLUT9) protein expression in HUA group; H-RRT alleviated these changes. Serum metabolomics showed that HUA increased guanosine, inosine, and 5-hydroxyisourate while decreasing L-kynurenine, quinolinic acid (QA), and xanthurenic acid (XA) in tryptophan metabolism. H-RRT reversed these alterations. Cecal 16S rRNA sequencing indicated higher *Bacteroides* and lower *Blautia* abundance in HUA group; H-RRT reduced *Bacteroides* ($P$ < 0.05). Furthermore, an in vitro HUA model was established in HepG2 cells using hypoxanthine and xanthine oxidase to investigate mechanisms of RRT active components (catechin, (-)-catechin) and the serum differential metabolite XA. Five groups were set: CON, HUA, HUA+Catechin, HUA+(-)-Catechin, and HUA+XA. Compared with CON, the HUA group showed significantly higher mRNA expression of *PPAT*, *PNP*, and *PRPS*, as well as elevated UA in supernatant. Addition of (-)-catechin or XA markedly reduced *PRPS* mRNA; XA and Cr significantly lowered supernatant UA levels ($P$<0.05), thereby alleviating HUA. In summary, RRT extract effectively reduces UA accumulation and serum Cr elevation in HUA goslings, decreases hepatic XDH and renal GLUT9 protein expression, modulates purine, tryptophan, and arginine/proline metabolism pathways, and may mitigate gosling HUA via gut microbiota alterations.

**Keywords:** Magang geese; Hyperuricemia; Rosa roxburghii Tratt; Uric acid; Xanthurenic acid

## Metabolism and Nutrition: General Nutrition II

**201 (A-F) (MTCC)**

**264 Evaluation of Brown Rice (At 362) as an alternative energy source to corn for growth performance, intestinal morphology, and nutrient digestibility in Broiler Chickens**

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Accurate evaluation of digestibility and growth performance is crucial for optimizing poultry diets amid rising feed costs and the need for alternative energy sources. Brown rice (BR), with its favorable nutrient profile and local availability, may serve as a viable substitute. Therefore, this study evaluated BR as an alternative energy source to corn in broiler chicken diets. A total of 120-day-old male broiler chicks (42.34 ± 2.96 g) were assigned to four dietary treatments in a randomized complete block design with pen location as the blocking factor (6 birds/pen; 5 replicates/treatment): corn-based control (CON) and diets with 20% (BR20), 40% (BR40), and 100% (BR100) BR replacing corn. The trial lasted 35 days (starter: d 1-21; finisher: d 22-35). Growth performance was measured throughout. Excreta were collected at the end of each phase, and coefficients of ap-

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parent ileal digestibility (CAID) and coefficients of apparent digestibility (CAD) of dry matter (DM), crude protein (CP), and gross energy (GE) were determined using acid-insoluble ash as a marker. Processing yields were measured, and on day 35, selected birds were euthanized to determine relative organ weights, lengths of small intestinal segments, and intestinal morphological parameters. Day1-35, weight and feed intake increased, while feed conversion ratio decreased (P < 0.05) in the complete replacement (BR100) compared to the control group. Similarly, CAID of DM, CP, and GE increased with increasing brown rice level in the diets (P < 0.05). Additionally, CAD of CP was higher (P < 0.05) in the BR100-fed group compared to other treatments during both the starter and finisher periods. CAD of DM was higher in the BR100-fed group compared to the control group during the finisher stage (P < 0.05).BR inclusion had no effect (P > 0.05) on the relative weights of crop, proventriculus, heart, liver, and pancreas or on the relative weights and lengths of the small intestinal segments. However, BR increased the relative gizzard weight (P < 0.05), and no differences were observed in the morphological parameters of the duodenum and ileum (P > 0.05) compared to the control group. However, a higher crypt depth was observed in the jejunum of the group fed the BR100 diet (P < 0.05) compared to other treatment groups. The L* value and drip loss of breast meat samples were higher in the BR100 group compared to the control (P < 0.05). In conclusion, brown rice can fully replace corn in broiler chicken diets without negatively affecting growth performance, while improving nutrient digestibility, although certain meat quality parameters, such as color and water-holding capacity, could be negatively affected.

**Keywords:** Brown rice ; Apparent ileal digestibility; Growth performance; Apparent digestibility; Broiler chicken

### 265 Influence of the origin of the soybean meal on protein and amino acid digestibility in broiler diets

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We studied the chemical composition, protein quality indicators [urease activity (UA), protein dispersibility index (PDI), KOH solubility, and trypsin inhibitor activity (TIA)], N retention, and standardized ileal digestibility (SID) of CP and amino acids (AA) of 27 soybean meals (SBM) from Argentina (ARG), Brazil (BRA), and USA, in equal number, in broilers from 14 to 26 d of age. On 88% DM basis, CP was not affected by the origin of the meal. Per unit of protein, Lys (6.14 vs. 5.99 vs. 6.07%), TSAA (2.74 vs. 2.65 vs. 2.69%), and the sum of the four key amino acids (Σ 4 AA: Lys, Met, Cys, and Thr; 12.8 vs. 12.5 vs. 12.7%; *P* < 0.05) were higher for the USA than for the BRA meals with the ARG meals being intermediate. All protein quality indicators studied, except for UA, had higher values for the USA meals than for the South American meals (*P* < 0.05). All the birds were fed a standard commercial diet from 0 to 14 d of age, and then a corn-based diet that included 35% of each tested SBM from 14 to 20 d of age (phase 1). From 21 to 26 d of age, broilers were fed a diet that contained 53% of a N-free diet based on dextrose and 47% of the experimental SBM (phase 2). Each SBM treatment was replicated 6 times and the experimental unit was a cage with 6 broilers. Data were analyzed as a completely randomized design using the MIXED procedure of SAS with origin as the main effect. Also, Pearson correlations (*r*) were performed between the protein quality indicators and in vivo data. In both phases, N retention was higher for the USA meals than for the BRA and ARG meals (63.9 vs. 61.5 and 60.9%, and 54.7 vs. 51.8 and 50.6%, respectively; *P* < 0.01). In phase 2, no differences were detected for the SID of CP of the SBM. However, the SID of TSAA (78.7 vs. 76.7 vs. 75.3%; *P* < 0.05) and of Σ 4 AA (81.7 vs. 80.3 vs. 79.4%; *P* = 0.07) were greater for the USA than for the BRA and ARG meals. Similarly, broilers fed the USA meals grew faster (69.0 vs. 62.6 and 64.3 g/d; *P* < 0.001) and had better FCR (1.867 vs. 1.990 and 1.957 g/g; *P* < 0.01) than broilers fed the BRA and the ARG meals. Independently of SBM origin, significant correlations were detected between the SID of TSAA and PDI (*r* = 0.470; *P* < 0.05), KOH (*r* = 0.480; *P* < 0.05), and TIA (*r* = 0.620; *P* < 0.01) values, but not for UA values. Moreover, the SID of TSAA was correlated with BW gain (*r* = 0.364; *P* = 0.06) and FCR (*r* = -0.446; *P* < 0.01). In summary, due to differences observed, it is recommended to classify commercial SBM by soybean origin prior to laboratory chemical analyses, reducing variability in reported values and improving prediction equations used to estimate its nutritive values.

**Keywords:** Amino acid digestibility; broiler performance; N retention; protein quality indicators; soybean meal origin

### 266 Increasing dietary inclusion of two faba bean (*Vicia faba*) varieties differing in their vicine and convicine contents: Effects on broiler growth performance and carcass traits

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The nutrient profile of western Canadian faba bean (*Vicia faba;* 27% CP; 34% starch) suggests its suitability as a feedstuff for growing broilers, however there are several knowledge gaps that serve to limit their use as such. One important knowledge deficit pertains to the biological significance of two related anti-nutritional factors present in faba bean – vicine and convicine (VC). Metabolites of VC increase oxidative stress, placing an increased burden on antioxidant systems. The present study compared the effect of increasing dietary inclusion of two varieties of faba bean differing in VC content on broiler growth performance and carcass traits. In a 40-d experiment, mixed-sex Ross 708 broilers (n=420) housed in floor pens received one of 7 dietary regimens in a randomized complete block design with 5 replicate pens per regimen. Dietary regimens consisted of phase-specific diets (starter, d 0 – 11; grower, d 12 – 25; finisher, d 26 - 40) containing 16%, 32% or 48% of one of two faba bean varieties differing in their VC content ('Nevado', Low VC; 'Snowbird', High VC), or a wheat soybean meal control. ADFI, ADG, and gain:feed (G:F) was measured in each phase and for the overall 40-d experiment. On d 41 of age, birds were slaughtered and the resulting carcasses were assessed for dressing percentage and yield of carcass components. Data were analysed as generalized linear mixed models using the GLIMMIX procedure (SAS Institute; Cary, NC, USA). Models included the main effects of variety, dietary inclusion level and the two-way interaction, with block as a random effect. Pen was specified as the subject of repeated measures in instances where measurements were taken on individual birds, carcasses or portions thereof. The significance level for all statistical analyses was set at 5% (A = 0.05). Aside from thigh yield (P < 0.05), which was lower for the 32% and 48% low VC regimens compared to all others, variety and inclusion level did not interact to affect any growth or carcass metric (P > 0.10). There was no effect of faba bean variety on any growth performance or carcass metric (P > 0.47). Broilers fed 16% and 32% dietary regimens had greater ADG than the those fed the 0% control or 48% regimens in the overall 40-d experiment (P < 0.01). Increasing dietary faba bean inclusion linearly reduced G:F in the finisher phase and overall 40-d experiment (P < 0.01) due to a numerical increase (P < 0.13) in ADFI without any change in ADG (P = 0.27). Proportionate yield of breast was greater for all faba bean regimens compared to the 0% control (P < 0.01). In conclusion, growing broilers were not adversely affected by dietary VC concentration, even at very high dietary inclusion of high VC faba beans.

**Keywords:** faba bean; broiler; antinutritional factor; growth; carcass

### 267 Impacts of enzymatically-processed soybean meal inclusion on poult performance intestinal morphology, and intestinal permeability

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In meat bird production, muscle accretion is supported through dietary amino acids. To meet amino acid requirements, soybean meal (SBM) is commonly added to turkey diets. High inclusion rates of SBM in early life can impact digestibility due to anti-nutritional factors (ANFs) and may contribute to disruptions of the intestinal barrier. Enzyme-treated soybean meal (ESBM) is a soy specialty protein produced through a patented process that reduces ANFs and increases protein compared to SBM, which may provide a better start to young animals, such as turkey poults. To determine if ESBM can be implemented into young turkey diets, a feeding trial was conducted to evaluate the effects of ESBM (HP 300; Hamlet Protein) on growth performance, intestinal morphology, and intestinal permeability of poults fed diets with 10% and 5% inclusions of ESBM, substituting SBM, for the first 14d of age and from 15 to 35d of age, respectively, comparing to iso-nutritious control diets fully based on SBM. For the trial, 1,178 poults were weighed and assigned to one of 21 pens (10 pens for control, 11 pens for ESBM; 56 poults/pen). Poult growth performance and feed efficiency were measured at the end of each dietary phase (d14 and 35). On d35, 2 poults per pen were sampled to determine differences in small intestine morphology and intestinal permeability, indicated by plasma D-lactate. Data were analyzed using lmer package in R with diet fit as a fixed effect and pens blocked within room fit as a random effect. In comparison with the control group at d14, ESBM-fed poults were heavier (P=0.016), and their feed intake was lower (P = 0.037). This resulted in more efficient poults, as measured by feed conversion ratio (FCR) from d0 to d14 for the ESBM diet (P < 0.001). This effect was not observed from d15 to d35 as poults fed the ESBM diets performed similarly to poults fed the control diet (P > 0.099). Livability of poults in this study was similar between dietary treatments for all phases (P > 0.790). Intestinal morphology at d35 was similar for all traits measured across the 3 segments of the small intestine between poults fed the dietary treatments (P > 0.075). Intestinal permeability was decreased in poults fed the ESBM diet compared to poults fed the control diet (P = 0.045). Overall, ESBM increased body weight, decreased feed intake, and improved FCR during the first 14d of life. Additionally, the lower intestinal permeability at 35d suggests that the nutritional interventions early in life may have lasting impacts on overall intestinal health later in life.

**Keywords:** turkey; early interventions; anti-nutritional factors; performance; intestinal permeability

### 268 Increasing dietary inclusion of two faba bean (*Vicia faba*) varieties differing in condensed tannin content: Effects on broiler growth performance and carcass traits

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Faba bean (*Vicia faba*) has tremendous potential as an alternative feedstuff for broilers, but most contemporary varieties grown in Canada and worldwide contain measurable levels of condensed tannins (CT). Due to their binding affinity for proteins, CT exert an antinutritional effect by reducing AA digestibility and negatively impacting palatability. The objective of the present study therefore was to evaluate the biological significance of faba bean CT for broilers in conjunction with dietary inclusion level. Mixed--sex Ross 708 broilers (n=420) housed in floor pens received one of 7 dietary regimens from d 0 to 40 of age in a randomized complete block design with 5 replicate pens per regimen. Dietary regimens consisted of phase-specific diets (starter, d 0 – 11; grower, d 12 – 25; finisher, d 26 - 40) containing 16%, 32% or 48% of one of two faba bean varieties differing in their CT content ('Nevado', Low CT: 0.02 mg/g CT; 'Fabelle', Moderate CT: 0.42 mg/g CT), or a wheat-soybean meal control. ADFI, ADG, and gain:feed (G:F) was measured in each phase and for the overall 40-d experiment. On d 41 of age, birds were slaughtered according to industry standard practice. The resulting carcasses were assessed for dressing percentage and a subset of carcasses were further broken down to determine yield of primal carcass components. Data were analysed as generalized linear mixed models using the GLIMMIX procedure (SAS Institute; Cary, NC, USA). All statistical models included the main effects of variety, dietary inclusion level and the two-way interaction; with block included as a random effect. Pen was specified as the subject of repeated measures in instances where measurements were taken on individual birds, carcasses or portions thereof. The significance level for all statistical analyses was set at 5% (A = 0.05). Variety and inclusion level did not interact to affect any growth performance or carcass trait metric (P > 0.33). Faba bean variety (Low CT vs. Moderate CT) had no effect on any live performance (P > 0.29) or carcass trait metric (P > 0.20). Increasing dietary inclusion of either faba bean variety linearly increased ADFI (P < 0.01) and therefore linearly reduced G:F in the finisher phase, and in the overall 40-d experiment (P < 0.01). Inclusion level had no effect on BW or ADG over any interval of the experiment (P > 0.10). Increasing dietary inclusion of faba bean linearly increased yield of both breast (P < 0.01) and breast fillet (P < 0.02). In conclusion, the present study suggests that high dietary inclusion of faba bean with measurable levels of CT does not adversely affect broiler growth performance or carcass traits.

**Keywords:** faba bean; broiler; antinutritional factor; growth; carcass

### 269 Mechanism of Fermented Bamboo Powder Regulating Growth Performance and Meat Quality in Magang Geese via the Microbiota-Amino Acid Metabolic Axis

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The global livestock and poultry industry faces feed shortages and human–livestock competition for grain. As a novel feed resource, the application value and regulatory mechanism of fermented bamboo powder (FBP) in meat geese remain unclear. In this study, 360 healthy 35-d-old Magang geese (initial body weight (BW): 2.08 ± 0.01 kg) were randomly divided into 6 groups with 6 replicates and 10 geese per replicate, fed diets supplemented with 0%, 3%, 6%, 9%, 12%, or 15% FBP for 35 d. Data were analyzed by one-way ANOVA and Tukey's multiple comparisons using SPSS 26.0. Results are presented as means ± SEM, with significance defined at $P < 0.05$. For growth performance, final BW, average daily gain (ADG), and feed-to-gain ratio (F/G) in the control group were 3.58 ± 0.06 kg, 42.76 ± 1.67 g/d, and 5.84 ± 0.37. The 3% FBP group had 3.69 ± 0.05 kg final BW, 46.00 ± 1.47 g/d ADG, and 5.50 ± 0.21 F/G. The 6% FBP group had 3.72 ± 0.05 kg final BW, 46.90 ± 1.37 g/d ADG, and 5.39 ± 0.20 F/G. Quadratic regression showed final BW and ADG peaked at 5.88% and 5.94% FBP, while F/G was lowest at 5.78% FBP. Meat quality analysis indicated that 45 min post-mortem breast muscle lightness ($L^*$) in the 3% FBP group was higher than in 0%, 9%, 12%, 15% groups ($P < 0.05$). Breast muscle shear force in the 6% FBP group was lower than all other groups ($P < 0.05$), and leg muscle shear force was lower than in 0%, 12%, 15% groups ($P < 0.05$). Serum antioxidant enzyme activities were increased ($P < 0.05$) and pro-inflammatory cytokines decreased ($P < 0.05$) in 3%–6% FBP groups. The 15% FBP group showed increased hepatic inflammatory infiltration ($P < 0.05$) and down-regulated mRNA expression of intestinal barrier-related genes ($P < 0.05$). Targeted amino acid metabolomics showed L-carnosine in breast muscle and aromatic flavor amino acids in leg muscle were up-regulated in the 3% FBP group ($P < 0.01$). In the 6% FBP group, trans-L-hydroxyproline in breast muscle and glutathione in leg muscle were increased ($P < 0.01$), as were essential amino acids including L-lysine and L-methionine ($P < 0.01$). 16S rRNA sequencing of cecal digesta revealed elevated *Parasutterella* in the 3% FBP group, positively correlated

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with ADG and negatively with malondialdehyde. The 6% FBP group had higher *Bacteroides* ($P < 0.05$) and lower *Desulfovibrio* ($P < 0.05$), *Bacteroides* correlated positively with antioxidant enzymes and negatively with interleukin-2. In summary, 3%-6% FBP is optimal in diets for 35–70-d-old Magang geese, reducing F/G and improving meat tenderness, flavor, antioxidant capacity and nutrition. The mechanism may relate to optimized intestinal microbiota and regulated muscle amino acid metabolism.

**Keywords:** Magang geese; fermented bamboo powder; meat quality; amino acid metabolism; cecal microbiota

### 270 Effects of palm kernel cake and decanter cake combinations on growth performance, carcass traits, growth and nutrient transporter gene expression and breast meat composition in broiler chickens

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Palm kernel cake (PKC) and decanter cake (DC) are agro-industrial by-products, available in large quantities in countries with palm oil production. These by-products can be used as ingredients in broiler diets. As an original research, the current study assessed the effects of varying inclusion levels and combinations of PKC and DC on broiler performance. A total of 1,260 one-day-old male broiler chickens (Ross 308) were randomly assigned to 18 groups: treatment 1 was the basal-diet control, while treatments 2 and 3 contained 5% PKC and 5% DC, respectively. Treatments 4 to 18 contained a combination of PKC and DC in different ratios (30:70, 50:50, 70:30) and inclusion levels (2%, 4%, 6%, 8%, 10%). The basal diet was formulated as a corn–soybean meal (SBM)-based diet. The feeding trial was conducted over a period of 35 days. Growth performance parameters were recorded weekly throughout the trial, while data collection and sampling for all other parameters were conducted at the end of the experiment. The study followed a randomized design to ensure reliable comparison among treatments. Data were analyzed with the General Linear Model (GLM) procedure of SAS software. Treatment differences were determined by one-way ANOVA, while Duncan's Multiple Range Test was applied for post-hoc mean comparisons at $p < 0.05$. Birds fed 6-10% PKC, and DC combinations had lower ($p < 0.05$) body weight (BW), body weight gain (BWG), and higher ($p < 0.05$) feed conversion ratio (FCR). Likewise, broilers fed higher inclusion levels (8% and 10%) of PKC and DC combinations had reduced ($p < 0.05$) carcass weight and the relative weight of the breast. In contrast, hepatic growth and intestinal nutrient transporter gene expression remain unaffected ($p > 0.05$) across all treated groups. Additionally, no effects ($p > 0.05$) were detected in broiler breast meat moisture, crude protein (CP), or ash content; however, higher inclusion levels (8% and 10%) elevated ($p < 0.05$) the ether extract (EE) content of the meat. In conclusion, low to moderate levels of PKC and DC maintain growth performance, while inclusion ≥6% reduces growth and carcass yield, with no effect on hepatic growth or intestinal nutrient transporter gene expression.

**Keywords:** broiler performance; carcass traits; decanter cake; sustainable feed ingredients; palm kernel cake

### 271 Effects of roasting on nutritional characteristics of Peanut Skin (PS) and potentials in animal feed formulation in Sub-Saharan Africa

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The nutritional characteristics of roasted and intact PS were determined and a study was conducted to determine the growth performance and carcass characteristics of broiler finisher fed diets containing varying levels of peanut skin (arachis hypogaea) . The aim was to assess the quality of tropical PS as effective feed ingredient in broiler production, and promoting feed cost reduction and agricultural by-product utilization. A total number of one hundred and fifty broiler chickens were randomly allocated to five dietary treatments in a completely randomized design (CRD) having three replicates with ten birds per replicate. The broilers were fed finisher diets containing varying levels of roasted and intact peanut skin. The treatments were: T1 (0.0% - control), T2 (2.5% roasted PS), T3 (5.0% roasted), T4 (2.5% intact ), and T5 (5.0% intact PS). Each treatment received diets for four weeks. Data collected included growth performance parameters, body weight gain, feed intake,and feed conversion ratio (FCR). Carcass traits such as dressed weight and organ yields were also measured. Data were analyzed using one-way anova, and treatment means were separated using Duncan's multiple range test at a 5% significance level. Roasted PS contained 13.11% of CP on an as-is basis, while intact PS 13.95% . The tocopherol, pyridoxine, thiamine and niacin for intact PS are 0.93mg; 0.22mg;,0.19mg ;and 0.08 mg respectively. These vitamins can improve growth rates and feed conversion, and they can also be affected by heat treatment. The birds on T4 (2.5% intact PS) and T1 (control) had significantly ($P < 0.05$) higher average daily gain compared to T2, T3, and T5. T3 and T4 recorded higher daily feed intake, suggesting improved palatability. The best feed efficiency values were observed in T5 and T1, while T2, T3, and T4 exhibited reduced feed efficiency . Carcass analysis revealed significantly higher ($P < 0.05$) dressed weights in T2 and T4, indicating better meat yield at moderate inclusion levels. However, higher levels, of intact PS, negatively affected performance,which is likely due to increased fiber and anti-nutritional compounds in intact PS which negatively affected nutrient absorption and utilization.. The nutritional characteristics and the inclusion of 2.5% level of intact PS has no adverse effects on broilers growth performance, and carcass characteristics. This study concludes that PS can be safely included in broiler finisher diets at 2.5%, whether roasted or intact. This study provided valuable insights into the potential use of PS as a feedstuff in Nigeria  

**Keywords:** Peanut skin ; Nutritional ; Broiler; Roasted ; Feed conversion ratio

### 273 Fermented Orange Peel Improved Productive Performance, Immune Status, and Hormonal Responses in Aged Laying Hens

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Nutritional strategies for maintaining laying cycles in aged hens are increasingly important for improving production efficiency and sustainability. Fermented orange peel (FOP), a value- added agro- industrial by- product, may serve as a functional feed ingredient capable of improving performance, physiological stability, and reproductive health in aged laying hens. This study evaluated the effects of dietary FOP on laying performance, jejunal mucosal immunity, and serum hormonal, antioxidant, and inflammatory indices in aged hens. A total of 180 hens (70 weeks old) were randomly allocated to three dietary treatments: a control diet, a basal diet supplemented with 5% fermented orange peel, and a basal diet containing 0.5% unfermented orange peel, with five replicates of 12 hens per treatment over a 12- week period. Data were analyzed using SPSS, one-way ANOVA was done with Tukey's post hoc test at $P < 0.05$, and GraphPad Prism for graphs to visualise results. Dietary inclusion of FOP significantly increased laying rate, egg mass, and improved feed- to- egg ratio compared with the control group ($P < 0.05$), whereas hens fed the control diet outperformed those receiving unfermented orange peel. Fermented orange peel modulated mucosal

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immune responses by reducing jejunal interleukin- 1 B and increasing interleukin- 10 ($P < 0.05$), whereas interleukin- 6 remained unchanged ($P > 0.05$). In serum, FOP markedly elevated insulin- like growth factor 1 and glutathione peroxidase 3 ($P < 0.05$). Concurrent reductions in corticosterone and heme oxygenase-1 ($P < 0.05$) suggest a lower physiological and oxidative stress status. Fermented orange peel also increased serum estradiol and follicle-stimulating hormone, reflecting improved reproductive endocrine function, while concurrently lowering serum interleukin-1B ($P < 0.05$), further supporting its anti-inflammatory effect. In conclusion, dietary FOP improved productive performance, enhanced antioxidant and endocrine status, and promoted a favorable immune profile in aged laying hens. These findings indicate that fermented orange peel may be an effective and sustainable nutritional strategy for supporting productivity and physiological stability in aged laying hens.

**Keywords:** Fermented Orange peel; Aged Laying hens; Performance; Immunity; Physiology

## Microbiology and Food Safety I

### 203 (B-D) (MTCC)

### 274 Antimicrobial Resistance and Control of Non-Typhoidal *Salmonella enterica* in Poultry: A "One-Heath" Perspective.

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Since their discovery about 70 years ago, antibiotics have reduced mortality and morbidity associated with infectious diseases and have increased food safety and life expectancy around the world. Currently, about 180 antibiotics are available for the treatment and prevention of infectious diseases. However, pathogenic bacteria and other infectious agents continue to cause death and important economic loss worldwide. More than 70 years ago, several classes of antimicrobial agents also have been widely used in modern animal husbandry to prevent infections and promote growth. Increasing antimicrobial resistance (AMR) in animals and its potential threat to human health led to the ban or restriction of some antimicrobial agents as feeding additives. The emergence and spread of AMR are a complex mechanism since, resistance can be intrinsic or acquired through several genetic determinants including mutations, the integration of foreign genetic materials or the transfer of plasmids and transposons. Given this complexity, tackling AMR requires a "One Health" approach, which is based on the principle that human and animal health are interconnected in relation with a heathy environment. Poultry associated *Salmonella enterica* serovars have been involved in human salmonellosis which treatment is becoming of concern due to the emergence of antimicrobial resistant strains. This presentation discusses AMR in NTS serovars found in poultry related to food safety and public health issues to develop rational use of antimicrobial agents and/or better practices in respect to food and environmental safety. It also presents the potential of some novel *Salmonella* control strategies including the use of berry fruit products, bacteriocins and vaccination. Promised results suggest the possibility of using these products as feed additives, food preservatives to interfere with the survival while improving bird's immunity against *Salmonella* to improve poultry safety.

### 275 Impact of simulated transport of rear-in place pullets on the persistence of *Salmonella* Enteritidis in a cage-free production system.

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Rear-in place is a layer management practice used to reduce stress on pullets that may result from the transportation of pullets from the rearing facility to the laying facility. The objective of this study was to examine if the age in which *Salmonella* inoculation occurred and experienced simulated transport would induce *Salmonella* shedding in the cage-free production environment of pullets. Two trials were conducted, which consisted of 280 DOH chicks placed into two cage-free rooms (140 chicks/room). *Salmonella* Enteritidis inoculation via oral gavage of all chicks/pullets occurred at 7 days of age in Trial 1 and at 10 weeks of age in Trial 2. Every 1-2 weeks after *Salmonella* inoculation, environmental samples (wall, perch and drag swabs and substrate samples) were collected from every room. At 14 weeks of age, half of the pullets from each room were placed into transport crates. The crates were then placed on a flatbed cart and pushed up and down a hallway while being constantly being exposed to environmental stressors (sounds and physical agitation) for five minutes to simulate transport; this simulation was repeated every hour for four hours. After simulated transport, these pullets were placed into new, clean cage-free rooms and were kept for three more weeks to examine Salmonella recovery after simulated transport. Recovery of *Salmonella* from each sampling type was analyzed using Chi-square ($P<0.05$). In Trial 1, recovery of *Salmonella* was 100% from substrate samples collected at 1 and 2 weeks post-inoculation ($P<0.05$) compared to other environmental samples. However, the recovery of *Salmonella* from environmental samples decreased over time reaching 0% before simulated transport. One week after simulated transport, only a substrate sample (3.1%) from a simulated transport group tested positive for *Salmonella* but no *Salmonella* was recovered for the remainder of the trial. In Trial 2, *Salmonella* recovery was the highest (50%-100%) from substrate samples compared to other environmental samples. One week after simulated transport, recovery of *Salmonella* was at the highest rate from substrate samples (17.6%; $P < 0.001$). At the end of Trial 2, *Salmonella* recovery was 12.5%, all from environmental samples belonging to non-simulated transport pullets. Further research is needed to clearly understand the impact of stress during transportation. The sampling of the substrate may also be the best indicator of *Salmonella* in cage-free production. Keyword: Eggs, Pullets, *Salmonella*, cage-free, environmental sampling

**Keywords:** Eggs; Pullets; Salmonella; cage-free; environmental sampling

### 276 Comparison of Two *Salmonella* Serotypes Following Albumen or Yolk Inoculation of Hatching Eggs Cold-Stored and Incubated to Day 18

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*Salmonella* spp. are important foodborne pathogens, and contamination of hatching eggs can occur through transovarian transmission during egg formation. Although *Salmonella* may contaminate any component of a developing egg, prior research indicates that the albumen and the vitelline membrane are the most frequently affected sites. Prior studies have evaluated the recoverability of *S.* Enteritidis (**SE**) from hatching eggs following experimental inoculation, and the objective of the current study was to compare recoverability between SE and another serotype, *S.* Infantis (**SI**). A total of 132 SPF hatching eggs were collected and randomly divided into six treatments (n = 22/treatment): albumen (**AL**) control, yolk (**YO**) control, AL SE, YO SE, AL SI, and YO SI. On day 1, control eggs were injected with PBS while $10^3$ CFU of SE or SI nalidixic acid–resistant marker strains (100 ppm) were injected into the albumen or yolk of treatment eggs; all eggs were cold-stored (**CS**) for 2 days (6°C; 54% RH) and then transferred to an incubator (37.5°C; 55% RH). On CS day 0 and embryonic day (**ed**) 0, contents from three eggs per treatment were sampled, diluted 1:1 in BPW, and direct and enriched samples were plated in

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duplicate on Brilliant Green Sulfur (BGS) with 100 ppm nalidixic acid. On ed5 (n = 3), ed11 (n = 3), and ed18 (n = 10), viable embryos were aseptically removed and sampled separately from egg contents; Salmonella recovery from contents and embryos was performed as described above, with the only modification that embryos were diluted 1:2 in BPW. Direct plate counts were recorded as log10 CFU/mL and analyzed using ANOVA, while enrichment results were analyzed with the Kruskal–Wallis test to determine significance ($p \leq 0.05$). On CSd0 and ed0, all inoculated groups (excluding controls) were 100% positive by enrichment. At all incubation days sampled, direct recovery from egg contents was significantly higher for YO-injected eggs (6.7–8.8 log10 CFU/mL) compared with AL-injected eggs (0–0.3 log10 CFU/mL) for both serotypes. For YO-injected treatments, no viable embryos were present for either SE or SI, and YO control embryos were negative. On ed18, there was no significant difference in embryo recovery between AL SE and AL SI groups, with both having 40% (4/10) positive embryos. These findings indicate that SI behaves similarly to SE in this model and further support that the albumen is the more likely location of *Salmonella* contamination in forming eggs.

**Keywords:** Salmonella; egg inoculation; egg cold storage; egg incubation

### 277 Biocide tolerance and eggshell transfer of *Salmonella* Enteritidis biofilms

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*Salmonella* Enteritidis (SE) in multispecies biofilms shows greater biocide tolerance and eggshell transfer than in single-species, especially on thermoplastic polyurethane (TPU) at 20°C under wet condition compared to high-density polyethylene (HDPE) at 10°C dry condition. To assess biocide efficacy against SE single/multispecies biofilms on HDPE, and TPU, and determine SE eggshell transfer under egg storage (10°C) and barn (20°C) conditions, at two seasonal humidity scenarios: winter (dry, 20–30% RH) and summer (wet, 50–60% RH). After screening 15 SE strains for planktonic and biofilm biocide susceptibility, one SE was selected for multispecies: C1 (*P. aeruginosa* and *S. aureus*) + SE and C2 (*P. aeruginosa*, *E. faecium*, *L. reuteri*, and *L acidophilus* + SE). Evaluate SE transfer from single/multispecies biofilms on HDPE and TPU surfaces to eggshells along with eggshell penetration and internal survival. Biocide susceptibility of 15 SE isolates in planktonic and biofilm states (formed in LB-NS at 10 or 20°C) was evaluated via 96-well microplate assays. Minimum bactericidal concentration and biofilm eradication concentration were determined using manufacturers recommended concentrations for peracetic acid (PAA; 1200 ppm), hydrogen peroxide (HP; 5,000 ppm), sodium hypochlorite (SH; 120,000 ppm), benzalkonium chloride-glutaraldehyde (QG; 4,000 ppm), and potassium peroxymonosulfate (PMS, 20,000 ppm). One SE strain was combined with C1 or C2 in microplates to assess multispecies biofilm formation and biocide susceptibility. C1 biofilms were formed on dry/wet HDPE and TPU surfaces. To assess SE transfer to eggshells, fresh unwashed eggs were exposed to the biofilms (5 min). Eggs were washed using chlorinated alkaline detergent (40°C, pH 10; 5 ppm), rinsed (50°C) dried and then refrigerated for 20 d. Biofilm formation and survival were analyzed via factorial ANOVA (strain, multispecies composition, temperature); phenotypes and survival frequencies via Chi-square tests ($p \leq 0.05$). Planktonic SE was susceptible to all biocides. Single and C1 biofilms tolerated HP, PMS and PAA, but not SH or QG ($p<0.05$). Multispecies C1/C2 reduced SE by 3-log at 20°C, but increased SE by 0.9 log at 10°C ($p \leq 0.05$). All biocides eradicated SE on HDPE. However, TPU allowed SE survival ($p<0.05$), only QG eradicated C1 on TPU. The highest SE transfer occurred at 10°C under wet/TPU (5.29 log) and dry/HDPE (2.4 log). Internal egg penetration was only observed at 20°C (33.3%) for dry TPU/HDPE, and wet HDPE. No SE was detected in eggs exposed to multispecies biofilms. Facilities cleaning and disinfection protocols must account for type of material, biocides and biofilm formation, as biofilm formation on TPU, may allow SE survival after disinfection.

**Keywords:** Salmonella Enteritidis; biofilms ; biocides; thermoplastic polyurethane; high-density polyethylene

### 278 Can extracellular particles from different *Bacillus* spp. strains contribute to inhibitory and synergistic activities against *Salmonella* Heidelberg strains isolated from poultry?

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**Objective:**To evaluate strain-dependent inhibitory and synergistic effects of EPs produced by *Bacillus cereus* (BC), *Bacillus subtilis* (BS), and *Bacillus licheniformis* (BL) against *Salmonella enterica* serovar Heidelberg (SH). **Experimental design:**EPs were separated from the water-soluble fraction of cell-free supernatants using ultracentrifugation and ultrafiltration, then characterized for particle concentration, size distribution, protein content, and metabolomic signatures. **Materials and methods:**Probiotic Bacillus strains were cultured individually and in combinations to assess inhibition of SH in liquid medium, followed by inoculation onto agar plates for CFU counts. Cell-free supernatants (CFS) and EPs were characterized by nanoparticle tracking analysis (NTA), bicinchoninic acid assay (BCA), transmission electron microscopy (TEM), and metabolomic profiling via LC-QTOF-MS. **Statistical analysis:**Experiments were performed in triplicate. Significance was determined using one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Metabolomic profiles were compared by MANOVA and visualized through PCA with 95% confidence ellipses. **Results:**EPs showed heterogeneity, with diameters ranging from 142 to 367 nm and yields exceeding 10^10 particles/mL. TEM confirmed vesicular and non-vesicular subpopulations. Dual EP combinations (BL+BS and BL+BC) synergistically inhibited SH strain 13, reducing growth by up to 99%. All EP treatments suppressed strain 18 by 99%, while inhibition of strain 06 was more limited (95%). Metabolomic profiling identified 61 metabolites, 23 significantly differing among groups, including compounds with antimicrobial and quorum sensing activity. Multivariate analyses confirmed distinct strain- and combination-specific metabolic signatures. **Conclusions:**EP-mediated inhibition of SH is strain-dependent and enhanced through synergistic interactions, with metabolite composition contributing to antimicrobial activity. These findings highlight the critical role of Bacillus EPs in pathogen inhibition and provide mechanistic insights into their active components, supporting their potential application in food safety and poultry health.

**Keywords:** probiotic bacteria; synergism; antimicrobial; strain-dependent; pathogen inhibition

### 279 LitterGuard AI: An Early-Warning Machine Learning Platform for Predicting Salmonella Risk in Commercial Broiler Houses

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*Salmonella* remains a major foodborne pathogen in poultry production, and current monitoring strategies are largely reactive and limited by labor- and cost-intensive approaches. The objective of this study was to develop and evaluate a data-driven, machine learning-based predictive model to estimate *Salmonella* risk in broiler litter using field-measurable parameters. A total of 610 litter samples were collected from 26 broiler houses across 13 broiler farms in four U.S. states, representing heterogenous production systems and litter management practices. Key variables, including litter moisture, pH, flock age, and litter type (fresh vs. reused), were recorded at the time of sampling. Eight supervised models, including generalized linear mixed models, random forest, XGBoost, and Gaussian process models, were trained and evaluated using a stratified repeated hold-out validation approach to ensure robust performance of prediction. Model performance was evaluated using receiver operating characteristic area under the curve (ROC-AUC), precision, recall, and F1 score. Variable importance analysis was conducted to identify key predictors associated with *Salmonella* presence. Overall, *Salmonella* positivity was ~29.9%, however, farm-level prevalence ranged from 10% to >50%. Among the machine learning models tested, random forest demonstrated the highest overall performance (ROC-AUC = 0.824, precision = 0.700, recall = 0.704, F1 = 0.702). Litter moisture, pH, litter type, and flock age were all significantly associated with *Salmonella* presence ($P < 0.05$). Across all models, litter moisture and litter type were consistently identified as the most influential predictors, suggesting that moisture-driven microbial dynamics and litter reuse practices play a critical role in *Salmonella* ecology. These findings demonstrated that LitterGuard AI can effectively identify high-risk broiler houses using simple, field-measurable variables and provide a practical framework for an early-warning decision-support tool to guide targeted intervention strategies and litter management decisions in commercial poultry production systems.

**Keywords:** Salmonella; Broiler Litter; Risk Prediction; Artificial Intelligence (AI); Machine Learning

### 280 Differences in *Campylobacter* detachment and death as a function of PAA concentration and exposure time on broiler meat

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*Campylobacter* infections related to contaminated and undercooked chicken meat products continue to be a public health concern. Understanding the time-dynamics of cellular detachment and death as related to currently used biocide interventions for *Campylobacter* could improve food safety outcomes and the efficient application of these interventions. We therefore hypothesized that cellular detachment and death would vary based on the concentration of peracetic acid (PAA) and exposure time. Briefly, we inoculated chicken wings with approximately $10^6$ *Campylobacter* cells and exposed them individually to either water or one of four concentrations of PAA (0ppm, 10ppm, 20ppm, and 50ppm), for either one, two, or three minutes, in bench-scale simulated chillers. A single wing was used for each trial (n=12 individual treatments), and each trial was replicated three times (n=3 replicates/treatment). In order to reisolate cells for enumeration, wings were rinsed in 40mLs of neutralizing buffered peptone water for a minute, for five successive rinses; the first, third, and fifth rinses were plated onto Campy-Cefex agar for enumeration. Data was analyzed preliminarily using a t-test comparing the growth recovered from each exposed concentration for each exposure time to establish differences between exposure concentration. Treatment effect and interaction will be analyzed using a statistical model at a later date. As expected, fewer *Campylobacter* cells were recovered from wings exposed to any concentration of PAA than those exposed to water, at all time points on the first rinses, though not significantly lower for trials where growth was detected (t-test, $0.19<p<0.26$). At the three-minute exposure, 10 ppm PAA significantly decreased the concentration of *Campylobacter* ($p<0.04$). The non-significance was expected because low concentrations of PAA were selected for testing. Interestingly, cells were still recovered from the fifth rinse of the wings at concentrations of PAA below 50ppm, but not in the highest PAA exposures at the longer exposure times, indicating that not all cells are detaching during rinses at lower exposure concentrations and times. In conclusion, PAA was effective at reducing the number of *Campylobacter*cells present on inoculated meat, however, it should be noted that cells were still recoverable after five rinses in some treatments. The data presented here will be used to model the detachment and kill rates in order to predict the most effective concentrations and times for the optimal disinfection of poultry meat products.

**Keywords:** Campylobacter ; PAA; efficacy

## Metabolism and Nutrition: Vitamins and Minerals II

**206 (A-D) (MTCC)**

### 281 Microencapsulated vitamins and trace minerals maintain broiler growth performance under reduced inclusion levels

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The objective of this study was to evaluate the effects of a microencapsulated vitamin and trace mineral premix (Jefo Gut Harmony concept; Jefo Nutrition Inc., Canada) supplying lower inclusion levels of vitamins and trace minerals, on growth performance, intestinal morphology, and plasma fat-soluble vitamins of broilers. It was hypothesized that microencapsulation (Jefo Matrix Technology) would improve nutrient utilization and allow for reduced supplementation of vitamins and trace minerals without compromising performance. A total of 1,008 day-old male Cobb 500 broilers were allocated to 48 floor pens with 12 replicates per treatment in a completely randomized design. Treatments consisted of a control diet with a conventional free vitamin and trace mineral premix (VTM) at full commercial dose (VTM100) and three diets in which the conventional VTM was entirely substituted by Jefo Gut Harmony delivering either 75%, 50%, or 25% of the conventional VTM content, treatments[GT1] were designates as GH75, GH50 and GH25, respectively. Birds were reared for 42 days with *ad libitum* access to feed and water. Body weight and feed intake were recorded on days 14, 28, and 42 to calculate body weight gain and feed conversion ratio (FCR). On day 28, one bird per pen was sampled for jejunum histology and plasma fat-soluble vitamins measure[GT2]ments. Data were analyzed using ANOVA in XLSTAT, and means were separated using Tukey's test with significance declared at $P \le 0.05$. Plasma concentrations of retinol, A-tocopherol, and 25-OH vitamin D3 were affected by treatment, with the lowest values observed in GH25 ($P \le 0.05$). Jejunal villus height was increased in birds receiving GH50 and GH25 compared to VTM100 ($P \le 0.05$). No significant differences were observed in body weight and FCR across treatments throughout the 42-day period ($P>0.05$). Despite reduced circulating vitamin concentrations at the lowest inclusion level (GH25), growth performance parameters (body weight and FCR) were not compromised compared to VTM100. Jefo Gut Harmony maintained broiler performance when vitamin and trace mineral inclusion was reduced to 25% of the conventional dosage, while increasing villus height and reflecting dose-dependent changes in circulating vitamin levels. These results indicate that microencapsulation may allow for substantial reductions in vitamin and trace mineral inclusion

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without compromising performance, supporting more efficient nutrient use and contributing to more sustainable broiler production systems.

**Keywords:** Broilers; Microencapsulation; Nutrition; Vitamins; Trace Minerals

### 282 MICRO-ENCAPSULATION OF ALL SIX TRACE MINERALS TO REDUCE INCLUSION WHILE MAINTAINING PERFORMANCE AND CARCASS TRAITS OF BROILER

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Trace minerals (Cu, Fe, Mn, Zn, I and Se) are essential components of numerous enzymes thus indispensable for metabolism, immunity, growth and reproduction. Inorganic forms of these minerals (ITM) are widely used in poultry industry often with very high safety margins, resulting in large proportions of the ingested minerals to be excreted into the environment. Many countries have set up limits of Cu and Zn used in feeds. This study explored the extent of reduction of Cu, Zn, Fe and Mn supplemented in broiler nutrition, by using 400 newly hatched Ross 308 male chicks, randomly assigned to four treatments with ten floor pen replicates per treatment and 10 birds per pen, to determine the efficacy of encapsulating all six trace minerals into a single micro-granule, namely Metal Polysaccharides Complex (MPSC, MinCo®) and supplemented at descending pattern, on growth performance, carcass traits and retention of these minerals in broilers. The control diet was supplemented with ITM at their classical levels (mg/kg: Cu 15, Fe 40, Mn 100, Zn 100, Se 0.3, and I 1.0) for the entire 42-day life. Treatments 2–4 tested MPSC in three formulas, at 300/250/200/150 g/mt, resp. for Starter, Growers 1 and 2, and Finisher phase. Feed intake, weight gain, feed conversion ratio (FCR) and mortality were measured by phases. Apparent digestibility was measured on days 33–35 using titanium dioxide. Footpad dermatitis (FPD), wooden breast (WB), white strip (WS), and mineral contents in liver and breast were determined on day 43. Data were analysed by analysis of variance (ANOVA) and treatment means were compared using Duncan's multiple range test, with significances marked at $P < 0.05$. The results showed all birds reached expected live weight 3.2 kg by day 42 at FCR 1.55, mortality < 5%. No significant differences were observed among treatments up to day 35. During Finisher phase, the birds fed on MPSC showed improved growth rate and FCR compared with those on ITM (P<0.05). FPD, WB and WS did not differ. Birds receiving MPSC showed higher Mn and Se digestibility. Calculation on trace minerals intake revealed that the birds on ITM consumed 1,255 mg/bird total minerals (Cu, Fe, Mn, Zn), whilst using MPSC on descending supplemental pattern reduced total input to 491 mg (MPSC Formula 1), 264 mg (Formula 2) and 312 mg (Formula 3). It can be concluded that using MPSC can reduce minerals consumption by 70-80% without compromising performance and carcass traits. **Key words**: Trace minerals, encapsulation, broiler, sustainability.

**Keywords:** Trace Minerals; Encapsulation; Broile; Sustainability

### 283 Assessment of organic chromium supplementation on performance, intestinal integrity, bone mineralization, and meat quality parameters in broiler chickens subjected to experimental cyclic heat stress

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Chickens are homeothermic animals, and when ambient temperature exceeds the thermoneutral comfort range, heat stress (HS) occurs, negatively impacting growth performance and metabolism. There are multiple strategies to mitigate HS, including management practices and nutritional approaches. Chromium (Cr) is a trace mineral present in raw ingredients commonly used in broiler diets. Multiple studies have shown the benefits of organic Cr supplementation in poultry, including cell preservation, antioxidant activity, immune response, and glucose metabolism. The objective of this study was to evaluate the effects of organic Cr administration on performance parameters, intestinal permeability, and bone and meat quality in broiler chickens subjected to cyclic HS. Day-of-hatch chicks (n= 350; 25 chicks/rep) were randomly distributed into three groups. Group 1: No HS (Thermoneutral-TN, n=4/rep); Group 2: HS, n=5/rep; Group 3: HS + organic Cr, n=5/rep (NutraTech, Cromofeed®; 100 g/MT). The HS groups were exposed to cyclic HS at 35°C for 12 h daily from day 10 to day 42 of age. The experiment followed a completely randomized design. All data were subjected to analysis of variance (ANOVA) with a statistical significance set at $P \leq 0.05$. Performance parameters were evaluated at d 0, 10, 28, and 42. Serum fluorescein isothiocyanate-dextran levels (FITC-d) and bone mineralization were measured on days 10 and 42 (n=20/group). Meat quality parameters and carcass yield were measured on day 43 (n=10/pen). Chickens receiving organic Cr significantly improved performance parameters throughout the study compared to the HS group (BW 2482 vs 2717g; $P < 0.05$). By day 42, chickens subjected to HS had increased serum FITC-d levels (114.5 vs 46.9 ng/mL; $P < 0.05$) and decreased bone quality parameters compared with Cr-supplemented groups (Load at break 22.3 vs 33.2 kg; $P < 0.05$). At processing, the HS control had the lowest breast yield compared to the organic Cr supplemented group (17.5 vs 18.35; $P < 0.05$). Similar values were observed in carcass yield between groups ($P > 0.05$). Animals consuming Cr showed lower woody breast (0.37 vs 0.74) and white striping (1.00 vs 1.18) scores in comparison to the TN group ($P < 0.05$). HS did not affect cooked meat quality parameters ($P > 0.05$). In conclusion, Cr supplementation could be an alternative to mitigate the negative impact of HS on poultry production parameters, improving intestinal integrity and bone quality. Future research will focus on determining the underlying metabolic mechanisms of action at the cellular level.

**Keywords:** Broilers; Heat stress; Chromium; Performance; Intestinal health

### 285 Effects of Hydroxychloride and Organic Trace Mineral Nutrition on Eggshell Ultrastructure and Quality in Late-Cycle Laying Hens

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This study aimed to evaluate the effects of replacing conventional inorganic trace minerals with a combined hydroxychloride and organic source of Cu, Zn, Fe, and Mn on eggshell ultrastructure and shell quality in late-cycle laying hens. A total of 480 Hy-Line Brown hens (62 wk of age) were assigned to 2 dietary treatments in a randomized complete block design with 10 blocks, in which each block consisted of 1 replicate from each treatment; the replicate was the experimental unit. Each treatment had 10 replicates of 24 hens, and the 45-wk study was conducted in conventional cages under 13 h light/d (30 lux). Treatments were 1) a basal diet supplemented with inorganic Cu, Zn, Fe, and Mn at commercial levels, or 2) a basal diet supplemented with proprietary hydroxychloride and organic trace mineral products (Prokel, Alura Inc., Durham, NC, USA) to provide 50% of breeder-recommended supplemental Cu, Zn, Fe, and Mn levels. The Prokel strategy was designed to provide a more stable and bioavailable trace mineral supply. In this strategy, supplemental Cu and Zn were provided as 70/30 hydroxychloride:bisglycinate blends, whereas supplemental Mn and Fe were provided as glycinates. Productive performance was recorded weekly. Every 4 wk, 6 eggs/replicate (±2% of average egg weight) were collected to determine eggshell breaking strength and thickness. Every 8 wk, 6 eggs/replicate were collected to determine relative shell weight. At 82 and 100 wk, 2 eggs/replicate were evaluated by scanning electron microscopy, and mammillary knob width and height, palisade layer height, and shell thickness were measured using ImageJ. Data were analyzed using repeated-measures and linear mixed models with block included as a random effect, and significance was declared at $P \leq 0.05$. Trace mineral source did not affect laying rate, feed conversion ratio, egg mass, or

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mortality (P > 0.05), but reduced feed intake and egg weight (P < 0.05). Compared with the inorganic treatment, the Prokel strategy increased eggshell strength from 3.871 to 4.117 kg (SEM = 0.086; P < 0.001) and relative shell weight from 8.99 to 9.40% (SEM = 0.086; P < 0.001), whereas shell thickness was unchanged (P > 0.05). These responses were associated with greater mammillary knob width and palisade layer height (P < 0.001) and a lower proportion of mammillary height (P < 0.05). At 100 wk, palisade layer height increased from 228.7 ± 5.69 to 254.5 ± 4.97 µm (P < 0.001). In conclusion, the Prokel strategy, formulated to provide 50% of breeder-recommended supplemental trace minerals, improved eggshell ultrastructure and shell quality in late-cycle laying hens while maintaining laying rate, egg mass, feed conversion ratio, and mortality.

**Keywords:** trace minerals; eggshell quality; eggshell ultrastructure; laying hens; late-cycle hens

### 286 Determining the Effects of KemTRACE® Chromium on Turkey Performance

Karen Vignale*[1] [1]*Kemin Industries, Fayetteville, Arkansas, United States*

The objective of this trial was to determine if chromium propionate from KemTRACE® Chromium (KT Cr; Kemin Industries, Inc., Des Moines, IA) supplementation improves turkey performance during typical stress events associated with production. A total of 528 Nicholas® (Aviagen Turkeys, Inc., East Lewisburg, WV) turkey toms were transported from the hatchery to the research farm. Birds received 10x the recommended dose of Immucox® (Ceva Animal Health Inc., Cambridge, ON, Canada) vaccine at the hatchery. Upon arrival, birds were randomly selected, weighed as a pen (22 birds/pen), and assigned to one of two dietary treatments: Control and KemTRACE Chromium (1 lb/ton;12 reps; 22 birds/trt). At the beginning and end of each phase, birds and feeders were weighed for growth performance parameters. In addition, mortalities were recorded throughout the duration of the trial. Statistical analyses were performed using JMP® (SAS Institute, Cary, NC) software using the GLM procedure, and means were separated using Tukey HSD with significance considered if *P*≤0.05. Adding KT Cr to the diet significantly increased body weight (BW) at 5, 7, and 13 weeks of age (*P*<0.05), and tended to increase BW at 9 and 15 weeks of age compared to control birds (*P*-value 0.07 and 0.06, respectively). Adding KT Cr to the diet improved average daily gain significantly at 5, 7, and 17 weeks of age compared to control birds (*P*<0.05). KT Cr significantly improved mortality adjusted feed conversion rate (mort. adj. FCR) at 5, 7, and 11 weeks of age compared to control birds by 6, 2, and 8 points (pts), respectively (*P*<0.05). Furthermore, birds that were fed KT Cr had significantly better mort. adj. FCR at 0-7 and 0-9 weeks of age when compared to the control birds by 4 and 2 pts, respectively (*P*<0.05). Because of the BW differences at the end of the trial, FCR was adjusted to BW by 0.03 pts of FCR/lb of meat. Feeding KT Cr significantly improved BW adjusted FCR at 5, 7, and 11 weeks of age compared to the control (*P*<0.05) by 7, 3, and 6 pts, respectively. Furthermore, birds that were fed KT Cr had significantly lower BW adj. FCR at 0-7 and 0-9 weeks compared to control birds by 5 and 4 pts, respectively (*P*<0.05). Even though not significant, KT Cr improved BW adj. FCR at 0-15 and 0-17 weeks by 4 and 2 pts, respectively, compared to control birds (P>0.05). Feeding KT Cr numerically decreased mortality in toms at 0-7, 0-9, 0-11, 0-13, and 0-15 weeks by 1.03%, 1.38%, 1.77%, 1.46%, and 1.1%, respectively, compared to the control birds (*P*>0.05). KT Cr numerically improved overall mortality (0-17 weeks of age) by 1.48% compared to the control (*P*>0.05). KT Cr improved overall performance of turkey toms when fed from 0-17 weeks of age (P<0.05).

**Keywords:** turkeys; feed conversion; chromium propionate; mortality; performance

## Immunology, Health and Disease: Immunology



### 287 The One Health implication of *Enterococcus* in poultry production

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From a One Health continuum, *Enterococcus specie* has emerged as a significant yet underrecognized pathogen in poultry production, contributing to conditions such as skeletal infections, septicemia, and increased mortality in broiler. Once considered a benign commensal of the avian gut, *E. cecorum* is now negatively associated with intensified production practices, climate change and antimicrobial misuse. Its persistence in the farm environment and potential for dissemination along the poultry value chain highlight broader implications for animal health, food safety, and antimicrobial resistance (AMR). Despite its growing impact, critical gaps remain in understanding host–pathogen interactions, particularly the mucosal and systemic immune responses that govern colonization, translocation, and disease progression in poultry. Addressing these knowledge gaps is essential for developing effective control strategies and advancing sustainable poultry production.

### 288 Modulation of cytokine production from avian T-cells by vitamin D metabolites

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Through the presence of 1A-hydroxylase (CYP27B1) and the vitamin D receptor (VDR), immune cells are connected with the vitamin D metabolism. In previous studies we could show that activation of T-cells changed VDR but not CYP25B1 expression in the first 24 h, indicating a regulatory function of vitamin D in chicken T-cell biology. To gain further insights into the effects of 25(OH)D3 and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) on the function of chicken T-lymphocytes, in vitro proliferation and cytokine expression assays were performed. Spleen cells were isolated from 4 white leghorn birds and T-lymphocytes were specifically activated by T-cell-receptor crosslinking. Proliferation was quantified using a commercial BrdU-assay. In addition, cytokine expression by activated T-cells was investigated. Spleen cells from the same animals as mentioned above were snap frozen at -80°C immediately after preparation. For cytokine induction, T-cells were stimulated as for the proliferations assays but harvested after 16 hours as previous studies showed peak mRNA expression 16 - 20h after stimulation. Unstimulated cells served as control. Stimulated cells were either cultured without (stimulated control) or with 25(OH)D3 or 1,25(OH)2D3 (experimental groups). Cytokine expression was analyzed by qRT-PCR using validated in-house primer pairs and protocols. Duplicates were set up for each bird. Treatment comparisons were done by GLM (two sided T-Test). 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 compared to the control treatment (p < 0.001) as well as compared to 25(OH)D3 (p < 0.001). Activation of T-cells lead to a significant increase in IL-2, IL-10, IL-13, IL-17 and IFN-Γ expression. No statistically significant effect was observed if cells were stimulated in the presence of 25(OH)D3 or 1,25(OH)2D3 for IL-2, IL-17 and IFN-Γ, while there was a statistically significant reduction in cytokine expression for IL-10 and IL-13 in 1,25(OH)2D3 treated cultures. This study showed that the proliferative activity of T-cell receptor activated chicken T-cells is inhibited by 1,25(OH)2D3 but not by 25(OH)D3 which is similar to observations in mammalian immune cells. It can thus be concluded that 1,25(OH)2D3 has T-cell regulatory properties leading to an anti-inflammatory response in vitro. Fur-

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ther studies are planned to test whether the observed effects are also present in vivo and when using physiological dosages of 1,25(OH)2D3.

**Keywords:** Inflammation; Interleukins; Poultry; 25(OH)D3; 1,25(OH)2D3

### 289 Chicken blood alloantigen system H is encoded by the CD146 (MCAM) gene

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Alloantigen H is a genetically determined blood group system found on the red blood cells of chickens, identified as one of the 13 major alloantigen systems. Little is known about this system which has two serological alleles. The objectives of this study were 1) to identify the genetic region encoding the chicken alloantigen H; and 2) to develop DNA detection-based methods to aid H system allele identification. SNP genotypes from 54K Axiom chicken SNP arrays were established for DNA samples having known H system serological types including two elite White Leghorn Hy-Line lines segregating for H, non-pedigreed samples from the Northern Illinois University (NIU) DNA bank, plus inbred line samples. Sequence information was also available for the commercial and inbred lines. GWAS results from the elite Hy-Line lines and NIU DNA bank samples showed a very strong peak in the same 4.20-4.30 Mbp region on chromosome 24. Predicted cell membrane expression and the presence of non-synonymous SNP were criteria to identify candidate genes. Seven genes in this region have membrane-associated products: MCAM (CD146), THY1, MFRP, CLDN25, KCNJ14L, ABCG4, and PDZD3. However, only CD146 had SNP-variation that matched the serological haplotypes. Elite lines segregating for the H system had concordance rates between serological results and SNP haplotype of 95% for both the elite HYL lines and 99% for the NIU samples, indicating that the CD146 gene encodes the chicken H blood system. Gene knockout or overexpression with serological or immunofluorescence assays showing loss or gain of H antigen reactivity would confirm identification. The CD146 gene product is expressed by erythrocytes and erythroid progenitor cells at the protein and cDNA levels. It is a cell adhesion molecule affecting multiple activities including angiogenesis, development, cell differentiation, cell migration, signaling transduction, and immune responses. Long and short isoforms are differentiated by alternative splicing of exon 14. Chickens also have a soluble isoform. Online haplotype protein sequences include long and short forms meaning that haplotype differences are not based on isoforms. The short isoform was found in the samples tested, whereas a long isoform is reported elsewhere; its relationship to H haplotypes is unclear, though it may affect lymphocyte rolling and adhesion. The described DNA-based typing methods facilitate future investigations to examine H haplotype 1) frequencies in lines with identified differential outcomes such as growth or immune responses; and 2) association with traits of economic importance will aid in revealing the significance of this immune related gene in overall poultry health.

**Keywords:** alloantigen H ; CD146; MCAM; chicken H blood system; GWAS

### 290 Impact of environmental cyclic heat stress on blood leukocyte profiles of modern random-bred and divergent high- and low-water conversion broiler breeder hens

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To address the need for sustainable use of water in poultry production systems, water-efficient broiler breeder chickens have been developed. For this, a parental modern random-bred (MRB) broiler breeder population was used to establish, via divergent selection, low-water conversion ratio (LWCR; high water-efficiency) and high (H)WCR (low water-efficiency) populations. A previous study revealed that selection for improved water efficiency in the LWCR line did not negatively affect local and systemic cellular and humoral immune responses to bacterial components (LPS and PGN) and a killed *Salmonella* vaccine, compared with the MRB control population. There is little research on the impact of heat stress (HS) on the immune status of broiler breeder chickens. The objective of this study was to determine the impact of environmental cyclic HS on blood leukocyte profiles in MRB, LWCR, and HWCR broiler breeder hens at production. Twelve environmental chambers were used to rear the broiler breeder hens, and at 31 weeks of age, they were subjected to either thermoneutral (TN; 22°C) or cyclic HS (32°C for 8 h; 16 h TN) until they were 34 weeks of age. At 34 weeks of age, heparinized blood samples were collected from 3 hens per line and chamber to determine blood leukocyte concentrations using immunofluorescent staining and flow cytometry. Data were analyzed using two-way ANOVA to assess the effects of temperature (TN, HS), line (MRB, LWCR, HWCR), and their interaction. Significance was set at $P \leq 0.05$. There were no effects of temperature, line, or interactions for blood heterophil, $CD8^+$ T cell, ΓΔ T cell, and B cell concentrations. However, regardless of line, the monocyte concentrations in the blood were higher ($P = 0.036$) in HS than in TN. Independent of temperature, the concentrations of blood T cells ($P = 0.006$), $CD4^+$ T cells ($P = 0.001$), and total lymphocytes ($P = 0.001$) in the LWCR line were higher than those of the MRB and HWCR lines. Taken together, our findings suggest that environmental cyclic HS had limited effects on blood cellular immune status, as indicated by increased monocyte concentrations, while the remaining leukocyte profiles were unaffected. Moreover, the selection for water efficiency (LWCR) did not negatively impact blood leukocyte profiles and showed higher concentrations of total T cells, $CD4^+$ T cells, and total lymphocytes than the MRB and HWCR lines, indicating a robust immune system status in hens at production. Future research should dissect the quantitative and qualitative aspects of local and systemic immune responses to immunogenic stimuli between the MRB, LWCR, and HWCR genetic lines to better characterize their immune system capabilities under environmental heat stress.

**Keywords:** broiler breeders; heat stress; blood leukocyte profiles; water efficiency; hens

### 291 Nutritional energy deficiency alters humoral immunity in broiler chicks

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Nutritional deficiencies affect the immune system of broilers, and studies evaluating humoral immunity during energy-deficiency are limited. The objective was to assess the systemic and mucosal humoral immune system of broiler chicks when provided energy deficient diets. From 0 to 9 days of age (doa), a nutrient adequate corn-soybean meal diet was provided ad libitum. From 10 to 22 doa, chicks were feed restricted to 70% expected ad libitum feed intake and dietary treatments (trt) were provided: energy-deficient basal diet (EDB; 2,547 kcal/kg metabolizable energy) and EDB+100, 200, or 300kcal/kg from dextrose (glucose monohydrate; N=10 chicks/trt). Peripheral blood and cloacal swabs were sampled prior to (0d) and at 3, 7, and 10d post-dietary trt provision (pp). Spleen and excreta were collected at 10d-pp. Leukocyte profiles in blood and spleen were assessed using immunofluorescent staining and flow cytometry. Antibody levels (μg/mL) in blood, cloacal swabs, and excreta were measured by ELISA. Data were analyzed by 1-way ANOVA (linear

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or quadratic regressions by trt or time) and by 2-way ANOVA (linear effect of time, trt, or their interactions) with significance at $P \leq 0.05$. There was a trt by time interaction ($P=0.01$) for blood B cell concentrations ($10^3$ cells/μL) where provision of EDB or EDB+100kcal resulted in maximal B cell levels by 3d-pp, and EDB+100kcal yielded higher B cell levels than EDB+300kcal at 3d-pp. Alternatively, provision of EDB+200kcal or EDB+300kcal resulted in highest blood B cell levels by 7d-pp, with all trt groups returning to pre-trt levels by 10d-pp. At 10d-pp, splenic B cell levels ($10^6$ cells/spleen) were greatest ($P=0.01$) in birds provided EDB+100kcal, with provision of EDB+200kcal or EDB+300kcal resulting in numerically higher splenic B cell levels compared to EDB. Blood IgM and IgA gradually increased to maximal levels by 10d-pp regardless of trt. There was a trt by time interaction ($P=0.04$) for blood IgY levels, where provision of EDB, EDB+100kcal, or EDB+300kcal resulted in a gradual increase to maximal levels by 10d-pp. During this progression, birds on EDB had higher IgY levels than those on EDB+300kcal at 3 and 10d-pp as well as higher IgY levels than those on EDB+200kcal at 7 and 10d-pp. Cloacal IgA increased ($P=0.003$) from 0 to 10d-pp regardless of trt. These results indicate that abrupt modification to dietary energy could alter humoral immune system activity in broiler chicks, potentially impacting vaccine- or disease-related responses. Further research is needed to understand how leukocytic metabolic shifts could affect humoral immunity during feed restriction and dietary energy deficiency periods. This project was supported by USDA-NIFA SAS #2019-69012-29905.

**Keywords:** Broiler; Metabolizable Energy; Humoral Immunity; Antibodies; B cells

### 292 Broilers grown in the absence of adults have lifelong deficiencies in serum IgY and cecal SCFA levels compared to chicks exposed at hatch to adult bacteria

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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. However, it is not known when and if chicks grown in the absence of adults reach microbiota compositional and functional maturity. To emulate vertical transmission from hens, newly hatched chicks (n=42) were exposed on the day of hatch to adult cecal content, while controls received PBS (n=42). Animals were grown for a full growth cycle of 5-weeks. Every week some animals were sacrificed, and microbial cecal colonization was evaluated by 16S rRNA sequencing. Body weight, total serum IgY, total gallbladder IgA, and cecal short-chain fatty acids (SCFA) levels were also measured. Differences in microbial composition were assessed using relative abundance, and Bray–Curtis dissimilarity (PERMANOVA), while SCFA, IgY, IgA, 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. 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). Considering higher SCFA levels imply better utilization of nutritional fiber, it could be expected that weight would increase following treatment. Yet, 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. Total IgA levels were similar in treated and control chicks. Finally, serum total IgY levels were significantly higher in treated chicks throughout the 5-week period ($P < 0.05$ or better in 3 of 5 weeks) and followed a different trajectory compared to control chicks. Of note, treated chicks did not display the classical drop in IgY levels following depletion of maternal antibodies and instead showed a lifelong increase. To conclude, these results show that growing broiler chicks in the absence of adults results in a lifelong disruption in the composition and function of the cecal microbiota. Furthermore, these results point out bacterial phylogenies that can be potentially developed as colonizing probiotics to correct desired microbiota functions. Finally, these results imply that some of the characterized chicken physiology is only relevant to chicks grown in the absence of adults.

**Keywords:** Gut microbiota; growing chicks in absence of adults; broilers; short chain fatty acids; IgY

## Management and Production II

**205 (A-D) (MTCC)**

### 293 Interaction between photoperiod and mash diet energy levels on broiler performance

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Providing broilers with longer dark periods and feeding mash diets alter growth rate. The study objective was to determine if mash diets (low energy (LE) or high energy (HE)), in combination with a longer dark period, impair performance. Ross 308 as-hatched broilers (n=5,952) were reared to 35 d in 8 identical rooms with 2 pens per room. Photoperiods of 20L:4D and 18L:6D were tested in 4 rooms each, with each room having one pen of each diet. Body weight and residual feed were weighed at 7, 14, 28, and 35 d. Mortalities and culls were necropsied to determine cause of mortality. Room was the replicate unit for photoperiod and pen for diet. Data were analysed as a completely randomized design using an analysis of variance. Mortality data were log transformed. Significance was declared when $P \leq 0.05$ and a tendency noted when $P \leq 0.10$. A tendency for an interaction ($P=0.09$) existed for BW at 28 d, with the LE diet birds having lower BW, but a larger reduction in BW was noted under 6D. For BW gain (BWG) from 14-28 d, the interaction tended ($P=0.07$) to show that under 6D the LE diet birds had higher BWG, however under 4D the HE broilers had higher BWG. Broilers reared on the HE diet had higher BW at all ages and higher BWG for all periods. At 7 and 14 d, broilers reared under 4D had heavier BW ($P<0.01$) and from 0-7 and 7-14 d had higher BWG ($P<0.01$). At 28 and 35 d, 4D birds tended to be heavier ($P=0.08$ and $P=0.06$, respectively) and have higher BWG ($P=0.10$ and $P=0.06$, respectively). There was a tendency ($P=0.10$) for broilers on the LE diet to have better uniformity at 33 d. Birds reared under 4D were more uniform ($P<0.01$). Broilers fed HE diets consumed more feed from 14-28 ($P=0.01$), 28-35 ($P<0.01$), and 0-35 d ($P<0.01$), while feed intake was highest under 4D for all periods except 0-7 d. A tendency for an interaction ($P=0.07$) existed for feed efficiency from 14-28 d, with better efficiency on the HE diet, however under 4D the difference between diets was smaller. The interaction ($P=0.05$) from 28-35 d showed that under 6D the LE diet birds had better efficiency, but under 4D the HE diet birds were more efficient. Feed efficiency was improved on the HE diet from 0-7, 7-14, 14-28, and 0-35 d. Mortality was higher under 4D ($P=0.05$). A tendency for an interaction for the percentage of mortalities due to omphalitis ($P=0.08$) and infectious mortalities ($P=0.10$) existed, where under 6D more mortalities occurred in the HE group, whereas under 4D more mortalities occurred in birds fed the LE diet. Feeding a mash diet tended to alter broilers responses to photoperiod in terms of growth rate, feed efficiency, and mortality. Producers feeding mash diets may consider adjusting their lighting programs to optimize performance.

**Keywords:** mash feed; photoperiod; growth rate; mortality; broiler

### 294 Insights from a comparative life cycle analysis of bacterial xy-

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**lanase supplementation to advance sustainable broiler production**

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Multiple studies have shown that supplementing broiler diets with xylanase improves feed efficiency and nutrient utilization. In this study, the effects of bacterial xylanase (*B. subtilis* endo 1,4-B-xylanase, Belfeed) supplementation, particularly on feed formulation and efficiency, were integrated with life cycle assessment (LCA) to provide a holistic evaluation of its impact on sustainable broiler production. At the feed preparation stage, the impact of reformulating regional representative basal diets for North and South America, Europe and Asia, with starter, grower and finisher diets supplemented with xylanase (nutrient matrix value of 100 ME(kcal/kg)) was assessed. At the farm level, trial data were integrated into an attributional LCA, evaluating a conventional broiler system in France under four feeding strategies: basal, basal with xylanase, nutrient reduced (NR), and NR with xylanase. The study included more than 15 years of trial data from 11,634 birds subjected to two to four treatment programs, with average initial and final weights of 44 g and 2.8 kg (adjusted). The LCA followed the European Union's Product Environmental Footprint method, the Food and Agricultural Organization's Livestock Environmental Assessment and Performance and the Intergovernmental Panel on Climate Change guidelines, in all relevant aspects. Climate change impact (expressed as greenhouse gas emissions, GHGEs (kg CO2-eq)) was assessed using the Environmental Footprint v3.2 method. The results showed that reformulating basal diets, to include xylanase, lowered GHGEs from 0.6–11.4% per kg of feed, from starter to finisher phase. At the farm level, the net GHGEs (methane, nitrous oxide and carbon dioxide) associated with the production of 1 kg live weight broiler chicken were 2.26 kg CO2-eq and 2.48 kg CO2-eq for NR and basal feed strategies. However, compared with NR and basal feed strategies, supplementing diets with xylanase further reduced GHGEs by 3.5–12.1% per kg live weight broiler chicken. These reductions were largely driven by a better feed conversion ratio with xylanase supplementation (basal: 1.57; basal + xylanase: 1.48; NR: 1.62; and NR + xylanase: 1.53), reformulated diets, particularly, changes in soybean meal and rapeseed oil, and decrease in emission intensities such as nitrogen excretion, nitrous oxide and manure methane, by up to 21.7%. These positive effects were statistically confirmed ($p<0.05$) based on the comparative outcomes of 1,000 Monte Carlo simulation runs, conducted as part of an uncertainty analysis on the LCA study. Overall, the supplementation of broiler diets with bacterial xylanase is a valuable strategy for improving productivity while moving toward sustainable broiler production.

**Keywords:** Bacterial Xylanase; Feed Conversion Ratio; Enviornmental life cycle assessment; Greenhouse gas emissions ; Broiler production

**295 Broiler sampling strategies for estimation of mean body weight**

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The broiler industry traditionally utilizes a 1% sample size to estimate average body weight (BW) during live production. However, this standard approach may not suit all operational needs and can lead to estimation errors. Accurate flock data is important for decision-making regarding feed formulation, health treatments, and processing plant configurations. This study aimed to evaluate the accuracy of various sample sizes in predicting the true average BW and uniformity of a broiler flock. The individual BWs of 3,070 42-day-old broilers were recorded to represent the true population, which had an average BW of 3,097 g and a CV of 15.6%. Sample sizes representing 0.5%, 1.0%, 2.0%, 5.0% of the flock were drawn using a probabilistic simulation model with 10,000 simulations per scenario. Differences between the sample estimates and the true population mean BW and CV were calculated, and confidence intervals (CI) were established at 95% (representing practical targets) and 90% (representing precision approaches). Results demonstrated that for the 0.5%, 0.75%, 1.0%, and 2.0% sample sizes, 78.46%, 92.38%, 99.00%, 100% of the simulated sample means fell within the 95% CI, respectively. Correspondingly, 47.28%, 62.74%, 79.27%, and 95.7% of the sample estimates fell within the 90% CI. While these data represent a specific biological distribution, they show that sampling more than 1% provides marginal improvements in accuracy for a practical target. A larger sample size is still needed for high-precision approaches. However, there is an unavoidable trade-off between estimation accuracy and the labor and cost constraints of larger samples. Ultimately, this simulation approach could be adapted into a customizable tool to help producers determine optimal sample sizes for their specific operational scenarios.

**Keywords:** Broiler; Body weight; Sample size; Probabilistic simulation; Uniformity

**296 Broiler breeder feathering between 45 and 60 weeks of age is affected by target body weight and the length of the starter feed period**

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Feather loss during the laying phase can decrease fertility, as increased scratching may reduce voluntary mating. Target body weight (BW) dictates feeding amounts allocated during rearing. The length of the starter (ST) phase affects amino acid intake and potentially feathering development. This study evaluated the effects of two target BW: Low (L) and High (H), and offering ST feed for 4 or 6 wk in a factorial experiment. A total of 1,600-day-old Cobb pullets were split into 16 pens. Corn-soy-wheat bran diets were fed in a dark-house during the rearing phase, with feed amounts allocated to achieve target BWs and adjusted weekly to maintain them. Pullets were moved to a laying house with 2/3 slats at 23 wk. Seven Cobb MX roosters and 72 hens were placed in each pen. Pullets were fed starter, grower, and developer diets. Feed was allocated to achieve the target BW and was adjusted weekly based on actual values. Hens were fed breeder 1, 2, and 3 following Cobb's nutrient recommendations according to egg production and BW. The male-to-female ratio fluctuated between 9.3 and 10.5% due to spiking and mortality. Feathering was scored at 49, 55, and 60 wk of age by experienced personnel. Scores ranged from 0 to 8, with S0 = fully feathered, and S5 = bald thighs, S6 = scratches, S7 = small wounds, S8 = large wounds. Data was analyzed using a completely randomized design with ordinal logistic regression and contingency analysis. The BW was used as a covariate. At 49 weeks of age, no treatment effects ($P > 0.05$) were observed. At 55 and 60 wk, interaction effects of treatments were observed ($P < 0.001$) in feathering. At 55 wk of age, 11.2% of H hens fed starter for 6 wk had S0 or S1, while 9.2% of the ones fed starter for 4 wk had similar scores. Only 42.3% of H hens fed starter for 6 wk have S4 and S5, while 50.8% of the H hens fed starter for 4 wk had similar scores. In the L hens, 8.9% of hens fed starter for 4 wk had S0 and S1, and 55.5% had scores S4 and S5, while only 4.02% of L hens fed starter for 6 wk had these good feathering scores, and 63.5% had bad feathering (S4 and S5). At 60 wk, the effects were similar, 10.3% of H hens fed starter for 6 wk had S0 and S1, and 45.6% had S4 and S5, while only

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5.5% of H hens fed starter for 4 wk had good feathering and 58.3% had S4 and S5. In the L hens10.2% of the hens fed starter for 4 wk had S0 and S1, and 55.1% had S4 and S5, while only 4.8% of the hens fed starter for 6 wk had good feathering and 59.1% had S4 and S5. In conclusion, interaction effects were observed in feathering. Heavy hens benefited from feeding starter for 6 wk, while light hens had better feathering at the end of the production cycle when starter was fed for 4 wk during the rearing phase.

**Keywords:** Feathering; broiler breeders; target body weight; starter phase; reproduction

### 297 Ultraviolet (UV-A) light effects on welfare and performance in slower-growing broilers

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Lighting is a key management tool in broiler production. While chickens perceive ultraviolet (UV) light, its effects are mainly studied in fast-growing broilers. This study evaluated UV-A light in slower-growing broilers, hypothesizing increased foraging, exploration, and activity. 192 Hubbard JA757 broilers were assigned to 12 pens (6 UV-A [UV], 6 control [CON]; 16/pen; 1:1 sex) and reared to 52 d (11.4 kg/m2). Pens contained wood shavings, ad libitum feed and water, an elevated platform, and an alfalfa bucket. Behaviour, enrichment use, and a free space test were assessed at 14, 20, 29, 35, 42, and 49 d, with a novel object test conducted at 14, 29, and 42 d. Clinical welfare (gait, footpad dermatitis, feather cover, hock condition, and skin lesions) was assessed at 51 d, and body weight and feed intake were recorded at 0, 10, 24, 37, and 52 d. The study followed a completely randomized design with pen as the experimental unit (n=6). Data were analysed using linear mixed models (SAS, proc mixed), ordinal regression (R, ordinal), and Cox proportional hazards models (R, survival). Significance was set at P≤0.05. Footpad dermatitis and skin lesions were not observed (all scores were 0). Light treatment had no effect on gait, feather cover, hock condition, novel object responses, body weight, weight gain, or feed intake. UV light exposure decreased locomotive (P=0.002) and standing (P<0.001) behaviour, while increasing inactivity (P=0.001) compared to CON. UV birds performed less locomotion around the alfalfa bucket (*P*=0.032) but more comfort behaviour under the platform (*P*=0.040) compared to CON birds. Treatment × time interactions were observed for play around the alfalfa bucket (*P* = 0.008), play on the platform (*P* = 0.029), and foraging under the platform (*P*=0.036). Play behaviour peaked in CON birds at midday around the alfalfa bucket and in the afternoon on the platform, exceeding both other time points and the UV group, while UV birds showed no differences across time. In contrast, foraging under the platform was higher in UV birds at midday compared to CON birds, with no time differences in UV birds, whereas CON birds showed reduced foraging at midday compared to morning and afternoon. During the free space test, UV birds spent a greater proportion frolicking (*P*=0.036), although no differences were observed in running, sparring, or wing flapping. Overall, UV light had mixed effects on behaviour and resource use, without impacting clinical welfare or performance. Contrary to our expectation, UV provision stimulated inactive behaviour in slower-growing broilers. Further research is needed to clarify strain-dependent behavioural responses and their welfare implications.

**Keywords:** broiler; UV light; behaviour; welfare; performance

### 298 Replacement of Soybean meal with Black Soldier Fly (*Hermetia illucens*) larvae meal on growth performance and meat quality traits of broiler chickens

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A feeding trial was conducted at Poultry Research Center in the research shed of Bangladesh Livestock Research Institute to know the effects of replacing soybean meal with Black Soldier Fly Larvae (BSFL) meal on growth performance, and meat quality traits of broiler chickens. A total of 408 day-old broiler chicks were randomly allocated to six dietary treatments in a Completely Randomized Design with four replications of 17 birds each. The treatments included a control diet (T0) and diets where soybean meal was replaced with BSFL meal at 12.5% (T1), 25% (T2), 50% (T3), 75% (T4), and 100% (T5). Birds were reared under standard management practices with *ad libitum* access to feed and water. All data were subjected to one-way ANOVA using R-Studio (version 2025.05.01). Dietary treatments significantly affected body weight from the second week onward (P< 0.05). At the end of the trial, the highest final body weight was observed in T2 (1462.9 g), which was statistically comparable with T0, T1, and T3, while birds receiving complete replacement (T5) had significantly lower body weight (1126.6 g). Feed conversion ratio was significantly influenced during the first, third, fourth, and fifth weeks (P< 0.05), with poorer efficiency observed in T5. Breast meat quality analysis indicated significant effects of dietary treatments on pH (P<0.05) and water holding capacity (WHC) (P<0.01), while drip loss, cooking loss, and color parameters (L*, a*, and b*) were not significantly affected (P>0.05). In contrast, thigh meat quality traits including drip loss, cooking loss, color attributes, pH, and WHC were not significantly influenced by dietary treatments (P>0.05). Texture profile analysis of broiler meat in this study revealed that significant differences among treatments in minimum force, fracture force, maximum force, gumminess, and chewiness (P<0.05), whereas cohesiveness, springiness, and resilience remained unaffected. Lipid oxidation of fresh and preserved meat was also analyzed by TBA analysis and better result was observed in moderate levels of BFSA meal. Birds receiving moderate inclusion levels of BSFL meal exhibited better carcass traits including meat quality parameters comparable to the control group, whereas complete replacement resulted in reduced carcass yield. These findings highlight the potential of BSFL meal as a sustainable alternative protein source in broiler diets when included at moderate levels.

**Keywords:** Broiler chicken, ; Black Soldier Fly larvae meal,; Soybean meal replacement, ; Growth performance, ; Meat quality

### 299 Optimizing Feed Withdrawal Duration to Modulate Physiological Responses of Broilers During Heat Stress in a Tropical Environment

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Heat stress is a major constraint to broiler production in tropical environments where high ambient temperatures impair thermoregulation, reduce productivity, and compromise animal welfare. Feeding activity increases metabolic heat production, suggesting that strategic feed withdrawal during peak heat periods may mitigate thermal stress. This study evaluated the effects of different durations of daytime feed withdrawal on physiological responses, metabolic indicators, and antioxidant status in broiler chickens raised under hot tropical conditions. A total of 240 Ross 204 broiler chicks were reared under conventional management until 21 d of age and then allocated to four treatments with four replicates of 15

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birds each. Birds were either fed ad libitum (AD) or subjected to daily feed withdrawal during the hottest part of the day for 2 h (THW; 12:00–14:00), 4 h (FHW; 12:00–16:00), or 6 h (SHW; 12:00–18:00). Growth performance, serum biochemical indices, antioxidant enzymes, hematological parameters, rectal temperature, and respiratory rate were evaluated at day 42. The data obtained were analysed using one-way analysis of variance in a completely randomised design. Results showed that feed withdrawal reduced feed intake but improved feed conversion efficiency ($p < 0.05$) compared with AD birds. Birds subjected to FHW and SHW showed lower feed conversion ratios, indicating improved feed efficiency. Physiological indicators of heat stress were also moderated by feed withdrawal. Rectal temperature and respiratory rate were reduced ($p < 0.05$) in birds exposed to longer withdrawal periods, with the lowest values observed in the SHW group. Antioxidant responses were enhanced ($p < 00.05$) in feed-withdrawn birds, as indicated by higher superoxide dismutase and catalase activities relative to the control group. Several haematological parameters associated with oxygen transport and immune function were also improved in birds subjected to longer withdrawal periods. These findings suggest that strategic daytime feed withdrawal can improve thermophysiological stability and antioxidant responses in broiler chickens raised under hot tropical conditions without negatively affecting growth performance.

**Keywords:** heat stress; broilers; feed restriction; physiological responses; oxidative stress

### 300 Effects of Medical Plants on rectal temperature, respiratory rate, blood parameters, and intestinal histology in broilers exposed to a heat wave (37°C).

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Heat waves (HW) seriously harm broiler production and jeopardize its sustainability, which has spurred scientists to look for novel and practical remedies. Therefore, there is a need to find appropriate feed additives like medicinal plants to sustain the performance of the broiler during summer. Using *Artemisia Sieberi*, *Achillea Fragrantissima*, and *Olea Europaea* to decrease the effect of HW in broilers is limited in the literature. The present study aimed to determine the effect of medicinal plants on rectal temperature (Tr), respiratory rate (RR), blood parameters, and intestinal histology in broilers exposed to a heat wave (37°C). A total of 180-day-old chicks were randomly assigned to six dietary treatments in a completely randomized design (CRD) with 3 replicates per treatment. Ctrl/TN: the chicks were kept under thermoneutral conditions and fed a basal diet. The other treated groups were exposed to HW and fed a basal diet (Ctrl/HW) or supplemented with *Artemisia Sieberi* (1.25 g/kg ; ARTE), *Achillea Fragrantissima* (15 g/kg ; ACHI), *Olea Europaea* (10 g/kg ; OLEA), and all additives combined (COMB). The chicks were subjected to HW (day 35 to 39; at Ta = 37±1°C, RH% = 59±1; 4 h/day). Ctrl/HW broiler chickens' treatment had increased ($P < 0.01$) Tr and RR (0.3 and 0.5%) relative to Ctrl/TN treatment. Wonderfully, chicken treated with medical plants had decreased ($P < 0.01$) Tr and RR relative to Ctrl/HW treatment. Overall, Ctrl/HW broiler chicken treatment decreased feed intake (FI) and body weight gain (BWG) and increased feed conversion ratio (FCR) ($P < 0.01$) compared with Ctrl/TN treatment. The OLEA treatment had a better effect than other groups at maintaining an optimal FI and BWG during the heat challenge, while all medical plants maintained a better FCR in broilers. HW increased ($P<0.05$) the cortisol level (17.54%) in Ctrl/HW relative to Ctrl/TN broiler chickens. Interestingly, heat-stressed broiler chickens treated with medical plant treatments had decreased cortisol levels by 4.3, 8.0, 4.5, and 1.7% relative to the Ctrl/HW treatment. Meanwhile, heat-stressed broiler chickens treated with ARTE had increased glutathione peroxidase levels by 29% relative to the Ctrl/HW treatment. The OLEA- and COMB-treated chickens have increased villus height in the duodenum and ileum relative to the Ctrl/HW treatment. In addition, ACHI treatment decreased crypt depth in the duodenum relative to the Ctrl/HW treatment. Moreover, the villus surface area of the duodenum and ileum was increased in OLEA, COMB, and ACHI treatments relative to the Ctrl/HW treatment. In conclusion, these data indicated that medicinal plant supplementation might alleviate HW in broilers through improved intestinal health.

**Keywords:** Medical plants; antioxidant; broiler; heat wave; rectal temperature

## Microbiology and Food Safety II

**203 (B-D) (MTCC)**

### 301 Host–Diet–Pathogen Crosstalk: Shaping the Future of Poultry Gut Health

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Gut health is a critical determinant of animal welfare, production efficiency, and food safety in poultry systems, yet it remains poorly defined due to the lack of standardized, quantifiable indicators. Intestinal integrity is frequently challenged by enteric pathogens and dietary contaminants. *Campylobacter jejuni*, a leading cause of bacterial gastroenteritis in humans, commonly colonizes the avian gut and represents a major source of foodborne transmission. Feed-borne mycotoxins such as deoxynivalenol (DON) can impair gut barrier function and act as predisposing factors that enhance pathogen colonization and translocation. Therefore, this study aimed to evaluate whether replacing DON with its metabolite DOM-1 in broiler diets could mitigate DON-induced effects on growth performance, intestinal permeability, and *C. jejuni* colonization and translocation. A total of 180 broiler chickens were allocated into six groups (n = 30; 5 replicates/group): control, DOM-1, DON, DOM-1 + *C. jejuni*, DON + *C. jejuni*, and *C. jejuni*. Birds were housed in floor pens with ad libitum access to feed and water. On day 14, selected groups were orally inoculated with 1 × 10^8 CFU of *C. jejuni* NCTC 12744. At 7, 14, and 21 days post-infection, samples from intestinal segments and internal organs were collected for bacteriological analysis. Intestinal permeability was assessed using Ussing chambers by measuring paracellular mannitol flux. Data were analyzed using a multivariate general linear model, ANOVA, and post hoc tests. Birds fed DON-contaminated diets, with or without *C. jejuni* infection, showed significantly reduced body weight compared to other groups. Co-exposure to DON and *C. jejuni* resulted in higher bacterial loads in the intestine as well as increased translocation to the liver and spleen, which was associated with elevated paracellular permeability in the duodenum, jejunum, and cecum. In contrast, DOM-1 supplementation improved growth performance and feed conversion ratio and did not adversely affect intestinal barrier function. Moreover, DOM-1 reduced *C. jejuni* colonization in the gut and its dissemination to internal organs. In conclusion, these findings demonstrate that DON compromises intestinal integrity and promotes *C. jejuni* colonization and translocation in broiler chickens. In contrast, the DON metabolite DOM-1 mitigates these adverse effects, supporting gut barrier function and reducing pathogen burden. Nutritional strategies incorporating mycotoxin detoxification may therefore represent an effective approach to improve poultry health and reduce foodborne zoonotic risk.

**Keywords:** Campylobacter jejuni; Poultry gut health; Mycotoxins; Deoxynivalenol (DON); Feed additives

### 302 Dietary Fiber Modulates Caecal Microbiota Dysbiosis and Enhances Intestinal Barrier Integrity in Broiler Chickens Infected with *Campylobacter jejuni*

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*Campylobacter jejuni (C. jejuni)* is a major foodborne pathogen in poultry that disrupts gut microbiota and impairs intestinal health. Its high prevalence highlights the need for microbiome-targeted nutritional strategies. This study evaluated the effects of oat-derived dietary fiber on intestinal barrier integrity, microbiota diversity, and functional potential in broiler chickens under *C. jejuni* infection. A total of 180 one-day-old Ross 308 broilers were randomly assigned to six groups (n=30; 5 replicates/group) and fed a corn-based basal diet or diets supplemented with 10% or 20% oats, with or without *C. jejuni infection.* At 2 days of age, infected groups were orally inoculated (1×10^8 CFU/mL), while controls received PBS. At 7, 14, 21, 28, and 35 days post infection (dpi), five birds per group were euthanized. Caecal contents were analyzed for bacterial quantification and shotgun metagenomics, while tissues were used for tight junction and MUC2 gene expression (RT-qPCR). Gene expression was analyzed by two-way ANOVA. Alpha and beta diversity were assessed using the Shannon index and Bray–Curtis dissimilarity, with NMDS and PERMANOVA for ordination and group comparisons. Differential abundance at species and pathway levels was determined using MaAsLin3 with FDR correction. Dietary fiber significantly reduced caecal *C. jejuni* colonization at 21 and 35 dpi ($p<0.05$). It also upregulated tight junction genes (claudin-1, zonula occludens-1, occludin) at 21 and 35 dpi, and increased MUC2 expression at 21 dpi. Infection reduced alpha diversity ($p<0.001$), whereas fiber supplementation restored diversity in infected birds ($p<0.05$). Beta diversity showed distinct microbial community structure between infected and control groups ($R^2=0.21$, $p=0.001$), this dissimilarity was reduced by fiber supplementation ($R^2=0.05$, $p=0.01$). Differential abundance analysis revealed that infection decreased beneficial taxa, including *Bacteroides, Parabacteroides,* and *Butyricimonas faecalis (q<0.01), w*hile fiber supplementation preserved these taxa ($q<0.1$). Functional analysis indicated enrichment of virulence-associated metabolic pathways in infected birds fed the basal diet, whereas fiber supplementation promoted carbohydrate fermentation and host-beneficial metabolic pathways ($q<0.1$). Overall, oat-derived dietary fiber modulated caecal microbiota, enhanced gut barrier integrity, and reduced *C. jejuni* load. Notably, 10% inclusion tended to show stronger protective effects than 20%, supporting its potential as a nutritional strategy to enhance poultry gut health and food safety.

**Keywords:** Campylobacter jejuni; dietary fiber; food safety; gut microbiota; intestinal barrier function

### 303 Effects of Chronic Exposure to Dietary Mycotoxins on Cecal Microbiota, Short-Chain Fatty Acids, and Predicted Functional Pathways in Broiler Chickens

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The gut microbiome is essential for nutrient metabolism and overall gut health in poultry. Broilers in commercial production are routinely exposed to *Fusarium* mycotoxins through contaminated corn and grain by-products. Chronic exposure to subclinical levels of multiple *Fusarium* mycotoxins can alter microbial composition and function. The objective of this study was to evaluate the effects of chronic dietary exposure to fumonisins (FUM), deoxynivalenol (DON), and zearalenone (ZEN) on cecal microbial diversity, taxonomic composition, short-chain fatty acid (SCFA) production, and predicted microbial metabolic pathways in broilers. Birds were assigned one of four dietary treatments: a basal diet (CON), MTX1 (FUM 9.0 + DON 3.5 + ZEN 0.7 mg/kg diet), MTX2 (FUM 17.0 + DON 1.0 + ZEN 0.2 mg/kg diet), and MTX3 (FUM 2.0 + DON 2.5 + ZEN 0.9 mg/kg diet). On d35, cecal content samples (n=6) were analyzed by 16S rRNA gene sequencing, SCFA quantification, and functional pathway prediction using PICRUSt2 and MetaCyc. Statistical analysis was performed using the Kruskal–Wallis test with false discovery rate correction, and the relationship between variables was assessed using Spearman's rank correlation. On d35, no significant differences were observed at the phylum level ($p > 0.05$). However, the Burkholderiaceae and Enterobacteriaceae families and the genus *Ralstonia* were completely absent in all MTX treatments compared with CON ($p < 0.05$). Alpha-diversity indices did not differ among treatments ($p > 0.05$). Significant treatment effects were observed in SCFA levels, including acetate, propionate, isobutyrate, butyrate, and valerate ($p < 0.05$). Birds in MTX3 had lower acetate and higher propionate, isobutyrate, butyrate, and valerate compared with CON ($p < 0.05$). Lachnospiraceae correlated negatively with acetate and positively with propionate, butyrate, and valerate, whereas Ruminococcaceae showed positive correlation with acetate and negative correlation with propionate, isobutyrate, butyrate, and valerate. Lactobacillaceae had a positive correlation with isobutyrate ($p < 0.05$). Predicted functional pathways, related to aromatic compound degradation, amino acid fermentation, pyruvate fermentation, and glucuronate metabolism, were inferred using PICRUSt2 and were reduced or absent in the MTX groups compared with CON. These predicted functional changes were consistent with the observed alterations in SCFA profiles, suggesting shifts in microbial metabolic activity. In conclusion, chronic dietary exposure to *Fusarium* mycotoxins altered cecal microbial composition and metabolic function, resulting in shifts in SCFA profiles and suppression of key microbial metabolic pathways.

**Keywords:** Mycotoxins; V3-V4 16s Sequencing; Gut microbiome; Short chain fatty acids; Broiler Chickens

### 304 Comparing performance and microbiota of laying hens fed US and European style diets between 35 and 45 weeks of life

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Diet formulation aims to result in a feed that supplies all nutritional requirements of an animal according to life stage and physiological status. This is particularly important for laying hens in production as their nutrient demands are high and changes can impact production efficiency and health. Typical US diets rely mostly on corn-soybean (CS) while EU diets usually include corn-wheat (CW) and by-products. This study compared the impact of these diet types on layer performance parameters and microbiota between 35 and 45 wk. It was hypothesized that performance would be similar although CW diets would have more lactate and SCFA-producing bacteria due to fiber compared to CS. The CS diet had corn as the only cereal (54% inclusion), and the CW diet had a combination of 35% corn, 15% wheat and 12.5% wheat bran. Each diet was fed to 8 replicates with 9 Dekalb White hens each. Performance parameters evaluated were body weight, feed intake, weight gain, feed conversion, laying rate, egg weight and egg mass. Data was subjected to a two-way ANOVA in a randomized block design. Microbiota was evaluated by analyzing 18 cloaca swab samples per treatment at 35, 39, 42 and 45 wk using Galleon™, Cargill's microbiome intelligence platform, measuring a list of pre-defined bacterial biomarkers by fluorescence microarrays. Microbiome data was submitted to ANOVA, and differences between standardized LS-means were considered significant after adjusting for FDR with P=0.05. There were no significant differences between diets for any performance parameter. CS had significantly more *Intestinibacter bartlettii*, *Peptostroptococcaceae*, *Turicibacter* and *Bifidobacterium gallinarum* compared to CW at 35wk. At 39wk, CS had significantly more *Enterobacter*

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and *Parabacteroides* while CW had more *Lactobacillus frumenti* and *Clostridium perfringens*. There were no differences due to diets at 42 or 45 wk. It was possible to train a machine learning model with 85% accuracy to classify samples into correct diet groups at 39wk. The top biomarkers of this model confirmed the association of CS with *Parabacteroides* and *Enterobacter* while the profile of CW included different lactic acid producing bacteria and *Lachnospiraceae*. So, diet type impacted microbiota composition in the first 4 weeks of the trial without impacting egg production, but long-term impact should be investigated. The hypothesis that CW diets promote cross-feeding between lactic acid bacteria and SCFA-producing bacteria was confirmed but adjustments should be made to control increased risk associated with *Clostridium perfringens*. These results demonstrate the added value of microbiota information to improve diets towards better gut health before performance is compromised.

**Keywords:** layer; diet; formulation; performance; microbiota

### 305 Microbial diversity and succession associated with poultry processing stages

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The microflora of poultry is highly tolerant to the rigorous steps encountered during processing. These interventions, combined with antimicrobial treatments, can create selective pressures that enrich microbial communities capable of surviving and ultimately persist in final products. Therefore, understanding bacterial diversity associated with poultry processing will advance the implementation of mitigations to prevent cross-contamination and extend the shelf life of poultry products. Using 16S rRNA sequencing, we characterized the microbial communities of a poultry processing facility over multiple flocks using chicken carcasses (pre-slaughter and post-chill) and processing equipment at pre-evisceration (bleeder, scalder, and picker) and second processing (debone line). Genomic DNA from 88 samples representing 22 flocks was extracted and the full length 16S rRNA was sequenced using an Oxford Nanopore Technology P2S sequencer. The raw reads were quality-filtered and taxonomically classified using Emu v3.6.2. The data was analyzed and visualized in R v 4.5.0. Alpha diversity decreased after chilling, with significant changes in evenness ($p$<0.05) and dominance ($p$<0.01). Pre-slaughter samples were also clearly separated from post-chill samples ($p$=0.001) in microbial composition. Factors such as flock origin and seasonal variation had no influence on alpha and beta diversity. This dynamic continued to sanitation, where pre- and post-sanitation samples were similar in richness but variable in composition ($p$<0.001). At pre-sanitation, second processing samples had a significantly lower microbial diversity ($p$<0.05) compared to pre-evisceration samples, but both had similar distribution patterns ($p$=0.05) at pre- and post-sanitation. Furthermore, at pre-slaughter, the top five genera were *Psychrobacter* (18%), *Staphylococcus* (16%), *Carnobacterium* (13%), *Pseudomonas* (8%), and *Enterococcus* (7%). Four of these persisted to post-chill where *Pseudomonas* (30%) dominated and *Escherichia* (6%) emerged as a notable constituent. On the equipment surfaces, *Psychrobacter* (19%), *Pseudomonas* (10%), and *Escherichia* (7%) were retained after processing and persisted post-sanitation. Notably, pathogenic genera like *Bacillus* (15%) and *Escherichia* (13%) were prominent on both pre-evisceration and second processing surfaces after sanitation. This study demonstrates substantial microbial diversity at critical points in poultry processing, with evidence that microbial populations entering the facility could influence those that persist on processing surfaces. These findings support the use of culture-independent microbiome profiling as a complementary tool for monitoring microbial hazards across poultry processing operations.

**Keywords:** microbiome profiling; poultry processing; food safety; shelf life; 16S rRNA

### 306 Potential of probiotic *Bacillus* and *Escherichia coli* strains to acquire resistance to aminoglycosides and quinolones under selective conditions

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**Objective:** To evaluate whether selected probiotic strains can acquire antibiotic resistance under selective pressure and whether resistance is influenced by antibiotic exposure or generation time. **Experimental design:** *Bacillus velezensis* BV01UNICLON, *Bacillus clausii* ENTPro, *Escherichia coli* CEC 15, and *E. coli* Nissle were exposed to sublethal concentrations of aminoglycosides and fluoroquinolones (amikacin and norfloxacin) over successive generations. **Materials and methods:** Strains were divided into control and treated groups, with the control group not receiving antibiotics; *E. coli* ATCC 25922 was used as a positive control. Bacteria were serially subcultured for up to 240 generations, with antibiotic supplementation every 50 generations. Minimum inhibitory concentration (MIC) was determined at each interval to monitor susceptibility changes. At the end, *Bacillus* cultures were exposed to heat and acidification to eliminate vegetative cells, and spore formation was evaluated using malachite green staining. MIC values were log 2 transformed. **Statistical analysis:** Pearson correlation and linear regression were used to assess the relationship between generations and resistance. Effects of bacterial species and antibiotic type were analyzed using two-way ANOVA followed by simple effects analysis (A = 0.05). **Results:** A significant positive correlation was observed between generations and MIC increase (r = 0.221; p = 0.005), although generation explained only 5.8% of variability (R^2 = 0.058). No significant main effect of bacterial species was detected (p = 0.103), whereas antibiotic type significantly influenced resistance (p < 0.001; H^2 = 0.167). Following heat and acidification treatments, *Bacillus* sporulation was confirmed by malachite green staining and spore visualization. A significant interaction between species and antibiotic was observed (F(4,122) = 5.278; p < 0.001). Differential susceptibility was most pronounced in *E. coli* ATCC 25922 (p < 0.001), while *Bacillus clausii* (p = 0.409) and *B. velezensis* (p = 0.261) maintained stable MIC profiles. **Conclusions:** Sporulation in *Bacillus* was confirmed and may be associated with stable resistance profiles and *Bacillus* spp. maintained consistent MIC patterns under selective pressure, suggesting low propensity to develop resistance and supporting their safety for poultry production.

**Keywords:** Probiotics; Antibiotic; MIC; Mutation; Poultry

### 307 Comparison of the infectivity of cattle adapted Highly Pathogenic Avian Influenza (HPAI) virus to Low Pathogenic Avian Influenza (LPAI) virus in a food matrix

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The objective was to compare the survivability and infectivity of cattle adapted Highly Pathogenic Avian Influenza (HPAI) virus to Low Pathogenic Avian Influenza (LPAI) virus in a beef slurry food matrix compared with water. HPAI and LPAI were inoculated into beef slurry or water and

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held at a controlled temperature and pH. Samples were collected over time to assess loss of infectivity and persistence of genetic material. Two independent trials were conducted. Virus was added to 100 mL of 10% beef slurry or water at 6.5 log10 50% embryo infectious doses (EID50) per mL. Samples were incubated at 37 C and pH 5.0 and collected at 0, 3, 7, 9, and 15 h. Samples were clarified by centrifugation and tested by qPCR for genetic material. Virus viability was evaluated using 10-day-old specific pathogen free (SPF) embryonated chicken eggs, four replicate sets of titrations per experimental condition. Embryo survival was monitored for six days; chorioallantoic fluid from sampled embryos between two and six days were harvested and tested for hemagglutination activity. Differences between HPAI and LPAI at each timepoint were assessed using Welch's T-test for log10-transformed titers among detected samples, analyzed separately within each slurry condition. At 0 h, HPAI in water showed no reduction, while HPAI in beef slurry showed a significant 4.0–4.3 log reduction (p 0.0048) in infectivity. There was no significant difference for LPAI between slurry and water. From 3 to 15 h, both viruses showed zero detectable infectivity in beef slurry, except for HPAI at 7 h in trial 1. Across both trials, qPCR Ct values averaged 22.2 ± 1.7, indicating consistent presence of genetic material regardless of infectivity loss. A beef slurry matrix rapidly reduces cattle adapted HPAI infectivity immediately upon contact, while LPAI remains unaffected under the same conditions. No infectious pathogen was detected at 9 h or later in beef slurry samples. Both avian-adapted LPAI and bovine-adapted HPAI grow well in embryoned SPF eggs. These viruses grow comparably in eggs when they come out of a water sample. However, bovine-adapted HPAI virus viability is reduced when it comes out of a beef slurry matrix. This may have consequences for passage back into birds or potential risk profiles of food products. We are following up on this study with a comparison of these viruses and an avian-adapted HPAI in a poultry meat matrix to characterize this phenomenon further.

**Keywords:** HPAI; LPAI; influenza; food safety; infectivity

## Animal Well-Being and Behavior

### 206 (E-F) (MTCC)

### 308 White hens are from Venus, Brown hens are from Mars

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Most commercial laying hens are from one of two phylogenetic lineages. Brown-feathered strains of laying hens derived mainly from Rhode Island Reds lay brown eggs, are generally heavier bodied and have poorer feed conversion than their white-feathered counterparts, which were derived mainly from White Leghorns and lay white eggs. Apart from differences in egg characteristics and production traits, brown- and white-feathered hens differ physically and behaviorally, which can affect their housing requirements and how we manage them, especially in non-cage systems. Brown and White hens differ in their body proportions, with White hens having proportionally longer and narrower bodies, larger wings, keels and pectoral muscles and lighter leg muscles than browns. Consequently, White hens have lower wing loading and greater ability for short flights and more controlled landings and differ significantly in their use of 3-dimensional space in aviaries. White pullets in rearing aviaries run more, perch more and perform more aerial transitions (flying or jumping between different vertical tiers) compared to Browns. These load-bearing exercises can affect skeletal development and promote the growth of stronger bones that are less susceptible to breakage. Browns and Whites express fear and stress differently and react distinctly to various stimuli. Brown strains tend to show freezing response fear in some standardised tests, whereas birds from white strains show a greater flight response. White strains also show a higher physiological response (corticosterone) to restraint, although Brown hens often have higher heterophyl:lymphocyte ratios. Strain differences need to be considered when designing experiments, generalising research results from experiments involving only one type of hen, and even setting space, housing or management requirements in animal care guidelines.

### 309 Dopamenergic manipulation affects vocalizations but does not elicit feather pecking behavior in Pekin ducks

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Feather pecking (FP) is an unwanted behavior that damages skin and feathers, which are valuable components of duck commodity. FP in ducks is the act of mutilation to a conspecific's feathers, primarily focused on the wings and tail and is associated with altered brain dopamine (DA) and serotonin (5HT) activity in chickens. The cause and motivation for FP in Pekin ducks is unknown. DA agonists have also been shown to alter vocalizations in chickens. Thus, we set out to elicit these behaviors with the use of pharmacologically manipulating DA signaling using receptor-specific agonists. We hypothesized that ducks given a DA agonist would perform FP behaviors and alter their vocalizations. We utilized 300, 13-day-old Pekin ducks obtained from a local producer and placed them into 8 pens in one room at Purdue's research farm, with 2 pens/treatment randomly placed throughout the room (~35 ducks/pen). The experiment started when the ducks were 20 days of age and continued until they were 25 days of age (days 1-5). The treatments and controls were: D1 (SKF-38393, 0.1mg/kg/mL), D2 (Quinpirole, 0.1mg/kg/mL), D2/3 (Pramipexole Dihydrochloride tablets, 0.25mg), and control (CON; saline, 0.1mL/kg; sham oral), n=12/treatment and were given every day for 5 days. Body condition scores (BCS) were taken on days 1, 3, and 5 of the experiment on all ducks in each pen. Ducks who received the treatment were placed into an anechoic chamber in pairs from their respective pens on days 1, 3, and 5. They were recorded for 3 mins and the latency to vocalize once the chamber door was shut and the total number of vocalizations was recorded. BCS were analyzed using the PROC LOGISTIC procedure (SAS v9.4) and vocalization data were analyzed using a repeated measures mixed model (PROC MIXED, SAS v9.4) and Tukey's test for post-hoc analyses. P≤0.05 considered significant. No significant differences were observed for BCS. Number of vocalizations was significantly influenced by treatment (p=0.0006), where the D2 ducks vocalized significantly less (35.28±6.23) than the CON ducks (207.33±16.39, p=0.006) and the D1 ducks (199.78±21.5, p=0.0018). The D2/3 ducks vocalized significantly less (113.83±9.51) than the CON ducks (p=0.0164) and the D1 ducks (p=0.0446). Latency to vocalize was significantly influenced by day (p=0.0022), where the ducks took longer to vocalize on day 1 (2.07±0.84) compared to day 3 (0.62±0.17, p=0.0068) and day 5 (0.54±0.12, p=0.0057). The results show that though the DA treatments did not induce FP, they did affect the ducks' vocalizations, suggesting that the DA agonists may act on DA receptors in different parts of the brain, such as the tectum versus the limbic system, in Pekin ducks.

**Keywords:** vocalizations; Pekin ducks; dopamine receptor agonists; feather pecking

### 310 Effect of replacing string-tied straw enrichment bales with plastic wrapped bales on broiler behaviour and welfare

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Meeting legislative and consumer expectations for high broiler welfare standards while maintaining strict biosecurity requires an integrated management approach. Enrichment bales enhance broiler welfare by stimulating exploratory and comfort behaviours and increasing activity levels but traditional straw bales can pose higher bio-security risks.

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Wrapped bales improve hygiene but equivalance of their enrichment value is not known. This study investigated how slower growing broiler chickens interacted with two types of enrichment bales. Trials were conducted at Nottingham Trent University following favourable ethical approval. Redbro broilers (n=600) were allocated as hatched to four pens (4.8m x 2.8m, 150 per pen) and reared to d42. Birds were fed *ad libitum* and each pen was provided with 2 enrichment bales, either processed wrapped straw with the packaging slit to allow birds to straw access (PROC) or traditional string tied straw (TRAD). Bale interaction was determined by scan sampling twice a day (5 minutes am and 5 minutes pm) per pen (d1-42,). Each minute an observer noted the number of birds interacting with the bales in the following ways: pecking at bales, pulling at straw, carrying straw or running with straw. The number of birds perching on the 2 bales within each pen was also recorded. Ten birds from each pen were selected at random and weighed every week. Litter was scored on d42 on a 10-point scoring system. Statistical analysis was carried out using SPSS v.30. Data was analysed using a one-way ANOVA to compare litter scores between bale types and also for weekly weights (g). The total number of birds observed interacting with bales during the 5-minutes (meaned from am and pm) was compared using a 2-Way ANOVA with bale type and bird age as factors. Bird growth and litter quality were not impacted by bale type. Broilers interacted with both bale types, with no differences in number of pecks (11.3 PROC, 10.1 TRAD, SEM 0.53, p = 0.122) occasions of pulling straw , (1.8 PROC, 2.1 TRAD, SEM 0.28, p = 0.446) straw carrying occassions (1.64 PROC, 1.72 TRAD, SEM 0.207, p = 0.786) or occassions running with straw (0.02 PROC, 0.09 TRAD, SEM 0.05, p = 0.373) with the 5 minute observation period for each pen. Perching behaviour (22 birds on 2 bales in 5 minutes for PROC, 36.6 birds on 2 bales in 5 minutes for TRAD, SEM 0.97, p < 0.001) occurred more on the traditional bales, though interaction effects (p < 0.001) show more perching on processed bales for younger birds. Overall, the study suggests wrapped processed bales designed for improved biosecurity could deliver the same welfare benefits as traditional string-tied bales.

**Keywords:** Broiler; enrichment; straw; bales; behaviour

### 311 A comparison of nest use in four cage-free system designs for laying hens

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Cage free housing systems for egg production provides increased space and supports expression of natural hen behaviors with enrichments. Since few eggs are laid in nests late in the day, we were interested in nest occupancy during this period and effects of nest design. The objective of this study was to examine nest use in commercial cage-free egg housing systems. Two cage-free systems with two nest designs were compared: Fienhage with (FK) and without (FO) kickout nest function, and Big Dutchman Natura with (DK) and without (DO) kickout function. Dekalb White 16-week old pullets were placed in each of the Fienhage (n=1,000 hens/room) and Dutchman Natura systems (2,160 hens/room) in accordance with certified cage-free guidelines space allowances. Direct behavior observations were performed twice weekly during select weeks from placement through 69 weeks of age at four time points (TP), 0800, 1100, 1730, 2130, and after lights out at 60 weeks of age. The ethogram included nest occupancy (number of hens) in each nest location and hen postures (standing or sitting). We hypothesized that nest use in TP3 and TP4: 1) would be rare versus TP1 and TP2, 2) would be less frequent in kickout nest vesus open nest designs, and 3) hens would not use nests for roosting at night. Data were analyzed using a generalized linear mixed model. Number of hens in the nests was converted to percentage of flock (PoF) due to hen population differences between systems. The PoF was square root transformed to achieve normality and analysed as a mixed model with TP * week * system, and system as subject. In D systems, PoF was greatest at TP1 than other TP ($p<0.01$), whereas TP1 differed only from TP3 and TP4 in F systems ($p<0.03$). PoF was greater in D than F system at TP1 ($p<0.01$), but this effect reversed in TP2 and TP3 ($p<0.01$). At peak lay, standing occurred more frequently in FO than FK nests ($p<0.01$), but DO and DK did not differ ($p<0.07$). Nest use during the dark period was extremely rare (PoF range 0.2 to 1.2). In conclusion, this data supports the hypothesis that nest use outside the typical laying period is rare for DO, DK and FK systems. However, hens used FO nests throughout the day, likely due to differences in nest design. Analysis of floor and system eggs is pending and may inform impacts of non-nesting hens on settled nesting behavior in the early egg laying stage of production.

**Keywords:** nest design; environmental enrichment; animal welfare; housing systems; egg laying hens

### 312 From worms to welfare: Can inducing play behavior give us a window into chick welfare?

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Incubation conditions can shape broiler behavior, health, and production outcomes. This study evaluated whether incubation stress influenced play behavior, specifically worm-running, and whether this reflects broader activity patterns. Two hatches (H1, H2) of broilers from the same parent stock were compared. Embryos from H1 experienced cold stress during early incubation, resulting in delayed hatch and reduced hatchability. Hatchability, chick quality, early growth, and activity levels (before and during worm-running tests) were measured. Unhatched eggs were broken out to determine the fertility and developmental stage of embryo mortality. Chicks were weighed and evaluated for quality at hatch, and individual body weights were collected on D7 and D14. H1 consisted of 12 pens with 15 broilers per pen; H2 consisted of 24 pens with 15–17 broilers per pen. Worm-running tests were conducted on D6 and D13 by placing tissue paper "worms" in each pen for one hour, with video recording during the test and the preceding hour as a control. Activity was quantified using frame-by-frame pixel change in EthoVision XT17, averaged per minute and normalized for pen weight. Data were analyzed in R using generalized linear and additive mixed models using hatch as a fixed factor for all models, interacting with day and worm treatment as added factors for activity data. For proportion data, odds ratio (OR) used H1 as the reference group. Activity variability is expressed as effective degrees of freedom (edf), where edf=1 describes a linear pattern and edf>1 indicates increasing variability. Hatchability of H1 was markedly lower than H2 (21.1 % vs 56.4 %). Early embryo mortality was more likely in H1 (OR=3.90, $p<0.001$), but H1 chicks were less likely to have unhealed navels (OR=0.42, $p<0.001$) and red hocks (OR=0.43, $p<0.001$). Body weight was lower in H1 than H2 for D0 (44 vs 49 g, $p<0.001$), D7 (129 vs 138 g, $p<0.001$), and D14 (346 vs 364 g, $p<0.001$). Activity was higher in H1 during the testing periods on D6 (0.045 %) and D13 (0.044 %) than H2 (0.032 and 0.026 %, $p<0.001$). Generally, activity was higher on D6 than D13 (0.034 vs 0.032 %, $p=0.005$) and in H1 than H2 (0.038 vs 0.028 %, $p=0.03$). Interestingly, there was only increased activity in the test compared with control periods in H1 (0.045 vs 0.032 %, $p<0.05$), but not in H2 (0.027 vs 0.029 % $p>0.05$). Activity was more variable during the test compared with their respective control periods (edf=1.83-3.77 vs 5.89-7.83). Despite cold stress during incubation resulting in poor hatchability, H1 chicks had better hatch quality scores and were more active than H2 chicks during worm-running tests.

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**Keywords:** Broiler; Welfare; Activity; Worm-running; Behavior

## Metabolism and Nutrition: Enzymes I

### 201 (A-F) (MTCC)

### 313 Pushing the Limits of Carbohydrase: Do Fiber and XOS Deliver?

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Extensive research has enhanced phytase and carbohydrase efficiency in poultry nutrition. Super-dosing phytase further enhances phosphorus digestibility, reducing the levels of inorganic P supplementation. Despite these advances, optimizing xylanase (or carbohydrase) use in broiler diets remains a key gap. Xylanase is widely adopted in wheat-based diets with well-established efficacy, whereas responses in corn-based diets in variable and less consistent. To address this variability, recent research has explored strategies to enhance enzyme efficacy, including the use of enzyme combinations and the incorporation of higher-fiber feed ingredients in broiler diets. This presentation focuses on the combined use of carbohydrase enzymes with functional feed additives, such as xylo-oligosaccharides (XOS), alongside the inclusion of high-fiber feedstuffs, and their collective impact in broilers. Current findings indicate that supplementing xylanase in combination with XOS does not consistently yield additional improvements in performance or digestibility compared to xylanase alone. However, when diets are enriched with non-starch polysaccharide rich ingredients such as wheat bran or soybean hulls, a synergistic effect may emerge. This is likely due to enhanced proliferation of fiber-fermenting microbiota, supported by increased substrate availability. The resulting fermentation activity promotes the production of short-chain fatty acids, which are associated with improved gut health. These benefits appear to be more pronounced under enteric challenge conditions, such as coccidia infections. However, responses remain inconsistent, as elevated dietary fiber levels under challenged conditions may exacerbate gut health issues, including increased susceptibility to necrotic enteritis. This creates practical challenges in commercial settings, where dietary adjustments cannot be easily implemented once feeding programs are established. Another strategy to enhance enzyme efficacy in high-fiber diets is the reduction of particle size in fibrous feed ingredients. Finer particle size improves enzyme accessibility and facilitates substrate fermentation in the ceca, thereby enhancing the availability of nutrients for beneficial microbial populations. In conclusion, while the integration of carbohydrase enzymes and fiber-rich ingredients presents a compelling approach to improving broiler gut health and nutrient utilization, further research is required to define optimal inclusion levels that do not compromise performance. A key priority is developing advanced analytical methods to better characterize fiber composition and functionality in feed ingredients, enabling more precise and cost-effective diet formulation.

### 314 Effect of B-mannanase application on the growth performance and immune response of turkeys

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Beta-mannans, a type of non-starch polysaccharides (NSP) found in the fiber fractions of soybean meal and grains, cannot be hydrolysed by endogenous enzymes, resulting in reduced nutrient digestibility and performance. Additionally, mannans can elicit localized or systemic immune response in birds, resulting in a negative impact on growth performance. Supplementation of mannanase can reduce these negative effects through several mechanism. This study evaluated the effects of a B-mannanase (Natupulse® TS) on the immune response and growth performance of turkeys under two dosing regimens. In this experiment, 780 one-day-old male turkeys Hybrid Converter from a commercial hatchery were divided into 3 treatment groups with 20 replications of 13 birds each. Birds were reared in a 5-phase feeding system over a period of 20 weeks. All birds received the same wheat-corn-soy based basal diet, containing phytase at 1500 FTU/kg and a xylanase/glucanase blend at 100 g/t of feed. Treatment (T) 1 did not receive an additional mannanase, T2 received 100 g/t mannanase in all phases and T3 received 200, 200, 150, 150, 100 g/t for phase 1-5, respectively. Feed intake (FI) and body weight (BW) were recorded on 4, 8, 12, 16 and 20 weeks for BW gain (BWG) and feed conversion ratio (FCR) calculations. At 4 weeks, blood sample was collected from one bird per pen for analysis of antioxidative and inflammatory status. No significant effect on performance was observed up to 8 weeks. However, FCR was significantly better (lower) at 12 weeks (P=0.004) and overall phase (*P*=0.001), in the mannanase groups (T2 and T3) in comparison to T1. At 4 weeks, significant differences in blood cytokines and antioxidant markers were observed among treatments. IL-10 concentration was higher in T3 than in T1 and T2 (*P*=0.013), IL-1b concentration was lower in T2 and T3 than in T1 (*P*=0.039), but IL-6 concentration was lower only in T3 compared to T1 (*P*=0.027). Significant increase in Catalase (*P*<0.001), and Superoxide dismutase concentration (P=0.001) was observed in T3 in comparison to T1 and T2. Total antioxidant status in the blood was significantly higher in T2 and T3 in comparison to T1 (*P*=0.001). In conclusion, this study showed that both single (T2) and stepwise (T3) B-mannanase supplementation improved the oxidative and inflammatory status of the birds. This improvement may have resulted in the improved FCR observed in the mannanase groups (T2 and T3).

**Keywords:** beta-mannan; immune response; enzymes; performance; turkey

### 315 In-feed supplementation of a new mannanase improves turkey growth and improves jejunal barrier integrity at day 49 through enhancement of stress- and immune-response

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The objective of the present study was to assess the effect of beta-mannanase and delineate its mode of action on growth performance and gut integrity of young 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 49 days with *ad libitum* access to diet and fresh water. Body weight (BW), BW gain (BWG), feed intake (FI), feed conversion ratio (FCR), and intestinal permeability using fluorescein isothiocyanate-dextran (FITC-d) were measured on days 49. 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 $P < 0.05$. Dietary supplementation of NPU increased BWG (*P*=0.028) and FI (*P*=0.061), but not FCR (*P*=0.550). NPU significantly reduced serum FITC levels (*P*<0.05), indicating improvement of gut integrity. The jejunal expression of HSP70 protein was significantly increased by NPU compared to the control diet. Jejunal expression levels of IL8, *Tjp1*, and *CLDN5* were significantly decreased ($P < 0.05$), whereas *ZO-1* expression was significantly increased ($P < 0.05$) in the NPU group relative to the control group. The jejunal expression of the adherens junction AFDN1 decreased (*P*<0.05) by NPU sup-

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plementation compared to the control diet. In summary, this study showed that dietary NPU supplementation can enhance growth performance by improving gut integrity and ameliorating stress- and immune-response.

**Keywords:** Turkey; mannanase; growth; stress markers; inflammation

### 316 Feeding the microbiome: xylanase supplementation in early life broilers

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Broilers have little time for development of intestinal functioning, which includes digestion and absorption, as well as building a robust intestinal microbiome. Non-starch polysaccharide degrading enzymes, e.g. xylanases, have been originally used for performance gains. Targeting application to the starter period may offer economic advantages by driving microbiome development. In this study, a commercial nutrient adequate (2850kcals) wheat-soy based diet (Control) was compared to the same diet with three xylanase levels; 2000, 4000, and 8000 U/kg (Danisco® Xylanase) with a 100kcal reduction, fed *ad lib*. Ross 308 male broilers were placed in 32 pens divided over 8 houses (34 birds/pen, 4 pens/house). In each house, treatments were randomly assigned to pens. Body weight (BW) and feed intake were determined on d 11 and mortalities recorded. On d 4, 8 and 11, 2 birds/pen were sacrificed and cecal content collected for 16S community analysis using Illumina 16S rRNA V4 amplicon sequencing to evaluate overall xylanase effect on microbial community. On d 11 microbiomes were evaluated using targeted quantitative-PCR for *Escherichia coli* (*E. coli*), *Candidatus arthromitus* and *Blautia spp*. Performance data was analyzed using JMP (v. 16) with treatment as a fixed effect and house as random effect. Kruskal-wallis was used for Q-PCR data. Differences in microbial composition were analyzed by Shannon A-Diversity, PERMANOVA and differentially abundant taxa analysis (DeSeq2). On d 11, BW significantly increased between the Control and 8000U (P = 0.016, 300g and 323g, respectively) with the 2000U and 4000U being intermediate (306g and 309g, respectively). Feed intake did not differ. FCR was improved between the Control and 8000U (P = 0.042, 1.19 vs 1.11, respectively), with the 2000U and 4000U being intermediate. There were no significant differences in microbiome A-diversity, however, a significant increase was seen as early as d 4 with *Mediterraneibacter spp.* (8.67 to 14.8% abundance; P=0.01) in xylanase groups and sequential significant decreases in *Enterococcus spp.* at d8 (3.78 to 1.85%, P=0.02). *Mediterraneibacter* is a member of the *Lachnospiraceae* family and produces short-chain fatty acids. *Enterococcus spp* (and *E. coli*) account for 50% of early mortalities. On d 11 reductions in *E. coli* (0.40% in control and 0.24% in 8000U treatment, P = 0.21) and numerical increases in *Blautia* and *C. arthromitus* populations were observed (2.7 vs 3.13 Log10 CFU copy number in Control and 8000U respectively for *C. arthromitus*). Both populations are known to drive gut development. These outcomes demonstrate performance gains and beneficial microbiome developments resulting from early xylanase supplementation.

**Keywords:** Xylanase; feed efficiency; broilers; microbiome; intestinal development

### 317 The effect of xylanase supplementation on performance, digesta viscosity, carcass traits, and immune organ of broiler fed wheat based diets

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Arabinoxylan, one of the major non-starch polysaccharides (NSPs) in plant ingredients, is a major component of the plant cell walls that encapsulates intracellular nutrients, acting as an antinutritional factor, through increasing viscosity of the intestinal digesta, hindering digestion and absorption of nutrients, and altering gut microbial community. Xylanase is commonly used in feeds to hydrolyze xylan and mitigate its antinutritional effects. One of important considerations in the xylanase application is its recovery rate in pelleted feeds and its effectiveness in improving growth performance and gut health. The objective of this study was to evaluate the effect of an exogenous xylanase preparaton on growth, digestibility of nutrients and gut health of broiler fed on wheat and soybean meal based diet. A total of 320 male Cobb 500 day-old chicks were randomly divided into two treatments, with 8 pen replicates per treatment and 20 chicks per pen. The basal diets contained wheat by 45%, 45%, 30% and 40% during starter, grower, finisher 1, and finisher 2 phases, respectively. The control (CON) diets received no supplemental xylanases whilst the xylanase group was supplemented with an exogenous xylanase (40,000 U/g) at 200 g/mt feed. The starter diet was provided in crumble form, while the grower and finisher diets were pelleted at a conditioning temperature of 80-85 °C. The birds had free access to water and feed throughout the trial. The data were analyzed using one-way ANOVA, and A level of $P \leq 0.05$ was used as the criterion for statistical significance. The recovery rate of xylanase was determined to be 63% after feed pelleting at 80-85 °C. The supplementation of the xylanase did not significantly affect weight gain (WG), feed intake (FI) and FCR ($P>0.05$), but significantly decreased mortality from 6.88% to 2.50% ($P<0.05$), and numerically increased Europen Production Index (EPI) from 565.8 to 586.6 ($P>0.05$). No changes were observed on carcass yield, immune organ index and gastrointestinal pH ($P>0.05$). However, ileal digesta viscosity was significantly reduced by the addition of the xylanase ($P<0.05$). Other parameters, including digestibility and gut morphology are still under analyses. In conclusion, supplementation of the exogenous xylanase in wheat-based diets did not affect growth performance or organ indices, but markedly reduced mortality, improved EPI and reduced ileal digesta viscosity, suggesting the enzyme may have improved digesta flow and gut health.

**Keywords:** xylanase; wheat-based diet; broiler; growth; digesta viscosity

## Monday, July 13, 2026

### Metabolism and Nutrition: Enzymes II

**201 (A-F) (MTCC)**

### 318 Bacterial xylanase supplementation improves early feed efficiency, intestinal integrity, nutrient digestibility, and cecal microbiota in broilers fed reduced-energy diets

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Corn–wheat-based broiler diets contain substantial levels of non-starch polysaccharides, which can impair nutrient utilization, particularly when dietary energy is reduced as a cost-saving strategy. This study evaluated the effects of bacterial xylanase supplementation on growth performance, nutrient digestibility, intestinal permeability, and cecal microbiota composition in broilers fed reduced-energy diets. Two trials were conducted. In Trial 1, a total of 1,050 broilers were assigned to 3 treatments: a basal control diet (CON), a negative control with reduced metabolizable energy (−85 kcal/kg; NC), and the NC diet supplemented with bacterial

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xylanase (100 g/t; NCX). Birds were fed from 0 to 35 days of age, and growth performance was measured across starter (0–10 d), grower (11–28 d), and finisher (29–35 d) phases. On d 35, birds were orally gavaged with fluorescein isothiocyanate–dextran (FITC-d) and blood serum and cecal contents were collected to assess intestinal permeability (serum FITC-d) and microbiota composition, respectively. In Trial 2, a total of 192 broilers, housed in metabolic cages, under the same treatments as trial 1, were used to determine ileal nutrient digestibility and apparent metabolizable energy (AME) using titanium dioxide as an indigestible marker. Growth performance and nutrient digestibility were analyzed by ANOVA. Microbiota diversity was assessed using Kruskal–Wallis and PERMANOVA tests. Statistical significance was set at $P < 0.05$. At the starter phase, broilers fed the NC diet had higher ($P < 0.05$) feed intake (329 vs 323 g/d) and poorer ($P < 0.05$) feed conversion ratio (FCR; 1.12 vs 1.10) compared with NCX diets, whereas xylanase improved ($P < 0.05$) FCR similar to the CON fed birds. The NCX-fed broilers had lower ($P < 0.01$) serum FITC-d concentrations compared with NC birds (0.38 vs 0.41 mg/mL) indicating improved intestinal barrier integrity. Cecal microbiota analysis revealed increased ($P < 0.05$) relative abundance of *Bifidobacterium pseudolongum* and *Dietzia aerofata* and reduced ($P < 0.05$) abundance of *Corynebacterium simulans* in NCX-fed birds compared to the NC and CON birds indicating a beneficial microflora population. Xylanase supplementation increased ($P < 0.05$) ileal digestibility of neutral detergent fiber, acid detergent fiber, and AME by 62.4, 71.8 and 2.31%, respectively compared to NC-fed birds. In conclusion, bacterial xylanase supplementation in reduced-energy broiler diets enhanced early feed efficiency, improved fiber utilization and energy availability, strengthened intestinal integrity, and promoted a beneficial cecal microbiota, supporting its role as an effective nutritional strategy to mitigate the negative effects of dietary energy reduction.

**Keywords:** bacterial xylanase; broilers; cecal microbiota; intestinal integrity; reduced-energy

### 319 Bio-optimized xylanase improves feed efficiency in broilers fed energy-reduced corn–soybean meal diets

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This study evaluated the efficacy of a bio-optimized (BO) and wild type (WT) xylanase supplementation in broiler chickens fed corn–soybean meal (SBM) diets with reduced dietary energy. Both enzymes were included at 100 g/MT in diets formulated with a 130 kcal/kg reduction in apparent metabolizable energy (AME). A total of 432 one-day-old male Ven-Cobb 430Y broilers were randomly assigned to 4 dietary treatments with 9 replicates per treatment and 12 birds per replicate over a 42-day trial. The treatments consisted of a positive control diet (PC), a negative control diet with 130 kcal/kg less AME than the PC (NC), NC supplemented with BO xylanase, and NC supplemented with WT xylanase. Birds were fed using a 3-phase feeding program, and no antibiotic growth promoters were included. Growth performance and intestinal histomorphometry (villus height, crypt depth, and villus:crypt ratio) were evaluated. Data were analyzed by one-way ANOVA, and means were separated using Tukey's test. Results showed that from 0-42 d, there were no differences in feed intake or mortality by treatment. Reducing dietary energy increased feed conversion ratio (FCR) by 5 points relative to the PC ($P = 0.02$). Supplementation with BO xylanase fully recovered the lost FCR, whereas WT xylanase recovered only 2 points. FCR values were 1.457, 1.510, 1.461, and 1.486 for PC, NC, NC + BO xylanase, and NC + WT xylanase, respectively. European production index tended to improve with BO xylanase supplementation compared with the NC treatment ($P = 0.07$), whereas the WT xylanase showed an intermediate response. Caloric efficiency was improved by 145 kcal/kg gain with BO xylanase supplementation ($P = 0.01$), while the WT xylanase improved caloric efficiency by 70 kcal/kg gain. No significant differences were observed in duodenal histomorphometry. In the jejunum, crypt depth was lower in birds fed the BO xylanase diet than in birds fed the PC or WT xylanase diets ($P = 0.01$). In the ileum, villus height tended to be greater in birds supplemented with BO xylanase than in birds fed the PC or WT xylanase diets ($P = 0.09$), with the other treatments showing intermediate values. These findings demonstrated that reducing dietary AME by 130 kcal/kg impaired broiler performance in corn–SBM diets, whereas xylanase supplementation mitigated these negative effects. The BO xylanase was more effective than the WT xylanase in improving feed efficiency and caloric utilization.

**Keywords:** Broiler; bio-optimized xylanase; energy reduction; caloric efficiency; histomorphometry

### 320 Selective effects of a protease treatment on trypsin inhibitor activity and inhibitor fractions in soybean meal.

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Trypsin inhibitors (TI) in soybean meal (SBM), mainly Kunitz trypsin inhibitor (KTI) and Bowman–Birk inhibitor (BBI), are major antinutritional factors that limit protein digestibility and animal performance. This study evaluated the effects of protease treatment on total trypsin inhibitor activity (TIA) and on the degradation of the principal TI fractions in SBM with differing baseline TI levels. SBM samples characterized by low (40.3 TIA/mg of sample), medium (43.8 TIA/mg of sample), and high (61.1 TIA/mg of sample) TI levels were evaluated with and without protease addition under *in vitro* conditions. The enzymatic reactions were conducted at 37 °C, pH 7.0, for 120 min, using enzyme-to-substrate ratios adjusted to simulate practical feed inclusion levels. Protein concentration was determined by the Lowry method to standardize sample loading. Protein fractionation was performed by SDS-PAGE under reducing conditions, followed by semi-quantitative densitometric analysis of bands corresponding to approximately 30 kDa (KTI) and 8–10 kDa (BBI). TIA was quantified according to the AOCS Ba 12-75 method. Data were analyzed by one-way ANOVA followed by Tukey's test, considering $P < 0.05$ as statistically significant. Protease treatment significantly reduced TIA across all soybean meal samples, regardless of the initial level of trypsin inhibitors ($P < 0.05$). Mean TIA values (expressed as mg trypsin inhibited per g of sample) decreased from [40.3 ± 4.7], [43.8 ± 3.4], and [61.1 ± 1.9] in low-, medium-, and high-TI SBM, respectively, to [31.2 ± 2.3], [27.4 ± 1.9], and [37.1 ± 0.9] following protease treatment, corresponding to relative reductions of [23–39]% across samples. Densitometric analysis of SDS-PAGE gels revealed a marked reduction in the intensity of the ~8–10 kDa bands associated with BBI after protease application in medium-TI (treated vs. control: [49%] decrease; $P < 0.05$) and high-TI (treated vs. control: [50%] decrease; $P < 0.05$). In contrast, the ~30 kDa bands corresponding to KTI showed no statistically significant change in band intensity between treated and untreated samples ($P > 0.05$). Despite the persistence of KTI, the selective degradation of BBI was sufficient to promote a statistically significant reduction in overall TIA. The significant reduction in TIA demonstrates that partial and selective proteolysis of trypsin inhibitors can meaningfully improve the nutritional quality of SBM. These findings demonstrate that the tested protease effectively mitigated the antinutritional effects of trypsin inhibitors in monogastric animal diets. However, because enzyme characteristics vary, similar responses may not be observed with other proteases.

**Keywords:** Protease; Trypsin Inhibitors; Soybean Meal; Bowman–Birk ; Kunitz

### 321 Multi-component protease mitigates the negative impacts of under- and over-processed soybean meal on growth performance

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**and carcass characteristics in broilers**

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Soybean meal (SBM) is the primary plant protein source in poultry nutrition, but its nutritional value varies widely depending on thermal processing. Under-processing leaves anti-nutritional factors that impair digestibility, while over-processing reduces amino acid availability. This study evaluated whether multi-component protease (PRO) supplementation could mitigate the negative effects of varying SBM processing quality on broiler growth performance and carcass characteristics. A total of 960 one-day-old male Ross 308 broiler chicks were randomly assigned to 6 dietary treatments in a completely randomized design with 10 replicate pens per treatment and 16 birds per pen. Treatments included: Control (standard SBM), NCU (under-processed SBM), NCO (over-processed SBM), and each supplemented with PRO (Control+PRO, NCU+PRO, NCO+PRO) at 125 g/t. SBM quality was characterized by potassium hydroxide protein solubility: under-processed (93-95%), standard (81-84%), and over-processed (63-67%). Birds had ad libitum access to feed and water for 41 days. Corn-SBM diets were formulated according to Ross 308 recommendations and fed in three phases (starter: d 0-10, grower: d 11-21, finisher: d 22-41) in mash form. All diets contained phytase (1,000 FTU/kg) and xylanase (100 g/t). Body weight and feed intake were recorded on d 0, 10, 21, and 41. On d 41, three birds per replicate were evaluated for carcass yield. Data were analyzed using ANOVA with Tukey's HSD test; significance was set at P<0.05. At d 41, NCU and NCO decreased FI and BWG, and increased FCR, compared with the Control (P<0.0001). PRO supplementation increased FI and BWG and reduced FCR in NCU+PRO and NCO+PRO compared with their respective un-supplemented treatments (P<0.0001). In addition, Control+PRO increased FI and BWG and reduced FCR compared with the Control (P<0.0001). Carcass yield was reduced in NCU and NCO compared with the Control (P<0.0001), while PRO supplementation increased carcass yield in NCU+PRO and NCO+PRO relative to their respective controls (P<0.0001). Breast yield was also reduced in NCU and NCO (P<0.0001), with numerical increases observed in PRO supplemented groups (P>0.05). No differences were detected between Control and Control+PRO for carcass or breast yield (P>0.05). In conclusion, both under- and over-processed SBM significantly impaired broiler performance and carcass characteristics. PRO supplementation effectively mitigated these negative impacts across all SBM processing qualities and provided additional growth performance benefits even with adequately processed SBM.

**Keywords:** Protease; Soybean meal; Performance; Carcass yield; broilers

**322 Effects of increasing dietary non-phytate phosphorus levels and phytase supplementation on performance, and apparent ileal nutrient digestibility in broiler chickens**

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The objective of this study was to evaluate the effects of increasing dietary levels of non-phytate phosphorus (nPP) and phytase supplementation on growth performance and nutrient digestibility in 23-day old broiler chickens. A total of 392 one-day-old Cobb byproduct male broiler chicks were randomly assigned to cages in a randomized complete block design, with 7 replicate cages per treatment and 7 birds per cage. With the location in the room as the blocking factor, birds were allotted to 8 treatments in a 4 × 2 factorial arrangement, consisting of 4 diets containing 0, 1.4, 2.4, and 4 g/kg nPP from dicalcium phosphate and 2 phytase levels (0 vs 1,500 FTU/kg). Birds received a nutritionally adequate corn-soybean meal-based starter diet from d 0 to 9, while the experimental diets were fed from d 9 to 23. Birds and feed were weighed on d 0, 9, 14, and 23. On d 23, ileal digesta from the distal two-thirds of the ileum were collected to determine AME, AMEn, and apparent ileal digestibility of DM, N, Ca, P, and energy. The IML procedure was used to generate coefficients for orthogonal polynomial contrasts, which were applied to evaluate linear and quadratic responses to increasing levels of nPP in the diets. Data were analyzed as a 4 × 2 factorial using the MIXED procedure of SAS. Statistical significance was set at $P < 0.05$. Increasing dietary levels of nPP quadratically increased ($P < 0.05$) ADG and ADFI, and decreased FCR from d 9 to 23. Phytase supplementation increased ($P < 0.01$) ADG and ADFI and decreased FCR from d 9 to 23. Additionally, phytase supplementation increased ($P < 0.05$) DM and energy digestibility. A significant diet × phytase interaction was observed for N ($P = 0.0099$) and P ($P = 0.0034$) digestibility, indicating that the response to phytase depended on dietary nPP level. Specifically, phytase supplementation increases P digestibility by 34.9% and 28.9% in the 0 and 1.4 g/kg nPP diets, respectively. Calcium digestibility quadratically decreased ($P < 0.05$) with increasing nPP levels. No significant treatment effects were observed for AME or AMEn. These findings indicate that increasing dietary levels of nPP and phytase supplementation influenced broiler performance and nutrient digestibility. Higher nPP levels reduced Ca digestibility, which peaked at 1.4 g/kg of nPP without phytase supplementation. Under phytase supplementation, Ca digestibility peaked at 0 g/kg nPP. Phytase supplementation can effectively reduce the use of inorganic P to support growth performance and potentially reduce mineral excretion in low nPP diets.

**Keywords:** broiler; growth performance; non-phytate phosphorus; nutrient digestibility; phytase

**323 The effects of copper source and dose on ileal Ca and P digestibility and phytase enzyme efficacy in broilers**

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The inclusion of copper at therapeutic concentrations (125-250 ppm) as an antimicrobial and growth promotor in broiler diets has the potential to reduce phytase enzyme efficacy due to the formation of stable Cu-phytate complexes. The objective of the study was to determine if the magnitude of this antagonism was dependent on Cu concentration, Cu source, and dose of phytase. The trial utilized a 3 × 4 factorial incorporating three Cu treatments (trt), copper sulfate (CuS) at 10 and 200 ppm and 200 ppm from monovalent copper (Cu2O) and four dose levels of *E.coli* 6-phytase expressed in *Komatagaella phaffii* (0, 500, 1,000, and 2000 FTU/kg) per Cu trt. Ross 308 male broilers were reared in floor pens to 18 d and moved to battery cages. The 12 corn/soy trt diets containing Ti (5g/kg) as an indigestible marker were pelleted and fed for 36 hours to 6 replicate battery cages per trt (6 birds/replicate). Broilers were euthanized at 21 d and ileal digesta flushed from Merkels diverticulum to 3cm distal of the ilea-cecal junction. Digesta was freeze dried, analyzed, and apparent ileal digestibility (AID) of Ca and P calculated. Data were analyzed using JMP Pro 17.0 (SAS, Institute, Cary, NC) using a fit model platform to evaluate significance of main effects and interactions. Means were separated using a protected Tukey HSD test at $P<0.05$. Significant main effects of Cu trt and phytase ($P<0.001$) were observed for AID of Ca and P with a significant interaction of Cu trt and phytase on AID P ($P=0.015$). AID of Ca was lower ($P<0.05$) when diets contained 200 ppm CuS *vs*. diets containing 10 ppm CuS (56.19%) or 200 ppm Cu2O (58.27%). Increasing phytase dose from 0 to 500 and 1000 FTU increased AID Ca from 50.59% to 54.55% and 57.01%, respectively ($P<0.05$) with no further improvement (58.44%) at 2000 FTU phytase. There were no differences in AID P between Cu trt when diets contained no phytase. Increasing CuS from 10 ppm to 200 ppm reduced AID P ($P<0.05$) when phytase was in-

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cluded at 500 FTU (61.88% *vs*. 54.22%), 1000 FTU (66.09% *vs*. 60.01%), and 2000 FTU (75.65% *vs*. 61.80%), respectively. The AID P of trt containing 200 ppm Cu2O and 500, 1000, and 2000 FTU phytase was 62.76%, 65.03%, and 75.17%, respectively. At each phytase dose the AID P of trt with 200 PPM Cu2O was not different to trt containing 10 ppm CuS and was significantly higher than trt containing 200 ppm CuS ($P$<0.05). Collectively, these results support a mechanism that higher doses (200 ppm) of soluble Cu sources such as CuS promote Cu-phytate complex formation, with significant consequences of reduced exogenous phytase enzyme efficacy. In contrast, application of therapeutic doses of less soluble Cu sources such as Cu2O mitigated the Cu-phytase antagonism.

**Keywords:** Broiler; Copper; Phytase; Phosphorus digestibility; calcium digestibility

### 324 Total replacement of added inorganic phosphate by a consensus bacterial 6-phytase in pullet from day 1 till 18 weeks of age

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Recent studies showed that a consensus bacterial 6-phytase (PhyG) phase-dosing application totally replaced added inorganic P in broilers from day 1. As part of a current long-term layer nutrition trial (to 100 weeks of age), this study tested the hypothesis that with similar phytase dosing and feed formulation strategy, the PhyG can also maintain performance in diet without added inorganic P in pullets from 0 to 18 weeks of age to improve production economics and sustainability. One-day-old (Lohmann LSL-Lite) were distributed in a completely randomized design, to 2 dietary treatments with 8 replicates of 22 birds each. Treatments were: Positive Control (PC) = nutritionally adequate starter (from 0 to 4 wk), grower (from 4 to 8 wk), developer (from 8 to 16 wk) and pre-lay (from 16 to 18 wk) diets; IPF = PC with reduction of Ca, digestible AA and ME based on the phytase matrix and with 1,000 FTU phytase/kg feed (Axtra Phy GOLD), without out added inorganic P, and supplemented with PhyG at 3,000, 2,000, 1,500 and 1,000 FTU/kg, respectively in each of the pullet phases. The phytase phased dosing strategy is related to the P requirement in each feeding phase. Diets were based on corn, wheat, soybean meal, canola meal, and wheat middlings, with phytate P range of 0.341 to 0.289%. Pullets were fed with mash feed and water *ad libitum*. Feed disappearance for each dietary phase was recorded, and daily intake calculated (FI) per bird on a cage basis. Pullets were individually weighed on d 0 and at the end of each dietary phases to determine body weight gain (BWG) and mortality-corrected feed conversion ratio (FCR) was calculated. One-way ANOVA was performed using JMP 19.1. Pullets in each treatment group had performance consistent with that of the primary breeder management guide. The BW of the PC birds was 246 g, 606 g, 1,095 g and 1,183 g at 4, 8, 16 and 18 wk of age, respectively. The IPF+ PhyG treatment maintained all performance parameters vs. PC. Relative to the PC, the IPF+PhyG diet numerically reduced the starter through pre-lay feed cost, $ per kg BWG (1.302 to 1.249: $P$=0.089) and significantly reduced the carbon footprint (gCO2 eq)/kg BWG (5,482 to 5,020: $P$=0.001) In conclusion, the results showed that PhyG phytase is able to compensate for the reduction of Ca, dig AA and ME, together with total removal of added inorganic P, maintained performance vs. PC in cost effective and sustainable manners. This is the first work reporting total removal of added inorganic P in laying hen pullets from 0-18 weeks, and this approach would help to improve sustainable pullet production.

**Keywords:** Phytase; Inorganic phosphate free diet; Pullet; Production economic benefit; Sustainability

### 325 Growth performance of layer pullets supplemented with phytase and a plant-based vitamin D3 source from 0 to 18 weeks of age

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Phytase is widely used to enhance dietary P utilization and reduce the use of inorganic P in poultry diets. Active vitamin D, 1,25(OH)2D3 enhances Ca and P metabolism. The pullet phase influences subsequent egg production, eggshell quality and bone health of long-cycle laying hens. Consequently, the impact of pullet management has become increasingly important . As part of an ongoing layer trial (to 100 wk of age), the effects of phytase and a product containing 1,25(OH)2D3-glycosides extracted from *Solanum glaucophyllum* (Panbonis; PAN; Herbonis Animal Health), on pullet performance from 0 to 18 wk of age were investigated. A total of 880 one-day-old Lohmann LSL-Lite pullets were distributed in a completely randomized design, to 5 dietary treatments with 8 replicate cages with 22 birds each. Treatments were: T1 (Positive control; PC; calculated % available P in parentheses) = nutritionally adequate starter (0 to 4 wk; 0.480), grower (4 to 8 wk; 0.450), developer (8 to 16 wk; 0.370) and pre-lay (16 to 18 wk; 0.450) diets; T2 = T1 with phase-specific reductions of available P (calculated % available P of 0.281, 0.220, 0.160 and 0.231 for the respective dietary phases), as well as reductions in Ca, digestible AA and ME based on the phytase matrix with 1,000 FTU phytase/kg feed (Axtra Phy GOLD, IFF) added; T3 = T2 + PAN (0 to 18 wk; T4 = T2 (0 to 8 wk) and then T3 (8 to 18 wk); T5 = T2 (0 to 16 wk) and then T3 (16 to 18 wk). PAN was added to the respective diets at 1 µg 1,25(OH)2D3-glycosides/kg feed. Pullets had access to mash feed and water *ad libitum*. Within each dietary phase, individual ADG, ADFI and mortality-corrected FCR (on a cage basis) were calculated. One-way ANOVA was performed, followed by Tukey`s test, with a significance level of P < 0.05. Pullet performance in each treatment group was consistent with the primary breeder management guide. The average weight of the T1 birds was 246 g at 4 wk of age, 602 g at 8 wk of age, 1,093 g at 16 wk of age and 1,182 g at 18 wk of age. There were no treatment effects on body weight (P = 0.35), BWG (P > 0.31), FI (P > 0.52), nor FCR (P > 0.59) from 0 to 18 wk of age. Dietary phytase at 1,000 FTU/kg feed was able to compensate for the reduction of available P, Ca, digestible AA and ME and maintained pullet performance (T2 *vs.* PC). There was no effect of PAN on any of the pullet performance parameters measured, suggesting that dietary vitamin D activity was not limiting for growth in any of the diets. However, the effects on other parameters such as bone mineralization and body composition, as well as the long-term effects of these dietary treatments, may become more evident during the subsequent laying phase

**Keywords:** 1,25(OH)2 vitamin D3-glycoside; feed conversion ratio; feed intake; growth; phytase

### 326 Substrate-specific activity of invertase preparations and their efficacy in galactooligosaccharides degradation in plant feed ingredients for poultry

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Galactooligosaccharides (GOS), including raffinose and stachyose, present in plant-based feed ingredients and poultry diets, are traditionally regarded as antinutritional factors. However, both GOS and products of their enzymatic hydrolysis may exert prebiotic effects in the poultry GIT. Invertase is applied as an exogenous enzyme to hydrolyze GOS to fructose, melibiose, and manninotriose. Although invertase activity is typically quantified by its ability to hydrolyze sucrose, understanding the degradation kinetics of raffinose and stachyose is nutritionally rel-

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evant for poultry. The objective of this glycobolomic in vitro study was to investigate the substrate-specific activities of three different invertase preparations and to evaluate their efficacy in hydrolyzing GOS and releasing bioactive glycobolites from SBM, canola meal (CM), peas, and lupin. Invertase preparations IA, IB, and IC were initially evaluated using pure sucrose, raffinose, and stachyose (Sigma- Aldrich) as substrates. The activity unit was defined as the amount of enzyme hydrolyzing 1.0 Mmol of substrate per minute at pH 4.0 and 40°C. The highest lytic activity toward sucrose and stachyose was demonstrated for IA; IB showed superior raffinose-degrading activity, while IC was the least efficient across all three substrates. The sucrose: raffinose: stachyose activity ratios were 1: 0.38: 0.26 for IA, 1: 0.85: 0.17 for IB, and 1: 0.27: 0.08 for IC, revealing distinct substrate specificities. In a subsequent study, test feed ingredients were submerged in buffer and incubated with enzymes at 0, 0.01, 0.1, and 1.0 mg/g for 16 h, then analyzed for total sugar content by gas chromatography. Data were analyzed in RStudio using a one-way ANOVA followed by Tukey's HSD test. A dose-response pattern was observed across all enzyme-substrate treatments (p<0.01). Sucrose was hydrolyzed by all the invertase preparations at 0.1 and 1.0 mg/g. At 0.1 mg/g, IA and IB caused complete hydrolysis of GOS to simple sugars and manninotriose in SBM, CM, and peas, whereas IC caused this at 1.00 mg/g. Incubation of pea and lupin without enzyme led to partial autolysis of raffinose; however, its residual amount was still detected in enzyme-treated samples. The IB was the most effective at releasing the prebiotic manninotriose, particularly in SBM and CM. In summary, invertase can enhance the prebiotic structure of GOS present in the poultry diets. However, its activity to hydrolyze sucrose does not predict its ability to hydrolyze raffinose and stachyose. Therefore, invertase selection for monogastric diets, particularly those with high GOS content, should be based on a substrate-specific assay rather than the conventional sucrose-based assay alone.

**Keywords:** invertase; raffinose; stachyose; glycobolite; enzyme

### 327 Glucose oxidase, probiotic and their combination to replace AGP in broiler under tropical condition

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Recently, glucose oxidase (GOD) has attracted attention as a functional enzyme in improving gut health as a potential alternative to antibiotic growth promoters (AGP). However, it remains unclear whether GOD can effectively replace AGP under challenging conditions and whether it may or may not interact with dietary probiotics. This study aimed to evaluate the effectiveness of glucose oxidase, probiotics, and their combination as an alternative to AGP in fast-growing broiler chickens fed on corn and soybean meal–based diet under thermal stress. The experiment was conducted with a flock of 960 one-day old male broiler (Ross 308 AP95), under tropical condition with temperature range of 28-36°C, last for 42 days. A three-phase feeding was followed, and the feed was pelleted at 80°C ± 2°C. There were 5 experimental treatments and each consisted of 16 pen replicates with 12 chicks per pen. The control (CON) was without any growth promoter; the AGP group (AGP) was supplied with Enramycin 8% at 125 g/mt; the glucose oxidase group (GOD) was added with GOD at 250 g/mt, the probiotic group (PRO) was added with PRO at 200 g/mt and a combination of GOD and PRO (GOD+PRO) was provided with GOD 250 g/mt and PRO 200 g/mt. The data were analyzed by one-way ANOVA, and A level of $P \leq 0.05$ was used as the criterion for statistical significance. The results showed that, compared with the control group, supplementation with AGP, GOD, PRO, or their combination numerically improved body weight gain (BWG), although the differences were not statistically significant ($P > 0.05$). Notably, the combination of GOD and PRO increased BWG by 80 g/bird compared with the control group. Supplementation with either GOD or PRO significantly reduced FCR ($P < 0.05$), with the lowest FCR observed in their combined group ($P < 0.05$). In addition, the combination of GOD and PRO significantly improved European Production Index (EPI) compared with the control ($P < 0.05$). When compared with AGP, no significant differences in BWG, FCR, and EPI were observed with either GOD or PRO alone ($P > 0.05$). However, the combination of GOD and PRO numerically increased BWG ($P > 0.05$) and significantly improved both FCR and EPI ($P < 0.05$). It was concluded that either GOD or Probiotic can replace AGP without negative impact on performance. In addition, a combination of GOD and PRO increased BWG, significantly improved FCR with the highest EPI, which could be considered as an efficient alternative to AGP, to maintain or enhance growth performance of broiler fed on AGP-free diets.

**Keywords:** glucose oxidase; probiotic; AGP-free; broiler; performance

### 328 Evaluation of a multienzymatic product on laying hen productive performance and tibia mineral content.

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To evaluate the hen response to a multienzymatic feed enzyme (phytase, xylanase, protease, B-mannanase, and alpha amylase) product, BioMagic E20, compared to options commonly added to hen feed, 960 43-wk-old-Bovans White hens were randomly distributed to 6 treatments with 8 replicates each and 20 hens per replicate (4 cages with 5 hens each). With the following treatments: 1, Positive control (PC) a corn-SBM-Canola feed formulated to fulfill 40 to 60-wk-old Bovans White hen nutritional requirements, 2, Negative control (NC) as 1 applying the following feed formulation matrix: 140 kcal/kg ME, 0.2% P, 0.145% Ca, 0.043% Na, 0.9% Protein, 0.047% Lys, 0.050% SAA, 0.043% Thr, 0.050% Val, 0.047% Arg, 0.045% Ile, 0.024% Trp, 0.076% Leu, 0.083% P+T, 0.023% His, and 0.011% G+S, but with no feed enzyme addition, 3 as 2 + 120 g/T BioMagic E20, 4 as 3 + 100 g/T Lipase activity, 5 as 2 + 30g/T HiPhorius (phytase) + 75 g/T Ronozyme WX (xylanase) + 125 gt/T PoultryGrow (protease), and 6, As 5 + 100 g/T Lipase activity. Birds had free access to water and feed. The trial was conducted from week 43 to week 63 of hens' age. Data was analyzed with excel statistics tool, as 2-way-ANOVA under a Randomized Complete Block Design (RCBD), with one replicate per block, with pen location as blocking criteria. Hen weight, hen mortality, egg production, egg quality, and tibia mineral content where not affected by treatment ($P > 0.05$). Egg weight, g was statistically higher ($P < 0.004$) for T1 (61.2) vs T2, T3, T4, T, T6 (60.2 average), Feed conversion was statistically lower ($P<0.002$) for T1 (1.785) vs all other treatments (1.853), egg production cost as percentage of T1 was lower for all treatments compared to T1 (100%) vs T2, T3, T4, T5, T6 (93.3%), ME and NPP daily intakes were statistically lower( $P < 0.001$) for T2, T3, T4, T5, T6 vs T1, ME 293 vs 283 kcal/d, NPP 392 vs 188 mg/d. Feed formulation matrix decreased egg weight, and increased feed conversion, feed enzymes addition to the feed did not bring back these numbers to T1 observed performance. It is important to mention no difference in tibia and mineral content between treatments 1 and 2 with the following NPP daily intakes 392 vs 188 mg/h/d both with no feed enzymes addition. It is recommended the revision of the feed formulation matrix numbers for ME and protein and amino acids, because under the experimental conditions of the present trial, the addition of feed enzymes to the experimental diets, did not recover these numbers to the ones observed on the positive control treatment.

**Keywords:** Hens; Enzymes; carbohydrase; phytase; protease

### 329 Evaluation of several feed enzymes options on laying hen productive performance and tibia and femur mineral content.

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To evaluate the hen response to different feed enzymes activities combinations 980 51-wk-old-Bovans White hens were randomly distributed to 7 treatments with 7 reps per treatment and 20 hens per replicate (4 pens with 5 hens each). Treatments were: 1, Positive control (PC) a corn-SBM-Canola feed formulated to fulfill 50 to 70-wk-old Bovans White hen nutritional requirements, 2, Negative control (NC) as 1 applying the following feed formulation matrix: 110 kcal/kg ME, 0.2% P, 0.214% Ca, 0.044% Na, 0.7% Protein, 0.041% Lys, 0.019% SAA, 0.026% Thr, 0.033% Val, 0.053% Arg, 0.033% Ile, and 0.014% Trp, but with no feed enzyme addition, 3 as 2 + 30g/T HiPhorius (phytase) + 75 g/T Ronozyme WX (xylanase), 4 as 2 + 40 g/T Danisco Xylanase + 20 g/T Axtra Phy Gold 30t (phytase), 5 as 2 + 60 g/T SUNTAQ CEM (multicarbohydrase) + 150 g/T SUNTAQ phytase, 6 as 2 + 22 g/T SUNTAQ xylanase + 150 g/T SUNTAQ phytase + 70 g SUNTAQ Protein Plus (protease), and 7 as 5 + 70 g SUNTAQ Protein Plus. Birds had free access to water and feed. The trial was conducted from week 51 to week 72 of hens' age. Data was analyzed as 2-way-ANOVA under a Randomized Complete Block Design (RCBD), with one replicate per block, with pen location as blocking criteria. Hen weight, percentage egg production, feed intake, egg quality, and tibia and femur mineral content where not affected by treatment (P > 0.05). Egg weight, g was statistically higher (P < 0.001) for T1, T5, T6, T7 (61.7 average) than T2, T3, T4 (60.9 average ), egg mass, g/h/d was statistically higher (P < 0.02) for T1, T6, T7 (57.1) than T2, T3, T4, T5 (56.0), total kg of egg mass per hen was statistically higher (P < 0.02) for T1, T6, T7 (8.798) than T2, T3, T4, T5 (8.629), feed conversion was statistically lower (P < 0.04) for T1, T6, T7 (1.866) than T2, T3, T4, T5 (1.894), egg production cost as % of T1 was statistically lower (P < 0.0001) for all treatments vs T1 (100% vs 93%). All treatments showed statistically lower (P, 0.0001) consumption of ME and NPP vs T1, ME (298 vs 287 kcal/d), NPP (371 vs 160 mg/h/d). It is important to mention no difference in tibia and femur mineral content between treatments 1 and 2 with the following NPP daily intakes 371 vs 159 NPP mg/h/d both with no feed enzymes addition. The feed formulation matrix with no feed enzymes had a negative effect on egg weight, egg mass, and feed conversion, phytase and carbohydrases addition to T3, T4, and T5 did not recover the hen performance, whereas addition of phytase, carbohydrases and protease on T6 and T7 showed the same performance as positive control T1.

**Keywords:** Hens; enzymes; carbohydrases; phytase; protease

### 330 Ahiflower seed and press cake, with or without multi-enzyme supplementation: influence on nutrient digestibility and gut development in broiler chickens

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Ahiflower seed (AS) and ahiflower press cake (APC) are rich in Ca and P and contain moderate levels of energy and crude protein (CP); however, their bioavailability in broilers and laying hens has been reported to be low. Dietary supplementation with exogenous enzymes improves nutrient utilization in broiler chickens. This study evaluated the effects of exogenous multi-enzyme supplementation (ES; containing carbohydrases, phytase, and protease) with 10%AS or 10%APC on nutrient digestibility (P, Ca, and CP) and intestinal morphology (villus height -VH, crypt depth -CD, and villus height-to-crypt depth ratio -VH: CD) in the duodenum, jejunum, and ileum. A total of 540 one-day-old Ross 308 broiler chicks were randomly allocated to 1 of 6 dietary treatment, which include a corn-soybean based control (CON), CON + 10%AS or 10%APC with or without ES, in a 3 × 2 factorial arrangement (diet × enzyme) in a randomized complete block design with six replicates (each replicate consisted of a pen housing 15 birds) per treatment for 35 days. In the duodenum, VH was increased in birds fed 10%AS compared with CON or 10%APC (*P*=0.0064), whereas CD and VH: CD were not influenced by treatments. In the jejunum, VH (*P*=0.0488) and VH:CD (*P*= 0.0284) were increased by 10% AS, compared with CON, but did not differ from 10% APC. In the ileum, VH:CD was influenced by diet (*P*=0.0332), with 10% APC showing the highest ratio. Enzyme supplementation and diet × enzyme did not affect morphometric indices. P digestibility was reduced by 10% AS compared to CON (*P*=0.0381), but did not differ from 10% APC, whereas ES reduced P digestibility (*P*=0.0396). Ca digestibility was not significantly influenced by treatment and ES. CP digestibility was significantly reduced by 10% APC compared to the CON but did not differ from 10% AS (*P*=0.0010) and showed a diet × enzyme interaction (*P=0.0211),* with ES increasing CP digestibility in CON, as in 10% APC diets, but not in 10% AS. In conclusion, the findings demonstrate that dietary inclusion of 10% AS and 10% APC supports intestinal development, as evidenced by improved VH:CD in the ileum and jejunum, and increased villus height in the jejunum. The inclusion of 10%AS enhanced CP digestibility, whereas 10% APC improved CP digestibility when combined with ES, highlighting their value as alternative protein sources in broiler nutrition.

**Keywords:** Ahiflower seed; broiler chickens; nutrient digestibility; gut morphology; exogenous multi-enzyme supplementation

## Processing and Products

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### 331 Processing Innovation and Technology: Industry Impacts and Opportunities

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Over the past five decades, the poultry industry has experienced consistent and substantial growth in the United States and globally. Continued consumer demand for poultry products is driven by their affordability, convenience, versatility, and perceived health benefits. During this period, the average broiler weight has increased from approximately 1.7 kg to more than 3 kg. Today, the large bird market segment (>2.7 kg) accounts for nearly 70% of broiler meat production based on weight. This shift toward larger birds has been enabled by advancements in genetics, nutrition, management practices, and extended grow-out periods. Research within poultry processing has focused on improving welfare and operational efficiency through enhanced processing technologies, as well as advancing meat quality, food safety, and product formulation and innovation. Significant progress has been made in humane slaughter practices through improved stunning methods, in tenderness enhancement through techniques such as electrical stimulation and marination, and in mitigating quality defects including pale, soft, exudative meat and emerging myopathies. Despite these advancements, further innovation is needed in the intelligent automation of processing tasks. Expanded automation has the potential to reduce labor intensity and demand, enhance operational efficiency, and strengthen food safety outcomes. Continued research is also needed to address consumer preferences for clean labels, sustainability, and quality.

### 332 The protective effect of different condiment recipes on marinated duck meat against the production of carcinogenic PAHs during natural gas grilling.

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Meat is frequently processed by variety of heat treatments in food in-

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dustry, worldwide. Such heat treatment methods may lead the generation of polycyclic aromatic hydrocarbons (PHA's) during pyrolysis, as a major source of carcinogenicity. The purpose of the current research aimed to evaluate impact of natural gas grilling on genesis of polycyclic aromatic hydrocarbons (PHA's) in tissues of five organs (leg, chest, liver, heart and wings) of black-headed (*Heteronetta atricapilla*) and falcated (*Mareca falcata*) ducks after marination with different condiment recipes; Normal condiment (NC: salt 30g, chili powder 15g, turmeric powder 20g coriander powder 20g, cumin seed 15g), Modified condiment-I (MC1) containing additional onion 25g and garlic 25g to the NC, Modified condiment-II (MC2) composition same as MC1 with addition of 25g ginger and Modified condiment-III (MC3) composition same as MC1 with additional 10g clove and 10g lemon juice. Five grams of each tissue sample was obtained from both duck varieties (15 each variety) after slaughter. The samples were marinated overnight with different recipes before they grilled over natural gas open air yellow flame (1000 °C). All marinated/non marinated control tissue samples were extracted to isolate PHA's. The final solution was reconstituted in acetonitrile and then introduced (20 µL) into reverse phase high performance liquid chromatography (RP-HPLC) to identify and measure the concentration of certain PAHs that were unknown. Two way ANOVA ($P \leq 0.05$) was applied by PRISM 9 and Post Hoc test by Co stat to compare means. Natural gas grilled leg meat tissue sample of black headed duck without any condiment marinating showed higher ($P \leq 0.05$) concentration (61.4±0.2 ng/g) of overall PAH's as compared to all other grilled samples of both duck varieties. However, overall PAH's concentration (16.42 ng/g) in natural gas grilled tissues of leg meat marinated with MC3 found decreased by 75.36% ($P \leq 0.05$) as compared to all other samples of both duck varieties. In PAH's particularly fluorene (2.12ng/g), phenanthrene (4.2ng/g) and BaP (0.43ng/g) concentrations were reduced ($p \leq 0.05$) while naphthalene, acenephthalene and fluoranthane remained lowest ($p \leq 0.05$) in MC3 samples of both duck varieties as compared. Conclusively, all condiment recipes showed promising results in reducing the generation of total PAHs level in both duck tissues samples keeping them below MRL's of the international guidelines. Recipe MC3, serves as the most suitable recipe in reducing the PAH produciton due to its rich content of antioxidants and polyphenolic acids as secondary metabolites, thus ensure safety of the consumer in fast food industry.

**Keywords:** Polycyclic aromatic hydrocarbons,; duck meat; condiment; natural gas grilling; RP-HPL

### 333 Deep learning-based automated detection method for eggshell spotting traits

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Eggshell spotting, including translucent spots and speckles, is a common appearance defect, and reliable quantitative phenotyping is essential for commercial egg grading and poultry breeding. Conventional automated detection methods often rely on manual feature engineering, making it challenging to adjust parameters across multi-breed and multi-batch images, which is inadequate for large-scale and high-throughput detection. This study aimed to develop an efficient and generalizable end-to-end deep convolutional neural network model, named SAR-Net, for high-accuracy automated detection of eggshell spot area ratio (SAR). In this study, semi-automatic annotation was utilized to construct an eggshell speckle dataset (6,466 images) and an eggshell translucent spot dataset (3,578 images). SAR-Net adopted the lightweight ConvNeXt-V2-Nano as its backbone network. To address variable spot sizes, a progressive Spatial Feature Pyramid Fusion module was incorporated to effectively integrate multi-scale features. In addition, a cascaded structure combining windowed self-attention and Squeeze-and-Excitation channel attention was employed to enhance the perception of spotted regions. Finally, a Smooth MAPE loss function optimizing relative error was designed for the robust regression prediction of SAR. Evaluation on the speckle validation set showed that SAR-Net achieved an $R^2$ of 0.9827, outperforming comparable lightweight models such as MobileViT-S ($R^2$ = 0.9749) and EfficientNet-B3 ($R^2$ = 0.9742). For the bright-field translucent spot validation set, SAR-Net also reached a reliable $R^2$ of 0.8295. With only 7.47 million parameters, the proposed model achieved an inference speed of 25.2 FPS in a pure CPU environment. These results demonstrate that SAR-Net can achieve high accuracy and robustness while maintaining a lightweight architecture, providing an efficient high-throughput phenotyping tool for evaluating eggshell spotting traits.

**Keywords:** egg quality; deep learning; automated detection; Eggshell defect; eggshell spotting

### 334 Pellet Durability Index (PDI) Prediction Using Machine Learning Models across Feed Mills

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Pellet quality, which is a major concern for feed mills, refers to the ability of pellets to withstand fragmentation and abrasion during handling and transport while maintaining their integrity. Pellet quality is commonly measured using the Pellet Durability Index (PDI), which can be influenced by multiple interacting factors, including ingredient composition, nutrient levels, processing conditions, environment, etc. Controlling pellet quality in the commercial setting represents an ongoing challenge, as it may be affected by many factors that change simultaneously. Several studies have developed machine learning or statistical models to predict PDI using datasets from single feed mills. However, these models may not perform well when applied to datasets from different feed mills because manufacturing equipment and processes, ingredient usage, and environmental conditions can vary substantially among mills, and even among production lines within a single feed mill. Therefore, developing generalized models capable of predicting PDI across feed mills would be highly beneficial. In this study, generalized regression models were developed to predict PDI, based on seven independent datasets representing seven feed mill production lines collected from four feed companies. Variables used to predict PDI included within-mill variables, which varied across pellet runs within a production line (e.g., conditioning temperature, and dietary nutrient levels, ingredient usage, etc.), and mill-level variables (e.g., conditioner capacity and expander capacity), which remained constant within a production line. Four machine learning regression algorithms were applied to build the models, including Support Vector Regression (SVR), Random Forest (RF), Gradient Boost Regression (GBR), and Multilayer Perceptron (MLP). Global pooled (GP) models were constructed using the training sets from all feed mills and evaluated on the testing set of each feed mill. Additionally, to evaluate the generalization ability of the models to new feed mills, a leave-one-mill-out (LOMO) approach was applied, in which data from one mill was excluded during model training and used only for testing, while the model was trained using data from the remaining mills. The evaluation results indicated that the GBR model was the best GP model, achieving a Concordance Correlation Coefficient (CCC) of 0.657 ± 0.093. Under the LOMO approach, the SVR model exhibited a higher CCC (0.218 ± 0.072) compared to the other models. The models developed in this study captured the general relationships between factors and PDI, which could reflect the global patterns across different feed mills, but struggled with considerable between-mill variation and differences.

**Keywords:** Pelleted feed; Pellet Durability Index (PDI); Generalized model; Regression

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### 335 Genome-wide variations detected in spaghetti meat myopathy of chicken breast

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Spaghetti meat (SM), a recently identified muscular abnormality in chicken breast meat, is characterized by a loss of muscle fiber integrity, resulting in a spaghetti-like appearance. The incidence rate of SM is estimated to reach up to 20% in some instances, but few studies have been conducted on SM due to its recentness. The etiologies of SM are not fully understood. A recent multi-omics study with SM fillets showed physiological alterations associated with beta-oxidation, energy metabolism, lipid formation, and protein metabolism in SM fillets. While processing practices may exacerbate SM characteristics, it is important to also understand the genetic susceptibility of breast muscle to develop the poor muscle integrity observed in SM. The purpose of this study was to investigate potential genetic variations in SM fillets using a genome re-sequencing method. Nineteen SM and 3 normal fillets were collected from a commercial processing plant. Small pieces of each fillet were used for genomic DNA extraction. Genomic DNA was subjected to genome resequencing analysis, which was conducted by PCR free library construction, 150 bp pair-end read method with Illumina NovaSeq X plus technology. Raw sequencing reads were aligned with broiler chicken reference genome (GRCg7b) and single nucleotide polymorphism (SNP), insertion/deletion (InDel), copy number variation (CNV), and structural variation (SV) were identified for each individual data. Genome variation data sets were compiled and compared among the SM and normal samples. The mapping rate of each sample ranged from 99.03% to 99.28%. Based on the reference genome, the average depths were between 3.88X and 4.73X, and 1X coverages ranged from 94.62% to 96.73%. Average numbers of SNPs, InDels, CNVs, and SVs for each sample were 5.03 million, 588135, 2940, and 1702, respectively. Through filtering group-specific variants and Fisher's exact test, genetic variations uniquely found in SM compared to normal fillets included 353 SNPs, and 54 InDels, but no commonly detectable SM specific- CNVs and SVs were found. While all InDels were found in intergenic regions, two SNPs resulted in non-synonymous amino acid substitutions in the annotated proteins: lysosomal cystine transporter (CTNS, exon11:c.A1076G:p.Q359R) and sedoheptulokinase (SHPK, exon5:c.C800A:p.T267N). The results in this study may provide insights into potential genetic effects on weakening muscle integrity leading to SM syndrome.

**Keywords:** Spaghetti meat; genome resequencing; genetic variation

### 336 Enzyme activities linked to mitochondrial function altered in multiple organs in broilers with wooden breast

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The development of growth-related metabolic and oxidative stress in the breast muscle of broilers is thought to be related to the development of the wooden breast (WB) myopathy. Mitochondrial function plays a key role in cellular energy production and has been investigated in muscle tissue with the WB myopathy. It is unknown if mitochondrial function in tissues other than muscle is altered in broilers exhibiting the WB myopathy. The objective of this study was to compare indicators of mitochondrial function across multiple organs in normal broilers (no WB) and those exhibiting the WB myopathy. Sixty 8-week-old male Ross-308 broilers were slaughtered via electrical stunning and exsanguination procedures. Immediately following bleed-out, breast muscles were assessed for WB condition. Ten carcasses with severe WB and 10 normals (no WB) were selected and tissues (breast muscle, liver, pancreas, and three sections of intestine) were immediately collected (<15 min postmortem) and frozen in liquid nitrogen. From these tissues, mitochondria were isolated for total mitochondria protein content, citrate synthase activity, glutathione, mitochondrial complex II activity, and total ATP content measurements. One breast muscle from each carcass was used to assess meat quality. Data were analyzed using the t-test method to determine differences between normal and WB samples. As expected, WB meat exhibited greater ultimate pH ($P<0.05$), compression force ($P<0.05$), and drip loss ($P<0.01$) than normal meat. Total mitochondria protein content was lower ($P<0.05$) in pancreas and liver tissue from WB birds. Mitochondria citrate synthase activity was lower in muscle ($P<0.05$) and duodenum ($P<0.01$) from WB birds. Mitochondria glutathione concentrations were lower in pancreas ($P<0.05$), liver ($P<0.05$), duodenum ($P<0.05$), jejunum ($P<0.001$), and ileum ($P<0.05$) tissue from WB birds. Mitochondria complex II activity was lower in muscle ($P<0.001$), pancreas ($P<0.05$), liver ($P<0.01$), and duodenum ($P<0.05$) tissue from WB birds. Total ATP concentrations were lower in muscle ($P<0.05$) and liver ($P<0.05$) tissue from WB birds. Overall, data suggest that mitochondrial function is impaired in tissues beyond just the breast muscle in broilers with the WB myopathy and may be involved in the complex etiology of this myopathy.

**Keywords:** broiler; wooden breast; myopathy; meat quality; mitochondria

## Genetics and Molecular Biology

Balancing Discovery and Application: The Evolution of Industry–Academic Partnerships in Poultry Science

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The evolution of academic based Poultry Science research is inseparable from its long-standing yet often shifting relationship with the commercial poultry industry. Early in the twentieth century, academic research and industry development were closely aligned by necessity, with land-grant institutions providing practical solutions that enabled the rapid establishment of modern production systems. As the industry advanced, collaboration yielded remarkable improvements in efficiency, growth, and egg production, but increasingly narrow, performance-driven objectives also exposed correlated traits that challenged production success. There were periods of partial separation between industry and academia, most notably during the expansion of proprietary breeding programs and the early molecular era. During the 1970s through the early 1990s, the poultry industry and academic programs experienced tremendous consolidation pressure, leading to a survival-of-the-fittest mentality for both. While these intervals reduced short-term integration, they proved critical for preserving exploratory, mechanism-based research that later became essential for studying complex industry challenges including meat quality defects, metabolic disorders, and immune system trade-offs. In recent decades, the re-emergence of strategic, bidirectional partnerships has underscored an important lesson: the most robust advances arise when industry defines real-world problems while academic research retains the freedom to investigate underlying biological mechanisms. From my perspective, it is clear that neither complete alignment nor strict independence is optimal. Instead, productive balance between application and discovery has driven progress. As poultry science confronts multifactorial challenges related to vigor, welfare, sustainability, and societal expectations, future success will depend on collaborations that value long-term biological understanding alongside commercial relevance. The current presentation will attempt to reaffirm the importance of strengthening the industry/ academic relationship as we enter yet another period of technological opportunity.

### 337 Profiling of alternative polyadenylation reveals post-transcriptional regulatory divergence between chicken ESCs and PGCs

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The study of germline development and differentiation is important for poultry production and developmental biology. Alternative polyadenylation (APA) is a key post-transcriptional mechanism that generates mRNA isoforms with distinct 3,Ä≤ ends and modulates transcript stability, translation, and regulation. However, its role in germline specification remains elusive, especially for 3,Ä≤UTR regulatory potential or isoform architecture. We isolated ESCs and PGCs. Each of the three replicates represented a pool of embryos or tissues from the same sex. ESC samples were generated by pooling blastoderms from 100 fertilized eggs. PGC samples were generated by pooling gonads from 30 embryos. Here, we used APA-centered analysis to investigate transcript-end regulation during the transition from chicken ESCs to PGCs. By integrating WTTS-seq with Nanopore long-read RNA sequencing across both sexes, we identified 12,843 genes with APA sites, including 11,732 coding genes and 1,015 lncRNA genes. Poly(A) site usage showed a pronounced bimodal distribution, consistent with widespread proximal-versus-distal cleavage bias and 3,Ä≤UTR remodeling during the ESC-to-PGC transition, whereas sex-biased APA differences were comparatively modest. Long-read data further resolved isoform-level APA events and transcript-end heterogeneity not captured by WTTS-seq alone. Integration identified both concordant and divergent APA patterns at the gene and isoform levels. Among 560 APA genes, 259 showed differential expression. Only 65 APA genes showed alternative splicing changes, and 30 genes exhibited isoform switching, supporting a model in which APA primarily remodels the 3,Ä≤UTR landscape rather than the coding structure. A PAS-enriched miRNA-like motif corresponding to gga-miR-1653 was identified, including FGFR2, OTUD3, PLA2G10, SBDS, SEPTIN8, and ZBED4, consistent with gene-specific coupling between APA and miRNA-mediated regulation. Mechanistically, canonical PAS strength, GC content, and UGUA motifs emerged as major positive cis determinants of PAS usage, while multiple 3,Ä≤ end processing factors showed significant global associations with APA. However, the persistence of a cell-type effect after cis adjustment, together with the absence of significant cis × trans interactions, suggests that ESC–PGC APA divergence reflects both local cis architecture and broader shifts in the 3,Ä≤ end processing environment. Together, these findings demonstrate that APA is a pervasive and functionally important post-transcriptional regulatory layer during the chicken ESC-to-PGC transition, and establish a complex, sex- and cell-type-resolved APA atlas in an avian system that highlights APA as an important regulator of early germline development.

**Keywords:** Embryonic stem cells; Transcriptome ; alternative polyadenylation ; development and differentiation ; post-transcriptional mechanism

### 338 Evaluating Alternative Reference Genes to GAPDH for qPCR Normalisation in Chickens (*Gallus gallus domesticus*)

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During gene expression quantification using quantitative PCR (qPCR), the normalisation of the values against a reference gene is fundamental. These reference genes should be constantly and stably expressed by the host cells, regardless of the external conditions. Over the last decades, the expression of the chicken version of the gene encoding the Glyceraldehyde-3-phosphate dehydrogenase (chGAPDH) has been extensively utilised in chicken gene expression research. However, recent studies indicate that its expression shows high variability between conditions and tissues. This indicates the requirement for alternative reference genes that are more stably expressed by chicken cells. In this study, the optimal RNA extraction kit for chicken tissues and homogenisation solution (RNA Shield versus kit lysis buffer), in terms of RNA yield, was initially evaluated using Nanodrop. The selected kits were NEB Monarch, QIAGEN RNeasy and Zymo Quick-RNA. Then, multiple potential reference genes, including chGAPDH, were assessed for their stability across chicken tissues, utilising the RNA from the best extraction method and subjected to reverse transcription qPCR (RT-qPCR). Then, the Cq values were compared using the software package NormFinder Excel Add-in, in which a lower S value indicates a higher stability in the expression of that gene. The tissues selected were jejunum, caecal tonsils and liver, collected in previous experiments and preserved at −80 °C. From the evaluated RNA extraction kits, NEB Monarch showed the highest RNA yield, 10 times higher than the other two kits used. Surprisingly, the use of RNA Shield yielded higher RNA yields than the respective kit-included buffers. In terms of gene expression, GAPDH, TRFC, TBP, GUSB, RPLP1, Beta-actin, HMBS, ACTA and RPL4 genes were selected. The genes RPLP1 (S = 0.013) and GUSB (S = 0.015) were the most stable across tissues and yielded the best combined stability value of 0.011. Meanwhile, chGAPDH and TRFC exhibited the greatest variability in tissues, suggesting instability of expression and poor suitability as reference genes. Overall, RPLP1 and GUSB were deemed the most reliable genes in this study for qPCR normalisation in chicken gene expression studies. Additionally, results suggested that using dual-genes in normalisation increases accuracy when compared to single-genes.

**Keywords:** Reference genes; Chickens; Gene expression; mRNA; qPCR

### 339 Dissecting Dynamic Transcriptome in Chicken Ovaries during Primordial Follicle Assembly via Single-Cell RNA Sequencing

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Objectives: Primordial follicle assembly in chickens is crucial for ovarian development and reproductive capacity, yet the regulatory mechanisms remain unclear. This study analyzed regulatory mechanisms and interactions between germ and granulosa cells during key developmental stages. Materials and methods: Single-cell RNA sequencing (scRNA-seq) was performed to construct cellular transcriptional profiles from ovaries of Hyland's brown-shelled laying hens at critical developmental periods (12.5 d embryonic, 18.5 d embryonic, and 5 d post-hatch). Morphological changes of chicken ovarian germ cells at the three critical periods were characterized by H&E staining. The expression changes of germ cell-specific genes and the temporal expression of key regulatory factors during primordial follicle assembly were analyzed using qRT-PCR. The activation status of the WNT4 signaling pathway during primordial follicle assembly was assessed by Western blot. One-way ANOVA was used to compare the expression differences in chicken ovaries at different developmental stages. Results: H&E staining showed that during primordial follicle assembly, germ cells formed cystic lineages and ruptured, with a marked decrease in the number of oocytes. Using UMAP, germ cells were categorized into five subtypes: mitotic S, mitotic G2/M, pre-meiosis, meiosis, oocyte, and dying oocyte. qRT-PCR validation demonstrated that germ cell-specific genes *Kif15*, *Crabp1*, *C14orf39*, *Lhx8*, *Sqstm1* synergistically regulate the normal progression of meiosis and ensure the formation of high-quality primordial follicles. Temporal trajectory analysis and transcriptional network analysis showed that oocytes undergo autophagy and apoptosis, coordinated by two fate regulators (MCM5, SMC1B, GDF9, SQSTM1), to remove aberrant cells and maintain germ cell quality. Granulosa cell differentiation was driven by GATA4 and WT1, thereby activating steroid hormone synthesis (*Cyp17a1*) and maintaining follicular homeostasis. CellChat cellular communication analysis revealed that germ cells and granulosa cells

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mediated synergistic activation via the WNT signaling pathway during primordial follicle assembly, precisely regulating the spatial arrangement of cells and the timing of assembly. Conclusion: This study provides a comprehensive molecular framework for chicken primordial follicle assembly, identifying key genes and pathways essential for ovarian development.

**Keywords:** Chicken; Germ-granulosa; Ovary; Primordial Follicle; scRNA-seq

### 340 Developmental isoform switching in broiler breast muscle across embryonic and post-hatch stages

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The objective of this study was to characterize developmental changes in transcript isoform usage and isoform switching consequences in breast muscle of meat-type chickens. We hypothesized that major transitions in muscle development from late embryogenesis to market age are associated with widespread isoform switching events affecting protein function. Publicly available RNA-seq data from breast muscle samples of Cornish, White Plymouth Rock, and their crossbred progeny at embryonic day 17 (ED17) and post-hatch days 1, 21, and 42 were reanalyzed to investigate developmental isoform switching (n = 6 samples per genetic background and stage, total = 72 samples). PCA analysis of gene expression data indicated that the three genetic backgrounds shared a similar developmental trajectory, leading to a statistical model that utilized developmental stage as the primary factor while accounting for breed as a covariable. Isoform switch analysis was conducted using the R package IsoformSwitchAnalyzeR with transcript-level counts generated by Salmon, with significance testing performed via the isoformSwitchTestSatuRn statistical framework. Significant switches were defined by an absolute isoform fraction difference (dIF) > 0.1 between stages and a false discovery rate (FDR) < 0.05. External bioinformatic tools were integrated to predict functional consequences of isoform switches, including changes in protein domains, signal peptides, and open reading frames (ORFs). Developmental stage strongly influenced transcript isoform usage in breast muscle. A total of 935 significant isoform switches affecting 742 genes were identified across all developmental transitions. The highest number of switches occurred during the transition from ED17 to D1 (565 switches), followed by D1 to D21 (385 switches), with fewer switches observed between D21 and D42 (25 switches). Functional consequence analysis revealed that 602 of these switches were predicted to impact protein function. Common consequences included gain or loss of protein domains, changes in intrinsically disordered regions (IDR), and ORF length modifications. Alternative transcription start and end sites (ATSS and ATTS) and exon skipping (ES) were the most frequent splicing events driving these switches. These results demonstrate that post-transcriptional regulation through isoform switching is a prominent feature of muscle development in meat-type chickens. Characterizing these isoform-level regulatory events provides new insight into the molecular mechanisms underlying muscle growth and maturation in poultry.

**Keywords:** alternative splicing; isoform switching; RNA-seq; transcript usage; broiler muscle development

### 341 Genetic Selection Alters the Satellite Cell Secretome in Turkeys

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Satellite cells are adult myogenic stem cells that play a critical role in post-hatch skeletal muscle growth, repair, and regeneration. Beyond responding to extrinsic cues, satellite cells actively shape their microenvironment by secreting signaling molecules that facilitate communication with neighboring cells and support coordinated muscle development. Decades of intensive genetic selection in commercial turkeys for rapid growth and enhanced breast muscle yield have profoundly altered satellite cell biology. Compared with satellite cells from random-bred control lines, those derived from growth-selected lines exhibit accelerated proliferation and differentiation and form longer myotubes. Consistent with these functional differences, molecular profiling of satellite cells from the Nicholas Commercial Turkey (NCT) and Random-Bred Control (RBC) lines has revealed substantial divergence in gene expression patterns. In the present study, we examined the satellite cell secretome to test the hypothesis that genetic selection has modified the repertoire of proteins secreted or released by these cells, thereby contributing to differences in muscle growth between commercial and random-bred turkey lines. Satellite cells were isolated from one-week-old NCT and RBC poults, and serum-free conditioned media were analyzed using data-independent acquisition mass spectrometry. A total of more than 3,400 proteins and peptides were identified across both lines. Of these, 3,103 were common to both groups, while 297 proteins were unique to NCT satellite cell media and 368 were unique to RBC media. Proteins enriched in NCT-conditioned media were predominantly associated with extracellular matrix remodeling, niche-mediated signaling, stromal and fibro-adipogenic support, and paracrine communication. In contrast, proteins enriched in RBC-conditioned media were largely intracellular in nature and were involved in RNA processing, protein trafficking, vesicle-mediated transport, and general cellular regulation. Collectively, these findings suggest that long-term growth selection has altered satellite cell function, at least in part through changes in their secreted protein profiles, which may drive line-specific differences in satellite cell proliferation, differentiation, and ultimately muscle growth potential.

**Keywords:** satellite cell; secretome; genetic selection; tukey

### 342 Genomic analysis of avian *Salmonella* spp. using Oxford Nanopore Technologies: serotyping, genotyping, and antimicrobial resistance profiling

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The objective of this study was to characterize *Salmonella* spp. isolates from poultry environments using third-generation sequencing and to identify genotypes and antimicrobial resistance (AMR) genes of zoonotic relevance.A cross-sectional observational study was conducted using four litter samples collected from broiler production systems. The sequences were analyzed using Oxford Nanopore Technologies (ONT). Genome assembly was performed with Flye, and quality control was assessed using Minimap2, Nanoplot, and Samtools Coverage. Genotyping was conducted using multilocus sequence typing (MLST) based on seven housekeeping genes. Serotypes were identified using SeqSero2 and SISTR. AMR genes were detected using ABRicate, abriTAMR, and ResFinder v4.5.0. Results were compared with PubMLST and EnteroBase databases, and phenotypic antimicrobial susceptibility was evaluated using the disk diffusion method. Sequences included zoonotic serotypes such as *S.* Typhimurium (ST19), *S.* Infantis, and *S.* Corvallis. *S.* Infantis exhibited a multidrug-resistant profile, harboring genes including *blaCTX-M-65*, *fosA3*, *tet(A)*, *floR*, *dfrA14*, *sul1*, and *aadA1*, located on a megaplasmid, along with a mutation in the *gyrA* gene. Additionally, *S.* Corvallis carried the plasmid-mediated *qnrB19* gene. Discrepancies between genotypic and phenotypic resistance were observed, particularly for aminoglycosides, beta-lactams, and fosfomycin. These findings show that ONT sequencing, together with complementary molecular techniques, are fast, accurate, and reliable tools for the genomic characterization of *Salmonella* spp.. Combining these approaches allows not only the identification of serotypes and ge-

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netic virulence factors, but also the timely detection of antimicrobial resistance profiles, supporting continuous surveillance strategies within a One Health approach that integrates animal, human, and environmental health.

**Keywords:** Salmonella spp.; Antimicrobial resistance; Oxford Nanopore sequencing; Zoonotic pathogens; MLST

## Metabolism and Nutrition: Amino Acids

**202 (A-D) (MTCC)**

### 343 Determination of Aviagen amino acid specifications

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Amino acids and protein continue to be a cost pressure point for nutritionists in day to day formulation. The primary challenge lies in matching amino acid dietary concentrations with amino acid requirements to optimizing performance at a cost effective basis. In addition, primary breeding companies continue to focus their development strategies to enhance key traits such as feed efficiency, meat yield, robustness and reproductive parameters. Amino acid specifications play a key role in optimizing the advancement of these key performance traits. Aviagen's balanced protein (BP) amino acid profile is a critical aspect of our broiler nutrition program, focusing on the balance of essential amino acids (EAAs) to digestible lysine (dLys). With continued advancements in genetic progress, it is essential that BP be routinely evaluated to maintain alignment with the broiler's genetic potential. Recent efforts have focused on defining ideal amino acid profiles across different growth phases and recommended energy levels, including titration studies for key amino acids such as lysine, methionine, arginine, and threonine, as well as central composite design studies for branched chain amino acids. Published literature from other research groups also provides valuable reference points. Specific adjustments to any recommendations may be required depending on final product, raw material pricing and availability, processing age and live weight, carcass yield, market specific requirements, and sex separate growing strategies. Aviagen's BP profile reflects extensive internal research efforts combined with published literature and is considered an optimal amino acid profile for achieving strong biological performance that optimize margins across diverse market scenarios.

### 344 Crystalline vs Encapsulated Glycine in Low-Protein Diets: Benefits on Performance, Albumen Quality, Blood Parameters, and Nitrogen Utilization in Laying Hens

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Reducing dietary crude protein (CP) is an effective strategy to lower nitrogen (N) emissions in poultry, but it compromises production performance and egg quality due to limitations of conditionally essential amino acids (EAA) such as glycine (Gly). The research objective was to determine the effects of crystalline and encapsulated Gly in low crude protein (LCP) diets in relation to production performance, egg quality, N utilization, and physiological parameters in laying hens. A total of 280 Hy-Line Brown laying hens (44 weeks old) were randomly allotted to 5 dietary treatments having 7 replicates of 10 birds each for 8 weeks. A control diet (CON; 16.50% CP), and LCP diet (14.50% CP), further supplemented with 0.2% Gly (0.2Gly), 0.5% encapsulated Gly (0.5GlyE), and 0.4% encapsulated Gly (0.4GlyE) were formulated. Encapsulated Gly had 40% purity with levels adjusted accordingly. The standardized ileal digestible EAAs in all LCP diets were balanced. Research data were analyzed using one-way ANOVA (SAS) with Tukey's post hoc test. Results showed that the LCP diet had a significant negative effect ($P < 0.05$) on average egg weight (AEW), laying rate (LR), egg mass (EM), and feed efficiency (F:E) along with reduced albumen height (AH) and Haugh unit (HU). Supplementation of 0.2% crystalline Gly completely restored ($P < 0.05$) production performance and internal egg quality similar to the CON diet. Similarly, the 0.5GlyE and 0.4GlyE supplementation also significantly improved ($P < 0.05$) LR and EM. However, the 0.4GlyE diet exhibited better recovery of AH and HU. Moreover, the addition of Gly in LCP diet also reduced ($P < 0.05$) serum uric acid and aspartate aminotransferase, increased ($P < 0.01$) antioxidant capacity and immunoglobulin (IgG, IgA, IgM), and trypsin activity in intestinal segments. The LCP diets decreased ($P < 0.01$) N excretion by 23–24% primarily due to lower N intake, with 0.2% Gly achieving the highest (P = 0.001) apparent N digestibility. In conclusion, the findings support that Gly acts as a conditionally essential AA in LCP diet to maintain the production performance, egg quality, and physiological conditions thereby decreasing N excretion and environmental footprint.

**Keywords:** Glycine; Encapsulation; Low crude protein diets; Albumen quality; Nitrogen retention

### 345 Effect of added L-Tryptophan on the performance and mortality of turkey toms

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Tryptophan is an essential amino acid in turkeys that influences hormones, stress regulation, immune function, antioxidant status, and behavior. Dietary changes in tryptophan benefit production performance in poultry, but its effects on turkey toms specifically are understudied. We aimed to investigate the effect of 0.1% added L-tryptophan (TRP) throughout brooding and grow out in three strains of large white turkey toms. At 1 day of age, 1,008 turkey tom poults were assigned to six treatments in a 3 x 2 factorial design with three genetic strains (N=19 pens of Strain A, N=16 pens of Strain B, and N=13 pens of Strain C; 21 toms/pen) and two six-phase diets consisting of a model estimated requirement for amino acids that approximated breeder recommendations (CON; N=24 pens) or the same diet with 0.1% added L-tryptophan (TRP; N=24 pens). Toms were reared from 1 day of age to 19 wks, with diet phase changes occurring approximately every 3 wks. Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality (including culls) were recorded by feed-phase. Data were analyzed with linear models and general linear models in R Studio. There were no significant strain X diet interactions observed, but significant diet effects were observed on growth performance and cumulative mortality throughout the trial. At 10 wks, toms fed TRP were a heavier BW than toms fed CON (p=0.036; CON: 7.14 ± 0.07 kg/bird, TRP: 7.35 ± 0.07 kg/bird; mean ± SE). From 7 to 10 wks, toms fed TRP also had a higher BWG than toms fed CON (p=0.034; CON: 3.56 ± 0.04 kg/bird, TRP: 3.69 ± 0.04 kg/bird). Diet did not affect BW or BWG at any other age, and diet did not affect FI or FCR throughout the trial (p>0.05 for all). The TRP toms had lower cumulative mortality throughout the trial (p<0.001; CON: 18.1 ± 2.4%, TRP: 13.1 ± 2.3%). When mortality was assessed by phase, the TRP toms displayed lower mortality than CON toms across all phases (p=0.034). The primary cause of mortality was impaired mobility due to foot and leg abnormalities, where the TRP-fed toms notably had lower mortality the CON-fed toms (p<0.001; CON: 5.5 ± 1.1%, TRP: 2.7 ± 0.7%). In conclusion, dietary tryptophan supplementation resulted in modest improvements in growth performance but substantially reduced mortality throughout the production cycle, especially for toms with impaired mobility.

**Keywords:** turkey; mortality; body weight; tryptophan; performance

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### 346 Effects of Guanidinoacetic Acid Supplementation on Growth Performance and biomarkers of Heat-Stressed Broilers: A Meta-Analysis

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Heat stress (HS) is a major constraint to global poultry production, impairing growth performance through reduced feed intake and constrained cellular energy metabolism. Guanidinoacetic acid (GAA), the direct precursor of creatine, supports cellular energy metabolism by enhancing the creatine–phosphocreatine system responsible for maintaining high cytosolic ATP levels and may improve resilience to HS. This meta-analysis quantified the effects of dietary GAA supplementation on growth performance and selected metabolic indicators in heat-stressed broilers. A systematic literature review identified six independent peer-reviewed studies (published between 2017 and 2025). Across studies, HS was induced using ambient temperatures ranging from 32 to 36 °C with 50–65% relative humidity, applied for 6–24 h/day, predominantly during the grower-finisher period. The mean age at HS onset was 27 days, with exposure continuing until 42-44 days of age. Dietary GAA inclusion levels ranged from 0.6 to 1.2 g/kg feed. Only heat-stressed control groups without GAA supplementation were used as comparators. Meta-analysis was performed using a random-effects model with inverse-variance weighting. Effect sizes were expressed as standardized mean differences (Hedges' g). Mean effect sizes (MES) were evaluated using two-sided statistical tests. Between-study heterogeneity was modeled ($\tau^2 > 0$) and quantified using $I^2$. Publication bias was assessed using Egger's regression intercept test when more than two studies were available. GAA supplementation significantly increased average daily gain (ADG) under HS conditions (MES = 1.13; SE = 0.23; P = 0.004), with moderate heterogeneity across studies ($I^2$ = 35.0%; P = 0.174). Feed conversion ratio (FCR) was also significantly improved (MES = −1.23; SE = 0.42; P = 0.031), with substantial heterogeneity ($I^2$ = 78.9%; P < 0.001). Average daily feed intake (ADFI) was not significantly affected by GAA supplementation (MES = 0.50; SE = 0.54; P = 0.397) despite high heterogeneity among studies ($I^2$ = 88.3%; P < 0.001). Creatine showed no overall effect (MES = 0.02; SE = 0.25; P = 0.954), with no detectable heterogeneity ($I^2$ = 0.0%; P = 0.998). Uric acid levels significantly increased with GAA supplementation (MES = 4.49; SE = 1.39; P = 0.032), with very high heterogeneity ($I^2$ = 94.9%; P < 0.001). Overall, GAA supplementation at 0.6-1.2 g/kg feed significantly improved ADG and FCR in heat-stressed broilers. The ADG response showed moderate heterogeneity, indicating a relatively consistent effect across studies, whereas heterogeneity was higher for other outcomes, likely reflecting differences in the severity and design of HS challenge models, which influence the magnitude of the response.

**Keywords:** broiler; guanidinoacetic acid; heat stress; meta analysis; growth performance

### 347 Assessing Essential Amino Acid Requirements in Broiler Breeders at Peak Production Using a Refined Indicator Oxidation Approach

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Quantifying digestible essential amino acid (AA) requirements in broiler breeders at peak production requires approaches capable of capturing metabolic responses under controlled but stable intake conditions. An Indicator Amino Acid Oxidation (IAAO)-based protocol was applied to derive requirement estimates for seven indispensable AA and the total of dispensable amino acids. Twelve 30-week-old hens from a modern genetic line (CDP5) were housed individually in open-circuit respiratory chambers and adapted for 2 d to a common control diet. Birds were exposed to six dietary concentrations per AA (three deficient, three excessive) across six consecutive days in a repeated Latin-square-type arrangement, evaluating LYS, TSAA, ARG, ILE, THR, VAL, TRP, and CP (GLU), with two AA tested per cycle. Feed intake was controlled: 80% of the daily allocation was provided as thirteen 8-g meals every 30 min, and 20% once the IAAO procedure was concluded. L-[1-$^{13}$C]phenylalanine was administered as a priming dose (4.5 mg/kg BW; meal 5) followed by incremental doses (1 mg/kg BW; meals 7–13). Breath samples were collected every 30 min (13 per bird/day) and analyzed for $^{13}$CO2 enrichment. For each bird and dietary level, $\Delta^{13}$C enrichment curves were used to define baseline and plateau phases, and oxidation responses were calculated as $\Delta\Delta^{13}$C. Requirement estimates were obtained by fitting nonlinear models to oxidation responses across dietary concentrations, identifying breakpoints associated with maximal AA utilization. Two approaches to define baseline and plateau enrichment (full vs. reduced sampling) yielded comparable oxidation responses and requirement estimates (P>0.05), with prediction error <10% and strong agreement ($R^2$>0.85). Variability around estimated breakpoints was also similar between approaches (P>0.05). The applied IAAO framework enabled consistent estimation of digestible AA requirements across multiple indispensable AA in broiler breeders at peak production, supporting its use to determine AA requirements under varying physiological conditions.

**Keywords:** Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; isotope; Peak production

### 348 Optimizing broiler feed formulations across different growth phases by adjusting guanidinoacetic acid level and its energy matrix value

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Feed cost is a major driver in poultry production, with dietary energy representing the most expensive component. Guanidinoacetic acid (GAA), a precursor of creatine, enhances cellular energy efficiency and provides economic benefits through its energy sparing effects. A more comprehensive understanding of the suitable reductions in dietary energy and the optimal GAA inclusion levels across feeding phases is essential for maximizing its functional and economic benefits. Therefore, this study evaluated the economic and performance parameters of 1,500 one-day-old Ross 308 male broiler chicks raised from 1 to 42 days of age fed with different doses of GAA (600 and 1200g/t) and with apparent metabolizable energy (AME) matrix values equivalent to 50, 75 or 100 kcal/kg of feed. Birds were randomly allocated to 6 treatments with 10 replicates of 25 birds each. The experimental diets were formulated for three feeding phases: starter (1 to 10d), grower (11 to 24d), and finisher (25 to 42d). Treatments consisted of: T1) Control diet formulated according to the breeder recommendations; T2) T1 with 50, 75, and 100 kcal/kg reduction of AME in starter, grower and finisher phases, respectively; T3) as T2 + 600 g/t GAA in each phase; T4) as T3 but 1200g/t GAA in starter phase; T5) as T3 but 1200 g/t GAA in starter and grower phases; T6) as T2 + 1200 g/t GAA in each phase. Body weight (BW) and feed intake (FI) were measured on 10, 24 and 42d. Statistical differences among the treatment groups and the control group were analyzed using one-way ANOVA, followed by Dunnett's post hoc test ($p < 0.05$). No significant differences were observed between the treatment groups and the control groups for any of the performance parameters in starter phase. From 1 to 24d, BW, body weight gain (BWG), and feed conversion ratio (FCR) were significantly worse in T2 compared to the control group T1 (1.11 vs. 1.17kg; 1.07 vs. 1.13kg; and

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1.24 vs. 1.21, respectively). From 1 to 42d, no significant differences were observed among the treatment groups or the control group (T1) for FI, BW, and BWG. The FCR was significantly greater in T2 and T5 compared to T1 (1.43 and 1.44 vs. 1.41; P < 0.001) from 1 to 42d. On the economic side, T3 and T6 demonstrated a significant improvement in income over feed costs (IOFC), with T3 achieving the greatest IOFC— 5.40% greater than T1 ($2.93 USD/bird compared to $2.78 USD/bird) while T2 had an IOFC of $2.82 USD/bird. In conclusion, supplementing the feed with 600 g/t of GAA, while applying energy matrix values equivalent to 50, 75, and 100 kcal/kg feed in the starter, grower, and finisher phases respectively, effectively maintained bird performance with the best income over feed costs.

**Keywords:** feed cost; guanidinoacetic acid; metabolizable energy; profitability

**349 Early biomarker relationship to breast muscle myopathies in broilers fed arginine silicate inositol and guanidinoacetic acid**

Michael Carroll*[1], Elizabeth Bobeck[1] [1]*Iowa State University, Department of Animal Science, Ames, Iowa, United States*

Woody breast, white striping, and spaghetti meat are economically important breast muscle myopathies in broiler production. Further understanding the relationship between early biomarkers and breast muscle myopathies at market weight can give insight into the development and possible prevention of these myopathies. Supplements such as arginine silicate inositol (ASI) that increase systemic blood flow and nitric oxide production or guanidinoacetic acid (GAA) that support energy metabolism could potentially alter these early biomarkers. Therefore, a phase-specific 99.85% complete basal diet was used to formulate three experimental diets: 1) control (CON; basal + 0.15% sand), 2) basal + 0.15% arginine silicate inositol (ASI), or 3) basal + 0.12% guanidinoacetic acid (GAA) + 0.03% sand. A total of 1,560 one-day-old male Ross 708 broilers were randomly assigned to 39 floor pens (40 birds/pen, 8 x 4 ft). One bird per pen (n=13/treatment) was euthanized on d 0, 4, 7, 11, 14, and 21 for collection of serum and breast tissue to analyze protein content, lactic acid content, and gene expression. On d 49, twenty birds per treatment were euthanized for scoring woody breast, white striping, and spaghetti meat presence. All data were analyzed with a one-way ANOVA in SAS 9.4 with significance set at $P \leq 0.05$ for the effect of diet. Partial Spearman rank correlations controlling for dietary treatment were used to evaluate associations between early biomarkers and breast muscle myopathies with significance set at $|\rho| \geq 0.30$ and $P \leq 0.05$. There were no significant differences due to dietary treatment for lactic acid, protein content, or gene expression at any timepoint ($P > 0.05$). Serum lactic acid at d7 and 11 was negatively associated with woody breast ($\rho$ = -0.334 and -0.344; P = 0.0463 and 0.0398, respectively). Gene expression of *MYOD* and *MSTN* at d0 was positively associated with spaghetti meat ($\rho$ = 0.394; $P$ = 0.0159 and $\rho$ = 0.377; $P$ = 0.0214, respectively). At d4, expression of *MYOG* was positively associated with spaghetti meat ($\rho$ = 0.361; $P$ = 0.0357). By d14, expression of *MSTN* at was negatively associated with spaghetti meat ($\rho$ = -0.380; $P$ = 0.0268). Gene expression of the myogenic regulatory factors is indicative of muscle development and lactic acid may provide insight into muscle metabolism. The lack of dietary impact by ASI and GAA on the selected biomarkers sampled at early timepoints may be due to their modes of action altering other pathways or early sampling did not allow for sufficient time for them to take effect. Measurement of these biomarkers early in a broiler's life may provide data for a predictive model of breast muscle myopathies at market age.

**Keywords:** lactic acid; myogenic regulatory factors; woody breast; white striping; spaghetti meat

**350 Nutrient-specific regulation of IGF-1–mTOR axis and growth under dietary restriction in Japanese quail (*Coturnix japonica*)**

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Nutritional conditions in poultry production vary in feed quantity and nutrient, requiring birds to adjust physiological responses. We tested how quantitative and qualitative dietary restriction affect growth through the IGF-1–mTOR pathway in growing Japanese quail, and whether targeted amino acid supplementation modifies responses to restriction. A total of 56 Japanese quail were assigned to seven treatments: control (full fed), 20% feed restriction, feed restriction with leucine, methionine, or combined leucine and methionine supplementation, as well as 20% protein restriction and 20% energy restriction. Control and feed-restricted birds differed only in feed quantity, whereas amino acid-supplemented restricted diets contained 20% extra leucine, methionine, or both. Birds were housed individually under uniform controlled conditions, with treatment as the only difference. After 14 days, birds were euthanised and liver samples collected for gene expression analysis. After RNA isolation and cDNA synthesis, expression of nutrient-sensing and downstream genes (GHR, IGF1, IGF1R, MTOR, FASN, SOD2, ATG5, ATG9A) was quantified by qPCR. Expression was normalized using selected reference genes and calibrators, with amplification efficiencies ranging from 97% to 100%. Data were analyzed using R (v4.5.2). Growth trajectories were analyzed using linear mixed models. Gene expression differences were tested using linear models with Tukey-adjusted comparisons. Bootstrap-averaged Bayesian networks (R = 1000) and path analysis were used to evaluate gene–gene and gene–body weight relationships. Feed restriction reduced growth ($p = 0.002$), and energy restriction showed a comparable reduction ($p = 0.006$), while protein restriction had a smaller, non-significant effect ($p = 0.577$). Feed restriction downregulated anabolic genes, with significant reductions in IGF1 ($p = 0.025$) and MTOR ($p = 0.020$). Amino acid supplementation shifted IGF1 and MTOR expression toward control levels and reduced ATG9A upregulation ($p \leq 0.092$), although growth recovery was modest. Methionine increased SOD2 expression ($p \leq 0.049$), a key oxidative stress response gene. Energy restriction had limited effects on anabolic gene expression but increased ATG9A ($p = 0.052$). Network analysis identified MTOR as a central integrator linking anabolic and maintenance pathways to body weight. Feed and energy restriction produced similar growth suppression but distinct molecular responses. Amino acid supplementation partially modified nutrient-sensing pathways without fully restoring growth. These findings indicate that nutrient-specific constraints differentially regulate growth and maintenance through the IGF-1–mTOR pathway.

**Keywords:** dietary restriction; amino acids; IGF-1–mTOR signalling; Gene expression; Japanese quail

# SYMPOSIA PRESENTATIONS

## Friday, July 10, 2026

### Metabolism and Nutrition Symposium: Understanding Supply Chain and Quality of Ingredients in the Modern Era

**John W. H. Bassett Theatre (MTCC)**

**1000S** *Insights into the changing landscape of supply chains in poul-*

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*try production and meat markets*

Roy D. Brister*[12] [1]*Texas A&M University, Poultry Science, Springdale, Arkansas, United States;* [2]*Alltech, Nicholasville, Kentucky, United States*

Meat production and consumption are constantly evolving and are influenced by global forces. In this session, we will explore the changing landscape through a SWOT analysis (Strengths, Weaknesses, Opportunities and Threats) of meat and feed production, examining how these factors influence markets and what stakeholders should consider staying prepared. As we bring together poultry industry experts, industry liaisons, suppliers and researchers, we will discuss how we can collaborate to meet demand in a cost-effective and sustainable way.

**Keywords:** supply chain; poultry production; meat markets

**1001S Corn, SBM, Wheat (Macro** *Ingredients); Global challenges and opportunities due to the supply chain*

Roshan Adhikari*[1], Tim Albright[1], Jason T. Lee[1] [1]*CJ Bio America Inc, Downers Grove, Illinois, United States*

The global feed industry has entered a new era where geopolitical shifts and supply chain volatility—rather than simple market forces—dictate trade flows and production costs. This presentation provides a comprehensive analysis of the global landscape for macro ingredients, such as corn, soybean meal (SBM), wheat. This talk provides a bird's-eye view of the global macro ingredient market, comparing the scenarios and trends between traditional exporters like the USA and the expanding influence of Brazil and Argentina, alongside shifting demand across Europe, Asia, and Africa. The talk will examine how current trade barriers, maritime disruptions, regional quality variations, regional conflicts, and fluctuating input costs (such as urea and energy) impact the price and global demand of raw materials. Beyond simple supply-and-demand metrics, we will analyze how growing conditions and soil health in the USA versus Brazil and Argentina result in divergent amino acid profiles, energy values, and mineral content. We will discuss how these regional differences—coupled with trade barriers and maritime disruptions—complicate formulation strategies. We will discuss practical strategies for nutritionists and purchasing teams to move from reactive buying to proactive decision-making. Attendees will engage in a forward-looking discussion on bridging the gap between "pen trials" and commercial reality in an increasingly fragmented global market. This talk serves as a brainstorming platform for industry professionals to rethink supply chain management through the lens of both economic reality and nutritional performance.

**Keywords:** Macro ingredients; feed; supply chain; nutritional quality; global market

**1002S Assessing the potential effects of vitamin and amino acid supply chain disruptions on U.S. poultry production and food security**

Yuan-Tai Hung*[12], Spencer Parkinson[3], Dave Miller[3], Philip Lobo[4], Lara Moody[1] [1]*Institute for feed education and research, Arlington, Virginia, United States;* [2]*American feed industry association, Arlington, Virginia, United States;* [3]*Decision Innovation Solutions, LLC, Urbandale, Iowa, United States;* [4]*Lobo Consulting Solutions, LLC, Ballwin, Missouri, United States*

The U.S. poultry industry provides an affordable and nutritious source of animal protein, producing over 21 million metric tons (MMT) of broilers, 2 MMT of turkeys and 7.5 billion dozen of table eggs annually. This impressive production is achieved through genetic improvement, nutrition and supply of quality feed (approximately 81.6 MMT of poultry feed consumed annually). Because quality feed is the key driver of poultry production, steady access to feed ingredients, particularly those that are sourced internationally, is extremely important for industry viability. This study evaluated the supply chain of vitamins and amino acids (AAs) and quantified the potential impacts of supply disruptions on U.S. poultry production. Global supply and trade data were summarized. Representative poultry diets (RIDs) were formulated to estimate performance and productivity impacts, assuming a linear response with 20% dietary shortage of vitamins and AAs as references. Results indicated that China is the dominant supplier for both vitamins and AAs globally. In 2024, China accounted for 99%, 87%, 89% and 64% of U.S. imported lysine, methionine, threonine and tryptophan, respectively. China also supplied an average of 76.5% of U.S. vitamin imports, including vitamins A, D, E, C and B vitamins during 2020–2024. Simulated performance and productivity were negatively affected by limiting supplemental vitamins and AAs under scenarios of disruptions. Limiting supplemental methionine in broiler and layer RIDs (38.31% and 49.10% dietary short, respectively) reduced daily gain by 43% and egg production by 17%, respectively. Limiting supplemental vitamins weakens bird welfare in addition to performance impacts. Limiting supplemental vitamin A in broiler and turkey RIDs (39.38% and 67.72% dietary short, respectively) increased the incidence of food pad lesions to 11% in broilers and 23% in turkeys. Other supplemental nutrients (e.g., lysine, threonine, B vitamins and vitamin D) could be short in RIDs, thereby decreasing poultry production efficiency. While diet reformulations partially balance some supplemental nutrients under restricted availability, they increase diet costs and cannot balance all supplemental nutrients, such as vitamins B2 and D. The U.S. dependency on imported vitamins and AAs jeopardizes the ability of U.S. poultry production and its downstream sectors to provide accessible, nutrient-rich protein.

**Keywords:** Feed; Ingredients; Micronutrients; Supplements

**Break/Poster Viewing**

**1003S Practical Management of Ingredient Variability Across Regions in Broiler Breeder Nutrition**

Antonio Beitia*[1], Justina Caldas[1] [1]*Aviagen, Huntsville, Alabama, United States*

Broiler breeder performance depends on the consistent delivery of nutrients, yet this consistency is challenged by variability in global and regional ingredient supply. Nutritionists operating across the Americas formulate with common major raw materials such as corn, soybean meal, and plant and animal byproducts, with variability, cost, and availability depending on origin, season, and processing conditions. For example, metabolizable energy in corn can vary by 100–150 kcal/kg depending on origin and growing conditions, while soybean meal crude protein ranges from 44 to 48%, with lysine digestibility influenced by processing. These differences are observed in commercial production systems and require practical adjustments in formulation strategies, directly influencing growth, body composition, egg production, fertility, hatchability, and overall productivity (Zuidhof et al., 2017; Arrazola et al., 2019). Ingredient variability arises from differences in climate, agronomic practices, storage, and processing, resulting in fluctuations in energy, amino acid digestibility, fiber, and anti-nutritional factors. While the use of diverse, regional available ingredients improves cost competitiveness and supply flexibility, it increases the complexity of maintaining nutritional consistency. In commercial settings, the nutritionist operates as a risk manager, balancing ingredient cost, variability, and performance outcomes. In broiler breeders, where feed intake is controlled, small inconsistencies in nutrient composition can result in disproportionate biological responses. Variability in nutrient supply can impair body weight, metabolic efficiency, and reproductive performance, while reducing persistency of lay. Consequently, managing finished feed variability becomes as critical as ingredient selection itself, since birds respond to what is delivered rather than what is formulated (Rubio et al., 2023; Gous, 2018). Minimizing variability requires a understanding of regional raw materials and their limitations, supported by quality control systems. In practice, this in-

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cludes continuous evaluation of ingredient sources, adjustment of matrix values, and strategic use of alternative ingredients based on availability and cost. Routine analytical evaluation including proximate composition, digestible nutrients, and contaminant screening is essential to ensure accurate nutritional matrices. Tools such as NIRS and dynamic formulation systems enable adjustments to ingredient variation. Ultimately, broiler breeder performance is defined not by the diet on paper, but by the consistency of nutrients delivered to the bird, making variability control central to achieving optimal performance and predictable outcomes.

**Keywords:** Broiler breeder nutrition ; Nutrient consistency ; Ingredient variability ; Feed formulation ; Broiler breeder performance

### 1004S Feed mill strategies to mitigate supply chain disruptions: implications for efficiency, pellet quality, and nutritional integrity

Wilmer J. Pacheco*[1], Jose I. Vargas[1] [1]*Auburn University, Poultry Science, Auburn, Alabama, United States*

Global supply chain disruptions are redefining feed manufacturing by increasing ingredient cost volatility, limiting raw material availability, and introducing greater variability in ingredient quality. These constraints directly challenge feed mill efficiency, pellet quality, and the ability to deliver consistent nutritional value to the birds. As a result, feed mills must move beyond traditional practices and adopt more dynamic, integrated strategies to sustain bird performance and overall economic efficiency. This presentation will provide a comprehensive overview of how changes in the supply chain influence feed mill operations, including grinding, mixing, and pelleting. Emphasis will be placed on the pelleting process, where changes in particle size distribution, fiber content, moisture, and fat inclusion can significantly impact conditioning efficiency, pellet durability (PDI), fines generation, and throughput. Therefore, targeted adjustments in processing conditions such as conditioning temperature and retention time, steam pressure, and die specifications (e.g., compression ratio and open area) will be discussed to maintain pellet quality and pelleting throughput. In addition, examples from commercial and research settings will demonstrate how feed mills can proactively manage variability while maintaining consistency in feed production. A key message of this talk is that feed mills can no longer treat formulation and processing as independent systems. Instead, resilience to supply chain disruptions depends on aligning nutritional strategies with process optimization. Practical tools including ingredient characterization, mixer uniformity testing, and pellet quality monitoring, will be presented as critical components for decision-making under variable conditions. By integrating nutrition, processing variables, and quality assurance, feed mills can mitigate the negative impacts of supply chain disruptions, maintain operational efficiency, and consistently produce high-quality feed that supports the genetic potential of birds. This systems-based approach provides a framework for improving both biological performance and economic outcomes in an increasingly uncertain global supply environment.

**Keywords:** feed manufacturing ; supply chain disruptions; pellet quality; process optimization ; nutrient availability

**Panel Discussion**

## Metabolism and Nutrition: Amino AcidsInformal Nutrition Symposium: Care and Feeding of Modern Broiler and Layer Breeders

**John W. H. Bassett Theatre (MTCC)**

### 1005S Symposium Introduction

Roselina Angel*[2], Doug Korver[13] [1]*Alpine Poultry Nutrition, Inc., Edmonton, Alberta, Canada;* [2]*University of Maryland, College Park, Maryland, United States;* [3]*University of Alberta, Edmonton, Alberta, Canada*

Advances in broiler chicken and laying hen productivity have been accomplished through genetic selection over many generations. The achievement of 500 eggs per laying hen to 100 weeks of age is an amazing feat, but what are the impacts on how modern layer breeders should be fed and managed? In broilers, the primary focus has been on growth rate, breast muscle yield and feed efficiency. Although other traits such as overall health, welfare and reproduction are also part of breeding decisions, the consequences for broiler breeders have been dramatic. A reduction in body fat prior to sexual maturation, the increased degree of feed restriction relative to ad libitum consumption, and a reduction in fertility have created new challenges for breeder managers and nutritionists.

**Keywords:** Broiler breeders; layer breeders; precision feeding; fertility; hatchability

### 1006S State of the Art in Layer Breeder Nutrition and Management

Xabier Arbe*[1], Emilio Scappaticcio[1] [1]*H&N International, Cuxhaven, Germany*

**Objective** The objective of this presentation is to describe recent advances in layer breeder nutrition and management that contribute to extended productive cycles, increased hatching egg output, and improved day old pullet quality, with emphasis on housing systems and early production nutrition strategies. **Description of the production**Layer breeder performance has significantly improved in recent years, with productive cycles extending beyond 75 weeks and hatching egg output frequently exceeding 110 day old pullets per female breeder. While genetic progress is a major driver, practical challenges remain related to flock size, housing systems, male fertility, and nutritional strategies during rearing and onset of production. **Updates we can apply and new feed for layer breedersHousing**: large scale commercial layer breeder farms are adopting family cage systems. The benefits in productivity are significant however the male fertility could be affected if the number of females per cage unit are below 40. **Adjustments in energy needs:** Producers who have moved to cages have different nutritional requirements because flock activity decreases. Diets must be reformulated and the energy to lysine ratio adjusted. **Implementation of hybrid feed in parent stock:** Hybrid feed, developed by H&N International for layers, aims to maintain feed intake development during start of production, support body weight growth, improve bone mineralization, and increase egg size faster than current standards. A trial was conducted in Italy using Brown Nick parent stock. Birds were managed according to standard body weight targets during rearing, with controlled lighting programs. **Results**The birds receiving hybrid feed exhibited a significantly faster increase in egg size compared with standard expectations. Despite a slight delay in light stimulation, egg production increased rapidly, and total hatching egg output improved, resulting in at least six additional day old pullets per hen. Larger early production eggs also produced heavier chicks with improved early viability. **Conclusions**Recent progress in layer breeder productivity is driven not only by genetics but also by optimized housing systems and targeted nutritional strategies during rearing and early production. Family cage housing and hybrid feed implementation can enhance egg size development, hatching egg output, and chick quality when properly managed. Future improvements will require continued integration of commercial layer knowledge into layer breeder management to support longer productive cycles as the direct research in layer breeders seems difficult to happen.

**Keywords:** Layer breeders; Housing; Hybrid feed; Egg size

### 1007S Optimizing nutrition and feeding strategies for female broiler breeders today

Shivaram K. Rao*[1] [1]*Cobb Genetics, Atlanta, Georgia, United States*

This symposium presentation aims to briefly explain how broiler genetic companies determine nutrition and feeding recommendations for their

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various genetic lines and to provide an overview of current nutrition and feeding practices for broiler-breeder hens. The nutrition and feeding plan (NFP) for a female broiler-breeder flock of a specific genetic line is tailored to the flock's life-cycle body weight profile. It includes different feeds used and their daily feed allocation schedule throughout the female breeder's life cycle, along with the nutrient specifications for each feed. The NFP is designed to achieve targeted body weight gains and age-specific body compositions of skeletal tissue, muscle, fat, and feathers. Meeting these body weight and body composition targets is essential for optimizing performance. Until the mid-1990s, NFPs for breeder hens across different genetic lines remained quite similar, with only minor variations; however, over the last thirty years, these plans have started to diverge. This divergence occurs because genetic companies develop broilers suited for different markets, such as those with high appetite and rapid growth, and others with high breast meat yield. With at least two major genetic companies and multiple lines within each, producers now face the challenge of managing various broiler-breeder NFPs. This presentation, instead of reviewing the NFPs of different genetic lines one by one, offers a quick overview of how nutrition and feed allocation are planned for the seven key stages of a breeder hen's life. These stages generally involve important, sometimes minor, changes in the objectives for nutrient requirements and feeding strategies. The seven stages or phases include: 1 to 6 weeks of age (WOA), 7 to 14 WOA, 15 to photo-stimulation (PS, around 21 WOA), PS to 5% egg production, from 5% to peak egg production, from peak production to 45 WOA, and finally from 46 WOA to depletion. For each stage, differences in nutrition and feeding among genetic lines are explained, along with the reasons for these differences. Understanding how to plan nutrition and feeding by stage makes it easier to optimize the FNP throughout a genetic line's entire life cycle and helps managers fine-tune or adapt it to their specific field conditions.

**Keywords:** Broiler-breeder hen; female breeder; nutrition; feeding plan; body composition

### 1008S New Feeding Strategies for Male Breeders

Justina Caldas*[1] [1]*Aviagen, Global Technical Team, Huntsville, Alabama, United States*

Feeding modern male broiler breeders presents a complex challenge due to improved growth efficiency and strong feeding motivation, which intensify competition and make it difficult to maintain uniformity. Thus, optimal feeder space, appropriate feed volumes, and rapid feed delivery (< 3 minutes) are crucial for uniform flock development. At the same time, a decline in fertility and hatchability has been observed across the USA industry over the past five years. This multifactorial issue highlights two key contributors: poor uniformity during rearing and excessive breast fleshing during production. Grading has become common in the USA in the past 2–3 years, allowing partition of males by body weight to reduce competition and improve feeding strategies. Most companies use a two or three-way grading, meaning the roosters are divided into 2 or 3 groups. Also, this practice is typically performed twice at 4 and 8 weeks. Before grading, larger roosters consume more feed and maintain that advantage. After grading, however, they receive lower incremental feed increases, while smaller males receive higher weekly increases to recover body weight. By the next grading, feed allocation between groups becomes more similar, aiming to improve uniformity within a short period of time. It's advisable to start grading as early as possible to recover the males sooner. Outside the USA, grading is extensively used and it's more complex. In addition to uniformity, controlling male body composition is also critical. Controlling male breast yield, also called breast fleshing, has become increasingly important. As the parent stock of broilers, roosters have the potential to achieve higher levels of breast meat yield if feed is not properly managed. While broilers at 10 lb (4.5 kg) can achieve 30% breast meat yield, to maintain good reproductive fitness, breeder males must maintain a fleshing score between 2–4 during their reproductive life (25–65 weeks, typically 2–3 for 25–40 weeks, and 3–4 up to 65 weeks). These fleshing scores correspond to 15–25% breast meat yield for similar weights as the broilers. Roosters with score 1 and 5 should be removed from the flock due to impaired mating ability. A separate male feeding with lower protein (12–13%), lower digestible lysine (0.35–0.40%) than a female breeder feed provides an opportunity to control fleshing and support fertility in males. This feed is a tool that needs to be managed by feed volume at the proper stages of production. Together, early grading and targeted male nutrition represent a practical approach to improve uniformity, control body composition, and support reproductive performance in modern broiler breeder males.

**Keywords:** male broiler breeders ; grading; breast fleshing; hatchability; efficiency

**Awards**

**Break/Poster Viewing**

### 1009S What precision feeding has revealed about changes to nutritional requirements for sexual maturation in broiler breeders

Martin Zuidhof*[1] [1]*University of Alberta, Edmonton, Alberta, Canada*

Broiler breeder sexual maturation has historically been viewed as a coordinated response to photoperiod, age, body weight, feed allocation, and body composition. Earlier literature emphasized that, unlike laying hens, broiler breeders required photostimulation after controlled rearing to initiate puberty and lay. Studies showed that lighting program, photostimulation age, and body weight at photostimulation influenced first egg, ovarian development, egg size, and reproductive performance. However, light was not an independent switch. Pullets had to be prepared to respond, and target body weight became a readiness proxy. Precision feeding has refined this interpretation by allowing individual body weight control in group-housed birds, reducing variation that confounded effects of body weight, age, nutrition, and photostimulation. These studies indicate that body weight alone is an incomplete readiness indicator. Some pullets managed to recommended body weights have shown delayed or reduced reproductive performance, implying that body composition and metabolic status are also important. Conversely, broiler breeders fed ad libitum, or much more than conventional restriction, have matured before photostimulation. This challenges the older view that breeders cannot enter puberty without a photoperiodic cue and supports a dual-control model: photostimulation synchronizes reproductive development, while nutritional and metabolic status can activate the reproductive axis when restriction is relaxed. Genetic selection has intensified this issue. Modern broiler breeders have been selected for rapid growth, feed efficiency, and breast muscle yield. As broiler traits advanced, feed restriction became more severe to control obesity, skeletal problems, and follicular recruitment. One unintended consequence may be that conventional programs produce pullets that meet target body weight but remain too lean, or too metabolically constrained, for sexual maturation. Precision feeding has revealed that uniformity and target body weight are not enough; birds must reach a developmental state. Thus, nutrient requirements should be reframed as requirements to achieve both target growth and reproductive body composition at the correct age. Practical strategies include earlier pubertal growth, increased late-rearing energy intake, higher energy-to-protein ratios, and phase-specific diets that support controlled lipid deposition.

**Keywords:** Broiler breeders; Precision Livestock Farming; Sexual maturation; Reproduction; Body composition

### 1010S Impact nutrition and management on fertility

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*Wageningen, Netherlands*

Poultry meat remains the most important animal protein source worldwide, and the rapid growth of broiler production places increasing pressure on broiler breeder management. Because a relatively small breeder population produces all commercial broilers, optimal management of nutrition, housing, and reproduction is essential. Ongoing genetic progress has increased growth potential in both broilers and their parent stock, making it more challenging to maintain welfare, reproductive performance, and egg quality. Lowering dietary protein levels during the rearing and laying phase has been studied as a strategy to reduce the negative welfare effects of feed restriction. Diet dilution increases feed volume and reduces stereotypic object pecking behavior. Besides that, studies also show that lower daily protein intake increases abdominal fat reserves, which may positively influence egg production and offspring performance. These findings highlight the importance of body composition, particularly adequate fat deposition, in reproductive success. Feeding strategies during lay also affect breeder performance. Twice a day feeding improves behavior and increases mating activity, while split feeding (using different morning and afternoon diets) can enhance egg production with no effects on eggshell quality. Increased late day activity due to the different feeding pattern may support fertility. Field observations confirm higher voluntary mating rates during the second feeding moment in the afternoon. Male-to-female ratio management is another key factor. Commercial flocks often show excessive mating pressure, leading to injuries, fearfulness, and reduced female availability on the litter. Research suggests that starting a flock with fewer males (4–5%) and adding small number of males later in the laying period improves voluntary matings, feather cover, and fertility. This approach compensates for the natural decline in male reproductive capacity over time. Finally, breeder house design influences mating behavior and fertility. Systems with a central slatted floor and litter areas on both sides provide more space for males, reduce aggressive interactions, and result in fewer floor eggs. Because most matings (>95%) occur on litter, lower male density and better spatial separation support higher fertility. In contrast, central litter floor systems allow females to hide on slats behind the nests boxes, reducing mating opportunities. Together, these findings demonstrate that integrated nutritional strategies, feeding schedules, male management, and housing design are essential to optimize welfare, fertility, and reproductive efficiency in modern broiler breeders.

**Keywords:** broiler breeder; nutrition; management; fertility

### 1011S Practical Considerations when Feeding Broiler Breeders

Benton Hudson*[1] [1]*CSA Animal Nutrition, Hanceville, Alabama, United States*

Managing and feeding broiler breeders has become more challenging over the last 10-20 years. Many attribute this continued challenge to the genetic progress that has been made with broilers, inadvertently causing pullets and breeders to have stronger appetites and the potential for rapid or excessive growth. To deal with the shifting behavior, greater emphasis has been placed on providing sufficient feeder space, achieving rapid feed delivery, and ensuring all birds can access feed simultaneously. Ensuring broiler breeders meet their daily nutrient intake requirements is essential for achieving optimal flock performance and reproductive outcomes. Three major factors influence whether these needs are met: (1) the nutrient concentrations of the diets, (2) the feed allotments determined by Service Technicians, and (3) the uniform distribution of feed to individual birds within a house or pen. Because each of these components affect actual nutrient intake, strong communication among nutritionists, production teams, and growers is critical to ensuring birds consume what is intended. For slow feathering breeds, monitoring feather condition and flock behavior has become a practical tool. Feeding relatively low levels of nutrients that are linked to poor feather condition or adverse behavior is not worth the risk. Feather coverage may also be correlated to reproductive performance, making it a valuable indicator when evaluating feeding programs.

**Keywords:** Broiler breeder; Feed distribution; Nutrition

**Panel Discussion**

## Processing and Products Symposium: Poultry in Pet Food: Meeting Market Demands Though Science and Innovation

**105 Constitution Hall (MTCC)**

### 1012S From Bird to Bowl: Translating Poultry Meat Ingredients into High-Performing Pet Food Products

Leah Lambrakis*[1] [1]*Simmons Foods, Toronto, Ontario, Canada*

Poultry ingredients are foundational to modern pet food systems, contributing nutritional value, functional versatility, palatability, and cost competitiveness across a wide range of product formats. Their successful translation into high-performing pet food products depends not only on nutrient composition, but on how ingredient form, freshness, structural characteristics, and processing conditions interact to influence final product quality, sensory performance, and manufacturing consistency. This presentation examines how different poultry ingredient forms behave within pet food applications and how those differences shape formulation strategy and process design. Variability in macronutrient composition, fresh or frozen forms, particle size, connective tissue, and moisture content influences processing behavior, texture development, and finished product stability, particularly in wet pet food systems where product structure and appearance strongly affect consumer acceptance. Particular attention will be given to ingredient freshness as an important determinant of product performance. Recent work evaluating poultry raw material shelf life demonstrated that aged poultry inputs were associated with elevated microbial load, increased biogenic amine formation, lipid oxidation, and altered volatile compound development, all of which influenced finished product sensory characteristics. In paired palatability evaluations, feline subjects showed a preference for diets produced with fresher poultry ingredients, highlighting the downstream impact of raw material quality on palatability and feeding response. The presentation will also explore how processing shapes the relationship between poultry ingredient quality and finished product performance, helping to explain why ingredient quality alone does not guarantee product success. By connecting poultry ingredient characteristics to formulation behavior, process capability, palatability, and market performance, this session offers a working framework on how poultry science enables innovation and value creation in pet food.

**Keywords:** poultry ingredients; pet food; ingredient freshness; palatability; quality

### 1013S How pet food marketing trends impact food safety

Shannon Harrison*[1] [1]*Simmons Foods, Siloam Springs, Arkansas, United States*

The continued growth and competitiveness of the pet food industry have driven innovation in formulation and marketing, largely influenced by pet humanization and the perception of pets as family members. This trend has produced marketing claims aligned with human food health concerns, despite fundamental differences between human and pet nutritional needs. Pet food is regulated by the U.S. Food and Drug Administration under the Food Safety Modernization Act (FSMA) and 21 CFR Part 507, requiring implementation of Hazard Analysis and Risk-Based Preventive Controls (HARPC). While pet and human food production share similarities in processing, sanitation, and foreign material control, pet food presents unique risks related to ingredient sourcing, nutrient bal-

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ance, and intended use. Poultry byproducts, which are key ingredients in many pet food formulations, introduce additional safety and quality considerations. Because these materials originate as secondary or waste streams from poultry processing, maintaining raw material nutritional consistency is critical yet challenging. Variability in composition can directly affect nutrient balance, while the risk of foreign material contamination is increased because the materials used in pet food are sourced from waste streams in poultry processing. In addition, the risk of Highly Pathogenic Avian Influenza (HPAI) requires robust supplier verification, validated lethality steps, and strict environmental controls. This review outlines key food safety risks associated with ingredient variability, formulation complexity, and processing controls, with emphasis on nutrient degradation, microbial contamination, batching errors, and foreign material hazards. It further evaluates how marketing trends including organic, limited ingredient diets, raw diets, clean label initiatives, label modernization, and human-grade claims alter risk profiles and regulatory expectations. In particular, raw diets pose a disproportionately high food safety risk, accounting for a significant share of pet food recalls despite limited market penetration, largely due to pathogen hazards such as *Salmonella*, *Listeria monocytogenes*, and emerging risks including Highly Pathogenic Avian Influenza (HPAI). While marketing claims shape consumer perception, they must be scientifically justified and evaluated through a comprehensive food safety lens. Effective risk management requires alignment between upstream animal protein suppliers, pet food manufacturers, and regulatory requirements to ensure nutritional adequacy, product safety, and protection of both pets and pet parents.

**Keywords:** Marketing; Food Safety; Pet Food

### 1014S Quality of Poultry Meat Ingredients for Pet Food Processing: Impact on Final Product Stability and Acceptability

B.J. Bench*[1] [1]*Tyson Foods, Inc., Ingredient Solutions Group, Springdale, Arkansas, United States*

The pet food industry continues to expand, driving increased demand for high-quality, consistent, and nutritionally robust ingredients. Poultry-derived materials—including rendered protein meals, fresh and frozen meats, and other wet ingredient streams—serve as foundational inputs across a wide range of pet food applications. As utilization of these diverse inputs increases, understanding the factors that influence quality during the transition from raw materials to ingredients is increasingly important. The quality of poultry-based ingredients is fundamentally established by the condition and freshness of incoming raw materials. Variability in raw material age, composition, and time–temperature exposure initiates degradation pathways prior to further processing, regardless of whether materials are directed toward rendering systems or fresh and frozen product streams. These early-stage conditions define the baseline for ingredient quality and introduce variability that persists through subsequent handling and processing. Both lipid oxidation and protein degradation begin at the raw material stage and evolve as a function of handling, storage, and processing conditions. Lipid oxidation progresses through primary and secondary products, while protein degradation alters structural integrity, reactivity, and amino acid availability. These concurrent and interdependent pathways influence key quality attributes of poultry-derived ingredients, including compositional consistency, oxidative status, and functional characteristics. Current industry practices often rely on discrete analytical measurements, such as proximate composition or isolated indicators of oxidative status, to evaluate ingredient quality. While informative, these approaches do not fully capture the dynamic and interconnected nature of degradation processes across varying raw material conditions and ingredient types. This presentation will examine the relationship between raw material freshness and the development of poultry-derived ingredients across both rendered and fresh/frozen product streams. Emphasis will be placed on how early-stage degradation mechanisms influence ingredient composition, oxidative status, and functional properties, as well as the limitations of commonly used analytical approaches. The findings highlight the need to prioritize raw material management and adopt multi-dimensional quality frameworks to improve the consistency and predictability of poultry-derived ingredients used in pet food applications.

**Keywords:** lipid oxidation; protein degradation; petfood; analytical testing; quality

**Break/Poster Viewing**

**Panel Discussion**

## Saturday, July 11, 2026

### Immunology, Health and Disease Symposium: Inter-Generational Nutrition and Gut Health

**John W. H. Bassett Theatre (MTCC)**

### 1015S Impact of breeder nutrition on progeny performance

Martin Zuidhof*[1] [1]*University of Alberta, Edmonton, Alberta, Canada*

Research on broiler breeder nutrition and progeny performance began with studies testing whether maternal dietary energy and protein changed broiler growth and carcass composition. Early work showed that higher breeder energy intake could increase male progeny growth, increase carcass protein, reduce carcass fat, and reduce carcass downgrades, whereas responses to maternal protein were less consistent and often depended on offspring sex. Later studies with modern breeders showed that maternal energy, protein, energy:protein ratio, amino acid balance, breeder age, and breeder growth trajectory can influence economically important progeny traits such as chick quality, early growth, market BW, feed intake, efficiency, livability, carcass, breast meat and abdominal fat yield, and meat quality. Important intergenerational effects have also been reported for essential amino acids, vitamins, minerals and antioxidants. These nutrients have been studied mainly for effects on embryonic survival, chick robustness, immune and antioxidant status, skeletal development, muscle growth, breast meat yield, and meat quality. Across studies, the most consistent pattern is that progeny responses are strongest when maternal nutrition changes breeder body composition, egg size, or egg nutrient deposition, rather than simply increasing or decreasing breeder nutrient intake. Higher breeder energy:protein ratios have sometimes reduced hatch weight but increased posthatch growth, feed intake, market weight, and feed efficiency. In contrast, excessive dilution of rearing diets has reduced progeny BW and carcass yield in some studies. These inconsistencies suggest that maternal nutrition must be interpreted in relation to breeder age, body composition, egg composition, offspring sex, and timing of the nutritional intervention. This research is challenging because treatments are applied to hens, but responses are expressed after a considerable time lag in eggs, embryos, and chicks. Thus, small maternal effects can be masked by variation in hatchery and grow-out contexts. The critical implication is that breeder nutrition should be optimized for total system output, not breeder reproduction alone. Promising nutritional strategies include phase-specific amino acid and energy programs, breeder-age-specific vitamin D and trace mineral supplementation, precision feeding to body composition targets, and using egg composition as an indicator of progeny performance potential.

**Keywords:** Maternal nutrition; Offspring performance; Supply chain optimization

### 1016S Developmental programming and in ovo strategies in broiler

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**chickens**

Eugeni Roura*[1] [1]*The University of Queensland, Nutrition and Chemosensory Group, Centre for Animal Science, Brisbane, Queensland, Australia*

The neonatal period is the most critical phase in the life of a chicken; in the initial days post-hatch, up to 5% of birds may perish or exhibit suboptimal performance characterized by heightened disease susceptibility. A primary determinant of this early-life robustness is embryonic development. While substantial expertise exists regarding the optimization of maternal diets for reproductive performance, specific metrics of embryonic development -beyond hatchability- remain difficult to quantify and are frequently overlooked in breeder diet formulation. Furthermore, feed-restriction programs in breeders can compromise the nutritional status of the embryo, resulting in depleted nutrient stores in the yolk, liver, and muscle. Conversely, optimizing embryonic development offers a significant opportunity to accelerate the maturation of the gastrointestinal and immune systems. This can be achieved by enhancing the mobilization of albumen and yolk nutrients or through in ovo feeding (IOF). Recent advances in our group have focused on the in ovo delivery of plant-derived functional compounds with the potential to improve embryonic development. Like primary nutrients, essential oils (EOs) and plant extracts can be transferred into egg compartments to modulate metabolic events. The in ovo application of carvacrol —the primary bioactive in oregano and thyme— has demonstrated potent immunomodulatory activity, upregulating the gene expression of key cytokines, chemokines, and their receptors immediately post-hatch. Furthermore, low doses of in ovo carvacrol may enhance lipid metabolism during late-stage incubation. However, a narrow therapeutic window exists; high doses elicit a stress response, characterized by the elevated expression of detoxification enzymes and altered lipid metabolism in the jejunum. Our systematic testing of 27 EOs suggests that precise in ovo delivery can significantly upregulate biomarkers for gut integrity, digestive enzyme activity, and nutrient transport. In conclusion, IOF interventions with functional plant compounds show high potential for improving embryonic development to increase nutrient utilization, intestinal barrier function, and systemic immunity in broiler chickens.

**Keywords:** in ovo; broiler chickens; essential oils; immune system; gastrointestinal tract

**1017S Vertical Transmission of Microbiota and Pathogens**

Lisa R. Bielke*[1], Thaciane L. Amaral[1], Tracy Omasanuwa[1], Muhammad Wajeeh Ul Hassan[1] [1]*North Carolina State University, Raleigh, North Carolina, United States*

Initial intestinal microbial colonization has a major influence on early animal health, making the intergenerational transmission of microbiota from primary breeders to production flocks of utmost importance to efficient growth. Hatchery-acquired bacteria influence microbial colonization, starting quality, and health of young poultry. Previous work has shown that pioneer colonizing bacteria, altered during the hatching process, can influence immune development, response to infection, and colonization by opportunistic pathogens. Specifically, exposure to Enterobacteriaceae resulted in measurement of more inflammatory pathways ($p<0.05$) with a decreased response to inflammation ($p<0.05$), whereas probiotic-exposed chicks expressed more appropriate response to inflammatory processes. Further experiments revealed that this was accompanied by differences in response to colonization by *Clostridium perfringens* after inoculation with *E. coli*, and that ducklings exposed to probiotics during the hatching process possessed fewer bacteria that fit within potential pathogen classifications such as Proteobacteria and Enterobacteriaceae. Additionally, intergenerational bacteria have been shown to affect malleability of pioneer colonizing bacteria with parent-flock source associated with differences ($p<0.05$) in beta-diversity changes associated with hatchery-applied probiotics in ducklings. Microbiota of ducklings from parent flocks that transmitted more Proteobacteria to offspring did not respond ($p<0.05$) to probiotic treatment at the same extent ($p<0.05$) as those from parent flocks that transmitted fewer potentially pathogenic bacteria. More recent studies investigating the role of parental transmission of *Enterococcus cecorum* to chicks verifies that vertical transmission of pathogens is a substantial concern for control of diseases that affect young birds. Correlation analysis mapped the small intestine as a major source of bacteria to the eggshell and gastrointestinal tract of neonatal chicks. Intergenerational transfer of bacteria to poultry plays a major role in the health of flocks, making breeder level application of probiotics and control of pathogens an important aspect of efficient and sustainable production systems.

**Keywords:** Enterococcus; probiotics; pathogens; microbiome; inflammation

**1018S Post-hatch: low crude protein strategies in the starter period**

Shemil Macelline*[1] [1]*The University of Sydney, School of Life and Environmental Sciences , Brownlow Hill, New South Wales, Australia*

Growth performance during the starter phase is critical for achieving target performance at market age in broiler chickens. Low crude protein (CP) diets are gaining interest for sustainable chicken meat production, especially in regions with limited soybean cultivation. However, reducing CP in starter diets may be detrimental, and providing standard-CP diets during this phase may be a safer strategy when low-CP diets are intended for subsequent feeding phases. This strategy was evaluated in a study at the Poultry Research Foundation. A total of 1,000 Ross off-sex male broilers were allocated into five dietary treatments (8 replicates; 25 birds/replicate) with different dietary-CP levels in the starter (S) and grower phases (G). T1 (S23%, G21.5%), T2 (S22%, G20.5%), T3 (S21%, G19.5%), T4 (S23%, G20.5%), T5(S23%, G19.5%). The starter and grower phases concluded at 10 and 24 days post-hatch, respectively. The data were subjected to analysis by One-Way ANOVA. In the starter phase, dietary-CP influenced weight gain ($P < 0.001$), feed intake ($P < 0.001$), and FCR ($P = 0.010$). Reducing dietary-CP from 23% to 21% compromised weight gain by 4.76% and feed intake by 3.56% without affecting FCR. At day 24, treatments influenced weight gain and FCR ($P < 0.001$). Broilers offered T3 and T5 exhibited lower weight gain than T1 by 5.82% and 3.48%, respectively. T3 and T5 had a higher FCR compared to T1 by 3.76% and 3.26%, respectively, whereas T2 and T4 exhibited statistically comparable growth performance to T1. A second study (144 Ross 308 males; 3 treatments; 8 replicates) tested whether optimising the starter diet would facilitate the utilisation of low-CP diets in grower and finisher phases. The dietary treatments included: T1 (standard-CP diet), T2 (T1+10% higher lysine, TSAA, and threonine to Ross recommendations), T3 (T1+10% higher essential amino acids Ross recommendations). T1 received standard-CP diets throughout, while T2 and T3 received low-CP diets in later phases. At day 14, T3 had improved FCR compared to T1 ($P = 0.013$). In the grower phase, both T2 and T3 had lower FCR than T1 ($P < 0.001$). In conclusion, optimising nutrient density in starter diets facilitates better utilisation of low-CP diets in subsequent feeding phases in broiler chickens.

**Keywords:** Crude protein; Stater diets; Broiler chickens; Amino acids

**1019S Low CP strategies and gut health: Post-starter periods**

Michael Kidd*[1], Kenneth B. Nelson[1], Pablo Escovar Ruidiaz[1], Garrett J. Mullenix[2] [1]*University of Arkansas, Poultry Science Department, Fayetteville, Arkansas, United States;* [2]*Cargill Animal Nutrition, Elk River, Minnesota, United States*

Average U.S. broiler slaughter weight is showing signs of stabilizing, but feed utilization is not. Hence, modern broilers consistently have improved feed utilization as measured by an annual reduction in feed conversion. To take advantage of the former efficiency, formulation matrices need

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adequate minimums for all essential amino acids, and to some extent, nonessential amino acids. Research herein represents live performance, processing traits, and gut health, as measure by intestinal permeability, from 9 experiments dealing with dietary essential and nonessential amino acids in male broilers post starter period. An initial experiment was designed to decrease CP by 3% resulting in His being the first limiting constraint (7 dietary feed grade amino acids added) and demonstrated that gut permeability was lowest in the low CP diet and highest in the low CP diet with 10% *Spirulina*, and intermediate in the control CP diet. A subsequent experiment was designed to further decrease CP by 4% (8 dietary feed grade amino acids added), resulting in either His or Gly being the first limiting constraint. A combined treatment of 6 amino acids added back (His, Phe, Gly, Pro, Ala, and Gln) restored feed conversion, breast yield, and peritoneal leaf fat responses to that of the control diet, but not gut permeability. An additional experiment was conducted with a reduced CP diet containing 10 feed grade amino acids with Gly or Pro being the first limiting constraint. Progressions of Ala, Glu, Gln, Gly, and Pro were added back to the reduced CP diet to meet control levels. The reduced CP diet worsened gut permeability and feed conversion, and as nonessential amino acids were added both feed conversion and gut permeability were restored. Finally, six experiments assessed dose response titrations with either dietary Arg or Leu. Increasing dietary Arg from a digestible Arg:Lys of 1.05 to 1.40 either in a dose response manner with L-Arg or a combination of L-Arg and soybean meal did not affect gut permeability. To test the effect of Leu, 4 dose response experiments with digestible Leu:Lys graduations from 1.15 to 1.75 via L-Leu and dietary variations in digestible Val: Lys (0.76 or 0.80), digestible Ile:Lys (0.65 or 0.68), and a control diet (1.35 Leu:Lys) were fed. In the combined assessment of the 4 Leu experiments, a Leu:Lys need for live performance and processing traits was not found. However, as Leu:Lys increased, a linear worsening of intestinal permeability occurred. These results point to the importance of both essential amino acid balance and nonessential amino acid N in maintaining gut permeability in broilers post starter period.

**Keywords:** Amino acid; Gut permeability; Nonessential amino acid; Leucine; Low crude protein

**Panel Discussion**

## Processing and Products Symposium: Poultry Meat - Global Issues

**105 Constitution Hall (MTCC)**

### 1020S An overview of breast myopathies from South America

Sergio L. Vieira*[12] [1]*Federal University of Rio Grande, Porto Alegre, RS, Brazil;* [2]*Instituto Brasileiro de Pesquisa Animal, Farroupilha, RS, Brazil*

Broiler meat production in South America has expanded markedly in recent decades, driven by increasing global demand for affordable animal protein and the region's competitive production systems. Brazil dominates regional production, exceeding 14 million metric tons annually and accounting for approximately 14% of global broiler meat output. Countries such as Argentina, Peru, and Colombia also contribute, primarily serving domestic markets. Overall, South America produces approximately 19–22 million metric tons of broiler meat per year, with Brazil responsible for about 70–75% of this total, while Argentina contributes around 2.4–2.5 million tons and other countries, including Chile, add a further 3–4 million tons. In a global context, total broiler meat production was at 110 million metric tons in 2025, meaning South America represents approximately 18% of worldwide output. In addition, Brazil is the world's leading poultry exporter, accounting for about 35–39% of global chicken meat trade with more than 5 million metric tons. This growth has been supported by genetic selection for rapid growth, improved feed efficiency, and vertically integrated systems. However, increased productivity has been accompanied by rising concerns over meat quality, particularly breast muscle myopathies such as wooden breast and white striping. These conditions are linked to rapid growth and high breast yield, leading to structural and metabolic changes in muscle tissue. From the consumer perspective, wooden breast is typically perceived as a clear quality defect: affected fillets often present abnormal hardness to the touch, a pale or uneven appearance, and reduced water-holding capacity, which translates into a dry, fibrous texture after cooking. Consumers frequently associate these visual and tactile abnormalities with poor freshness or processing issues, even though the meat is safe for consumption, leading to reduced purchase intent and potential rejection at retail. In some cases, the inconsistency in eating quality may also diminish repeat purchases and erode brand confidence. Consequently, while South American broiler production plays a key role in global protein supply, addressing meat quality issues is essential to ensure sustainability and market acceptance. The overall-perception of breast myopathies in South America varies with the local markets from fresh and frozen deboned breast meat to whole live birds.

**Keywords:** Broiler chicken; myopathies; wooden breast

### 1021S North American poultry trends and challenges

Craig Bacon*[2], Amy Matsler[1] [1]*Simmons Prepared Foods, Research and Development, Noel, Missouri, United States;* [2]*Simmons Foods, Technical Services, Siloam Springs, Arkansas, United States*

The North American poultry sector continues to demonstrate resilience amid broader animal protein markets. Broiler meat production in North America is only expected to increase for the foreseeable future. A prevalent challenge accompanying this growth trajectory are breast meat myopathies — principally woody breast (WB), white striping, and spaghetti meat — in high-yield broiler lines. There is a fundamental tension in modern broiler production: the genetic trajectory toward heavier carcass weights and higher breast meat yields is the same force driving myopathy prevalence upward. Mitigation strategies across genetics, nutrition, and processing are the best way to address these myopathies. Simmons Prepared Foods recognizes the path the poultry industry has been on the last several years to produce meat with the best texture possible. Topics of discussion in this presentation will include specific research Simmons has conducted to guide our breed selection and processing parameters, and how measuring texture is more important now than ever to maintain or improve customer experiences.

**Keywords:** Poultry; breast meat quality; woody breast; texture; myopathy

### 1022S Poultry Meat – Global quality issues: an Asia Perspectives

Yuwares Malila*[2], Yanee Srimarut[1] [1]*BIOTEC, Thailand, Bangkok, Thailand;* [2]*Food Science and Technology Association of Thailand, Bangkok, Thailand*

The global broiler industry is currently undergoing a profound structural realignment driven by macroeconomic volatility, geopolitical shifts, and a transition toward domestic agricultural sovereignty. This report described an industrial dynamic of the 2024 to 2026 period, focusing on the Asian nodes of the poultry network, particularly China, Japan, the Republic of Korea, and Southeast Asia, against a global growth deceleration and conflict-induced commodity price volatility. In East Asia, the sector is characterized by intense consolidation and technological modernization. China has transitioned into a net chicken meat exporter, leveraging genetic sovereignty to mitigate reliance on imported grandparent stock and benefiting from a substitution effect as price-sensitive consumers trade down from pork to affordable avian protein. Conversely, Japan and South Korea face structural constraints, including an aging labor force and persistent biosecurity threats from Highly Pathogenic Avian Influenza. These markets are increasingly dependent on value-added heat-pro-

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cessed imports from regional hubs to bypass raw-poultry bans and address domestic labor shortages. Southeast Asia, led by Thailand-origin integrators such as the Charoen Pokphand Group, exemplifies the shift toward full-chain vertical integration and precision livestock farming. By implementing climate-controlled housing, the region has achieved high-efficiency feed conversion ratios of 1.5–1.6 while elevating food safety standards to meet global certifications. Furthermore, shifting consumer demographics in urban centers are driving demand for premiumization, including hormone-free and "clean-label" products. In 2026, the conflict in Middle East has introduced significant downside risks into the feed market. This volatility has accelerated food nationalism, driving aggressive tax reforms and subsidies, such as those in the UAE and Saudi Arabia, designed to decouple food security from vulnerable international shipping lanes and maritime logistics. Collectively, the Asian poultry landscape in 2026 is defined by food nationalism and the regionalization of trade. The strategic pivot toward genetic autonomy in China and advanced industrialization in Southeast Asia provides a resilient framework against the risk premiums of international feed and shipping markets. Future sector viability depends on the integration of modern feed science, stringent biosecurity protocols, and the capacity to provide high-margin, specialized protein products in an increasingly volatile global economy.

**Keywords:** Meat quality; Asian perspectives

### 1023S Omics technologies in poultry meat quality: Advancements in the molecular basis of breast myopathies

Martina Bordini*[1], Francesca Soglia[1], Massimiliano Petracci[1], Shai Barbut[2] [1]*University of Bologna, Cesena, Italy;* [2]*University of Guelph, Guelph, Ontario, Canada*

The broiler industry represents one of the most biologically and technologically advanced sectors in the global production of terrestrial animal-derived foods. Its success is closely linked to the remarkable improvements achieved in the past 60 yrs in genetic selection, nutrition, housing, and meat processing, from slaughter to product packaging. Today, these advancements allow the birds to fully express their genetic potential while ensuring exceptional outcomes throughout the supply chain in terms of product quality and production efficiency. Among these aspects, meat quality is a key factor in the global success of chicken meat, owing to its cost-competitiveness, nutritional profile, versatility, and acceptability to diverse culinary traditions. In this context, understanding the molecular and physiological pathways involved in determining meat quality is essential for implementing integrated management strategies aimed at preserving and enhancing the production chain. In this regard, the outstanding progress in high-throughput (HT) technologies, which has greatly advanced the investigation of complex phenotype and conditions in other species (including humans), is currently also finding important applications in meat quality research. Indeed, these technologies, such as HT sequencing, can help unravel the molecular mechanisms underlying major meat quality traits as well as some of the most relevant quality issues and abnormalities. More specifically, several omics approaches (e.g., genomics, transcriptomics, proteomics, and metabolomics) have been widely used to investigate the complex biological processes underlying meat quality. For instance, studies applying omics technologies have deepened our understanding of the molecular mechanisms involved in growth-related breast myopathies affecting fast-growing broilers (i.e., white striping, woody breast, and spaghetti meat). Over the past 15 yrs, a substantial body of literature has documented the concurrent and sequential occurrence of multiple biological, physiological, and metabolic events associated with the development of these myopathies, thus highlighting the complexity of their etiology. These advances have been made possible by remarkable progress in omics technologies, especially transcriptomics and proteomics, combined with powerful bioinformatic approaches (e.g., Weighted Co-expression Network Analysis; WGCNA). Comparing to 30 yrs ago, these developments have enabled unprecedented insight into the dissection of complex biological traits. This talk therefore aims to provide an update overview of the mechanisms underlying these conditions, with particular emphasis on recent transcriptomic and proteomic findings.

**Keywords:** Pectoralis major; Transcriptomics; Proteomics; Network analysis; Growth-related abnormalities

**Break/Poster Session I**

### 1024S Chicks are cool

Theo Hoen*[1] [1]*Technical University, Mechanical Engineering, Bennekom, Netherlands*

Poultry meat is increasingly positioned as the protein source of the future, driven by its affordability, efficiency of production, and lower environmental impact compared to other animal proteins. As global demand for protein continues to rise, poultry consumption is expected to grow steadily, reinforcing its central role in future food systems. This presentation highlights four key trends shaping the poultry industry. First, global poultry production is projected to increase by approximately 25% over the next decade, reflecting strong and sustained demand. Second, the industry is facing a significant labour shortage, creating challenges across the value chain and accelerating the need for automation. Third, there is a clear shift among processors toward higher value-added products, including cooked and ready-to-eat poultry items, in response to changing consumer preferences for convenience. Finally, the sector is moving toward a smarter, more digitally driven value chain, where data, automation, and technology play an increasingly critical role in improving efficiency, traceability, and sustainability. Together, these trends indicate a rapidly evolving poultry sector that must adapt to both growing demand and structural challenges, positioning innovation and digital transformation at the core of its future development.

**Keywords:** Market poultry industry; Trends in Poultry market; Data in poultry chain

### 1025S Selection strategies for meat quality and yield

Richard a. Bailey*[1] [1]*Aviagen Ltd., Research and Development, Edinburgh, United Kingdom*

Chicken meat is an important source of sustainable and affordable animal protein for human consumption. For this reason biological efficiency and breast yield continue to be key selection criteria in broiler breeding, in a balance breeding goal including health and welfare outcomes. In addition, to deliver high-grade retail products to the consumer, meat quality traits, including the breast myopathies, are prioritisedin order to reduce genetic propensity.. As technology continues to advance, an increasing number of tools for measuring breast meat yield and quality, along with their proxies, are being incorporated into breeding strategies, enabling more precise and comprehensive selection decisions Where meat quality issues such as breast myopathies occur, it is essential that they are properly understood and characterised in order to develop an effective strategy to reduce their incidence. This begins with establishing the genetic basis of the condition by estimating the genetic parameters (i.e., heritability) of the individual myopathies, along with their genetic correlations with other traits in the breeding goal. The reliable estimation of these parameters, and the subsequent implementation of a selection strategy, depends on the accurate definition and detection of the trait. For breast myopathies, heritabilities are low to moderate, ranging from 0.04 for spaghetti breast, 0.024–0.2 for wooden breast and 0.185–0.338 for white striping, which indicates that although a genetic component exists, non-genetic factors play a major role in their expression. With regards to selection strategies, selecting against the genetic predisposition to breast myopathies is feasible; however, this should be regard-

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ed as a long-term approach. More immediate progress can be made by addressing non-genetic factors, including environmental, nutritional, and processing conditions, which exert a considerable influence on meat quality outcomes. The identification and optimisation of these factors is therefore critical in reducing both the incidence and severity of myopathies. Genetic correlations between myopathies and performance traits such as breast yield and body weight are generally low to moderate, indicating a limited shared genetic background. This demonstrates that concurrent improvements in breast yield, broiler performance, and a reduced incidence of myopathies are achievable through balanced breeding programmes combined with appropriate management interventions.

**Keywords:** broiler; myopathy; genetics; meat quality

**Panel Discussion**

## Metabolism and Nutrition Symposium: Nutrition and Management Strategies for Extended Laying Cycle

**John W. H. Bassett Theatre (MTCC)**

### 1026S Persistence in the extended laying cycle. Trends, challenges and opportunities in productivity, bird health, welfare and adaptation to diverse production settings

Ian Dunn*[1] [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, Midlothian, Scotland, United Kingdom*

This presentation will introduce the long-life layer, a concept coined over 10 years ago (Bain *et al.*, 2016). The ideas introduced then are still current and extended laying cycles are here. Some of the drivers such as genomic selection were not named specifically, but were referred to as "selection indices, linked to molecular genetics" which has been particularly important for male selection. The result of selecting hens over a longer laying period has the potential to improve the profitability and, importantly from a societal point of view, improve the sustainability of egg production. The reduction in the environmental footprint is due in a large part to reduced replacement rearing. It was recognised that there would be quality, nutritional and welfare challenges. The egg quality and reproductive issues are being addressed by existing genetic selection and nutrition over the longer laying period. It was harder to foresee how welfare challenges would be addressed and approaches have been developed for the issues of bone health and "injurious pecking". Although not unique to the long-life layer, these issues influence economics especially in alternative systems. Overall, there is rarely a single approach to a problem, with the design of alternative systems, improved awareness and management approaches as well as genetics and nutrition needing to be practiced. In the case of bone quality, we have found very short periods of low dietary calcium, which might be due to poor gut health or diet availability, rapidly results in keel bone damage. We will hear of nutrition strategies in this session, whilst recognition of the importance of rearing and the onset of lay in maintaining production over the period of production is becoming increasingly apparent for long production periods. In conclusion there are obstacles to producers adopting longer cycles. But as genetic and supporting science advance, it may be that it is only confidence that product quality and welfare can be maintained over long-term production that may be holding producers back. Because it is clear that the long-life layer is here in terms of performance if bird management is handled appropriately. That is why we will be focussing on management in this symposium. Funded by the Foundation for Food and Agricultural Research and BBSRC. BAIN, M.M., NYS, Y. & DUNN, I.C. (2016) Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? British Poultry Science, 57: 330-338.

**Keywords:** Long Life Layer; Bone; Keel; Injurious pecking; Calcium

### 1027S Growth trajectory before sexual maturity? Exploring the impact of body composition at the point of lay.

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Following decades of genetic selection, laying hens are now capable of producing over 500 eggs per cycle. This was achieved by advancing the age at first, increasing the duration of peak production, and significantly improving laying persistency to over 100 weeks of age. We previously reported that, in fact, the key determinant controlling sexual maturation in layer pullets is a metabolic threshold, and that selection for early entry into lay resulted in birds reaching that threshold earlier. Our recent data strongly suggest that this metabolic threshold is associated with the level of adiposity and is independent of photoperiod. However, photostimulation can still induce lay on its own even if the threshold is not met, and molecular analyses point to the pituitary gland as the primary integrator of metabolic and photoperiodic cues. Why would the timing of sexual maturation matter? Well, once the ovarian follicular pool is activated, increased steroid production, in particular estradiol from small white follicles during puberty, switches the overall physiology from growth to reproduction. Specifically, this includes changes in liver metabolism to focus on the production of egg yolk components, and alterations in calcium metabolism and bone physiology to sustain eggshell formation. Although it is possible to manipulate growth trajectory and adiposity through dietary intervention, the development of some key tissues and organs is age-dependent, and early entry in lay may alter their maturation and subsequent production, as well as health and welfare parameters. For example, switching long bones from cortical to medullary accretion may result in a smaller, weaker skeleton, which may predispose hens to osteoporosis and calcium deficiency later in the cycle, and result in poor eggshell quality. As well, premature entry in lay may impact the repositioning of the keel bone at a time when ossification is far from complete, thereby putting it at risk of injury. Some of our data also suggest that selection for an extended laying cycle may have resulted in hens capable of undergoing "silent" recurrent activation of the reproductive axis to sustain continuous egg production while replenishing calcium stores. Nonetheless, adequately preparing pullets for an extended laying cycle remains a prerequisite for success and can be achieved through careful management practices.

**Keywords:** Sexual maturation; Laying hens; Extended lay

### 1028S Nutritional considerations for setting up pullets for long-life production

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Primary breeding companies have selected laying hens for long production cycles, with a goal of producing 500 eggs per hen by 100 weeks of age. Selection for greater persistency and a slower rate of egg size increase with age have been largely responsible for the more frequent achievement of these targets in commercial production. However, the longer productive life of a hen (without a molt) places additional stressors on the bird. Simultaneous genetic selection for health, livability and welfare traits have offered some protection for the birds, but proper management and nutrition have become even more critical in table egg production. Achieving 500 eggs by 100 weeks of age reuires careful attention to the nutritional needs of the pullet, ensuring that appropriate nutrient reserves are acheived by the point of sexual maturity. Pullets must be managed according to their specific needs based on body weight, conformation and composition, rather than strict adherence to dates and ages provided by the management guide. Once in lay, skeletal health must be maintained through proper supply of calcium over each

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24-hour day. Egg size needs to be managed through nutrition to ensure the eggs do not become too large by the end of the cycle. Nutrients and energy must be provided at adequate levels, with neither deficiencies nor inappropriate excesses. These key points can become more diffficult to address under stressful situations such as high environmental temperature, and poor quality or mycotoxin-contaminated feed. Modern laying hens are capable of exceptional levels of egg production, but require closer attention and care than in the past in order to reach their potential.

**Keywords:** Laying hen; Layer pullet; Nutrition; long production cycles; nutrient intake

### 1029S The role of dietary protein and amino acids in modulating egg production, bone health, and feathering in the extended laying cycle

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The extension of the laying cycle in modern commercial hens has intensified the need for precise nutritional strategies to sustain productivity, health, economic benefit, and welfare. Dietary protein and amino acids play a central role in optimizing egg production, bone integrity, and feather condition in extended laying cycle hens. Adequate and balanced amino acid supply supports persistent egg mass and production by optimizing protein turnover and metabolic efficiency, particularly with respect to limiting amino acids such as lysine, methionine, threonine, isoleucine, and tryptophan. Recent research has connected proper branched chain amino acids and large neutral amino acids particularly tryptophan balance to optimized bone health, feathering and increased egg weight. Improper amino acid balance may exacerbate bone resorption and increase the risk of osteoporosis and fractures in late lay. Strategic formulation approaches to include proper amino acid ratios, are essential to meet the evolving physiological demands of hens in extended production cycles. Overall, optimizing dietary protein and amino acid nutrition is critical to maintaining productivity, skeletal strength, and feather cover in long-lived laying hens.

**Keywords:** Amino Acid; laying hen ; bone; feathering

### 1030S Nutritional strategies for modulating eggshell quality and skeletal integrity in the extended laying cycle?

Woo K. Kim*[1] [1]*University of Georgia, Athens, Georgia, United States*

The extension of laying cycle for modern laying hens has been implemented to increase egg production and profitability for the industry and eliminate the molting practice for the wellbeing of laying hens. Prolonged egg production and skeletal health are highly correlated with each other. High egg producing hens typically have skeletal integrity issues at older age because over 40% eggshell calcium comes from bones including medullary bone. During the lifetime of hens, medullary bone increases at the expense of structural bones such as cortical and trabecular bones. Thus, it is critical to identify and develop nutritional strategies that 1) promote optimizing bone structural frame and mineral accumulation in bones before onset of egg production (sexual maturity) and 2) provide hens with sufficient nutrients for egg production and skeletal modeling/remodeling. Because Ca and P are the most important nutrients for strong eggshell formation and bone health, balanced Ca and P supplementation in pullet and hen diets is critical. In addition, phytase and vitamin D supplementation is equally important to enhance Ca and P digestion and absorption. Furthermore, vitamin D has a dual function: 1) absorption Ca and P absorption in the intestine and 2) Ca and P deposition in bone by stimulating bone cell formation. The bone mineral density can be modulated by the balance between bone formation by osteoblasts and bone resorption by osteoclasts. At younger age, pullets have a positive balance (higher bone formation and lower bone resorption) to make more mineralized dense bones, whereas older hens have a negative balance (lower bone formation and higher bone resorption) for less bone density and weaker bones. Such balance can be influenced by dietary imbalance, environmental conditions (heat stress) and pathogen infection/enteric diseases. Heat stress, pathogen infection, and enteric diseases can cause inflammation and oxidative stress which tilt the balance more toward higher bone resorption to make weaker bone, eventually influencing egg production and quality. Thus, minimizing inflammation and oxidative stress by feed additives is essential for older laying hens to maintain prolonged egg production and bone health.

**Keywords:** Nutritional strategies; Eggshell quality; skeletal integrity; Laying hens; Bone health

### 1031S Reappraisal of management approaches for an extended laying cycle

Teun van de Braak*[1] [1]*Hendrix Genetics, Boxmeer, Noord Brabant, Netherlands*

**Objective:** The objective of this presentation is to discuss management interventions that support egg quality, hen health, and welfare in extended and very extended laying cycles, building on recent genetic progress that enables production well beyond traditional cycle lengths. **Description:-** Genetic improvement has resulted in laying hens capable of sustained production far beyond 100 weeks of age, creating new opportunities for efficiency and sustainability. However, realizing this potential requires a reappraisal of flock management, particularly during rearing and toward the end of lay. Key challenges include excessive egg weight, declining eggshell quality, skeletal stress, metabolic load, and increasing flock variability. These challenges are influenced by housing system, with additional complexity in cage free production, but are fundamentally management driven across all housing systems. Commercial experience indicates that maintaining egg quality and flock stability beyond 100 weeks of age depends strongly on proactive management throughout the cycle. Attention to egg weight development, timely nutritional and management adjustments, and continuous flock monitoring can limit late cycle egg quality losses. With appropriate genetic potential and management alignment, production levels approaching 600 eggs per hen housed in a single cycle become a realistic long term perspective. During this talk, practical experience from commercial layer operations managed for extended laying cycles across different housing systems will be presented. The presentation will begin with an overview of the contribution of genetic progress to improved persistency of lay, followed by a discussion of key management intervention points. Topics include balanced breeding program directions, pullet preparation, body weight and egg weight trajectories, feeding phase transitions, practical management strategies, and housing system specific aspects. **Statistical analysis:**Not applicable**Conclusions:**Extended laying cycles require closer integration of genetics and management. Proactive, system adapted management strategies are essential to maintain egg quality, welfare, and productivity throughout extended laying cycles.

**Keywords:** extended laying cycle; genetics; egg quality; flock management; late lay

**Panel Discussion**

## Sunday, July 12, 2026

### Extension and Education Symposium: The Role of Generative AI and LLMs in Poultry Education, Extension, and Operations

**John W. H. Bassett Theatre (MTCC)**

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**1038S Artificial Intelligence in Poultry and Agriculture: Practical Applications, Opportunities, and the Future of Work**

Aidan Connolly*[1] [1]*Agritech Capital, Wilmington, North Carolina, United States*

Artificial intelligence (AI) and large language models (LLMs) are rapidly becoming part of modern agriculture and food production systems. While AI is often discussed in highly technical terms, many of the most impactful applications are practical tools designed to improve efficiency, communication, decision-making, and operational management. This keynote presentation will provide poultry scientists, educators, extension specialists, and industry professionals with an accessible overview of how AI technologies influence poultry and agricultural systems and their future implications. It will explore current examples of AI use across agriculture, including precision livestock and crop management, predictive analytics, supply chain optimization, automated monitoring systems, and AI-assisted decision-making. Particular emphasis will be placed on how AI technologies are already being integrated into agricultural operations to improve productivity, reduce waste, enhance sustainability, and support more informed management decisions. Examples from commercial agriculture will demonstrate how relatively straightforward AI tools can generate meaningful operational improvements when paired with quality data and effective workflows. Rather than focusing on the technical inner workings of AI systems, the presentation will emphasize practical implementation and organizational adaptation. A central theme will be that the greatest value of AI may not come from isolated tools or pilot projects, but from rethinking how information flows through agricultural operations and how decisions are made across production systems. It will discuss how AI may reshape workflows, communication, planning, and operational coordination within poultry and agri-food systems. It will also draw on recent work examining how AI may influence agri-food supply chains, workforce dynamics, organizational readiness, data quality, scalability, and responsible implementation. The acronym DRIVE will be introduced as a framework describing actions poultry executives and managers can take to improve AI readiness within their organizations. Attendees will gain perspective on both the opportunities and limitations of AI technologies while developing a clearer understanding of how these tools may realistically support research, extension, education, and commercial poultry operations. Overall, this presentation provides an approachable and forward-looking introduction to AI in poultry and agriculture, helping attendees better understand the rapidly evolving technological landscape and identify practical opportunities for future application within their own professional environments.

**Keywords:** Artificial Intelligence (AI); Poultry Production; Operational Efficiency; DRIVE Framework; Agri-Food Systems

**1039S ExtensionBot: Trusted, Cited Answers for Agricultural Extension Using Generative AI**

David Warren*[1] [1]*Oklahoma State University, Bixby, Oklahoma, United States*

Generative artificial intelligence (AI) and large language models (LLMs) offer new tools for agricultural communication and stakeholder engagement, but general-purpose systems raise concerns about accuracy, hallucination, source transparency, and the reliability of recommendations reaching producers. These concerns matter in Cooperative Extension, where delivery of trusted, research-based information defines the mission. This presentation examines ExtensionBot as a case study for responsible AI deployment in agricultural outreach. ExtensionBot is a generative AI platform built for the U.S. Cooperative Extension System through a partnership of the Extension Foundation, USDA-NIFA, Oklahoma State University, the University of New Hampshire, and many other land-grant universities in the US. ExtensionBot operates primarily within a curated corpus of peer-reviewed extension and agricultural publications. Every answer cites its sources, allowing users to verify the underlying research and reducing the risk of misinformation reaching producers. The presentation will cover the technical framework, including retrieval-augmented generation (RAG) and the MERLIN data infrastructure that ingests and structures extension content from contributing institutions. Rather than relying on a model's memorized outputs, ExtensionBot retrieves "chunks" of relevant publications at query time and grounds responses in current institutional source material. Localization logic lets state systems prioritize their own recommendations and optionally nearby states while retaining access to the broader national knowledge base. ExtensionBot can interact in 60 languages include Spanish, German, French and many more. Wider testing is needed to verify the accuracy of languages interpretation of Extension content. A test version of image-based diagnostics is now available, allowing users to submit photos of crops, livestock, pests, or facilities for AI-assisted identification and guidance drawn from the same curated knowledge base. Poultry-specific applications will be discussed, including support for county extension agents, integrators, and producers seeking rapid access to research-based recommendations on flock health, biosecurity, housing, and production management. The talk will also address current limitations, the importance of institutional data readiness for any AI deployment, and why human extension expertise remains essential for validating AI outputs. The session closes with practical guidance for individual researchers and departments looking to build similar tools using approaches such as custom GPTs, NotebookLM, or do-it-yourself RAG platforms.

**Keywords:** Generative AI; Cooperative Extension; Agricultural Outreach; Retrieval-Augmented Generation (RAG); Poultry Production Management

**1040S From Data to Decisions: How Artificial Intelligence is Transforming Poultry Production and Sustainability**

Padmini Persaud*[1] [1]*Speria, Atlanta, Georgia, United States*

Artificial intelligence (AI) and integrated data systems are rapidly changing how poultry companies manage production, efficiency, and sustainability. While many poultry professionals associate AI with robotics or highly technical computer systems, some of the most impactful applications involve using existing operational data to improve decision-making, predict outcomes, and increase efficiency. This presentation will provide an approachable overview of how AI-driven technologies are being integrated into modern poultry systems and how these tools may influence the future of poultry production. Modern poultry operations generate enormous amounts of data across breeder farms, hatcheries, feed mills, grow-out facilities, processing plants, and supply chain systems. Historically, much of this information has existed in disconnected databases or isolated management systems, limiting the ability to identify relationships between production variables and final outcomes. This presentation will explore how integrated AI platforms are helping unify these data streams into connected systems capable of improving traceability, forecasting, and management decisions across the poultry production chain. The presentation will discuss how AI technologies and Internet of Things (IoT) sensor systems are being used to transition poultry production from reactive problem-solving toward predictive management. Examples will include automated environmental monitoring, bird weight tracking, feed consumption analysis, and forecasting of flock performance and processing yields. By analyzing large volumes of operational data in real time, AI systems can help identify inefficiencies, optimize logistics, improve resource utilization, and support more consistent production outcomes. A major focus of the presentation will be the growing relationship between AI, sustainability, and operational efficiency. The session will examine how AI-assisted systems can support carbon tracking, resource conservation, waste reduction, and environmental reporting while improving bird performance and economic effi-

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ciency. Rather than viewing sustainability and profitability as competing goals, the presentation will demonstrate how improved data integration and AI-assisted decision-making may support both production efficiency and environmental stewardship within modern poultry systems. Overall, this presentation will provide poultry scientists and industry professionals with a practical introduction to how AI technologies are being applied within commercial poultry operations. Emphasis will be placed on real-world implementation, operational value, and the growing importance of integrated data systems in supporting the future of poultry production.

**Keywords:** Artificial Intelligence; Precision Poultry Farming; Integrated Data Systems; Poultry Production; Sustainability

### 1041S Workshop: Building AI-Powered Teaching Tools: Custom GPTs, NotebookLM, and Emerging AI Workflows for Poultry Education

Andrew P. Benson*[1] [1]*University of Georgi, Poultry Science , Jefferson, Georgia, United States*

Generative artificial intelligence (AI) and large language models (LLMs) are rapidly changing higher education and creating new opportunities for teaching, learning, and student engagement. While much of the discussion surrounding AI in education has focused on concerns regarding academic integrity, these technologies also provide powerful opportunities to enhance active learning, improve accessibility to educational resources, and create customized learning experiences for students. This workshop will provide an approachable and hands-on overview of how generative AI tools can be integrated into poultry science and agricultural education to support teaching, extension, and academic workflows. The workshop will begin with an overview of how customized AI chatbots are being incorporated into undergraduate poultry science courses to support active learning, case studies, stakeholder simulations, and personalized student assistance. Examples will demonstrate how course-specific AI tools can be developed to reinforce concepts, generate practice materials, facilitate role-playing activities, and provide students with accessible academic support outside of the classroom. The workshop will emphasize practical implementation strategies rather than technical programming knowledge, making the session accessible to faculty with little or no prior experience using AI tools. Participants will also be introduced to practical workflows for creating customized AI assistants using platforms such as OpenAI ChatGPT and Google Gemini Gems. In addition, the workshop will demonstrate how NotebookLM can be used to organize course materials, generate study resources, and support student interaction with uploaded content. Emphasis will be placed on how these tools can improve content delivery, increase student engagement, and support flipped or active-learning classroom models. The workshop will further introduce emerging “vibe coding” and simulation-building workflows using AI-assisted development platforms such as Claude Code and ChatGPT. Demonstrations will highlight how faculty can rapidly generate simple simulations and adaptive learning activities without requiring extensive coding experience. Potential applications for teaching, research, and extension programming for poultry scientist will be discussed. Overall, this workshop is designed to help attendees better understand the practical capabilities of generative AI in academic settings while providing approachable examples of how these technologies can be used to support instruction, communication, and educational innovation in poultry science.

**Keywords:** AI; ChatGPT; Gemini; NotebookLM; Education

## Management and Production Symposium: Poultry Litter Management and the Environment – Where Have We Been and Where are We Going?

### 1042S Commercial Litter and Poultry House Management Strategies

Dana K. Dittoe*[1] [1]*University of Wyoming, Animal Science, Laramie, Wyoming, United States*

Poultry litter management plays a critical role in flock health, production efficiency, and environmental stewardship. Over the past several decades, in-house litter management practices have evolved in response to increasing production demands, economic pressures, and environmental concerns. This presentation will provide a historical perspective on the development and adoption of in-house composting and litter management strategies designed to reduce costs, minimize pathogen loads, decrease ammonia volatilization, and maintain healthy flock environments. Practices such as in-house composting, moisture management, ventilation, and downtime between flocks influence litter quality and bird performance will be examined with emphasis on how in-house management decisions affect downstream land application practices and environmental outcomes. In addition, current challenges and future directions for poultry litter management will be explored. Particular attention will be given to the potential advantages and limitations of extended downtime between flocks compared with traditional short downtime systems. These management approaches may influence pathogen reduction, litter stability, ammonia control, bird welfare, and nutrient management outcomes. Ultimately, integrated overview of historical and current poultry litter management practices while identifying future opportunities to improve flock performance, environmental sustainability, and long-term litter utilization strategies within modern poultry production systems will be discussed.

**Keywords:** Poultry litter management; In-house composting; Ammonia control; Ammonia control; Environmental sustainability

### 1043S Assessing Litter Quality: Economics and Management Decisions

Luis R. Munoz*[1], Johnathon Moon[2] [1]*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States;* [2]*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States*

Poultry litter quality directly influences broiler health, performance, and production efficiency, yet decisions regarding its management remain highly variable across operations. In commercial production systems, litter management is often delegated to individual growers, further contributing to inconsistency in how litter quality is assessed and managed. Despite its importance, standardized, measurable criteria to define litter quality in practical and economic terms remain limited, restricting the ability to make informed and consistent management decisions. This presentation will discuss key measurable indicators of poultry litter quality and their relevance to production performance and economic considerations, with emphasis on how these factors can inform litter management decisions. Topics to be discussed include key physical and environmental indicators of litter quality, such as moisture, caking, and ammonia, along with general biological aspects of litter dynamics. These factors will be considered in relation to production performance and economic outcomes within the context of practical litter management. A more consistent approach to evaluating litter quality may enhance decision making and support the development of more sustainable and economically efficient poultry production systems.

**Keywords:** Poultry litter quality; litter management; broiler production; economic decision-making; environmental sustainability

### 1044S Pathogen Occurrence in Poultry Litter

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Commercial poultry broiler production requires large-scale housing facili-

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ties and comprehensive management input in all phases during the grow out period. An important element of the overall management operation is the bedding material used in the poultry house. While poultry bedding material can consist of different starting materials, once birds are introduced, it accumulates excreta, feed waste, feathers, broken eggs, and residual bedding as it transitions to litter. In addition to the mineralization of organic components into elements such as nitrogen and phosphorus, litter becomes populated by a wide array of microorganisms. Litter microorganisms can originate not only from birds but from the house environment as well as sources external to the house. While some of these organisms are nonpathogenic and can be indirectly or directly involved in the metabolism and breakdown of litter components other organisms may be pathogenic to the birds. Since birds depending on the growth phase can harbor different pathogens these organisms can all enter the litter at any time and become part of the litter microbial consortia. Potential pathogens include Salmonella, pathogenic Escherichia coli, Staphylococcus, Streptococcus, Listeria monocytogenes, Enteroccus, and Clostridium, among others. Of the pathogens that occur in poultry litter, Salmonella has been the subject of numerous studies. The focus on Salmonella is not surprising given its importance as a foodborne pathogen and the food safety risks associated with this pathogen. Salmonella can originate from poultry as well as a myriad of other sources such as rodents, insects, feed, and wild birds. Regardless of the source, a primary contact point in the poultry house is the litter where Salmonella has demonstrated the capability to survive under a variety of environmental conditions. Identifying these factors that could potentially reduce the level and frequency of Salmonella in poultry litter represents an important management strategy.

**Keywords:** Poultry litter; pathogens; poultry house; Salmonella; broilers

### 1045S Poultry Litter as an Ecological Archive: From Bird–Environment Interactions to Ecological Intervention

Elena G. Olson*[12], Steven C. Ricke[3], Brett Hale[4] [1]*UW-Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States;* [2]*Supreme EGO LLC, Middleton, Wisconsin, United States;* [3]*University of Wisconsin, Animal and Dairy Sciences, Madison, Wisconsin, United States;* [4]*AgriGro, Inc, Doniphan, Missouri, United States*

Poultry litter is often viewed primarily as a byproduct, yet it functions biologically as a dynamic ecological archive within the poultry house. As litter accumulates, it reflects the continuous interaction among birds, housing conditions, and the surrounding microbial environment. This presentation will explore poultry litter as a biological interface that links bird health, environmental conditions, and microbial risk across production systems. Emphasis will be placed on how litter ecology can be used to better understand spatial heterogeneity within the house, the connection between the gastrointestinal tract and the surrounding environment, and the broader outcomes of these relationships for flock health and biosecurity. The presentation will also highlight how controlled *in vitro* litter microcosm models can extend these ecological insights by providing a practical platform for testing litter-focused interventions under defined conditions. Together, these perspectives support a broader framework in which poultry litter is understood not as passive bedding, but as an active ecological system that can be interpreted, monitored, and strategically manipulated to improve microbial management, bird health, and production outcomes.

**Keywords:** poultry litter; microbial ecology; bird-environment interactions; ecological interventions; production microbiome

### 1046S Applications of 16S sequencing for profiling the poultry litter microbiome- an industry perspective

Brett Hale*[1], Elena G. Olson[2], Steven C. Ricke[3] [1]*AgriGro, Inc, Doniphan, Missouri, United States;* [2]*UW-Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States;* [3]*University of Wisconsin, Animal and Dairy Sciences, Madison, Wisconsin, United States*

Poultry litter is a dynamic polymicrobial habitat with implications for bird health, human safety, and environmental integrity. Amplicon sequencing of the 16S rRNA gene has emerged as the dominant approach for characterizing poultry-associated microbial communities, offering a scalable and reproducible framework for exploring litter microbial ecology. Complementary to biosurveillance and the expansion of foundational knowledge, 16S-based profiling holds substantial translational value from an industry perspective. Microbiome data can inform the identification and mechanistic characterization of pre-, pro-, post-, and synbiotic intervention candidates, including elucidating indirect modes of action mediated through shifts in the native microbial community or litter environment. Profiling efforts also facilitate the discovery of indicator organisms, enteric pathogens, keystone taxa, and core microbiome members whose presence and relative abundance may signal broader community dynamics and guide targeted intervention strategies. When litter is profiled alongside adjacent matrices such as soil, dust, and aerosols, comparative analyses can illuminate microbial passage and shared community members across compartments, informing sanitation and biosecurity practices. Overlaying microbial composition with physicochemical parameters may improve understanding of acidifier performance, ammonia mitigation, and litter fertilizer value. Additionally, 16S datasets provide a framework for the development of targeted molecular assays for routine monitoring and decision-making. This presentation highlights practical applications of 16S-based poultry litter microbiome profiling from an industry perspective, demonstrating how microbiome-informed approaches can be leveraged to develop targeted, data-driven intervention strategies.

**Keywords:** Poultry Litter; Microbiome; 16S Sequencing; Biosurveillance

### 1047S Comprehensive biosurveillance: tracking DNA and RNA pathogens using shotgun metagenomic sequencing of environmental samples

Jake Byrnes*[1], Cassandra Ettinger[1], Elena G. Olson[2], Genevieve Roberts[1], Steven C. Ricke[3], Michael Rhys[1], Casey McGinley[1] [1]*Barnwell Bio Inc., San Diego, California, United States;* [2]*UW-Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States;* [3]*University of Wisconsin, Animal and Dairy Sciences, Madison, Wisconsin, United States*

Infectious disease remains the single biggest problem for the US poultry industry, with a long list of causal microbial pathogens contributing to production losses. The most salient example of this issue is the ongoing highly pathogenic avian influenza (HPAI) outbreak which has led to losses of more than 150 million birds since 2022. Despite the high economic costs of infectious disease response, the industry still lacks a comprehensive, scalable biosurveillance approach and instead growers and producers rely on reactive responses to clinical symptoms that arise in the barn. Barnwell Bio has been developing a biosurveillance platform relying on shotgun metagenomic sequencing of environmental barn samples to track DNA-based pathogens. In this talk we will review advantages and disadvantages of different sample sources (e.g. litter, boot sock swabs, egg wash water), the depth/breadth tradeoff of using a metagenomic sequencing approach, and how this type of monitoring fits into a broader health management strategy. We will share a few examples of success stories in which growers and producers using our platform were able to detect pathogens prior to symptom onset and adapt their disease mitigation strategies based on longitudinal monitoring. Finally, we will present our latest work in expanding our platform to track RNA viruses such as HPAI and avian metapneumovirus including sample source selection and optimization of laboratory processes.

**Keywords:** Pathogens; Biosurveillance; Metagenomics; Litter; virus

**Panel Discussion**

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## Metabolism and Nutrition Symposium: The Technologies and Nutritional Strategies that are Making Precision Nutrition an Affordable Reality

**105 Constitution Hall (MTCC)**

**Panel Discussion**

### 1048S Global Industry Trends to Shape the Future of Global and U.S. Broiler Production

Nan-Dirk Mulder*[1] [1]*Rabobank, Utrecht, Netherlands*

The global population is projected to grow from 8.3 billion today to 10.3 billion by 2050, with urbanization increasing from 58 to 68%. While Asia and Latin America will continue to drive near-term growth, Africa is expected to account for ~70% of population expansion between 2035 and 2050. These shifts, combined with rising incomes in developing regions, will significantly increase demand for animal protein, with poultry remaining the most accessible and preferred source. Global food consumption is expected to rise by 30–40% by 2050, with animal protein increasing by 40–50%. At the same time, agricultural land availability is declining and biofuel production is competing for resources. The industry's core challenge will be producing more with fewer inputs, requiring improvements in efficiency, productivity, and sustainability across the entire value chain. Growth in demand will be concentrated in resource-constrained regions such as East Asia (~50%) and the Middle East and Africa (~20%), intensifying pressure on global supply systems. Geopolitical tensions are increasingly reshaping food production, with governments prioritizing food and resource security. This is accelerating a shift toward localized poultry production systems, while feed inputs remain globally sourced. Many countries are also promoting domestic grain and oilseed production, altering global trade flows. At the same time, labor shortages are emerging as a key constraint, driven by urbanization and rising incomes, increasing reliance on automation and migrant labor. These macro trends are driving rapid transformation in poultry production systems. Advances in precision farming technologies including sensors, automated feeding, imaging systems, and integrated data platforms are improving efficiency and control. The adoption of artificial intelligence is enabling more data-driven decision-making, integrating performance, health, welfare, and environmental metrics. As a result, the industry is shifting from traditional, experience-based management toward more predictive and optimized systems. Structurally, the sector is expected to continue moving toward (semi-)vertically integrated models to improve scale and efficiency, while also adapting to regional differences in resources and consumer preferences. Technology-intensive systems will dominate resource-constrained regions, while developed markets favor more diversified models. Finally, younger, digitally native generations will further accelerate the adoption of technologies across the value chain, reinforcing a broader shift toward more data-driven, efficient, resilient, and sustainable broiler production systems.

**Keywords:** Poultry; Market; Outlook; Globa; Chicken

### 1049S The Dynamics of Amino Acid Nutrition in Lean Deposition in Broilers

Justina Caldas*[1] [1]*Aviagen, Global Technical Team, Huntsville, Alabama, United States*

We supply the world with animal protein, and such the genetic potential of lean deposition over the years has increased, lean deposition is expressed as eviscerated or carcass yield and breast meat yield, the latter has maintained the highest value of broiler meat parts in the USA, this is not the same in other countries where leg quarters have same or higher value than breast meat. Looking back over the last 25 years of all the breeds in the USA, the improvements of the first 15 years has been faster than the last 10 years, there has been an increase of 0.56% carcass yield per year (8% in 15 years) followed by a modest increase of 0.28% per year (2.8% - last 10 years). Similarly, to carcass yield, breast meat yield has increased in 0.48%/year (7% in 15 years) and 0.16%/year (1.6% last 10 years), these calculations are not adjusted to a common live weight (Industry reporting services, 2001-2026). Amino acids are the building blocks of protein; therefore, understanding the values and balance of amino acids have been a critical research focus of broiler nutrition to maximize biological performance and economic efficiency. In research conditions evaluating 80-120% of 2022 Aviagen amino acids recommendations at the recommended energy levels, there is a 2-3% carcass yield and 6-7% breast yield difference between the highest and lowest levels for processing weights of 2-5kg (4.4-11lb). These differences in carcass and breast yield due to amino acid nutrition is similar to years of genetic progress; providing broiler companies the flexibility to find the amino acid balance with the best return on investment. When evaluating specific amino acids, the latest update was on branched amino acids (valine, isoleucine and leucine). At 35d in 344x708 males, carcass (71.5%) and breast yield (26.7%) were maximized at the highest Leu:Lys ratio (190 Leu/Lys). This effect was complemented by increasing Ile:Lys ratio beyond 68. Lower Ile:Lys and Val:Lys ratios were required to maximize carcass and breast yield at the lowest Leu:Lys ratio (110 Leu/Lys). However, this strategy yielded less meat than providing a high Leu:Lys ratio diet. Optimum BCAA ratios to Lys may vary depending on response criteria and demonstrate the importance of maintaining proper Val and Ile ratios centered on dietary Leu (Kriseldi et al., 2022). Other factors such as high ingredient prices, ingredient availability, health status, farm conditions, management, etc.… can impact broiler yield responses, not all the operations can handle high amino acid profiles, but also many companies might be leaving money on the table by using lower level of amino acids.

**Keywords:** Lean deposition; carcass yield; breast yield; amino acids; genetic potential

### 1050S Navigating the competing metabolic demands of growth and immune competency & the consequences of getting it wrong

Rami A. Dalloul*[1] [1]*University of Georgia, Poultry Science, Athens, Georgia, United States*

Competing metabolic demands for growth and immune competence remain a major challenge in the modern poultry industry. In broilers, a nutrient-centered perspective helps explain why rapid growth and effective host defense often pull metabolism in different directions. Growth is directed toward desirable phenotypes such as breast muscle accretion, feather development, skeletal growth, organ maturation, feed conversion, and lean tissue deposition, all of which require substantial amounts of digestible amino acids, energy, minerals, and vitamins. Concurrently, immune competence imposes equally high nutritional demands, as leukocyte proliferation, antibody production, cytokine synthesis, acute-phase protein formation, nitric oxide generation, oxidative burst, regulation of inflammation, and tissue repair all depend on energy, amino acids, antioxidants, and intact mucosal barriers. Consequently, growth and immune reseponses draw from the same metabolic pools of energy, amino acids, fatty acids, vitamins, minerals, and other nutrients. Yet, this challenge becomes even more pronounced when birds are exposed to stressors such as infection, vaccination, heat or cold stress, or inflammation. Under these conditions, nutrient partitioning shifts away from growth and toward host defense. In broilers, true immune activation diverts energy and nutrients from productive functions, leading to measurable trade-offs in performance. Immune challenges stimulate cytokine production and stress hormone release, which suppress feed intake and downregulate growth-promoting pathways. Metabolically, activated immune cells shift toward glycolysis, while the liver increases synthesis of acute-phase

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proteins, thereby elevating demands for glucose and amino acids. As a result, body weight gain and feed efficiency typically decline during periods of immune stress. Of particular importance is the extent to which an extended immune challenge alters an optimal amino acid profile. Nutritional strategies such as balancing dietary protein and key amino acids, adjusting energy density, and incorporating functional additives can help mitigate these trade-offs by supporting immune function and gut health. Practical approaches include formulating diets to enhance immune resilience, using natural additives or microbiota modulators to reduce pathogen load, and timing nutritional interventions around vaccinations or other predictable stress periods. Important research gaps remain, including quantifying nutrient partitioning during acute vs chronic challenges, and developing integrative biomarkers and experimental designs that can disentangle nutritional and immunological effects under challenge conditions.

**Keywords:** immune competence; metabolic needs; precision nutrition; broiler

**Break/Poster Viewing**

### 1051S Precision animal performance diagnostics - how the combined strengths of animal gene expression and advanced AI are helping poultry producers unlock performance gains

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Precision nutrition and precision farming aim to fine-tune feeding and management by tailoring inputs to improve productivity, animal welfare, and sustainability. However, one major limitation remains unresolved: nutritional requirements are not static. They vary not only with age and genotype, but also with disease pressure, stress, toxins, environmental conditions, diet composition, and maternal effects. As a result, fixed nutrient tables cannot fully capture the biological variability that dictates commercial performance. A key challenge for precision is identifying what limits birds relative to their genetic potential. While biomarkers and omics have expanded biological insight, translating this into decisions remains difficult due to complexity and environmental effects. Transcriptomics is particularly promising, as it captures how animals adapt to their environment to maintain homeostasis. With advanced AI, these data can be translated into interpretable measures of functional status. This approach integrates sampling, mRNA sequencing, a reference database, and pathway modeling to generate standardized indices linked to health and performance, such as the Gut SAVVY™ Index (GSI; BIOFRACTAL), an intestinal health index which exemplifies this emerging approach. The GSI quantifies activation or inhibition across hundreds of biological pathways grouped into functional domains, such as digestion and absorption, nutrient metabolism, immune function, gut integrity, oxidative balance, microbial metabolites, and gut-brain axis signaling. It expresses the functional status of an individual relative to benchmark populations of birds with poor or robust health and performance on a continuous scale from -100% to +100%. Validation across independent datasets has demonstrated sensitivity to bacterial, viral, and metabolic challenges, as well as to nutritional ingredients and feed additives. In a field study, high-FCR farms scored GSI=-50% versus low-FCR counterparts, with strong correlations with 28-day villus:crypt ratio, adjusted FCR, and end-of-cycle mortality. Metabolic health indices have also shown potential to detect nutritional imbalances and deficiencies in specific amino acids, vitamins, and minerals. This paradigm shift could redefine research, product development, and field diagnostics by enabling functional evaluation of nutrition, health, vaccines, and genetics. Realizing that vision will require large, curated datasets and context-aware AI models trained across diverse conditions, enabling functional indices to become a powerful engine for precision nutrition and a step change in poultry health and productivity.

**Keywords:** precision nutrition; transcriptomics; AI; animal performance; diagnostics

### 1052S Precision feeding of functional ingredients for lean muscle and immunocompetency

Samuel Rochell*[1] [1]*Auburn University, Auburn, Alabama, United States*

Precision feeding is a nutritional strategy designed to deliver nutrients in amounts that accurately match the bird's dynamic requirements. Although not always named as such, nutritionists have been striving for this goal for decades through practices such as phase-feeding, formulation based on bioavailable or digestible rather than total nutrient content of feedstuffs, and defining the impact of various environmental factors on nutrient requirements. More recent technologies that markedly advance opportunities for precision feeding like real-time ingredient analyses and flock monitoring, rapid biomarker detection, data analytics, and dynamic feeding systems are becoming more accessible, but successful deployment of these technologies requires a fundamental understanding of the biological responses of birds to their environmental condition. In particular, a major opportunity lies in precision formulations of dietary amino acids and energy, as these components are key levers of lean muscle accretion and are also the primary determinants of diet cost. As such, the energetic and amino acid costs of immune system activation through its consequences on nutrient intake, digestion, absorption, and metabolic fate have long been a topic of interest for poultry scientists. Along with increased availability of feed-grade amino acids, this research has created opportunities for utilizing individual amino acids as functional ingredients to enhance gastrointestinal health, immunocompetence, and growth performance of both healthy and stressed flocks, though knowledge gaps remain, especially regarding the benefits and limitations of dietary protein reduction with feed-grade amino acids. Moreover, the impact of immune activation on energy partitioning and responses of modern broilers fed different sources (i.e., lipid or carbohydrate) and planes of energy are even less understood. Despite these open questions and the inherent challenges of applying data generated in controlled experiments to the multifactorial challenges of commercial flocks, convergence of emerging technologies with fundamental nutrition research has great potential to advance the long-standing goal of precision poultry feeding programs.

**Keywords:** broiler; precision feeding; amino acids; energy; immunity

### 1053S Exploring the role that feed additives play in precision nutrition

Brooke Humphrey*[1] [1]*NutriQuest, Maple Grove, Minnesota, United States*

The poultry industry has an unwritten rule: if a feed additive cannot move feed conversion by three points, it rarely earns a second conversation. That threshold reflects a rational economic logic since feed represents 60–70% of total broiler production cost, and every point on the ratio matters across millions of birds. Yet as precision nutrition reshapes how the industry thinks about genetic potential, metabolic trade-offs, and flock diagnostics, the question worth asking is not simply whether an additive can move three points, but whether the program was designed to create the conditions for it to do so. The gap between controlled research efficacy and commercial field performance is one of the industry's most persistent and least discussed problems. Power calculations in pen trials are often insufficient to detect the differences we're trying to measure. When products transition to commercial barns, data becomes noisy, confounded, and difficult to interpret with confidence. Recent high-profile experiences in neighboring livestock sectors with a product with over 100 publications of sound mechanistic science yet generates unexpected outcomes when applied at commercial scale, is a timely reminder that the translation from controlled conditions to commercial reality is a scientific challenge in its own right. Precision, properly defined, is a discipline of asking the right question before deploying a solution. Efficacy data

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without context on conditions for success is not science, it is marketing. Response to a feed additive is a function of bird metabolic state, challenge level, genetics, and production environment. Challenge-stratified commercial trial data consistently demonstrates that the same product can produce meaningfully different performance outcomes depending on baseline flock status. This is not inconsistency. It is the biology communicating when it needs help. The program that cannot hear that signal will continue to mistake correlation for causation and habit for strategy. Three principles are proposed to help define the precision additive program of the future: targeted compound selection driven by mechanistic understanding of relevant biological pathways; diagnostic triggers that connect real-time flock metabolic state to additive intervention decisions; and success metrics defined by what matters to the customer, not by what scientists find easiest to measure. The tools to build this framework are becoming available and the opportunity to redesign programs around them provides an exciting future for precision nutrition.

**Keywords:** Nutrition

## Symposium: Methodology Matters

**John W. H. Bassett Theatre (MTCC)**

### 1054S Why methodologies are important in scientific work as well as for application of scientific information commercially?

Paul B. Tillman*[1] [1]*Poultry Technical Nutrition Services LLC, Buford, Georgia, United States*

The general and accepted application of the scientific method to research, typically starts with the basic steps of an initial observation leading to unanswered questions, a review of the literature, forming a hypothesis, testing the hypothesis via experimentation, data analysis, reaching proper conclusions through discussion and ultimately reporting the knowledge obtained from the trial. However, such an approach may be too simplistic in regards to research with poultry where additional decisions must be considered such as the species, genetic strain, sex, housing, welfare considerations and husbandry practices. In addition, power analysis to give insight on needed bird numbers, number of treatments, replicates and blocking as well as adequate data collection, data management, data calculations, along with inclusion or exclusion of individual data points for accuracy and precision within the trial. Then ultimately, the use of the correct and appropriate and acceptable data analysis which can be validated. Only then can an in-depth contemplation of the data lead to an understanding of those results and must be done before any conclusions or consideration of reporting can be considered. In short, the application of design, implementation, analysis and interpretation in any research trial is vital for the possibility of believability, repeatability and ultimately commercial adoption in an applied and practical manner. This symposium seeks to provide attendees with information to aid in developing research projects and trials and to write and read scientific papers more critically so it is possible to better understand the plethora of information we are exposed to on a daily basis.

**Keywords:** Research; Scientific Method; Trials

### 1055S Consideration of mineral homeostasis when conducting calcium nutrition work in poultry

Laura E. Ellestad*[1] [1]*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Optimal dietary calcium levels are necessary for efficient growth, bone mineralization, and eggshell calcification in poultry. To properly assess nutritional requirements at all physiological stages, a holistic understanding of mechanisms governing mineral homeostasis is necessary. Regulation of intestinal uptake, renal reabsorption and excretion, and bone remodeling involves the integrated action of several hormones that contribute to the maintenance of homeostatic calcium levels. These physiological processes will be discussed, with a particular focus on how they relate to nutritional studies.

**Keywords:** calcium; phosphorus; vitamin D3; bone; eggshell

### 1056S Broiler calcium digestibility work. Importance of length of studies, timing of sampling, nutrient interactions in experimental diet formulation in the context of calcium homeostasis

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Methodologies to determine digestible Ca of ingredients and or of a diet in broilers must be developed with an understanding of Ca homeostasis. Ca homeostasis is the tightest physiological control in vertebrate animals. Work in the 1960s and 1970s showed that broilers, after 16 days of age, significantly down or upregulated Ca absorption and/or digestibility within 36h of being fed a diet with excess or deficient Ca, respectively. In contrast, most digestibility assays for other nutrients are done feeding experimental diets for 72 to 96 h. For calcium digestibility studies based on physiological responses, diets formulated to meet the Ca and P requirements of broilers at the relevant age must be fed for at least 12 h prior to the start of the experiment. In addition, experimental diets must contain Ca levels close to those required by the broiler at the age of the trial, with distal ileal collection being done within a 36h window post feed administration of the experimental diets. Passage of a titanium dioxide marker in broilers shows that in 7 and 18 d old broilers, excretion of the marker is below analytical detection limit by 10 and 13 hours, respectively, post feeding of the marker. More recent work in 7 and 18 d old broilers shows that Ca and P digestibility is up-regulated after 24 and 40 h, respectively, when Ca deficient diets are fed. Similarly, when diets with excess Ca are fed, Ca digestibility increases significantly after 24 and 40 h in 7 and 18 d old broilers, respectively. Thus, for Ca digestibility work in broilers, digestibility methodologies developed for other nutrients are not applicable. Using experimental diets with Ca levels close to those required by the broiler at the age the study is being done, is essential and the length of time the experimental diet is fed will be important to minimized the impact of up or downregulation of Ca digestibility if the diet is in excess or deficient in Ca, that may occur when ingredient digestibility's are being determined.

**Keywords:** Calcium homeostasis; calcium digestibility; Broilers; Ingredients

### 1057S Layer calcium digestibility work. Importance of length of studies, timing of sampling nutrient interactions in experimental diet formulation in the context of calcium homeostasis.

Kyle M. Venter*[12], Kean G. Jacobs[1], Roselina Angel[4], Peter Plumstead[3] [1]*Neuro Livestock Research, Lanseria, South Africa;* [2]*University of Pretoria, Department of Animal Science, Pretoria, Gauteng, South Africa;* [3]*Neuro Livestock Research, Lanseria, South Africa;* [4]*University of Maryland, Department of Animal and Avian Science, College Park, Maryland, United States*

Digestible Ca systems in poultry have been developed primarily from broilers and have improved how Ca availability is quantified in feeds through ileal digestibility studies. The quantification of Ca and P digestibility can be extended to laying hens, but interpretation is more complex because Ca metabolism is tightly linked to egg-cycle physiology, including shell calcification and medullary bone turnover. As a result, Ca and P digestibility responses are dynamic across the day rather than fixed values. Layer trials indicate that digestibility shifts with physiological period relative to oviposition. During the evening, when shell deposition is

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maximized, Ca digestibility is upregulated while P digestibility is downregulated. During the morning, when medullary bone repletion is most active, P digestibility increases and Ca digestibility declines. This diurnal regulation means that sampling time must be standardized, because values generated at different physiological windows during the day are not comparable or standard. Diet context further modifies responses, particularly through Ca-phytate interactions and limestone characteristics such as particle size and solubility, which influence dissolution kinetics and downstream digestibility. Feed form should also be considered, notably in mash diets where feed selection and especially limestone particle selection can differ across the shell formation window. This becomes a concern with the use of marker-based approaches for digestibility with set sampling timepoints based on oviposition, due to the assumption of homogenous nutrient intake. In this context the use of pelleted diets where selection is minimized is essential and digestibility data and retention should be interpreted as complementary but physiologically distinct outputs rather than interchangeable metrics. Integrating oviposition-aligned sampling, and diet interaction effects will allow for more accurate feeding of Ca to the hen and improve interpretation of layer Ca digestibility and better align Ca supply with peak shell formation demand.

**Keywords:** calcium; egg; oviposition; retention; digestibility

### 1058S Importance of research facility set up. Uniformity trials and understanding power analysis and facility capabilities

Nickki Tillman*[1] [1]*Nutritional Statistics LLC, Buford, Georgia, United States*

Experimental variability in animal research arises not only from treatment effects but also from overlooked facility-related factors. Poor setup and lack of standardization introduce excess noise, reducing data quality, weakening statistical power, and increasing inconclusive or non-repeatable results. These inefficiencies raise costs and ethical concerns by requiring more animals than necessary. This presentation highlights the role of facility management in minimizing variation across trials. Key contributors among experimental units (e.g., pens, cages, tiers, birds) include chick quality, hatchery practices (e.g., fasting duration), environmental uniformity (temperature, airflow, humidity, lighting), equipment consistency, and management of water, feeders, litter, diets, and data collection. Chick quality and uniformity are primary drivers of success, as mortality inflates variance. Recommended practices include sourcing chicks from a single hatchery with defined breeder flock ages (37–43 weeks), selecting high-quality individuals from an excess pool (5–8%), and minimizing initial body weight variation (CV ~1–1.5%). Single-sex populations are encouraged to reduce variability. When mixed-sex designs are required, balanced allocation and inclusion of sex-specific mortality in analyses are essential. In multi-facility or multi-room trials, location and management effects should be quantified and included in models. When productivity is the main objective, unnecessary sampling should be minimized, as handling and regrouping introduce variation. Separate trials are recommended for organ or tissue sampling unless extra animals are pre-allocated and sampling aligns with routine procedures. Uniformity trials—studies without treatments—are emphasized to establish baseline variability. They identify environmental gradients, facility biases, and equipment inconsistencies, enabling better blocking, optimized replication via power analysis, and improved treatment allocation. For example, reducing 42-day feed conversion ratio (FCR) variation to an SD of 0.025 allows 80% power with 12 replicates and detection of ~2% differences. Facilities with higher inherent variability may fail to detect such differences even with similar replication. Overall, systematically understanding and controlling facility-driven variation enhances experimental precision, reduces animal use, and strengthens both scientific rigor and ethical standards in animal research.

**Keywords:** Experimental variability; Facility management; Animal trials; Uniformity trials; Statistical power

**Break/Poster Viewing**

### 1059S Importance of methods selected for microbiome studies: State of the science and challenges

Joshua M. Lyte*[1], Monika Proszkowiec-Weglarz[2] [1]*USDA-ARS Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States;* [2]*USDA, ARS, ABBL, Beltsville, Maryland, United States*

The microbiota has impact on virtually every aspect of poultry production, including performance, health, and food safety. It is not surprising, then, that considerable promise exists for the application of microbiota science in addressing current and future challenges faced across the poultry industry. Yet, methodological variation among poultry microbiota studies has had negative impact on reproducibility of findings, thereby slowing the application of effective microbiota-based solutions in a production setting. The experimental workflow in microbiota research involves many steps, ranging from sample collection to bioinformatic processing. Likewise, at each experimental stage there exists a vast methodological toolbox available to poultry microbiota researchers. As the technical decisions made at each experimental stage have demonstrable impact on each downstream step, outcomes in microbiota research are largely dependent on the methodologies employed. In order to improve translatability and reproducibility between different methodological pipelines, it is essential that a best practice framework be adopted in poultry science regarding microbiota research. This symposium presentation discusses the need for a robust set of evidence-based guidelines that streamline microbiota research in poultry. Attention will be given to each major stage of a typical poultry microbiota study, including sample collection, storage, extraction, library preparation, sequencing, bioinformatic and data analyses, as well as data reporting and repositories. The use of positive and negative controls, improved reporting of raw data, and other tangible aspects that improve consistency and reproducibility across poultry microbiota study designs are highlighted. Ultimately, it is the goal of this symposium not to advocate a single methodology, but instead to demonstrate the need for robust practices at each experimental stage that enable cross-study comparisons of findings in poultry microbiota research.

**Keywords:** Microbiome; Poultry; Methodology; Gut; Avian

### 1060S Necessary validation steps too often neglected in the use of immunoassays

Tom Porter*[1] [1]*University of Maryland, College Park, Maryland, United States*

Immunoassays are powerful tools to measure hormones and cytokines. Common immunoassays include radioimmunoassays and enzyme-linked immunosorbent assays. While some targets are identical across species, others differ greatly in their structure among species. The key element of any immunoassay is the antibody that recognizes the target, specifically structural epitopes of the target. For example, the structure of corticosterone is identical in every species, but the structure of most proteins differs between each species due to differences in its primary amino acid sequence. Unfortunately, too often results are published from a commercial assay for a hormone or cytokine in another species without any validation of the assay for use in poultry. There are four generally acceptable criteria that must be met for validation of any immunoassay in any species. These include parallelism, recovery, repeatability, and specificity. Parallelism refers to the fact that diluted samples must yield results parallel to the standards. Non-parallel curves indicate interference between something in the sample (e.g., heparin) and binding of the target to the antibody. Recovery is determined by spiking a sample with a known quantity, which must yield a measurement equal to the amount added. If not, then

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the results from the assay are not accurate. Repeatability is determined by assaying a control sample in each assay multiple times and is used to estimate variation within assay and between assays. If variation is too great, then the assay is likely to yield random results. Specificity refers to the measured amount of cross-reactivity from other molecules closely related to the target. Cross-reactivity must be determined to ensure that the assay is measuring only the target of interest. Parallelism, recovery, and repeatability are easily determined, but they can be affected by sample type. Investigators should perform these validation steps with any new species and sample type studied. Specificity is more difficult to determine, as it requires measuring the cross-reactivity with other closely related molecules (e.g., insulin in an insulin-like growth factor-I assay). This is more easily done for steroid and thyroid hormones, because closely related hormones are available commercially. Determining specificity of an assay for protein hormones and cytokines in poultry is difficult, because their closely related molecules in poultry, and often the target itself, are not available. Without validation of the specificity of any immunoassay, the results might be completely inaccurate. In conclusion, results from immunoassays should not be accepted on face value without proper validation.

**Keywords:** radioimmunoassay; ELISA; physiology; immunology

**Panel Discussion**

## Monday, July 13, 2026

### Presidential Symposium: The State of the Art in Poultry Bone Biology

**John W. H. Bassett Theatre (MTCC)**

#### 1061S Introduction and issues in bone metabolism in poultry

Doug Korver*[1] [1]*Alpine Poultry Nutrition, Inc., Edmonton, Alberta, Canada*

Bone metabolism is critical to efficient and profitable growth and egg production in modern poultry. The unintended consequences of genetic selection for production traits alone have led indirectly to a high incidence of skeletal deformities in the past, and in the present, increased risk of bone fractures and infections. Increased attention to skeletal health in genetic sleection programs has reduced the incidence abnormalities that had been a problem in the past. However, as broiler and layer productivity traits continue to increase, new pressures are put on the skeletal system. Bone issues in poultry, the impact of genetic selection on bone metabolism and selection for a healthy skeletal system will be reviewed, in addition to the current state of knowledge of the effects of nutrition and metabolism on bone biology. Finally, the digestibility of calcium and effective use of supplemental phytase, two critical areas of nutrition relevant to bone formation, will be discussed in light of the most recent scientific developments.

**Keywords:** bone metabolism; skeletal defects; genetic selection; nutrition; phytase

#### 1062S Genetic factors affecting bone metabolism

Martin Johnsson*[1] [1]*Swedish University of Agricultural Sciences, Department of Animal Biosciences, Uppsala, Sweden*

The genetics of bone metabolism in poultry is relevant both to breeding and to fundamental science. In poultry breeding, genetics may help reduce the risk of bone problem in hens that lay eggs, in particular keel bone pathology in commercial laying hens. In fundamental science, the domestic chicken serves as a model of avian bone metabolism, which is relevant both for specific differences from mammals and for shared features of vertebrate bone metabolism. In this talk, I will review the state of knowledge about the genetic basis of bone metabolism in poultry, focussing primarily on the domestic chicken and laying hens. The talk will discuss how bone traits are measured for genetics, estimates of genetic parameters, and major loci that have been identified by genetic mapping. Bone traits are often moderately heritable. There are unfavourable correlations between both long bone and keel bone traits and early egg production. While some major loci have been identified, genome-wide association studies suggest that bone traits are, generally, complex. Genetic studies require larges sample sizes and affordable trait recording. Digital phenotyping of bone has advanced, with several research groups publishing genetic estimates based on radiography. The combination of affordable phenotyping and moderate heritability bodes well both for fundamental research and applications in poultry breeding.

**Keywords:** bone metabolism; keel bone; laying hen ; genetics; genomics

#### 1063S Physiological regulation of calcium and phosphorus homeostasis in poultry

Laura E. Ellestad*[1] [1]*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

The primary component of bone, hydroxyapatite, accounts for over 99% of the calcium and 80% of the phosphorus in the body. As a result, understanding the dynamic physiological processes that govern homeostasis of these two minerals as they relate to skeletal growth, remodeling, and eggshell mineralization in poultry is of upmost importance to ensure optimal bone health. Multiple hormones – bioactive vitamin D3 [1A,25(OH)2D3], parathyroid hormone, calcitonin, and fibroblast growth factor 23 – maintain circulating levels of calcium and phosphorus within homeostatic ranges by acting on the intestine, kidney, and bone to mediate mineral uptake, reabsorption and excretion, and resorption and deposition, respectively. These integrated processes are regulated at multiple levels that include production of 1A,25(OH)2D3, hormone action through their respective receptors, and expression of plasma membrane and intracellular calcium and phosphorus transporters. Recent findings related to these processes in poultry will be discussed, with a particular emphasis on how they relate to bone.

**Keywords:** calcium; phosphorus; vitamin D3; bone; hormonal regulation

#### 1064S Impact of reproductive status on bone physiology in laying hens

Grégoy Y. Bédécarrats*[1], Alexander Chattoe[1], Ana K. Rentsch[2] [1]*University ofGuelph, Animal Biosciences, Guelph, Ontario, Canada;* [2]*University of Guelph, Animal Bioscience, Guelph, Ontario, Canada*

For the table egg industry, decades of intensive genetic selection for extended lay and earlier sexual maturation resulted in the development of high-performing commercial strains capable of producing over 500 eggs by 100 weeks of age. Since calcium requirement for egg shell formation cannot be sustained solely by dietary intake, bones serve as calcium reservoir for eggshell formation. In chickens, bones are made of two functionally distinct types, medullary bone and structural bone (cortical and trabecular). Cortical and trabecular bone provide structure and strength while medullary bone is a porous inner bone inside marrow cavities which serves as the dynamic calcium reservoir. The formation of these structures is intimately linked to the reproductive status as during the growing phase, pullets build up their skeleton and structural bone mass, while medullary bone develops only in pullets undergoing sexual maturation. At puberty, due to the role of estradiol on osteoblast function, structural bone growth largely ceases, and physiology switches to medullary formation. As a result, selecting for earlier age at first egg may compromise skeletal integrity by weakening structural strength and increasing the risks of injury, while extending lay may deplete medullary store resulting in osteoporosis and calcium deficiency. Beyond long bones, the development and health of the keel bone (sternum) has

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also become a major issue in modern layers, especially in the context of switching towards non-cage rearing systems. Unlike long bones, the keel bone is not fully ossified at the time of sexual maturation and rather than responding to the maturation, our recent data suggest it is mostly dependent on age. In other words, advancing sexual maturation will not advance keel ossification resulting in a more cartilaginous keel at first egg. Although puberty does not impact the development of the keel bone, it still results in its repositioning (tilting) to allow for egg passage, possibly further increasing the risk of injury, particularly at the distal tip.

**Keywords:** Sexual maturation; Laying hens; Extended lay; long bones; keel bone

**Break/Poster Viewing**

### 1065S Effects of using digestible calcium for layers and broilers

Roselina Angel*[2], Kyle M. Venter[1], Peter Plumstead[3] [1]*Neuro Livestock Research, Lanseria, South Africa;* [2]*University of Maryland, Department of Animal and Avian Science, College Park, Maryland, United States;* [3]*Neuro Livestock Research, Lanseria, South Africa*

Commercial implementation of a digestible calcium (dCa) system for broilers and layers is in its infancy. Ca digestibility methodologies both for broilers and layers must be developed with an understanding of Ca homeostasis and based on physiological responses of the birds. In layers, work must be done in relation to oviposition time, as Ca digestibility of a diet and/or ingredient is high during shell formation (15 h post oviposition) and low during bone repletion (1.5 h post oviposition). In broilers, feeding experimental diets that are close to the requirement for digestible Ca, at the age the experiment is done is essential. Because broilers, after 16 d of age, can up or down-regulate Ca digestibility when diets deficient or in excess of Ca are fed, within 40 h, the length of the experimental period should be less than 36 h. To implement a dCa system ingredient dCa values must be known and dCa requirements for the production phase must be established. The most variable ingredient in terms of dCa is limestone both in broilers and layers. In broilers dCa in limestone can vary between 20 and 75% and thus the use of an average value is not accurate. Prediction equations for dCa of limestone based on limestone characteristics (dynamic solubility (5, 15, 30 min) in a 3 pH buffered solution, Ca and Mg concentration, geometric mean diameter) and phytate P content of the diet have been developed and validated. Development of a prediction equation of dCa in layers is ongoing. Limestone accounts for more than 90% of the Ca in layer diets. Based on preliminary dCa work in layers, the inclusion of limestone in a diet with 2.3% dCa can be as low as 8.5% and as high as 12% if a low or high Ca digestibility limestone is used. In spite of limestone being the least expensive ingredient in the formulation, the ability to include limestone in diets on a dCa basis and optimize its inclusion has important implications for diet costs because of diet space occupied by the limestone. . In broilers, implementation of a dCa system in conjunction with dP, has demonstrated improved mineralization of bone in birds, improved performance and lower feed cost/kg of body weight gain.

**Keywords:** Calcium homeostatis; calcium digestibility; broiler; layer; limestone

### 1066S Endogenous and exogenous phytase effects on P utilization in poultry

Markus Rodehutscord*[1] [1]*University of Hohenheim, Institute of Animal Science, Stuttgart, Germany*

Phytate-bound phosphorus (P) in plant feedstuffs was long considered overall unavailable to poultry. This view shaped feeding strategies, P evaluation assays, and the development of exogenous enzymes. However, birds have an endogenous capacity to release P and *myo*-inositol (MI) from inositol-6-phosphate (InsP6) and other inositol phosphates. In broiler chickens, endogenous InsP6 degradation can exceed 60% and responds strongly to dietary mineral P and calcium (Ca) concentrations. Mineral P, especially when combined with excess Ca, reduces endogenous InsP6 degradation. Therefore, P digestibility values of feedstuffs are not additive in mixed diets. Exogenous phytases can compensate for these effects in a dose-dependent manner by degrading most of the dietary InsP6 in broiler chickens. The extent of InsP6 degradation is consistently associated with changes in ileal and plasma MI concentrations, prececal P digestibility, and bone mineralization. In laying hens, the Ca concentrations in feed and intestinal lumen are a stronger regulator of endogenous InsP6 degradation than dietary P. The endogenous InsP6 degradation of laying hens hardly exceeds 20%, and even complete renunciation of mineral P from the feed only modestly stimulated InsP6 degradation under standard dietary Ca levels. The endogenous enzymes showed a predominant 6-phytase activity. Exogenous phytases can increase InsP6 degradation to levels similar to those found in broiler chickens. Still, some of the released P may be excreted because of the low P requirements of laying hens. Comparative studies have shown distinct differences in traits related to P and MI metabolism between high-performing laying hen strains, including differences in the heritability of endogenous InsP6 degradation and quantitative genetic variation in P metabolism-related traits. This indicates that breeding led to different metabolic priorities in InsP6 degradation and mineral metabolism, rendering breeding-metabolism-nutrition interactions relevant for future research in chicken.

**Keywords:** Phosphorus; Phytate; Endogenous enzymes; Laying hen; Broiler chicken

## PSA Hatchery Workshop: From Research to Reality: Communicating Poultry Science to Diverse Audiences

**105 Constitution Hall (MTCC)**

**Hatchery Business Meeting**

### 1067S Food safety and biosecurity communication for small-scale poultry owners

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Research, Extension, and education are three central pillars of poultry science. While research advances scientific knowledge, its greatest impact occurs when findings are translated into practical strategies that support poultry production, flock health, food safety, and producer decision-making. Extension provides a critical bridge between laboratory-based research and field application, particularly for small-scale and backyard poultry owners who may have limited access to technical training and science-based resources. The New England poultry industry includes many backyard, small-scale, diversified, and direct-market poultry producers. These audiences play an important role in local food systems but face unique challenges related to biosecurity, disease prevention, safe egg handling, flock management, sanitation, predator control, and small-scale poultry processing. Communicating with these groups requires more than sharing scientific information; it requires accessible language, practical recommendations, trust-building, and an understanding of producer needs and limitations. Translating poultry science into effective food safety and biosecurity education for small-scale poultry owners is essential to keep the flock safe and healthy. Drawing from poultry Extension and food safety programming in Connecticut and the broader New England region, the presentation will discuss strategies such as workshops, trainings, webinars, factsheets, farm consultations, stakeholder partnerships, and applied educational resources. Emphasis will be placed on helping future poultry professionals to communicate poultry science beyond academ-

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ic audiences by identifying stakeholder needs, framing risk-based messages, and converting research findings into usable recommendations

**1068S The feed forward: Translating science from lab to flock**

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The poultry industry has been successful for many decades; however, it faces increasing global scrutiny regarding affordability, sustainability, animal welfare, and food safety. Translating technical research for diverse audiences is essential. A significant disconnect remains between advances in evidence-based poultry science and public perception, often leading to misinformation, slower progress in the field, and a lack of funding for research. This presentation highlights ideas to promote communication under 5 headings that could help bridge the gap between rigorous poultry research and real-world impact: 1. Connecting to the Big Picture, 2. De-Jargonizing Research, 3. Expanding the Communication Toolkit, 4. Handling Skepticism and hot-button issues, and 5. Prioritizing communication to secure resources to sustain university poultry-focused departments.

**Panel Discussion**

# POSTER PRESENTATIONS

## Student Poster Competition I

**351P Determining the conversion efficiency of phenylalanine to tyrosine in a modern broiler strain**

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The objective of these experiments was to determine the efficiency of converting Phe to Tyr in modern broiler chickens. In Experiment 1, the L-Phe requirement was determined using a purified cornstarch-amino acid-based diet that was devoid of Tyr and contained 0.50% L-Phe. Eight dietary treatments were created by supplementing L-Phe in graded increments of 0.10% to the basal diet, at the expense of Solka Floc, to achieve a range of 0.50 to 1.20% L-Phe. In Experiment 2, the L-Phe requirement was determined using a similar purified cornstarch-amino acid-based diet with the exception that the basal diet contained 0.20% L-Phe and excess Tyr (0.60% L-Tyr). Eight dietary treatments were created by supplementing L-Phe in graded increments of 0.10%, at the expense of Solka Floc, to achieve a range of 0.20 to 0.90% L-Phe. In Experiment 3, the conversion efficiency of L-Phe to L-Tyr was determined using a slope-ratio assay. Seven dietary treatments were created by supplementing 0.06, 0.12, and 0.18% of either L-Phe or L-Tyr to a purified cornstarch-amino acid-based diet that was devoid of Tyr and contained 0.60% L-Phe. In each experiment, birds were given ad libitum access to a common nutrient adequate corn-soybean meal-based starter diet from 0 to 11 d-of-age. Experimental diets were provided ad libitum from 12 to 19 d-of-age. There were 5 replicate pens of 5 male broiler chickens per treatment in each experiment. All data were analyzed using a 1-way ANOVA. Broken line, linear, and quadratic regressions were conducted for Experiments 1 and 2. Efficiency of Phe conversion to Tyr was determined in Experiment 3 by regressing BW gain against Phe+Tyr intake. The significance value for all analyses was $P < 0.05$. In Experiments 1 and 2, there was a quadratic effect of supplemental Phe on gain:feed ($P < 0.05$). The broken line regression for gain:feed yielded an estimated L-Phe requirement of 0.93% ($R^2 = 0.96$) in Experiment 1 (diet devoid of Tyr) and 0.44% ($R^2 = 0.96$) in Experiment 2 (diet with excess Tyr). In Experiment 3, BW gain increased linearly in response to supplementation of both L-Phe and L-Tyr ($P < 0.05$). Multiple linear regression of BW gain on total Phe+Tyr intake yielded an estimated efficiency of L-Phe conversion to L-tyrosine of 97% on a weight basis. In conclusion, modern broiler chickens are able to efficiently convert L-Phe to L-Tyr. Furthermore, these findings indicate that L-Phe and L-Tyr can be used interchangeably to meet the Tyr component of the Phe+Tyr requirement for modern broiler chickens.

**Keywords:** broiler; relative bioavailability; conversion; phenylalanine; tyrosine

**352P Release of phosphorus and inositol from soybean meal by broiler chickens fed diets with graded levels of microbial phytase**

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The primary objective of this experiment was to determine the release of phosphorus (**P**) and inositol from soybean meal (**SBM**) at different levels of supplemental phytase fed to broiler chickens. The effects of phytase on metabolizable energy and amino acid digestibility were also evaluated. Twenty diets were arranged in a 4 × 5 factorial design with 4 sources of SBM (i.e., sources 1, 2, 3, and 4) and 5 levels of supplemental phytase (i.e., 0, 500, 1,000, 2,000, and 4,000 units/kg diet). In the dextrose-cornstarch-SBM diets, SBM was the only source of P. A total of 600 Ross 308 broiler chicks were allotted to one of the 20 dietary treatments, with 6 replicate battery pens per treatment and 5 chicks per pen in a randomized complete block design. Chicks were fed a common starter diet from d 1 to 18 and experimental diets were fed from d 19 to 20. On the morning of d 21, all birds were euthanized with CO2 gas for collection of ileal digesta. Blood samples were obtained via cardiac puncture, and excreta samples were also collected. Deproteinized plasma samples were analyzed for *myo*-inositol concentration. Ileal and total tract P digestibility, apparent metabolizable energy (**AME**), nitrogen-corrected (**AMEn**), and standardized ileal amino acid digestibility (**SIAAD**) were calculated. Data were analyzed using the PROC MIXED procedure of SAS. The pen was the experimental unit. Fixed effects included SBM source, phytase level, and their interaction, whereas block and replicate within block were considered random effects. Differences were considered significant at $P \leq 0.05$. A SBM source × phytase interaction ($P < 0.05$) was observed for ileal and total tract P digestibility. Thus, phytase increased ($P < 0.05$) ileal and total tract P digestibility, and the effect of phytase varied among SBM sources (e. g., 29 to 54% unit increase in ileal P digestibility among SBMs). The concentration of plasma *myo*-inositol increased ($P < 0.05$) as phytase inclusion increased regardless of SBM source (mean inositol increase was 82% among SBMs). Neither AME nor AMEn was influenced by phytase supplementation; however, SBM source affected both diet AME and AMEn ($P < 0.05$). The SIAAD was not affected by SBM source, phytase level, or their interaction ($P > 0.05$). In conclusion, increasing phytase supplementation enhanced P digestibility and plasma *myo*-inositol concentration in a dose-dependent manner, but did not affect metabolizable energy or amino acid digestibility. The response in P digestibility to phytase differed among SBM sources, indicating that SBM characteristics may influence phytase efficacy.

**Keywords:** phytase; myo-inositol; phosphorus; broilers; soybean meal

**353P A blend mainly based on oregano essential oil and spices improved body weight distribution and growth performance in broilers**

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Essential oils are recognized for their positive effects on broiler gut health and growth performance, yet their impact on flock uniformity remains poorly documented. Phodé (France) has developed an Essential

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Oil blend (EOB) mainly based on oregano and spice-extracts with synergistic properties. The aim of this study is to investigate whether its performance-enhancing effects extend to improve body weight distribution. Three hundred thirty-six male Ross 308 broiler chicks were distributed across 24 pens (14 birds/pen; 14 birds/m2) in a randomized complete block design with 2 treatments and 12 replications. A deliberate challenge was maintained throughout the 35-day trial by combining high stocking density (for an experimental design) with reduced litter quantity (3.5 kg/m2 instead of 6) in a closed house with tunnel ventilation in Thailand (August–September 2025). T1 was a corn-soybean meal diet formulated to meet Ross 308 (2019) requirements across starter (0–10 d), grower (10–24 d), and finisher (24–35 d) phases. T2 included EOB at 125 g/t replacing 125 g/t of corn in the same diet formulations compared to T1. Growth performance was recorded at each phase transition. On d 35, all individual body weights (168/treatment) were collected to assess body weight frequency distribution across 200 g classes from less than 1.9 kg to more than 3.1 kg (8 classes in total). Pen means were subjected to one-way ANOVA in a randomized complete block design using SAS. Individual body weight distribution between treatments was compared by chi-square test. $P < 0.05$ was considered as significant. Over 0–35 d, EOB-supplemented birds had significantly greater feed intake (3,684 vs. 3,584 g/bird; $p = 0.036$) and body weight gain (2,545 vs. 2,467 g/bird; $p = 0.004$); feed conversion ratio was not affected (1.447 vs. 1.453; $p = 0.457$). Individual body weight distribution at d 35 was significantly shifted toward heavier weight classes in the EOB group ($p = 0.002$): 66.7% of birds reached ≥ 2.7 kg vs. 55.4% in controls, and 41.2% reached ≥ 2.9 kg vs. 25.9% in controls. Moreover, the 2 extreme classes (less than 1.9 or more than 3.1 kg) had no birds in the treatment group.EOB at 125 g/t improved broiler growth performance by significantly shifting the individual body weight distribution toward heavier weight classes, indicating a beneficial effect on flock productivity. Flock uniformity was also improved. These findings support the potential of EOB to address uniformity and productivity challenges in commercial broiler production.

**Keywords:** Broilers; essential oils; flock uniformity; body weight distribution; growth performance

### 354P A SINGLE AND COMBINED SUPPLEMENTATION OF 25-HYDROXYCHOLECALCIFEROL AND PROBIOTICS IN FEED ON PERFORMANCE, INTESTINAL AND BONE MORPHOLOGY IN BROILER CHICKENS

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This study investigated the effects of dietary supplementation with 25-hydroxycholecalciferol (25-OHD3) and probiotics (Bacillus amyloliquefaciens) on productive performance, intestinal morphology, and bone morphometry in broiler chickens. A total of 200 one-day-old male broiler chickens were randomly assigned to four dietary treatments: a basal diet (T1, control), a basal diet with probiotics (T2), a basal diet with 25-OHD3 (T3), and a basal diet with a combination of both (T4). The collected data were submitted to ANOVA under a Completely Randomized Design and for means separation, the Duncan Test was used. Significant differences were observed in body weight gain from 0-21 days, ($P<0.05$) where T1 and T3 showed superior weight compared to T2; other performance indicators were not significant. Additionally, T4 exhibited a significantly higher villus height (859 um) compared to T2 with 711 um ($P<0.05$) at 21days. Regarding bone morphometry, tibia length was significantly longer for T1 with 70.43 mm ($P<0.05$) compared to T2 with 68.46 mm. For tibia width, T4 showed higher values than T2 and T3. In conclusion, under the trial conditions, performance favored 25-OHD3 and the control treatment compared to probiotics supplementation and a combination of both. Then, for the intestinal morphology, villus height was better for the treatment that combined 25-OHD3 and the probiotic, compared to the probiotic treatment only, meaning the effect might be only from 25-OHD3. Finally, regarding bone morphometry, tibia length favored the control treatment compared to adding a probiotic, and tibia width was better for the combination 25-OHD3 and the probiotic vs compared to adding a probiotic alone or 25-OHD3 alone.

**Keywords:** 25-hydroxycholecalciferol ; probiotic; bone morphology; intestinal morphology; broilers

### 355P The impact of a *Quillaja saponaria* product on growth performance and ileal amino acid digestibility of broiler chickens

Kasey Young*[1GS], Rosana Hirai[1], Rahul Reddy[1], Addison Elstner[1], Abigail Flores[1], Camryn Wilder[1], Austin silva[1], Kyle Brown[2], Staci Cantley[2], Audrey McElroy[1] [1]*Texas A&M University, Poultry Science, College Station, Texas, United States;* [2]*Huvepharma, Peachtree City, Georgia, United States*

This study evaluated the effects of a saponin derived from *Quillaja saponaria* (QS) on growth performance and ileal amino acid (AA) digestibility in Cobb 500 by-product male broilers. A total of 1,560 day-of-hatch chicks received 1X dose of Advent® and were randomly allocated to 4 dietary treatments (trts), with 15 replicates (0.093 $m^2$/bird) in a randomized complete block design. The 4 trts were: a corn-soybean meal diet at 95% of breeder's AA recommendations, no additive (C); C+250ppm QS; C+125ppm *Yucca schidigera* saponin (YS); C+250ppm QS+250ppm *Bacillus licheniformis* probiotic (QP). Feeding phases were starter (d0-14), grower (d15-28) and finisher (d29-42). Performance data included feed intake (FI), mortality-corrected feed conversion ratio (FCR), mortality %, average body weight (BW) and BW gain (BWG) on d14, 28 and 42. Ileal digesta was collected on d14 and 28 to measure apparent ileal digestibility (AID) and digestible AA intake (AAI). Data were analyzed in SAS 9.4; means were obtained by LSMEANS and separated using Tukey's HSD ($P\leq0.05$). From d0-14, birds fed QS, YS or QP had lower FCR, higher FI, d14 BW and BWG than C ($P<0.0001$). From d15-28, birds fed QS, YS or QP had greater FI than C, birds fed QS had higher BWG and d28 BW than birds fed C with other trts intermediate ($P<0.0001$). Overall, QS inclusion increased FI ($P=0.0006$), BWG ($P=0.03$) and d42 BW ($P=0.03$) compared with C, while the other trts were intermediate. At d14, AID was higher for all essential AA (EAA) except threonine (Thr), methionine (Met) or tryptophan (Trp) in birds fed QS, QP or YS compared to C ($P\leq0.05$). Thr AID was higher in birds fed QS or YS than C, with birds fed QP similar ($P=0.001$). Met AID was higher in birds fed QS than those fed C, with YS or QP similar ($P=0.008$). Trp AID was higher in birds fed QS or QP than C, with YS similar ($P=0.001$). Birds fed QS had greater d14 AAI of all EAA, except Trp or Thr, while C had the lowest AAI ($P<0.0001$). Birds fed QS or QP had greater Trp AAI than those fed YS or C, with C birds having the lowest Trp intake ($P<0.0001$). Birds fed QS or YS had greater Thr AAI than those fed QP or C, while birds on C had the lowest of all trts ($P<0.0001$). At d28, Trp AID was higher in birds fed QP than QS or C, while YS was similar ($P=0.006$). Feeding QS or QP increased d28 AAI of all EAA except valine (Val), Met, arginine (Arg) or Trp when compared to YS or C ($P<0.0001$). Birds fed QS had the highest AAI for Val or Arg ($P<0.0001$). Birds fed QS or YS had higher Met AAI than those fed QP or C ($P<0.0001$). Birds fed QP had higher Trp AAI than those fed C, with QS or YS intermediate ($P<0.0001$). In conclusion, this study suggests positive effects of QS inclusion on broiler performance and AA digestibility.

**Keywords:** Quillaja saponaria; growth performance; digestibility; saponins

### 356P Evaluation of *Lippia origanoides* essential oil and fructooligosaccharides on amino acid digestibility in 21 d old broilers

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Improving nutrient utilization through dietary strategies has become a

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key focus in broiler nutrition, increasing interest in feed additives as potential alternatives to enhance gastrointestinal function. The objective of this study was to evaluate the effects of a *Lippia origanoides* essential oil–based product and a fructooligosaccharide (FOS) prebiotic on the apparent ileal digestibility (AID) of amino acids in broilers to 21 d post-hatch. A total of 288 Ross® YP×708 broilers were randomly assigned to 36 battery cages and allocated to 3 dietary treatments: Control (CTL), *Lippia origanoides* essential oil (EO), and a FOS prebiotic (PRE). Ileal digesta were collected at 21 d to determine AID of amino acids. Data were analyzed using a one-way ANOVA, and means were separated using Fisher's LSD test; significance was declared at $P < 0.05$. Apparent ileal digestibility differed ($P < 0.05$) among treatments for all individual amino acids evaluated. Total AA AID was lowest for CTL (79.6%), intermediate for EO (83.4%), and highest for PRE (84.5%). Most individual amino acids followed a similar pattern. For several essential amino acids, including methionine, threonine, isoleucine, leucine, phenylalanine, and histidine, CTL was lower than both EO and PRE, which did not differ from each other (CTL < EO = PRE; $P < 0.05$). In contrast, lysine, tryptophan, valine, and arginine exhibited a progressive improvement across all three treatments, with each treatment differing from the others (CTL < EO < PRE; $P < 0.05$). In summary, dietary supplementation with *Lippia origanoides* essential oil or FOS prebiotic enhanced apparent ileal amino acid digestibility in broilers compared with the control diet, suggesting that these additives may improve protein and amino acid digestion and/or absorption.

**Keywords:** Lippia origanoides; Essential Oils; apparent ileal digestibility; amino acids; broiler nutrition

### 357P Effects of dietary deoxynivalenol and zearalenone on pullet performance from 4 to 10 weeks of age

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Mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEN) are common contaminants of feed ingredients, including corn, wheat, and barley, which pose significant health risks to poultry. Over 80% of feed and feed ingredients have been found to contain multiple mycotoxins. The Food and Drugs Administration (FDA) has reported action limits for mycotoxins, including DON, Aflatoxins, and Fumonisins. However, ZEN, which has become one of the most prevalent mycotoxins, with over 70% prevalence, has no limit reported by the FDA. This study investigated the effects of feeding a combination of naturally occurring DON and ZEN to pullets. At 4 weeks of age, pullets of uniform body weight were assigned to cages, with each cage consisting of 5 birds. In a completely randomized design with 6 replicates per treatment, 120 Lohmann LSL-Brown were assigned to 4 dietary treatments: diet 1 = (0.10 + 0.03), diet 2 = (2.67 +2.05), diet 3 = (5.34 + 4.13), and diet 4 = (8.01+6.22) ppm DON + ZEN, respectively, over 6 weeks. Feed intake, body weight, body weight gain, feed conversion ratio (FCR), and uniformity were measured weekly to determine the performance of pullets. Data were analyzed using regression analysis in Minitab 22.1. Significance was considered when $p \leq 0.05$. Dietary treatments did not influence body weight and uniformity ($p > 0.05$) during the test period. However, body weight gain, feed intake, and FCR were affected by dietary treatments ($p < 0.05$). At 8 weeks, body weight gain showed a quadratic response ($p < 0.001$) to DON and ZEN, with optimal weight gain observed in birds fed diet 2. A Similar quadratic response was seen for feed intake at 7 weeks of age, where feed intake was maximized in birds fed diet 2. There was a linear reduction ($p = 0.007$) in week 8 FCR with increasing levels of DON and ZEN. These results indicate that a combination of DON and ZEN can influence growth and feed efficiency in pullets.

**Keywords:** Zearalenone; Deoxynivalenol; Mycotoxins; Feed intake; Laying hen

### 358P Description of a dynamic mechanistic protein digestion model for poultry

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As feed can comprise 60-70% of the cost of production for poultry farmers, it is valuable to improve prediction of nutrient digestibility to decrease waste through overfeeding of nutrients. Mechanistic digestion kinetic models have been developed for a number of species, typically used to help optimize feed formulation and reduce excess nutrient excretion. Therefore, the objective of this work was to develop a dynamic mechanistic model of protein degradation kinetics and digestion for poultry, to improve prediction of nutrient utilization and explore ingredient impacts on the dynamics of degradation. The model developed herein for broilers was inspired by recent approaches in swine and ruminants and a novel transient plug flow reactor (PFR) approach to simulate the tubular structure of the gastro-intestinal tract. Instantaneous mixing pools are used to represent the crop and proventriculus/gizzard (PVG), while the small intestine is represented as tubes. Fluxes through the pools in the small intestine are calculated based on modified mass-action kinetic equations, incorporating both volumetric flow rate and passage rate. Separate pools are used to represent the undigestible (UD), slowly-digestible (SD) and rapidly-digestible (RD) protein fractions, beginning from the outflow of the PVG. Fluxes from the SD to RD pools represent degradation while RD to the absorbed pool represent absorption. Protein in the ileum of each fractional pool are summed and expressed relative to protein intake to determine the apparent ileal CP digestibility. Inputs into the model include feed intake, the protein percentage of diet fed, the proportions of the UD, SD and RD protein fraction, and the fractional degradation rate of the SD fraction. Feed intake is modelled as small consecutive boluses to simulate feeding behaviour over days. A behaviour analysis was conducted to evaluate model predictions in response to changes in inputs, as well as comparison of predictions against literature data. Overall behaviour of the model was as expected, with the model demonstrating correct responses to changes in the driving variables and results comparable to literature data. This work represents one of the first digestion kinetic models for protein in poultry, as well as the first that incorporates both instantaneous mixing pools and PFRs. Future work includes representation of other nutrients in the diet, consideration of fermentation in the hind gut, and properties of protein ingredients such as antinutritional factors. This work supports the development of precision nutrition strategies in poultry, thereby reducing excess nutrient feeding and excretion via improved prediction of ingredient value and digestibility.

**Keywords:** apparent ileal protein digestibility; mechanistic modelling; plug flow reactor; broiler; digestion kinetics

### 359P Determination of relative bioavailability of calcium in four limestone sources varying in apparent ileal digestibility of calcium in broiler chickens

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An experiment was conducted to determine relative bioavailability values (RBV) for Ca in 4 commercial limestones that had been found previously to vary in apparent ileal digestibility (AID) of Ca (2 low AID limestones averaging 20% versus 2 high AID limestones averaging 44%). Ross 308

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male broilers (330 chicks in total) were fed a common starter diet in mash form from days 1 to 7 post-hatch. From day 8 to 20 days-of-age, birds were fed 1 of 11 diets which were either a Ca deficient corn-soybean meal base diet (0.3% Ca) or that diet supplemented with 0.15% or 0.30% Ca from either reagent grade CaCO3 or one of the 4 commercial limestones that varied in AID of Ca. The RBV of Ca was based on bone ash content (mg/tibia) as the response variable. At the end of the experiment, birds were euthanized using CO2 gas, and weight gain and feed intake were recorded. To determine Ca RBV, the right tibia was collected from all birds for tibia ash analysis. A completely randomized design was used with 5 chicks per cage (experimental unit), 11 treatments, and 6 repetitions per treatment. Data were initially subjected to a one-way ANOVA, and differences among treatments were determined using the least significant difference (LSD) test at $P < 0.05$. To determine Ca RBV in the 4 limestone sources relative to reagent grade CaCO3, multiple linear regression analysis of bone ash on supplemental Ca intake was performed. Regression coefficients or slopes for the 4 commercial limestones were then compared with the slope for the reagent grade CaCO3 (set at 100%) which yielded RBV ranging from 74 to 89%. When comparing RBV with previously determined AID Ca values, one of the low AID limestones had a lower RBV (74%) ($P < 0.05$) than the RBV of the 2 high AID limestones (84 and 88%) which indicated agreement between RBV and AID. The other low AID limestone (21%), however, had an RBV (89%) that was similar and not different ($P > 0.05$) than the RBV of the 2 high AID limestones. Thus, there was both agreement and disagreement between RBV values based on bone ash and digestibility values based on AID.

**Keywords:** Calcium; Limestone; Digestibility; Bioavailability; Ash

### 360P Bone parameters of laying hens in the early lay phase when fed with lower copper through proteinate supplementation

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The creation and preservation of bone tissue depend on copper (Cu), an essential trace mineral that functions as an enzymatic cofactor in antioxidant, structural, and metabolic activities. Because of interactions in the gastrointestinal tract, inorganic sources like copper sulfate (CuS) have decreased bioavailability, which may hinder absorption and increase environmental excretion. Organic sources, including copper proteinate (CuP), on the other hand, have higher metabolic efficiency and can be fed at a lower level without sacrificing bone integrity. This study was designed to investigate the bone parameters of laying hens at early laying phases (20 to 33 weeks of age) when fed with different copper sources, Cu sulfate (CuS) or Cu proteinate (CuP), and levels during the growth, and early laying phases. A total of 480 hens were randomly assigned into 4 treatments in a 2 × 2 factorial arrangement with 8 hens per experimental unit. The experimental factors were Cu supplementation source during the growing and early laying phases. In each phase, birds received either 100% of the recommended copper requirement (Brazilian tables) from CuS or 50% of the requirement using CuP (Bioplex Copper, Alltech Inc.). Treatments represented all possible combinations of the two Cu sources across the two phases. Diets were prepared according to requirements of 10.43 mg Cu/kg for early laying phase analyzed. The evaluated variables at 33 weeks of age were: live body weight (LBW), bone length (BL), bone weight (BW), bone relative weight (RW), bone Seedor index (SI), bone strength (BS), and bone density (BD). The data were analyzed by ANOVA in a 2 × 2 factorial arrangement, and the means were compared by Tukey test using SAS v.9.0. No evidence of interactions or main effects were observed for variables LBW (1592,08 g), RW (0.47%), BS (13.57 kg), and BD (2.06 mmAl) ($p>0.05$). However, main effect of Cu source was observed where hens receiving CuS had higher BW ($p=0.008$) and SI ($p=0.007$) during the growing phase, with no significant effect during the early laying phase ($p>0.05$). There was no significant difference ($p>0.05$) in BL in the analyzed phases. However, there was an interaction between the growing and early laying phases ($p=0.029$) when supplementation was continuous. Although the copper source did not differ statistically under continuous supplementation, changing from CuP to CuS resulted in a lower BL, which did not occur when the change was from CuS to CuP. In conclusion, supplementing Cu as CuP at 50% of the requirement during the growth and early laying phase (20 to 33 weeks) did not negatively impact bone parameters of laying hens.

**Keywords:** bone density; copper proteinate; cooper sulfate; Seedor index; strength

### 361P Role of 25-hydroxyvitamin D3 on broiler growth performance and tibia bone quality

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25-hydroxyvitamin D3 [25(OH)D3] has been shown to have better bioavailability than vitamin D3 due to improved intestinal absorption and bypass the first hydroxylation step. A study was conducted to evaluate the effect of 25(OH)D3 on broiler growth performance and tibia bone quality. A total of 756 off-sex male Cobb 500 day-old chicks were randomly housed in 36 floor pens. Chicks were randomly assigned to one of the three dietary treatments, with 21 chicks per pen and 12 replicates per treatment: Control, with standard vitamin premix containing 1,400 IU/kg of vitamin D3; VD, control + 2,760 IU/kg vitamin D3; 25D, control + 69 µg/kg of 25OHD3 NexPureD3, SAM Nutrition (bioequivalent to 2,760 IU/kg vitamin D3). The experimental diet was divided into three phases, including starter (d 0-14), grower (d 15-28), and finisher (d 29-42). Body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded on d 0, 14, 28, and 42. Serum was analyzed on days 28 and 42, 25(OH)D3 level. On days 28 and 42, one bird/pen was used for bone quality, the right and left tibias were collected for bone strength and bone ash, respectively. Data were analyzed using one-way ANOVA, and means were separated using Tukey's HSD in JMP 17 (significance level: $P<0.05$). Results showed that supplementing 25(OH)D3 in the feed increased serum 25(OH)D3 concentration on days 28 and 42 compared to the control and VD treatments, thereby improving vitamin D status. However, no differences in BW. BWG, FI, and FCR were observed across phases and the overall period between the treatments. At day 28 and 42, no differences were observed in bone quality among treatments in bone strength and bone ash. In summary, supplementing 25(OH)D3 increased circulating serum 25(OH)D3 concentration compared with vitamin D3 under standard conditions, but did not show effects on growth performance or bone quality. It shows the safety of application the current product in broilers, and additional evaluation can be done under the stress conditions or when vitamin D3 becomes limiting to the birds.

**Keywords:** 25-hydroxyvitamin D3; broiler; bone health; performance; vitamin D status

### 362P Dietary available phosphorus reduction in commercial laying hens

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This study hypothesized that available phosphorus levels could be reduced in laying hen diets without negatively affecting performance and egg quality. The objective was to evaluate the feasibility of reducing available phosphorus content to decrease feed costs, considering that dicalcium phosphate is a finite and expensive ingredient. The experiment was conducted as a completely randomized design with four treatments and nine replicates. A total of 288 Hisex White laying hens were used from 25 to 40 weeks of age, comprising a 16-week period. Treatments consisted of four available phosphorus levels: 0.420% (T1), 0.340% (T2), 0.260% (T3), and 0.180% (T4). The trial was conducted at the Poultry Research Laboratory, University of São Paulo, Pirassununga Campus. Corn-soybean meal diets were formulated according to Hisex White nutritional guide (Hendrix Genetics, 2020), isonutritive except for available phosphorus. The period was divided into four 28-day cycles. Performance parameters evaluated: feed intake, feed conversion per dozen eggs and per egg mass, egg production, egg weight, egg mass, and marketable eggs percentage. Egg quality was assessed using Eggtester® device measuring egg weight, shell strength and thickness, albumen height, yolk color, and Haugh Units. Data were analyzed using JMP 14 (SAS Institute Inc.) with 5% significance level and Tukey's test for mean comparisons. Performance parameters including egg production (P=0.36), marketable eggs (P=0.12), feed intake (P=0.98), egg mass (P=0.06), feed conversion per egg mass (P=0.23), and per dozen (P=0.47) were not affected by treatments. For egg quality, except egg weight (P=0.96), all characteristics differed (P<0.05). Shell strength was highest in T2 vs T1 (5.07 vs 4.78 kgf, P=0.03). Shell thickness was greatest in T2 (0.48 mm) vs T1 (0.45 mm), superior to T3 and T4 (0.43-0.44 mm; P=0.001). Albumen height was higher in T3 and T4 (9.77-9.92 mm) vs T1 and T2 (8.28-8.76 mm; P<0.001). Haugh Units were highest in T3 and T4 (96.02-97.04) vs T1 and T2 (89.26-91.96; P<0.001). All egg quality parameters remained within acceptable commercial ranges. Reducing available phosphorus levels in laying hen diets can be accomplished without negatively affecting performance. The requirement of 0.18% available phosphorus is recommended for 25 to 40 weeks of age, representing significant feed cost reduction potential while maintaining production efficiency.

**Keywords:** Laying hens; phosphorus nutrition; egg quality; feed formulation; mineral requirements

### 363P Early exposure to adult cecal microbiota alters gut microbiota maturation throughout the broiler growth cycle

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Direct interaction between adult hens and chicks is an important mechanism for vertical transmission of gut symbiotic bacteria, yet commercial broiler chicks are raised in the absence of adults. Previous work suggests that this practice disrupts the maturation and function of the cecal microbiota. We therefore hypothesized that the absence of adult microbial exposure might also affect microbial communities in other regions of the digestive tract during the five-week broiler growth cycle. To test this, newly hatched broiler chicks (n = 84) were divided into two groups. Chicks in the treatment group were exposed to adult cecal content (input), whereas the control group received PBS. Each week, a subset of chicks was sacrificed and samples from cecal content and mucosa, jejunum, and crop were collected for 16S rRNA sequencing. Body weight and cecal short-chain fatty acids (SCFAs) were also measured. Microbial composition was evaluated using Bray–Curtis dissimilarity with PERMANOVA and dbRDA. Differentially abundant ASVs were identified using ANCOM-BC2, and Shannon diversity, SCFA levels, and body weight were compared between groups using Wilcoxon tests. The strongest treatment effects were observed in the cecum. Shannon diversity in cecal content was higher in treated chicks at all time points, and Bray–Curtis analysis revealed clear separation between treated and control communities (PERMANOVA $R^2$ = 0.16, P = 0.001). Treated chicks also showed a smaller Bray–Curtis distance to the adult input microbiota. Cecal mucosa communities showed a similar but weaker response and remained compositionally distinct from cecal content (pseudo-F = 6.32, P = 0.001). In contrast, treatment had a minor effect on microbial communities in the jejunum and crop. Consistent with this pattern, treated chicks also exhibited higher propionate and total SCFA concentrations in cecal content during the experiment. Body weights were lower in treated chicks for the first two weeks but were similar between groups by the end of the experiment. These results indicate that early exposure to adult cecal microbiota alters gut microbiota maturation and composition and affects intestinal regions differently. The cecum showed the strongest response, affecting both content and mucosa, while the jejunum and crop showed minimal changes. Microbial cecal communities in treated chicks were more similar to the adult microbiota, indicating accelerated maturation. Higher propionate levels in treated chicks suggest altered microbial fermentation. These differences persisted throughout the broiler growth cycle. The results highlight bacterial groups associated with specific intestinal regions and developmental stages as potential probiotics.

**Keywords:** Chickens; Gut microbiota; Vertical transmission; Short-chain fatty acids (SCFA); Immune function

### 364P Prevalence of Microbial Contamination in Chicken Carcasses and Eggs from Backyard Poultry Farms in North Carolina

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Backyard poultry (BYP) production has increased in recent years; however, substantial variation in management practices among flocks may influence the prevalence of foodborne pathogens, with possible implications for consumer health. This study aimed to evaluate the prevalence and levels of indicator organisms (*Escherichia coli* and coliforms) and foodborne pathogens in BYP chicken carcasses and eggs collected from six North Carolina counties. Forty-five chicken carcasses were collected post-processing using the MT-mitt method from four counties, and eight pooled egg samples (6 eggs/pool) were collected from BYP farms in two counties. MT-mitt samples were homogenized in 200 mL of Buffered Peptone Water (BPW). Egg samples were processed aseptically by rinsing external shells in 10 mL BPW, homogenizing pooled contents with BPW (1:5), and blending pooled internal shell membranes in 200 mL BPW. Carcasses were evaluated for *Campylobacter*, while both carcasses and eggs were evaluated for *Salmonella*, *E. coli*, and coliforms. For *Campylobacter*, 30 mL BPW suspension was mixed with 30 mL Bolton Broth, incubated microaerophilically for 48 h, and screened using BAX® qPCR, with confirmation on Campy Cefex Agar. *E. coli* and coliforms were enumerated using 3M Petrifilm plates and reported as log CFU/mL. The remaining BPW was enriched at 37 °C for 18–24 h and screened for *Salmonella* using BAX® qPCR. Presumptive positives were further enriched in Rappaport–Vassiliadis broth at 42 °C overnight and confirmed on XLT4 Agar. Data were analyzed in JMP using a one-way ANOVA (carcasses) and t-tests (eggs). All carcasses were positive for *E. coli* (2.31 to 2.85 mean log CFU/mL) and coliforms (1.28 to 2.82 mean log CFU/mL). Mean coliform levels differed significantly among counties (P < 0.0001), while *E. coli* levels did not (P = 0.0857). *Salmonella* prevalence varied by county as 0% (n = 12)

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in Caswell, 10% (n = 10) in Richmond, 91.7% (n = 12) in Rockingham, and 100% (n = 11) in Hurdle Mills. Notably, *Campylobacter* was not detected in any carcasses. Egg contamination was primarily on external shells, where *E. coli* and coliforms were detected in 50–100% of samples, and mean coliform levels ranged from 0.90 to 1.03 log CFU/mL. No significant differences were observed between counties for egg samples ($P > 0.05$). Internal shell membranes and contents showed minimal contamination, and *Salmonella* was not detected. While indicator bacteria are commonly present in poultry production environments, high and variable *Salmonella* prevalence observed in BYP carcasses highlights the potential food safety risk of pathogen transmission to consumers. Additional sampling is needed to better characterize the microbial prevalence in these systems.

**Keywords:** Backyard poultry; Salmonella; Campylobacter ; chicken carcasses; eggs

### 365P Impact of electron beam treatment on pathogen reduction and resuscitation dynamics in refrigerated chicken breast fillets

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Foodborne pathogens, particularly, *Salmonella* and *Campylobacter* continue to be significant contributors to bacterial foodborne illness associated with poultry products, leading to major economic losses in the United States. Electron beam (eBeam) technology, approved by USDA-FSIS and FDA, can be used to inactivate these pathogens in meat. Therefore, the objective of this study was to evaluate the effect of different eBeam doses within the current regulatory limit and determine the most effective dose for reducing *Salmonella, Campylobacter*, and background spoilage bacterial populations in chicken breast fillets. Shelf life was also evaluated to assess resuscitation of these bacteria under refrigerated conditions. For this study, chicken breast fillets were inoculated with a multi strain cocktail of *Salmonella* and *Campylobacter* containing 7.6 and 6.3 Log10 CFU/mL, respectively. A non-spiked control group was also included to account for the background bacterial populations on the fillets. The breast fillets were grouped into five treatments: 1 kGy, 2 kGy, 3 kGy, 4 kGy, and untreated control, each consisting of 5 samples in triplicates per dose for each storage timepoint. The samples were stored at 4°C and analyzed on days 1, 7, and 14 post eBeam treatment. On each sampling day, breast fillets were aseptically processed and plated on Xylose Lysine Deoxycholate agar for *Salmonella*, *Campylobacter* Enrichment Agar for *Campylobacter*, and Tryptic Soy Agar for total aerobic bacteria. Samples were also enriched in Rappaport Vassiliadis Soya broth and *Campylobacter* Enrichment broth. Data was analyzed using two-way ANOVA in GraphPad Prism 10 and $p<0.05$ was considered for statistical significance. Results demonstrated that all the eBeam doses significantly reduced *Salmonella*, *Campylobacter* and aerobic bacterial counts ($p<0.05$). Doses as low as 2 kGy reduced *Campylobacter* counts to below detection limits (>3 Log reduction), while 3 kGy eBeam dose reduced *Salmonella* counts to below detection limit (>5 Log reduction) on all sampling days. However, 4 kGy eBeam reduced all the bacterial counts to below detection limits on all three sampling days. Post-enrichment analysis showed resuscitation of *Campylobacter* with 2 kGy, and *Salmonella* with 3 kGy dose, whereas no resuscitation was observed for either *Salmonella* or *Campylobacter* in any fillets treated with 4 kGy. These findings showed that lower eBeam doses significantly reduced initial microbial loads but 4 kGy dose of eBeam treatment is required to ensure the complete inactivation of *Salmonella* and *Campylobacter*. Implementing 4 kGy of eBeam treatment effectively reduces foodborne pathogens and enhances product shelf life by maintaining low aerobic counts.

**Keywords:** Electron beam; Salmonella; Campylobacter ; Chicken breast; Poultry

### 366P Comparing *Salmonella* Typhimurium strain virulence using chicken cecal enteroids and drop plating method

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Research on poultry enteroids continues to develop and offer unique avenues to study host-pathogen interactions *in vitro*. *Salmonella* remains a prevalent problem in poultry production, and such *in vitro* methods could be a promising and efficient means for testing virulence or chemical mitigation strategies. *Salmonella* is shown to invade poultry enteroids with an apical-out formation and can be modeled *in vitro*. The objective of this study was to measure the difference in virulence between two strains of *Salmonella enterica* serovar Typhimurium (ST) in poultry enteroids using an established drop-plating method for measuring intracellular bacterial invasion. Intestinal crypts from ceca harvested from 18-day-old chicken embryos were cultured for 48h at 37C until the crypts formed into an apical-out formation where microvilli face outward in contact with culture medium. Enteroids were suspended in 50mL conical tubes containing warm medium where approximately 6 Log10 CFU/mL ST expressing red fluorescent protein (IR715/pFPV-mCherry, referred to as red ST) or ST expressing green fluorescent protein (SL1344 ptet.gfp, referred to as green ST) were added. Enteroids and ST were allowed to statically incubate together for 2h at 37C whereafter the medium was treated with 50uL/mL gentamicin sulfate for 1h. ST that successfully invaded enteroids were unaffected by the antibiotics while extracellular ST were killed. Following antibiotic treatment, enteroids were washed, resuspended in fresh medium without antibiotic and vigorously triturated to break into fragments. Resulting enteroid fragments were serially diluted with sterile saline and drop-plated onto XLT-4 agar plates to quantify intracellular ST recovery. Statistical analysis was performed using a one-way ANOVA with means separated by Tukey's HSD on JMP Pro 17 software. Data from 10 trials was compiled and compared for differences in ST recovery from enteroid fragments. Trials showed that red ST (n=19 replicate tubes) when cocultured with enteroids averaged 2.93±0.15 Log10 CFU/mL recovered, while green ST (n=22 replicate tubes) averaged 3.37±0.12 Log10 CFU/mL recovered. There was a significant ($P<0.05$) difference in bacterial recovery between the two ST strains, where recovery of green ST was notably higher compared to red ST (0.44 Log10 CFU/mL difference). This result was also observed and reflected in photomicroscopy where fluorescent protein expressions are exploited for visualization. This data suggests that differences in bacterial virulence can be tested and quantified using enteroids and a drop-plate method to better understand host-pathogen interactions. Future research will include gene sequencing and comparison of gene expressions between strains.

**Keywords:** Chicken enteroids; Salmonella; Virulence; In vitro; Gastrointestinal disease

### 367P Impact of stocking density on litter conditions and microbial contamination in cage-free laying hens

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Cage-free laying hens may experience increased stress under higher stocking densities, potentially increasing fecal shedding of pathogens and the risk of environmental and egg contamination. This study evaluated the effect of higher stocking density, defined by including colony nest space as usable floor space, on eggshell, litter, drag swabs, crop and cecal content in laying hens. A total of 1,436 Tetra Brown laying hens were housed in cage-free aviary pens at 1 $ft^2$/hen: high density (192 hens/pen; nests included) or standard density (167 hens/pen; nests excluded), with four room replicates (n=4 pens/treatment). At early-lay and peak-lay (26 and 41 weeks), 144 visibly clean eggs were collected, and pooled egg rinse samples and litter samples were analyzed. *Escherichia coli* and coliforms were enumerated on Petrifilms, and *Salmonella* was detected using BAX qPCR after enrichment. Water activity (aw), the amount of free water available that supports microbial growth, was also measured in the litter. At termination, crop and cecal content, drag swabs and litter samples were tested for *Campylobacter* and *Salmonella*. A one-way ANOVA and Chi-Square test were used for data analysis, with significant *p*-value <0.05. Eggshell *E. coli* and coliform levels (<1 log CFU/mL) were not significantly different between treatments at either time point. Litter from standard-density treatment at early-lay had higher *E. coli* (*p*=0.0005; 5.64 ± 0.23 log CFU/g) and coliform counts than the high-density litter (4.25 ± 0.24 log CFU/g), with no significant differences observed at peak-lay. In contrast, litter aw was higher in the high-density treatment at both early-lay (*p*=0.0033; 0.92 ± 0.01 vs. 0.89 ± 0.01) and peak-lay (*p*<0.001; 0.88 vs. 0.83 ± 0.01). *Campylobacter* populations in the crop cecal content, and the prevalence in environmental samples was not significantly different between treatments, and *Salmonella* was not detected in any samples. Eggshell samples did not yield a significant amount of bacterial contamination, likely due to the selection criteria of visibly clean eggs from the egg collection belt. Despite higher litter aw under higher stocking density, the lower *E. coli* and coliform levels at early lay (winter) may be associated with increased ammonia levels due to reduced ventilation, creating unfavorable environment for coliform growth.

**Keywords:** stocking density; cage-free eggs; eggshell; litter; Escherichia coli

### 368P Comparing a laboratory-scale pellet mill and a laboratory-scale water bath on the thermal inactivation of *Enterococcus Faecium*, a *Salmonella* surrogate, in broiler feed

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Broiler feed is commonly pelleted to improve handling, animal performance, reduce ingredient segregation, and enhance feed hygiene. However, feed safety concerns have increased following the implementation of the Food Safety Modernization Act and the reduced reliance on antibiotic growth promoters. Laboratory-scale models are often used to simulate bacterial thermal inactivation; however, their ability to reflect pelleting conditions remains unclear. This study evaluated the effects of conditioning temperature and time on surrogate bacterial reduction using a laboratory-scale water bath and pellet mill. A corn and soybean meal-based diet was formulated to meet the nutrient requirements of Ross broilers during the finisher phase. Feed samples were inoculated with nalidixic acid-resistant *Enterococcus faecium* and conditioned at 70 or 80°C for 15 or 30s, resulting in eight treatment combinations with six replications per treatment. Pelleted feed was produced using identical treatment combinations. Samples were enumerated on tryptic soy agar containing 200 ppm nalidixic acid following incubation at 37°C for 24 h. Pellet durability index was also measured to evaluate the pellet quality of the pelleted feed. Data were analyzed using PROC GLIMMIX in SAS with method, temperature, and time as fixed effects and replicate as a random effect; least-squares means were separated using Tukey's adjustment (P ≤ 0.05). In the water bath only, a temperature-by-time interaction was observed (P<0.05), with greater bacterial reductions at higher temperatures and longer conditioning times; the greatest reduction occurred at 80°C for 30s (2.9 log10 CFU/g). In the pellet mill only, time influenced bacterial enumeration (P<0.05), with counts decreasing from 5.9 log10 CFU/g in the control to 2.3 and 1.8 log10 CFU/g at 15 and 30s, respectively. Pellet quality was not affected by conditioning temperature or time (P>0.05). When data from both methods were analyzed together, no temperature-by-time-by-method interaction was detected (P>0.05); however, significant temperature-by-time, temperature-by-method, and time-by-method interactions were observed (P<0.05). These results indicate that increasing conditioning temperature and time improves bacterial reduction, although laboratory-scale models may not fully represent reductions achieved during pelleting

**Keywords:** Feed; Hygiene; Enterococcus Faecium; Surrogate; Feed Microbiology

### 369P Water-Stabilized Pimenta and Eugenol Nanoemulsions and a *Salmonella* Typhimurium Vaccine Elicit Comparable Cecal Colonization and Liver Dissemination Resistance on *Salmonella* Infantis and *Salmonella* Schwarzengrund in Growing Turkeys

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The emergence of multidrug-resistant (MDR) *Salmonella*, including *Salmonella* Infantis and *Salmonella* Schwarzengrund, has become a critical challenge for the poultry industry, including turkeys. Evaluation of antibiotic alternatives as preharvest control approaches has become relevant to mitigate these pathogens. The objective was to determine the efficacy of nanoemulsions containing eugenol (EG) and pimenta essential oil (PEO), and an *S*. Typhimurium vaccine, to reduce cecal colonization and limit internal organ dissemination in 6-week-old growing turkeys challenged with a combination of *S.* Infantis and *S.* Schwarzengrund. Two experiments were conducted with a total of 72 one-day-old poults, which were raised to 6 weeks of age. In each experiment, 36 poults were distributed into six groups, each fed a ration that exceeded National Research Council recommendations and included alternatives. The groups were: negative control (NC: no *Salmonella* challenge), positive control (PC: *Salmonella* challenge), EG (*Salmonella* challenge+0.25% EG throughout study), PEO (*Salmonella* challenge+0.25% PEO throughout study), VAC [*Salmonella* challenge+*S*. Typhimurium vaccine (AviPro Megan Egg) on days 1, 21, and 35], and anolyte water (*Salmonella* challenge +AW; free available chlorine adjusted to 5ppm as nanoemulsion carrier; pH=7). In week 5, birds in all groups except NC were challenged with a combination of *S.* Infantis and *S.* Schwarzengrund (5.7 log10CFU/bird), via crop gavage. After 7 days, the ceca, livers, and spleens were collected. Cecal samples

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were homogenized, serially diluted, and plated for quantitative *Salmonella* recovery. To detect *Salmonella* dissemination to the liver and spleen, samples were enriched in selenite cysteine broth. Differences in cecal colonization and organ dissemination of *Salmonella* were tested using one-way ANOVA and Fisher's exact test, respectively ($P<0.05$), including 6 turkeys per group and 2 experiments in the analysis (n=12 turkeys/group). EG and PEO nanoemulsions, and vaccination, effectively reduced *Salmonella* colonization in turkey ceca. Compared to PC (~4.3 log10 CFU/g), a 1.8 to 2 log10 CFU/g *Salmonella* reduction was observed in EG, PEO, and VAC groups ($P<0.05$), while AW reduced *Salmonella* by 1.3 log10 CFU/g ($P<0.05$). EG, PEO, and VAC groups were more effective at preventing *Salmonella* dissemination to the liver compared to PC ($P<0.05$). Results indicate that EG and PEO nanoemulsions and the *S*. Typhimurium vaccine can effectively reduce *S*. Infantis and *S*. Schwarzengrund colonization and organ dissemination in 6-week-old turkeys and can be used as preharvest *Salmonella* control approaches, potentially enhancing turkey food safety.

**Keywords:** Salmonella Infantis; Nanoemulsions; Vaccination; Turkeys; Preharvest

### 370P *Propionibacterium freudenreichii* probiotic and *Salmonella* Typhimurium vaccine alleviate cecal microbiome dysbiosis in 12-week-old commercial turkeys exposed to *Salmonella enterica* Reading challenge

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*Salmonella* Reading (SR) is a major serovar of concern due to its consistent isolation from turkey products and its potential for drug resistance. Novel approaches are required to limit the persistence of SR in turkeys. The objective of this study was to determine the effect of a dairy-derived probiotic, *Propionibacterium freudenreichii* (PF), and an *S*. Typhimurium vaccine (VC), alone and in combination, on the cecal microbiome of SR-challenged turkeys. Thirty, one-day-old poults were randomly distributed into 5 groups of 6 birds, and the experiment was repeated three times (n=18/group). The groups were negative control (NC: -SR, -PF, -VC), positive control (PC: +SR, -PF, -VC), PF (+SR, +PF, -VC), VC (+SR, -PF, +VC), and PV (+SR, +PF, +VC). Birds in PF and PV received 5 log10 CFU/mL *P. freudenreichii* via drinking water throughout 12 weeks. Birds in VC and PV were vaccinated with AviPro Megan Egg on days 1, 21, 35, and 75. Birds in all groups except NC were challenged with 5 log10 CFU/bird SR on week 11. In week 12, all birds were euthanized to collect cecal contents for microbiome analysis. Following DNA extraction and quality assessment, library preparation (Illumina Nextera XT) was performed, resulting in deep shotgun metagenomic sequencing on a NovaSeq X+ platform at a depth of ~40M bp/sample. Bioinformatics analysis was performed using the FastQC-fastp-Bowtie2-AMR++ pipeline (Kraken2 database for taxonomic profiling and MEGARes database for resistome profiling). Further data and statistical analyses were performed in R using the phyloseq, microbiome, and vegan packages. Taxonomic and resistome A and B diversity between the groups were tested using pairwise Wilcoxon and PERMANOVA tests, respectively. Differentially abundant species and resistance genes were determined using ANCOM-BC2. P-values for all tests were kept at <0.05. No significant difference in taxonomic and resistome A diversity was found (P>0.05). Taxonomic B diversity was significantly different between the groups (P<0.05). The abundance of multiple species in the genera *Bacteroides* and *Parabacteroides* was higher in PC than in NC, suggesting a challenge-induced bloom (P<0.05). Interestingly, the abundance of these same species was lower in PF, VC, and PV than in PC (P<0.05), suggesting that the treatments reduced SR-induced dysbiosis. There were no differentially abundant resistance genes between the groups (P>0.05). Antibiotic resistance genes of classes aminoglycosides (*ant*6, *rrs*A, *rrs*C, *rrs*H), tetracycline (*tet*40, *tet*W, *tet*16S), MLS (*mls*23S), and oxazolidinone (*o*23S) were the most abundant in all the samples. Results suggest that both VC and PF could alleviate microbial dysbiosis caused by SR challenge in commercial turkeys.

**Keywords:** Salmonella; Microbiome; Antibiotic resistance; Probiotics; Propionibacterium

### 371P Effects of Corn Type and Coccidiosis Challenge on Broiler Meat Quality Traits: Composition, Texture, and Lipid Oxidation

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Coccidiosis represents a major enteric disease in poultry production, frequently resulting in diminished growth performance, compromised gut integrity, and adversely affecting meat quality. Nutritional strategies, including the supplementation of corn as a source of carotenoids, may enhance broiler resilience to intestinal challenges, thereby aiding in the preservation of meat quality. The objective of this study was to evaluate the effects of corn type (yellow vs. orange) and coccidiosis challenge (challenged vs. non-challenged) on broiler meat quality, including composition, instrumental texture, and lipid oxidation.A 2 × 2 factorial arrangement in a completely randomized design was used, with treatments defined by corn type and coccidiosis challenge. Broilers were allocated to treatments with 8 replicate pens per treatment (15 birds/pen). Breast fillets were analyzed for proximate composition (fat, moisture, protein, collagen, and ash). Texture was evaluated using the Warner-Bratzler shear force test (cooked) and the BMORS test (raw and cooked). Lipid oxidation was determined using the TBARS assay (mg MDA/kg). Data were analyzed using PROC GLM in SAS 9.4, with pen as the experimental unit. Means were separated using Duncan's multiple range test ($P < 0.05$). Coccidiosis challenge affected meat composition, reducing fat content ($P = 0.017$), with no corn type or interaction effects observed for composition traits. Instrumental texture was influenced by the interaction between corn type and coccidiosis challenge. Significant interactions were observed for BMORS shear energy (raw and cooked; $P \le 0.001$) and firmness (raw and cooked; $P \le 0.001$). Under no-challenge conditions, yellow corn exhibited the greatest shear energy and firmness values, whereas challenged treatments, particularly yellow corn, showed reduced values. Orange corn showed intermediate shear force and firmness values. Warner-Bratzler measurements were not affected by treatments. Lipid oxidation (TBARS) was not affected by corn type or coccidiosis challenge ($P > 0.05$). Coccidiosis challenge influenced broiler meat composition and texture, reducing fat content and altering BMORS-derived shear energy and firmness, with these effects interacting with corn type. Corn type alone had minimal effects, and no differences were observed in lipid oxidation. These results indicate that coccidiosis primarily affects structural meat properties rather than oxidative stability.

**Keywords:** Broiler; Corn type; Coccidiosis; Texture; Meat quality

### 372P Parameter Optimization of Pulsed UV Light in Liquid Egg White and Yolk and Initial Microbial Assessment

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Ultraviolet (UV) light, a non-thermal antimicrobial technology that damages the DNA and cellular structures of microbes, has been investigated as a potential strategy to improve food safety and extend shelf-life while preserving functional properties of foods. A pulsed xenon UV system emits high-intensity broad-spectrum light, but treatment conditions must be op-

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timized to avoid coagulation in liquid egg products. The purpose of this study was to determine maximal non-coagulating treatment conditions for a high-intensity pulsed xenon UV system and evaluate the antimicrobial efficacy in liquid egg white (LEW) and liquid egg yolk (LEY). A screening design was used to evaluate numerous parameter combinations, and samples (10 g; ~2 mm thick) were treated with a prototype pulsed xenon system (200-1200 nm). Twenty-two treatment conditions were assessed in LEY, and 3 showed no visible coagulation, while 13 were tested in LEW with 7 showing no visible coagulation. The selected condition (3500 V, 10 Hz, 10 s), delivering 146.5 mJ/cm$^2$ from the top lamp and 188 mJ/cm$^2$ from the bottom lamp, was then applied to LEW and LEY inoculated with 2 spoilage bacteria, approximately 1 x 10$^4$ CFU/mL of *Escherichia coli*, 2 x 10$^3$ CFU/mL vegetative cells of *Bacillus spizizenii* ATCC 6633, and 2 x 10$^5$ CFU/mL spores of *Bacillus spizizenii* ATCC 6633. The log reductions were calculated between before- and after-treatment samples. One-sample t-tests were used to determine whether mean log reductions differed from zero (A = 0.05). The mean log reduction in LEY was 0.19 log CFU/mL (P = 0.0275, SD = 0.0557), indicating a significant reduction of *E. coli* K-12. In LEW, the mean reduction was 0.20 log CFU/mL but was not significant (P = 0.1027, SD = 0.1185). The vegetative cells of *Bacillus spizizenii* ATCC 6633 were completely inactivated, with reductions of 3.97 log CFU/mL in LEY and 2.58 log CFU/mL in LEW. In contrast, spore treatments resulted in small mean reductions of 0.21 log CFU/mL in LEY (P = 0.2244, SD = 0.2060) and 0.25 log CFU/mL in LEW (P = 0.3188, SD = 0.3291) which were not significantly different from zero. These findings suggest that high-intensity pulsed xenon UV irradiation can inactivate vegetative *Bacillus spizizenii* to a 100% level and produce modest reductions of *E. coli* without causing visible liquid egg coagulation, but is not effective against bacterial spores under the conditions tested, and may be a promising non-thermal intervention to enhance the microbial safety and shelf-life of liquid egg products with an additional and optimal approach.

**Keywords:** pulsed UV; liquid egg white; liquid egg yolk; microbial inactivation; shelf-life

### 373P Effects of dietary unblanched high-oleic peanuts on meat quality of spent laying hens

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**Objective:** Unblanched high-oleic peanuts are a locally available feed ingredient with high energy and protein value that may improve the quality and utilization of spent laying hen meat. The objective of this study was to evaluate graded peanut inclusion in layer diets on meat quality traits of spent hens. **Experimental design:** A completely randomized design was used with 200 Hy-Line Brown hens assigned to 5 dietary treatments containing 0%, 5%, 10%, 15%, or 20% unblanched high-oleic peanuts for 8 wk (5 replicates/treatment; 8 hens/replicate). After feeding, 2 hens/replicate were processed (n = 10/treatment). **Materials and methods:** Diets were isocaloric and isonitrogenous. Measurements included live and carcass weights, breast muscle weight, pH, color, purge, thaw and cook loss, texture profile analysis, proximate composition, TBARS after 6 mo frozen storage, and trained-panel sensory evaluation. **Statistical analysis:** Data were analyzed by one-way ANOVA using PROC GLM in SAS, and means were separated by Duncan's New Multiple Range Test at $P < 0.05$. Sensory data were analyzed by GLIMMIX with treatment as a fixed effect and replicate and panelist as random effects. Tukey-adjusted least squares means were compared at $P < 0.05$. **Results:** Peanut inclusion up to 20% did not affect body weight, chilled weight, breast yield, purge loss, thaw loss, cook loss, or most texture traits ($P > 0.05$). Breast pH at 2 and 24 h was unchanged, whereas 6-mo pH differed among treatments ($P = 0.04$), with the 5% group greater than 0 and 15%. At 24 h, redness (a*) was greater in the 15% group than in the 0, 5, and 10% groups ($P = 0.007$). Moderate peanut inclusion improved composition: the 10% diet produced the lowest fat (2.55 vs. 3.57% in control) and greatest protein (23.44 vs. 22.67% in control), and collagen declined from 0.97% in control to 0.79% at 20% peanut ($P <= 0.02$). Springiness differed among treatments ($P = 0.02$), but hardness, cohesiveness, gumminess, and chewiness did not. TBARS remained low after storage and sensory texture, flavor, and taste attributes did not differ, with no rancid or off-flavor development ($P > 0.05$). **Conclusions:** Unblanched high-oleic peanuts can be included in spent laying hen diets up to 20% without adverse effects on yield or eating quality. Inclusion at 10% to 15% provided the most favorable balance of improved meat composition and product stability, supporting high-oleic peanuts as a sustainable, value-added feed ingredient.

**Keywords:** spent laying hens; high-oleic peanuts; meat quality; poultry nutrition; sensory evaluation

## Student Poster Competition II

### 374P Computer Vision–Driven Feather Scoring for Automated 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 reduce fertility, as increased scratches may decrease voluntary mating. However, current welfare assessments rely on subjective manual scoring, which is labor-intensive, inconsistent across observers, and impractical for large commercial flocks. Automated feather scoring systems are therefore needed to enable continuous, objective, and scalable welfare monitoring. This study developed and evaluated an AI-based vision system to classify body-feather scores (0–5) using affordable, farm-deployable cameras. Sixteen pens with 68 hens and 7 roosters per pen were used. From each pen, 24 hens were randomly selected and manually scored by trained observers at different ages. Birds were then placed individually in a custom imaging pen, where synchronized RGB videos were converted into images. A total of 1,200 RGB images were annotated and split into training (70%), validation (20%), and testing (10%) datasets. Multiple training configurations were evaluated, including three optimizers (SGD, Adam, AdamW), different input resolutions, and augmentation techniques. Model performance was assessed using precision (correct positive predictions), recall (ability to detect all true cases), F1-score (balance between precision and recall), and mean average precision (mAP), which measures overall detection and classification accuracy across different thresholds. These metrics were selected to provide a comprehensive evaluation of both classification accuracy and model reliability. Statistical comparisons (ANOVA with Tukey's HSD, A = 0.05) showed that SGD achieved significantly higher precision (82.0%) and mAP (59.7%) than Adam and AdamW, with improvements of approximately 4–8% over the alternatives. AdamW produced the highest recall (86.2%), indicating better detection of all feather conditions but with slightly lower precision. Among models, YOLOv8-cls achieved the best overall performance, with significantly higher F1 scores than other architectures, indicating a bet-

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ter balance between accuracy and completeness. Feather score "0" (no feather loss) consistently showed higher classification accuracy (mAP) than other scores. To further improve performance, future work will expand the dataset and refine model training. These results highlight the importance of optimizer selection, data augmentation, and model architecture in developing robust AI systems. The optimized YOLOv8-cls model demonstrates strong potential as a real-time, farm-ready tool for automated feather scoring and precision welfare assessment in broiler breeders.

**Keywords:** Broiler breeder; welfare; feather score; computer vision; precision livestock farming

### 375P Comparison of white LED lighting to poultry specific lighting with moon phase on fear and stress

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Artificial lighting used in poultry production is typical white light and lacks parts of the spectrum that might be more beneficial to poultry; furthermore, the effect of simulating moon light has not been investigated. To determine if adding "moon" lighting to more poultry specific lighting spectrum would affect stress and fear response of laying hens we reared laying hens from 18 to 72 weeks of age under either just a commonly used LED fixture (Control) or a poultry specific lighting program which included a moon phase (DYNM) which consisted of dim blue light during the scotophase. Birds were housed in the same barn but separated by a tarp to the same environmental conditions with the exception of the lighting program. For each treatment 30 cages were used with 3 birds per cage. To determine stress susceptibility bilateral asymmetry (ASYM), heterophil to lymphocyte ratios (HL), and plasma corticosterone concentrations (CORT) were measured at 3 time points during production (18, 48, and 70 weeks of age). Fear response was measured using tonic immobility (TI) and inversion (INV) also at the 3 same time points during production. Data was analyzed using GLM: a significant difference was considered $P < 0.05$. There were no differences between the treatments in any measure at the start of the study ($P > 0.05$). The DYNM birds at 48 and 70 weeks of age had lower CORT (1481.6 ± 248.2 ng/dl, $P = 0.02$; 1080.8 ± 206.5 ng/dl, $P = 0.001$), less ASYM (1.14 ± 0.10, $P = 0.007$; 1.75 ± 0.14, $P = 0.04$) and smaller HL (0.18 ± 0.01, $P < 0.001$; 0.24 ± 0.03, $P < 0.001$) than the Control birds (3944.9 ± 919.7 ng/dl; 2571.0 ± 313.7 ng/dl, 1.56 ± 0.12; 2.26 ± 0.21 and 0.40 ± 0.03; 0.54 ± 0.04). The DNYM birds righted faster during TI (142 ± 18 sec, $P = 0.02$; 156 ± 17 sec, $P = 0.047$) compared to Control birds (211 ± 22 sec; 201 ± 23 sec) at 48 and 70 weeks of age. There was no difference observed between treatments in flapping intensity during INV at either 48 or 70 weeks of age (4.2 ± 0.1 flaps/sec, $P > 0.05$; 3.1 ± 0.1 flaps/sec, $P > 0.05$). These results demonstrate that housing laying hens with DYNM can reduce their stress and fear response and once again indicate that it is important to use the correct lighting to optimize bird welfare and that having more natural lighting can help maximize that welfare via reduced stress and fear responses.

**Keywords:** Layer; Light; Fear; Stress; Moonphase

### 376P Effects of environmental enrichment on behavior and space use in cage-housed laying hens

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This study evaluated the effects of environmental enrichment on damaging behaviors, non-damaging behaviors, and space use in commercial white egg–laying hens (Hy-Line) maintained in a cage-housed environment. It was hypothesized that enrichment would reduce damaging behaviors and alter behavioral time budgets and cage space use. Approximately 42-wk-old hens (n = 20) were assigned to one of four treatments over a 5-wk period: control (no enrichment), dust bath, mirrors, and turf, with each treatment consisting of a single cage containing five individually identified hens. Observations were conducted three days per week at consistent times of day to minimize diurnal variation. During each observation period, hens were monitored for one hour, with trained observers assigned to specific cages for 30-min intervals. Behavioral data were collected using a standardized ethogram including feeding, drinking, resting, preening, and general activity. Scan sampling at 2-min intervals during 30-min sessions was used to record hen location and behavior, while continuous event sampling was used to record ethogram events including chases, feather pecks, aggressive pecks, and interactions with enrichment devices. Prior to data collection, observers completed training and calibration sessions to ensure inter-observer reliability. Feeding schedules were standardized across all treatments, with hens fed once daily to reduce management-related variability. Scan data were analyzed as binomial proportions, and event data were analyzed using generalized linear mixed models (PROC GLIMMIX) with a negative binomial distribution. Models included treatment, week, and their interaction, with hen included as a random effect. Significance was declared at $P < 0.05$. Feather pecking was not affected by treatment ($P = 0.3626$) but changed over time ($P < 0.05$), with no treatment × week interaction ($P > 0.05$). Aggressive pecking differed among treatments ($P = 0.0002$) and over time ($P < 0.0001$), with lower counts observed in mirror and turf treatments compared with control and dust bath treatments, and no treatment × week interaction ($P = 0.9932$). Space use and other non-damaging behaviors were influenced primarily by week rather than treatment. Dust bathing was observed only in hens provided with dust bath enrichment. In this preliminary study, environmental enrichment was associated with reduced aggressive pecking in mirror and turf treatments, whereas feather pecking and other behaviors were influenced primarily by time. Given the limited replication (1 cage per treatment), these findings should be interpreted cautiously, and additional research is needed to confirm these observations and account for cage-level variation.

**Keywords:** laying hens; enrichment; aggressive pecking; behavior; welfare

### 377P Monitoring Pecking of enrichment block pecking in Cage-free laying hens with deep learning

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The US egg production is in transition to a cage-free (CF) housing system. This transition has increased the need for automated monitoring tools to support welfare management and economic sustainability. While CF houses allow hens to perform natural behaviors such as dust bathing and foraging, a persistent challenge is severe feather pecking. Pecking block enrichment is used as a management approach to control severe feather pecking. However, manual quantification of such block use is subjective and labor-intensive. This study evaluated the performance of small and large variants of both YOLOv10 and YOLO11 computer vision models for automatic detection of enrichment block pecking in the CF research environment. We raised a total of 800 Lohmann LSL-Lite, divided equally in 3 cage-free research environments, and recorded using overhead cameras. A total of 1,061 RBG images were used to train and evaluate the models using a 70:20:10 split for training, validation, and testing. To ensure consistency, manual annotations were performed by the same trained individual. A random subset (10%) of the annotations was reviewed by a second individual, with agreement above 95%. Performance was assessed

using precision, recall, mean average precision at 50% intersection over union (mAP50), confusion matrices, and F1-confidence curve. All models demonstrated robust performance, with precision, recall, and mAP50 values greater than 0.94. YOLO1ll achieved the highest precision with 0.962 and mAP50 with 0.988, while YOLOv10s achieved the highest recall. Evaluation on test datasets showed the model's robust generalization capability, with high-confidence detections. Overall, the findings show that YOLO models provide a consistent, objective, and scalable method for automatic quantification of enrichment block pecking in the CF system. It offers practical potential for poultry researchers and commercial farmers.

**Keywords:** Animal welfare; Cage-free housing; Computer vision; Enrichment block; Pecking

### 378P Genetic diversity monitoring and baseline establishment for investigating suspected sex-linked lethal alleles in Korean native chicken purebred lines

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While historical observations in Korean native chicken (KNC) purebred lines raise concerns about suspected sex-linked lethal alleles associated with late-feathering, investigating such traits requires understanding the population's genetic structure. Thus, this study's objective was to evaluate genetic diversity and establish a robust baseline across two recent generations using microsatellite (MS) markers, providing a framework for future phenotypic validation and lethal genotype tracking. A total of 843 KNC birds across two generations (369 parents in 2024; 474 offspring in 2025) were genotyped to establish a reference genetic structure. Genomic DNA was extracted from blood samples. Population genetic structures and inbreeding levels were analyzed using 30 multiplexed MS markers. Expected heterozygosity (*He*), observed heterozygosity (*Ho*), and Polymorphic Information Content (*PIC*) were calculated to assess genomic stability. Individual genetic relationships were quantified using Identity By State (IBS) based on allele sharing. The IBS matrix was cross-validated using R (adegenet) and Cervus to handle potential duplicate samples. Allele frequencies and diversity indices were computed per locus. Differences in mean metrics between the 2024 and 2025 cohorts were evaluated using independent t-tests in SAS, with significance declared at $P < 0.05$. Overall genetic diversity was maintained across generations without severe bottlenecks. In 2024, mean *He*, *Ho*, and *PIC* were 0.61 ± 0.03, 0.56 ± 0.03, and 0.56 ± 0.03, respectively, remaining remarkably stable in 2025 ($P > 0.05$). The mean pairwise IBS was 0.481, typical of a closed purebred population. Cervus identity analysis identified two pairs of duplicate records (30 loci matches, $P < 10^{-24}$), which were removed to ensure a high-fidelity baseline. Specific loci (e.g., MCW0165) exhibited significant heterozygote deficiency, reflecting cumulative selection pressure. In conclusion, the KNC breeding population maintains stable genetic diversity, establishing a secure baseline for genomic investigations. Localized heterozygote deficiencies and exact genetic matches highlight the impact of closed breeding, underscoring the need for strict pedigree management. Using this framework, future research will incorporate targeted genomic screening—focusing on unmapped regions like the Z chromosome—paired with phenotyping to identify lethal mutations and optimize mating.

**Keywords:** Korean native chicken; Genetic diversity; Microsatellite; Population structure; Identity by state

### 379P Transduction efficiency of adeno-associated virus (AAV) serotypes in chicken DF-1 fibroblast and primordial germ cells

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Improved gene delivery methods are needed to enhance genetic engineering capabilities in poultry, as the currently available techniques yield inconsistent efficiencies. Adeno-associated viral (AAV) vectors have shown promise in the medical field for safe, effective gene delivery; however, AAV tropism and receptor availability have not been investigated in chicken cells. In this study, we evaluated the transduction efficiency of AAV serotypes 1-9 in chicken DF-1 fibroblast cells and primordial germ cells (PGCs). AAVs harboring a CAG-eGFP transgene were transduced in both types of cells at a multiplicity of infection (MOI) of 100,000. Transduction efficiency was analyzed by flow cytometry at 48h post-transduction to determine the percentages of GFP+ cells along with cell viability by DAPI staining. Among the nine AAV serotypes, only AAV1, AAV4, and AAV6 showed detectable GFP expression in both DF-1 and PGCs without antibiotic selection. In DF-1 cells, an average of 7.65%, 0.26%, and 7.81% GFP+ cells were detected after transduction with AAV1, AAV4, and AAV6, respectively, whereas in PGCs, the average percentages of GFP+ cells were 0.05%, 0.92%, and 5.8%, respectively. Taken together, DF-1 cells showed comparatively higher percentages of GFP+ cells than PGCs, suggesting differences in viral entry or receptor availability in somatic versus germline-derived cells. The remaining serotypes showed minimal or no detectable GFP expression. Flow cytometry analysis also revealed that AAV exposure did not affect cell viability. Our results suggested that AAVs might act as promising gene transfer vectors for avian genome editing research.

**Keywords:** Avian genome editing; adeno-associated virus (AAV); primordial germ cells (PGCs); gene delivery; viral vector

### 380P Comparative Genomics of ECOR and Avian Pathogenic *Escherichia coli* Reveals Plasmid-Associated Virulence and Antimicrobial Resistance Determinants

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Avian pathogenic *Escherichia coli* (APEC) is a major cause of colibacillosis in poultry, leading to significant economic losses worldwide. Understanding genomic differences between pathogenic and commensal *E. coli* populations is essential for identifying virulence determinants and improving disease surveillance. The *Escherichia coli* Reference (ECOR) collection represents the natural genetic diversity of *E. coli* and provides a framework for comparative genomic studies. This study aimed to generate high-quality whole-genome sequencing (WGS) data for ECOR isolates and use this genomic framework to compare APEC isolates. Nanopore long-read whole-genome sequencing was performed on 72 ECOR isolates to establish a genomic reference dataset. Genomes were assembled using Autocycler (v0.5.2), annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (v2025-05-06), and assembly quality was assessed using CheckM. These genomes were compared with 26 poultry *E. coli* isolates with existing WGS data, classified as APEC based on embryo lethality assays (>30% mortality). Phylogenetic relationships were reconstructed using genome-wide sequence data. Virulence genes were iden-

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tified using VirulenceFinder (v2.0.4), and antimicrobial resistance (AMR) genes were detected using AMRFinderPlus (v4.0.3). Gene enrichment between APEC and ECOR isolates was assessed using Fisher's exact test with odds ratios and FDR correction. Genome assembly and annotation produced high-quality ECOR genomes suitable for comparative analysis. Phylogenetic analysis showed that APEC isolates were distributed across multiple ECOR lineages rather than forming a distinct clade, indicating diverse evolutionary origins within the broader *E. coli* population. Odds-ratio analysis revealed significant enrichment of virulence genes in APEC isolates compared with ECOR strains (FDR-adjusted $p < 0.05$), particularly genes associated with iron acquisition, serum resistance, toxin production, and adherence. Many were plasmid-encoded, with some genes showing differential chromosomal and plasmid localization between ECOR and APEC isolates, suggesting increased potential for horizontal transfer and dissemination. AMR genes were predominantly detected in APEC isolates and included determinants for aminoglycoside, B-lactam, tetracycline, sulfonamide, trimethoprim, and fosfomycin resistance, several of which were plasmid-associated. These findings demonstrate that plasmid-associated virulence and AMR determinants differentiate APEC isolates from the broader ECOR population and highlight the value of the ECOR genomic framework for identifying APEC-associated genomic features.

**Keywords:** Avian pathogenic Escherichia coli; ECOR collection; Whole-genome sequencing; Phylogenomic; Plasmids

### 381P Effect of Sodium beta hydroxy butyrate on bacterial chondronecrosis and osteomyelitis induced by *Staphylococcus aureus* in broiler chickens

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Bacterial chondronecrosis and osteomyelitis (BCO) is one of the important health and welfare issue for broiler chickens in the poultry industry. *Staphylococcus aureus* is a common cause of BCO pathogenesis, but metabolic interventions using ketone salts like Sodium beta-hydroxybutyrate (Na-BHB) remain unexplored. This study aimed to evaluate the effects of dietary Na-BHB supplementation on bone health and pathogen load in broiler chickens with BCO induced by oral *Staphylococcus aureus* inoculation. A total of 240 42-d-old male Cobb 500 broiler chickens, with an initial average gait score of 2, were allocated to four treatments (T1-T4) with six replicates. The 42–56 d age group was selected to evaluate BCO and treatment effects in late-finisher birds, when body weight and skeletal load are higher and lameness is more clearly expressed. A non-challenged control (T1) and a *Staphylococcus aureus*-challenged control (T2; $10^9$ CFU/ml), both of which were fed a standard basal diet. The remaining challenged groups, T3 and T4, were fed the standard basal diet with 500 ppm and 700 ppm of Na-BHB, respectively. Growth performance, feed intake, BCO and gait scores, body composition by dual-energy X-ray absorptiometry (DEXA), bacterial counts in femoral heads and ceca were assessed on d 49 and 56. The data were analyzed using one-way ANOVA. Means were separated using Tukey's HSD test. For the nonparametric data (Gait & BCO scores), the Kruskal–Wallis test and the Steel–Dwass post hoc test was performed. $P < 0.05$ was considered statistically significant. Body weight gain and feed intake were unaffected by either the challenge or Na-BHB inclusion ($P > 0.05$), but Na-BHB supplementation significantly reduced gait and BCO lesion scores on d 49 and 56 ($P < 0.05$). On d 56, DEXA findings showed a significant increase in bone mineral density (BMD) in both Na-BHB-supplemented groups (T3 and T4), whereas bone mineral content (BMC) was significantly higher in the 700-ppm group (T4) than that in the other treatments ($P < 0.05$). Furthermore, cecal and bone *S. aureus* counts were significantly lower in Na-BHB-treated birds, particularly at 700 ppm ($P < 0.05$) on d 49 and 56. In conclusion, Na-BHB inclusion in broiler diets reduces *S. aureus*-induced BCO and bone weakness and bacterial load in both the ceca and femoral heads. Therefore, Na-BHB improved bird welfare without compromising broiler performance, highlighting its potential as a metabolic intervention to manage BCO in broiler chickens.

**Keywords:** staphylococcus aureus; chondronecrosis; femoral heads; sodium beta-hydroxybutyrate; broilers

### 382P Further assessment of 1,25(OH)2D3-glycosides supply duration on reducing bacterial chondronecrosis with osteomyelitis lameness in broilers

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The use of 1,25(OH)2D3-glycosides remains an effective nutritional strategy in the reduction of bacterial chondronecrosis with osteomyelitis (BCO) lameness. This may be achieved through beneficial effects such as increased functions of respiratory and intestinal epithelial tight junction proteins, bolstered immunological defense, and enhanced calcium mobility against BCO pathology. Leveraging previously documented inclusion timing studies, this experiment further examined the possibility of a shorter optimal inclusion timing of 0.01% Panbonis (Herbonis, Switzerland) on the reduction of clinical BCO lameness compared to the highly effective four-week period, using the BCO aerosol transmission model. Eight hundred forty Cobb 500 male chicks were allocated to each of the four following treatments at 60 chicks/pen as the initial stocking density and reared over 56d: T1 (Positive Control, 2 wire-flooring pens) – Basal diet (7,166 IU/kg Vit-D3); T2 (Negative Control, 4 litter pens) – Basal diet; T3 (4 litter pens) – 14-day inclusion (Basal diet + Panbonis in filler); and T4 (4 litter pens) – 28-day inclusion (Basal diet + Panbonis in filler). There were three feeding phases: Starter (d0-21), Grower (d22-42), and Finisher (d43-56). The light schedule was set at 23L:1D for the entire experimental period, with feed and water provided ad libitum. Starting from d21 onward, assessment of clinical lameness was conducted daily, followed by euthanasia and necropsy of clinically lame birds to determine BCO lesion severity. At the end of the trial on d56, all survival birds were recorded as clinically healthy. Cumulative lameness insidence rates between tretments were assessed using binomal logistic registration analysis in a generalized linear mixed (GLM) model, with presence of clinical lameness as the measured outcome. Tretment 1 was excluded from formal analyses due to different treatment design, statistics of which were presented for contextual purposes otherwise. All statistical significance was determined at ($P<0.05$). Compared to T2 (NC), the 28-day inclusion period (T4) remained highly effective in clinical lameness reduction (-31.7%; $P < 0.001$) while the 14-day inclusion period (T3) was not significantly different (-2.0%; $P = 0.89$). These results further affirm documented optimal inclusion timing and rate of Panbonis against clinical BCO lameness incidence, providing producers and researchers with a valuable tool against the disease, thus improving broiler welfare and sustainable poultry production.

**Keywords:** broiler; BCO; lameness; bone; feed supplement

### 383P Evaluating the impact of carotenoid-rich orange corn diet on

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**broiler performance under *Eimeria* Challenge**

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Coccidiosis continues to impair broiler growth and feed efficiency, increasing interest in nutritional strategies that support performance under challenge conditions. This study evaluated whether orange corn (OC) versus yellow corn (YC), differing in total carotenoids (11 vs. 17 µg/g), affected broiler performance during an Eimeria challenge. A total of 480 one-day-old male Cobb broilers were randomly assigned to 32 pens (4 treatments; 8 pens/treatment; 15 chicks/pen) in a 2 × 2 factorial design with two corn types (YC vs. OC) and two challenge statuses (non-challenged, NC vs. Eimeria-challenged, EC). Birds in the challenged groups received a 20× dose of commercial live Eimeria vaccine by oral gavage on day 14. Data were analyzed using a two-way ANOVA in JMP with corn type, Eimeria challenge, and their interaction as fixed effects. No significant corn × challenge interactions were detected for most growth performance variables ($P > 0.05$). From day 0–14, broilers fed OC had higher body weight gain and feed intake than those fed YC ($p < 0.01$), with no difference in feed conversion ratio (FCR). From day 15–21, challenged birds had significantly lower weight gain, feed intake, and reduced FCR than non-challenged birds ($p < 0.01$), while corn type had no significant effect on performance ($p > 0.05$). From day 22–42, a significant corn × challenge interaction was observed for feed conversion ratio ($p < 0.05$), indicating that dietary effects differed between challenged and non-challenged birds, with OC-fed challenged birds exhibiting improved feed efficiency. Previously challenged birds also showed higher weight gain ($p < 0.05$) and improved FCR compared with non-challenged birds, suggesting partial recovery of growth following the challenge period, whereas feed intake did not differ ($p > 0.05$). In this period, birds fed OC had higher weight gain ($p < 0.01$) and improved FCR ($p < 0.05$) than those fed YC. Over the entire 0–42 d period, challenged birds had lower weight gain ($p < 0.001$) and higher FCR ($p < 0.05$) than non-challenged birds, while OC increased weight gain ($p < 0.001$) and improved FCR ($p < 0.01$) compared with YC, without affecting cumulative feed intake ($p > 0.05$). In conclusion, Eimeria challenge negatively impacted overall broiler performance, but feeding an orange corn diet improved growth and feed efficiency across the production cycle, regardless of challenge status, indicating that carotenoid-rich orange corn may help support broiler performance under coccidia challenge.

**Keywords:** Coccidiosis; Broiler; Orange corn; Carotenoids; Eimeria challenge

**384P Detection of Early Symptoms of Respiratory Diseases in SPF Leghorn Laying Hen Using Deep Learning Techniques**

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Infectious Laryngotracheitis (ILT) is an acute upper respiratory disease of poultry that causes severe economic losses due to increased mortality, reduced weight gain, and reduced egg production, impacting welfare. Early detection of ILTV is critical yet challenging, as current surveillance relies on manual clinical observation and laboratory diagnostics, which are labor-intensive and time-consuming. Clinical signs, such as eye rubbing, head bobbing, tucking the head inside the wings, mouth breathing, and huddling, along with behavioral changes, often precede severe outcomes of the disease. This pilot study deployed a machine-vision-based object detection tool to detect ILT-associated clinical and behavioral signs and compared the performance of two object detection models: YOLO11-OBB (Oriented Bounding Box) and YOLO26-OBB. Fourteen specific pathogen-free (SPF) Leghorn hens were distributed in two negative-pressure HEPA-filtered isolation units; one group of hens was infected with ILTV (n=7), and the other group of hens remained uninfected and served as a negative control (n=7). High-definition videos were collected from each chamber between 1 and 7 days post-infection from 8:00 am to 5:00 pm per day. Videos were converted into images at the rate of 15 frames per second using Python, and 2,000 images were randomly selected. 1,214 qualified images were split into training (70%), validation (20%), and testing (10%) and annotated in CVAT. Model performance was evaluated using one-way ANOVA at 5% level of significance using evaluation metrics (precision, recall, and mean average precision). Among YOLOv26X-OBB variants, YOLO26x-OBB achieved the highest precision (0.98, 0.66, 1, 0.45, 0.63, 0.36, and 1), recall (0.90, 0.73, 0.47,0.14, 0.46, 1, and 0.25), mAP@0.50 (0.951, 0.67, 0.29, 0.21, 0.44, 0.99, and 0.63), and mAP@0.50-0.95(0.76, 0.49, 0.20, 0.21, 0.29, 0.70, and 0.56) for feeding, drinking, eye rubbing, head bobbing, tucking head, pack in a corner, and mouth breathing respectively. Among YOLOv11-OBB variants, YOLOv11n showed the highest precision (0.93, 0.87, 0.63, 0.18, 0.60, 0.24, and 0.54), recall (0.89, 0.60, 0.05, 0.29, 0.41, 1.0, and0.25), mAP@0.50 (0.95, 0.72, 0.26, 0.21, 0.54, 1.0, and 0.29), and mAP@0.50-0.95 (0.72, 0.52, 0.18, 0.16,0.36, 0.80, and 0.21) for same behaviors. Feeding and drinking behaviors were detected with 93-98% precision using the YOLO26X-OBB and YOLO11s-OBB. Detection of ILT-related clinical signs ranged from 0.36 to 1.0 with YOLO26X-OBB. The result showed a robust automated detection method for feeding and drinking, and establishes a base for real-time detection of ILT-associated respiratory signs, enabling earlier management intervention before flock-level spread.

**Keywords:** Poultry health; Infectious Laryngotracheitis; Machine Vision; Poultry respiratory disease; Deep Learning

**385P A High n-6:n-3 PUFA Ratio Primes Apical-Out Chicken Enteroids for Enhanced Susceptibility to RSL3-Induced Ferroptosis**

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Polyunsaturated fatty acids (PUFAs), particularly the n-6:n-3 ratio, play a critical role in regulating intestinal physiology, immune responses, and oxidative balance in poultry. Conventional corn–soybean-based diets are typically characterized by relatively high n-6:n-3 PUFA ratios, which increase the abundance of peroxidizable lipids and promote oxidative stress, as reflected by elevated malondialdehyde (MDA) levels in poultry. As essential components of membrane phospholipids, PUFAs serve as primary substrates for lipid peroxidation and are critical determinants of ferroptosis sensitivity. An increased pool of oxidizable lipids can lower the threshold for ferroptotic cell death by facilitating lipid peroxidation chain reactions and disrupting redox homeostasis. However, it remains unclear whether PUFA imbalance directly induces ferroptosis or instead modulates cellular susceptibility to ferroptotic stress. This study aimed to determine whether different n-6:n-3 PUFA ratios directly induce ferroptosis and whether a high ratio enhances susceptibility to ferroptotic stress. Apical-out intestinal enteroids derived from day 18 chicken embryos were used as an in vitro model. Enteroids were treated with different n-6:n-3 PUFA ratios (1:2, 1:1, 5:1, 10:1, 20:1, and 40:1), and cell viability,

tight junction proteins (ZO-1 and CLDN1), and ferroptosis-related gene expression were evaluated. To assess priming effects, enteroids were pretreated with a high n-6:n-3 PUFA ratio (20:1) for 24 hr, followed by exposure to the ferroptosis inducer RSL3. Four groups were included: control, PUFA alone, RSL3 alone, and PUFA pretreatment followed by RSL3. Cell viability, barrier function, inflammatory cytokines (IL-6, IL-1B, and TNF-A), and ferroptosis-related gene expression were analyzed, and statistical significance was determined using two-way ANOVA followed by Tukey's multiple comparison test. PUFA treatment alone did not significantly affect cell viability, tight junction protein expression, or ferroptosis-related gene expression, indicating that PUFA does not directly induce ferroptosis. However, PUFA pretreatment significantly increased cell death upon RSL3 exposure, and this effect was reversed by the ferroptosis inhibitor UAMC. Changes in inflammatory cytokines, tight junction proteins, and ferroptosis-related gene expression further supported activation of ferroptotic pathways. In conclusion, different n-6:n-3 PUFA ratios do not directly induce ferroptosis but act as critical modulators of ferroptosis susceptibility. A high n-6:n-3 PUFA ratio primes intestinal epithelial cells for enhanced ferroptotic response, highlighting the role of lipid composition in determining ferroptosis vulnerability and intestinal health.

**Keywords:** n-6:n-3 PUFA ratio; ferroptosis; priming effect; intestinal enteroids; lipid peroxidation

### 386P A chicken embryo challenge model reveals strain-dependent variation in the pathogenic potential of *Enterococcus faecalis*

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*Enterococcus faecalis* is a common member of the intestinal microbiota of poultry. Even some strains in *E. faecalis* have been proposed as potential probiotics, other certain strains can act as opportunistic pathogens and are associated with diseases such as amyloid arthropathy in poultry. In this study, a chicken embryo challenge model was used to evaluate the pathogenic potential of two *E. faecalis* strains, MS8004 and MS8005, isolated from the ceca of broilers. A total of 13 treatments were included, with 24 embryos per group: a saline negative control (NC), a positive control with a known opportunistic poultry pathogen *E. cecorum* (PC; $10^2$ and $10^3$ CFU/egg), and the two *E. faecalis* strains (MS8004 and MS8005) tested at five inoculation doses ranging from $10^2$ to $10^6$ CFU/egg. Bacteria were inoculated into the allantoic fluid on day 12 of incubation, and embryo mortality was monitored daily. Samples were collected at 6 days post-inoculation to measure egg weight and embryo weight. Embryo mortality was analyzed using Kaplan-Meier survival analysis with the log-rank test, and other variables were evaluated using the Kruskal–Wallis test. Embryos in the NC treatment maintained a high survival rate of 92%, whereas those inoculated with the PC declined to 17% at $10^3$ CFU/egg (All $P < 0.0002$). Embryos inoculated with strain MS8004 showed survival rates ranging from 67% to 88%, significantly higher than those observed in the PC group (All $P < 0.0002$) but not different from the NC group (All $P > 0.07$). In contrast, embryos inoculated with strain MS8005 showed markedly reduced survival rates of 21% - 33%, which were lower than the NC group (All $P < 0.0003$) but comparable to the PC group (All $P > 0.46$). Embryo ratios (embryo weight to egg weight) in both *E. faecalis*-inoculated groups were slightly lower but not statistically different from the NC group (All $P > 0.19$). However, embryos inoculated with strain MS8004 at doses of $10^2$-$10^4$ CFU/egg had higher embryo ratios than those in the PC $10^2$ CFU/egg group (All $P < 0.04$). These results suggest differences in the pathogenic potential of the two *E. faecalis* strains, with MS8005 showing pathogenic effects comparable to *E. cecorum* in the chicken embryo model, whereas MS8004 appeared to have minimal impact on embryo survival and development. Further studies are needed to functionally characterize these strains to better define their pathogenic potential and their implications for poultry health and production.

**Keywords:** Allantoic fluid; Inoculation doses; Survival rate; Embryo weights; Embryo ratio

### 387P Kinetics of vaccine response in broilers in the presence of maternal antibodies

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**Hypothesis and objective** This study aimed to (1) evaluate the effect of maternal antibodies on vaccine virus replication and immune responses following hatchery vaccination against IBV in broiler chickens and (2) determine whether maternal antibodies alone, or combined with vaccine-induced immunity, provide protection against infectious bronchitis virus (IBV) circulating in Alberta. We hypothesize that vaccine virus shedding is expected to be transient, as maternal antibodies decline and are subsequently replaced by vaccine-induced antibody responses. Vaccination is expected to reduce viral shedding and pathology following challenge, with maternal antibodies influencing responses at 2 weeks and vaccine immunity predominating at 4 weeks. **Experimental design**Obj. 1: A total of 60 one-day-old broiler chicks were assigned to vaccinated and unvaccinated groups and sampled on days 1, 7, 14, 21, and 28. Obj. 2: One hundred eighty chicks were divided into six groups: controls and vaccinated/unvaccinated birds challenged at 2 or 4 weeks. Birds were euthanized at 3, 7, and 14 dpi, and blood, swabs (OP, CL), and tissues were collected. **Material and methods**Chicks were obtained from Maple Leaf Foods. Vaccinated birds received live attenuated IBV vaccine (Mass and Conn) via oculo-nasal route; controls received PBS. Antibody titers were measured by ELISA (cut-off ≥396). RNA was extracted (TRIzol™ LS), quantified, reverse-transcribed, and IBV load quantified by SYBR Green qPCR targeting the nucleocapsid gene. **Statistical analysis**Data were analyzed using one-way ANOVA with Tukey's test (GraphPad Prism v10.3). Assumptions were verified; significance set at $p<0.05$. **Results**In Obj. 1, high antibody titers at day 7 declined over time, with unvaccinated birds falling below the ELISA cut-off by day 21, while vaccinated birds maintained higher titers, though not statistically significant. In Obj. 2, vaccinated birds showed protection at both time points with no clinical signs or shedding, whereas unvaccinated birds developed disease. **Conclusion**-This study indicates that hatchery vaccination wth IBV induces immune responses that may not be fully detectable at the systemic level due to the masking by maternally derived antibodies in young broiler chicks. Additionally, vaccination provided consistent protection against infection at both early and later challenge time points, whereas maternal antibodies alone were insufficient to protect broilers against IBV challenge.

**Keywords:** Infectious bronchitis virus (IBV); Maternal antibodies ; Broiler chickens; Hatchery vaccination ; Vaccine-induced immunity

### 388P Virulence-associated genes and inoculum dose influence Avian Pathogenic Escherichia coli colonization and pathogenesis in late-lay hens via intratracheal challenge

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, leading to mortality, reduced performance, and economic losses in laying hens. With industry trends toward extended production cycles exceeding 90 weeks, understanding APEC susceptibility in late-lay birds becomes critical, yet limited research exists for this population. This study investigated associations between virulence-associated genes (VAGs), inoculum dose, and APEC colonization patterns in 90-week-old Hy-Line® W-36 laying hens using an intratracheal challenge model. Seventy-two hens were housed in an ABSL-2 facility across 36 cages (2 hens/cage). Four treatment groups (n=18 each) were established: VS-1 (MS-5033 at $10^8$ CFU/mL), VS-2 (MS-5033 at $10^9$ CFU/mL), positive control (MS-1657, O2/O50:H1, ST429 at $10^8$ CFU/mL), and negative control (sterile PBS). The primary comparison evaluated MS-5033, which carries five VAGs (*iss*, *iroN*, *ompT*, *hlyF*, *iutA*) associated with APEC pathogenicity, at two different doses. Birds were monitored through 11 days post-inoculation (DPI) for clinical signs, performance parameters, and tissue colonization. Tissue samples from lung, liver, air sac, and spleen were collected at 4 and 11 DPI for bacterial prevalence assessment. Data were analyzed using PROC GLM (SAS 9.4) for performance parameters, Fisher's Exact Tests and logistic regression for bacterial prevalence, and Kaplan-Meier analysis for survivability. Mortality was numerically highest in PC (22.2%) and VS-2 (16.7%) compared to VS-1 (6.7%) and NC (0.5%), though not statistically significant (P=0.2). Performance parameters showed minimal differences among treatments, though VS-2 demonstrated trends toward reduced hen-day egg production at 5–11 DPI (P=0.069). Time post-inoculation was the primary factor influencing colonization patterns. Respiratory tissues showed high bacterial prevalence at 4 DPI in PC and both MS-5033 dose groups compared to NC, with significant declines by 11 DPI (lung: 64%, P=0.004; air sac: 39%, P<0.0001) across all treatments. Conversely, liver colonization increased significantly from 4 to 11 DPI (13.9% to 50.0%, P<0.0001), with PC reaching 100% prevalence and demonstrating significantly higher colonization odds versus NC (P=0.010). Spleen colonization declined markedly by 11 DPI (91.7% to 33.3%, P<0.0001), with VS-1 showing enhanced clearance compared to controls (P=0.018). These findings demonstrate that APEC colonization in late-lay hens follows complex, tissue-specific temporal patterns influenced by both pathogen characteristics and host factors, providing foundational data for understanding disease dynamics in extended production systems.

**Keywords:** Avian pathogenic Escherichia coli; intratracheal challenge; laying hens; bacterial colonization; colibacillosis

### 389P Non-invasive monitoring of vaccine-induced immunity in Layers using egg yolk antibodies

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Infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and avian encephalomyelitis virus (AEV) are the major viral pathogens of laying hens that cause respiratory, gastrointestinal, and neurological signs and can reduce egg quality and production. Monitoring antibody titres to these viruses is essential for evaluating vaccine response and flock health in commercial layer flocks. Serum-based antibody testing is widely used as it provides a quantitative assessment of immune responses against vaccines. However, blood collection is labour-intensive, compromises biosecurity, and raises animal welfare concerns due to the stress induced in birds. Therefore, alternative, non-invasive sampling approaches are needed. This study investigated the potential of using egg yolk as an alternative to serum for antibody titre determination. The objectives were to (1) collect serum and egg yolk samples, including paired samples, from commercial layer flocks at 30 and 60 weeks of age, and (2) evaluate egg yolk antibody testing as a population-level surveillance tool for monitoring vaccine-induced immunity in laying hens in Alberta. A total of 360 serum and egg samples were collected from 12 commercial layer flocks across Alberta, representing early (30 weeks) and late (60 weeks) stages of production. In addition, 200 individually paired serum and egg samples were collected on-farm from both age groups. Enzyme-linked immunosorbent assays (ELISA) were performed against IBV, NDV, and AEV as per manufacturer's instructions. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test to evaluate differences between groups, and Pearson correlation analysis to assess the relationship between serum and egg yolk antibody titres. Data from three representative farms demonstrated that antibody titres measured in egg yolk were comparable to those measured in serum for IBV, NDV, and AEV. No significant differences were observed between serum and egg yolk ELISA titres, except for IBV in farms 1 and 2 and NDV in farm 2. Despite these differences, the geometric mean titres remained within the acceptable protective range across all farms. Further analysis of paired samples (n = 43) demonstrated a strong positive correlation (r = 0.93) between serum and egg yolk antibody titres for IBV. The geometric mean titres also indicate comparable antibody levels between the two sample types. NDV and AEV data are currently being analyzed to further evaluate the applicability of this approach for disease surveillance and vaccine efficacy assessment. Overall, this approach may facilitate routine, welfare-friendly immunity monitoring and improve flock health management in commercial layer operations.

**Keywords:** Avian Encephalomyelitis (AE); Egg yolk antibodies (IgY); Newcastle Disease (ND); Infectious Bronchitis (IB); Non-invasive sampling

### 390P Mycotoxin-induced immune suppression and body composition changes in broiler chickens

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Subclinical exposure to combined fumonisin (FUM) and deoxynivalenol (DON) poses significant challenges to poultry production. These toxins can impair immune functions, nutrient utilization, and body composition, ultimately affecting overall production performance. Macrophages are key regulators of innate immune response; however, their role in mycotoxin-induced immunomodulation and the connection between how these cellular responses translate to whole bird physiology are not fully understood. Hence, the objective of this study was to evaluate the *in vitro* effects of FUM and DON on macrophage function and examine corresponding *in vivo* physiological outcomes in broiler body composition. For the *in vitro* study, HD11 macrophage cells were treated with increasing concentrations of FUM and DON (50, 100, 250, and 500 ng/µL), with or without lipopolysaccharide (LPS). Nitric oxide (NO) production and mRNA expression of interleukin-1 (IL-1), inducible nitric oxide synthase (iNOS), and transforming growth factor (TGF-B) were analysed. For the *in vivo* study, broiler chickens were fed diets containing 2.5 mg/kg FUM and 2.5 mg/kg DON for 35-days, and body composition was assessed using dual-energy X-ray absorptiometry (DEXA). *In vitro* data were analyzed using one-way ANOVA ($P \leq 0.05$) with means separation by Tukey's test; *in vivo*

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data were analyzed using Student's t- test. *In vitro*, combined FUM+DON exposure significantly suppressed NO production and decreased iNOS, IL-1, and TGF-B expression relative to the LPS-stimulated control group $P \leq 0.05$), indicating impaired macrophage mediated immune response. *In vivo* DEXA analysis showed that the 35 d exposure to combined mycotoxin significantly reduced fat percentage by 26% and fat mass by 34%, while increasing lean meat percentage by 3.8% ($P \leq 0.05$). No significant differences were observed in bone mineral density, bone mineral content, total tissue, or lean meat mass ($P > 0.05$). In conclusion, exposure to FUM and DON impaired macrophage immune responses and downregulated IL-1 and TGF-B cytokine expression. These *in vitro* results corresponded with *in vivo* DEXA analysis, where broilers exposed to FUM and DON showed reduced fat deposition and increased lean tissue percentage. Together, these findings suggest that an impaired immune response may contribute to altered nutrient partitioning and subsequent changes in body composition under subclinical mycotoxin exposure.

**Keywords:** Fumonisin; Deoxynivalenol; Macrophages; Body composition; Immunomodulation

### 391P Developing a Sensor-based Automated Weight Measurement System for Cage-free Pullets and Hens

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Accurate body-weight monitoring is essential for welfare and productivity assessment in cage-free pullet and laying hen houses. However, most commercial operations rely on manual weighing, which can be labor-intensive, stressful to birds, and susceptible to sampling bias. This study developed and validated a low-cost Internet of Things (IoT)-enabled automated weighing system designed for continuous body weight monitoring in cage-free poultry houses. The system integrates four load cells, a load-cell amplifier, and a microcontroller unit for data acquisition, along with a single board computer for data processing, filtering, and visualization. Weight measurements were recorded at a sampling rate of 1 Hz, and individual weighing sessions were identified using a threshold-based detection algorithm. A rolling-median filtering method was applied to reduce noise caused by bird movement and electronic fluctuations. The system was validated using Hy-Line W-80 pullets and hens randomly selected from a flock of 750 birds housed in a cage-free research facility. Each bird was weighed using both the developed system and a standard reference scale during two sessions conducted one week apart. Agreement between the two methods was evaluated using Bland-Altman analysis and a linear mixed-effects model. The body weight of the birds measured by the reference scale was 615.78 41.07 g and 733.18 46.43 g during the first and second sessions, respectively, indicating moderate variability among birds. Results showed that the automated system produced measurements closely matching the reference scale, with a small positive bias of approximately 6-9 g and most measurements fell within the 95% limits of agreement. The mean absolute percentage error (MAPE) remained below 3%, corresponding to an accuracy above 96%. Overall, the proposed system demonstrated potential as a low-cost solution for continuous and welfare-centric weight monitoring in cage-free poultry houses. Measurement consistency has been improved during the second session after relocating the platform to a more stable surface, suggesting that mechanical stability influences system performance. Future work will focus on robust calibration procedures, improving stability of the platform, integrating bird-level identification using electronic tags or computer vision systems, and improving the overall design of the system to further improve measurement accuracy and support precision poultry management.

**Keywords:** body weight; poultry farming; poultry welfare monitoring; precision poultry management; wireless data acquisition

### 392P Influence of divergent selection for water efficiency on hatchability, embryonic mortality, and hatch window characteristics in poultry

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Selection for water efficiency in poultry is important for improving freshwater resource utilization, reducing environmental impact, and enhancing production sustainability. However, the potential effects of this selection on embryonic development and hatch characteristics remain unclear. To assess this, a study was conducted to evaluate hatch parameters in two divergently selected broiler lines: the high-water efficiency (HWE) and low-water efficiency (LWE) lines, which have been selected for their respective traits for 7 generations. Eggs from two broiler lines divergently selected for water efficiency, high-water efficiency (HWE), and low-water efficiency (LWE), were collected over 2 weeks and incubated under standardized commercial conditions in a setter (d 0–18) and hatcher (d 18–21). The study was conducted as a comparative design with broiler line as the primary treatment effect. The individual egg was considered the experimental unit for fertility, hatchability, and embryonic mortality outcomes. A hatch window was recorded every 6 h from 474 to 510 h of incubation, and chicks were removed and wing-banded at each time point. Following hatching, unhatched eggs were subjected to residue breakout analysis to determine infertility or stage of embryonic mortality. Data for fertility, hatchability, hatch of fertile eggs, and embryonic mortality classifications were analyzed using chi-square tests of independence to evaluate differences in proportions between lines, with significance declared at $P \leq 0.05$. Ongoing analysis of hatch window distribution will further evaluate differences in hatch timing between lines. The LWE line exhibited higher fertility than HWE (84.58 vs. 80.71%). However, the HWE line exhibited both a greater hatchability (65.22 vs. 61.20%) and hatch of fertile eggs (80.81 vs. 72.36%) than the LWE line. Differences in embryonic mortality were observed between lines, with LWE showing an increase in both mid-dead (7.95 vs. 2.72%) and late-dead (9.64 vs. 5.98%) percentages compared to the HWE. Early embryonic mortality did not differ between lines. These results suggest that selection for water efficiency may influence embryonic survival and hatch outcomes, particularly during mid to late stages of development. Selection for a more water-efficient bird could lead to better hatchability, although the decrease in fertility may be of concern. Ongoing analysis of hatch window distribution will further determine whether these lines differ in hatch synchrony and timing, providing insight into optimizing hatchery performance in water-selected poultry lines and further identifying the impact of selection for water efficiency on broiler hatch performance.

**Keywords:** water efficiency; hatchability; embryonic mortality; hatch window; broilers

### 393P The effect of heat stress on thermal regulation and growth in broiler breeder hens selected for water efficiency

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Increasing global temperatures have heightened concerns about heat stress and water efficiency in broiler breeders, driven by growing water scarcity and decreased productivity. We aimed to determine if selection for water efficiency affects core body temperature regulation,

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body weight (BW), and body weight gain (BWG) in broiler breeder hens exposed to cyclic heat stress (HS). Low (LWE) and high (HWE) water-efficiency lines and a modern random-bred (MRB) line were evenly distributed across 12 environmentally controlled chambers (3/line/chamber). In a 3x2 split-plot design, 6 chambers were exposed to cyclic HS (32°C 0900-1700h per day from 31-34 wks), and 6 were maintained at thermoneutral conditions (TN; 22°C). Chamber temperature and relative humidity (RH) were recorded with loggers (2/chamber). Core body temperature (°C) was recorded in 30 hens (10 LWE, 11 MRB, 9 HWE) at the end of HS (5 min intervals for 2d). Thermal images were taken from 36 hens (6/line/treatment) to measure maximum surface beak and eye temperature (°C). Body weight (BW) was recorded pre- and post-HS to calculate body weight gain (BWG). Ambient chamber HS temperatures averaged 30.54±0.018°C, while TN averaged 22.74±0.005°C and RH averaged 33.48±0.06% and 55.03±0.08%, respectively. Thermal image and BW data were analyzed using a linear mixed effects model. Internal temperature data were analyzed with a generalized additive model, with separate analyses for 0900-1700h (8h) and 1700-0900h (16h) with line and treatment as fixed effects. Hens within the HS treatment had higher (P<0.0018) beak (37.2±0.44) and eye (34.2±0.14) temperatures than TN (Beak: 34.3±0.44, Eye: 33.6±0.14°C). Internal temperatures during the 8h and 16h periods had nonlinear changes in magnitude over time (P<0.0001), except for LWE-TN. There was an interaction at 8h (P<0.0001) with LWE-HS (41.78°C) having a higher core temperature than all other treatments (41.35±0.07°C to 41.59±0.07°C), and overall LWE maintained higher temperatures (P<0.0001) than MRB and HWE (LWE: 40.57±0.06°C, MRB: 40.50±0.06°C, HWE: 40.49±0.06°C). At both 30 (P=0.0038) and 34 (P=<0.0001) wks, HWE (3561g and 3918 g) had higher BW than MRB (3293 g and 3547 g) and LWE (3371 g and 3624 g). Surprisingly, at 34 wks, HS had higher BW (3777 g vs 3616 g; P=0.0127) and BWG (347 gvs 206 g; P=0.0069) than TN. Overall, cyclic HS increased ambient and core body temperatures across lines, with LWE showing an elevated thermal response. Cyclic HS increased BW and BWG in this study, potentially due to already restricted diets and increased fat deposition associated with HS.

**Keywords:** broiler breeder; thermoregulation; water efficiency; heat stress; body weight

### 394P Brooding Temperature and Hygiene Practices on Growth Performance and Respiratory Health of Poultry in Commercial Farms

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Poultry farming profession can be a vital venture for food security and rural livelihoods in Bangladesh. Commercial farms are facing challenges in brooding temperature and hygiene practices that affect growth performance and respiratory health. This study examined the associations between these management factors and poultry health outcomes in Shariatpur and Madaripur districts of Bangladesh through a One Health approach. A cross-sectional study was conducted among 30 commercial broiler farms using structured questionnaires, observational checklists, and farm record reviews. Data were collected on brooding temperature, hygiene, biosecurity, growth, respiratory signs, antibiotic use, and farmer perceptions. While all farms used digital thermometers and 90% relied on electric heating, 20% placed thermometers incorrectly, and power failure affected 86.70% of farms. Hygiene practices showed selective compliance: 100% disinfected sheds before new batches, but 70% lacked footbaths and only 13.3% practiced all-in all-out system. Farms reported respiratory signs, but severity varied significantly with hygiene status (Cramer's V = 0.560, p = 0.014). Farms with adequate hygiene had no cases of maximum symptom severity, whereas 35% of inadequately managed farms exhibited all five clinical signs. Brooding temperature management showed non-significant associations with growth performance, though adequate management was associated with numerically lower mortality (5.18% vs. 5.68%). Brooding temperature significantly predicted antibiotic awareness (B = 0.410, p = 0.019), explaining 27.1% of variance, and showed stronger association with One Health perceptions (B = 0.315) than hygiene scores. Hygiene practices are strongly associated with respiratory health in commercial poultry farms, while brooding temperature management predicts antibiotic awareness and One Health perceptions in Bangladesh.

**Keywords:** Brooding temperature; Hygiene practices; Respiratory health; Growth performance; Commercial broiler

### 395P Assessing bone quality changes in turkey breeder hens over a laying cycle

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The onset of egg production in avian species is associated with changes in bone morphology driven by an increase in estradiol (E2), which stimulates medullary bone formation to meet the calcium requirements of eggshell formation. This exploratory study characterized changes in bone mineral density (BMD) and E2 in turkey breeder hens throughout a laying cycle. The work was conducted as part of a larger study evaluating the effects of light spectrum (white or red) and intensity (high: 0.3 W/$m^2$ or low: 0.1 W/$m^2$) on reproductive performance. A total of 402 female grandparent line Hybrid Converter turkey hens were assigned to 24 pens across four rooms in a split-plot arrangement, with spectrum assigned by room and intensity by pen (N = 6 per spectrum-intensity combination). At 30, 33, 44, and 60 weeks of age (woa), one hen per pen was blood sampled before euthanasia and femur collection. Plasma E2 was measured by immunoassay, and femurs were autoclaved, defleshed, and assessed for BMD using dual-energy X-ray absorptiometry. Data were analyzed in SAS (PROC GLIMMIX) with the main effects of spectrum, intensity, age, and the interaction between spectrum and intensity, and Pearson correlations (PROC CORR), as appropriate. Overall, light treatment had no effect on E2 or BMD. However, BMD was strongly positively correlated with age ($r = 0.85$, $P < 0.001$), increasing approximately two-fold throughout lay from 0.39 ± 0.02 g/$cm^2$ at 30 woa to 0.80 ± 0.02 g/$cm^2$ at 60 woa. As expected, E2 rose sharply from 30 to 33 woa (23 ± 15 pg/ml to 225 ± 15 pg/ml), then remained elevated until the end of the experiment. E2 was also moderately correlated with BMD ($r = 0.48$, $P < 0.001$). These results support a role for E2-driven medullary bone deposition in increasing BMD. Micro-computed tomography analysis is currently underway to determine structural and medullary bone contributions. Improved understanding of this relationship may inform testable hypotheses related to the mechanisms of bone remodelling and egg production in turkey breeder hens.

**Keywords:** bone mineral density; dual-energy X-ray absorptiometry; management; turkey breeder

### 396P Evaluation of semen characteristics and testis and epididymis health of late cycle broiler breeder roosters fed hen or rooster specific diets

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Sustaining reproductive performance in broiler breeder flocks requires consideration of both hen and rooster contributions; however, nutritional management primarily focuses on hens. The physiological effects of sex-specific diets for roosters, particularly during late-cycle production, remain poorly defined. Increased epididymal lithiasis in roosters receiving hen diets higher in calcium has been hypothesized. Therefore, this trial aimed to 1) compare semen quality and 2) evaluate testes and epididymides histology in late-cycle roosters fed hen or rooster-specific diets. A total of 50 Cobb MX breeder males (49 wk) were obtained from a previous study, in which they were fed rooster specific diets and reared in group floor pens with hens. Males were randomly assigned to individual cages and given a pre-experimental adaptation period, with data collection starting at 52 weeks. Dietary treatments consisted of either continued rooster specific diet (RD) or an adjustment to hen specific diet (HD). Diets were formulated to meet breed guidelines for roosters and hens (energy, protein, Ca, P, vitamins, and minerals). Semen was collected and analyzed, using an Avian SQA-VT semen quality analyzer, 4 times at weeks 58, 60, 62, and 67. Four birds were removed due to injury or illness unrelated to treatment, with 46 roosters remaining for necropsy and sample collection. During necropsy, testes and epididymides were removed and preserved in 10% buffered formalin for histological evaluation. A linear mixed model assessed semen quality, with dietary treatment as a fixed effect and bird as a random effect. Histological count data were analyzed using a GLMM with a Poisson distribution and logit link. Data were analyzed in JMP Pro 19, with significance at $P \leq 0.05$. No differences in semen quality were observed between treatments ($P > 0.05$), for any time point. Similarly, no treatment effects were detected for total calculi ($P = 0.338$) or lymphoid lesion counts ($P = 0.643$) in epididymides. These results indicate that sex-specific feeding in late-cycle roosters does not negatively affect semen quality or lesion severity. However, evaluating only one epididymis rather than both left and right would have yielded different histological interpretations, highlighting the importances of bilateral assessment. In addition, substantial bird-to-bird variability in semen and histology suggests larger sample sizes are needed. Future studies should establish replicate baseline and periodic prevalence of calculi and lesions.

**Keywords:** Broiler Breeder; Rooster; Semen Quality; Reproduction; Histology

## Poster Session I

### 397P Assessment of climatic changes on broiler production in Ido local government of Oyo state

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This study examined effect of climate change on smallholder broiler production in Ido area of Oyo, Nigeria and recent adaptation options. We hypothesized a significant relationship between socio-economic status of respondents and effect of climate change on broiler production. Multi stage sampling selected 100 respondents. Data was analysed using descriptive statistics (frequency, mean and percentages); hypothesis was analysed with chi-square. Results shows that 56.0% of respondents were male aged 21-30years, 62.0% had working experience of 0-5years out of which 21% were female while majority (80.0%) of the respondents had tertiary education; however only 56.0% of respondents were broiler farmers, male and female . 90.0% practice deep litters system. Results further shows that all the respondents are aware of climate change and its effect on the bird. The past five years have seen a steady increase in heat and solar intensity. Also, majority (92.0%) strongly agreed that excessive heat makes the birds spread their wings, leading to lower feed intake and increase mortality. The climate has become unstable and inconsistent and weather is increasingly difficult to forecast. This has influenced the spread of diseases as stated by 56.0% of the respondents. This can be as a result of increase in the rate of development of certain pathogens or parasites that have one or more life cycle stages outside their animal hosts. Climate change is affecting the feed resources due to low harvest and hence scarcity of feed grains which can to high cost of feed, 54.0% agreed that the indirect effects of weather on feed have significant impact on productivity and marketing of broiler products. Most respondents (59.8%) are very interested in finding out more about climate change. Over 60% of respondents indicated that they or their communities did not take any actions or were not sure if they or their communities had taken action. Results on the hypothesis shows that high temperature (x2=68.048), feed resources used(x2=346.721,p=0.011),types of housing (x2=30.113,p=0.036) have significant relationship on the challenges of climate change among the respondents.. Farmers should be provided with practical knowledge of climate-smart techniques and adaptation strategies, supported by government initiatives, to improve their preparedness for climate change uncertainties. Also integrating climate change into the curriculum of agriculture in our institutions

**Keywords:** Climate change ; Smallholder farmers ; Broiler; Feed; Adaptation

### 398P Problems and prospects of precision feeding among broiler farmers in Ibadan metropolis of Oyo state, Nigeria

Johnson A. Olupona[1], Opeyemi J. Bolaji*[1] [1]*Federal College of Animal Health and Production Technology, Ibadan, Animal Production , Ibadan , Oyo, Nigeria*

This study was conducted to investigate the challenges of precision feeding among smallholder broiler farmers in Ibadan metropolis of Oyo state,Nigeria. Precision feeding is a modern approach to animal nutrition that involves tailoring the diet of each animal (or group of animals) to its specific needs at a particular time. The goal is to maximize feed efficiency, reduce waste, lower production costs, and minimize environmental impact. One of the key features is real- time data use which involves sensors and technology, Smallholder broiler farmers face significant challenges in adopting precision feeding practices due to limited access to technology, knowledge, and financial resources. There is a significant relationship between farmers' access to digital tools and the adoption of precision feeding techniques. Multi stage sampling techniques were used to select 120 respondents for this study. The survey questions were group into six sections namely; background information, farm characteristics, awareness and knowledge of precision feeding, adoption and practices, challenges and recommendations and support. Descriptive statistics such as frequency, mean and percentage were used to analyze the objectives while chi square was used for the hypothesis. The results showed respondents had an average age of 35years, majority (75.3%)of which were male and 70.8% had tertiary education, 55.0% of the respondents had farming as their secondary occupation with five and above years of experience. Results further showed that 65.8% of the farmers used deep litters system of management and 76.7% were feeding their birds with commercially produced feed. Most of the farmers (64.2%) are aware of precision feeding and that it has led to reduction of the feaces and high feed efficiency. Many of them (80.8%) were of the opinion that precision feeding techniques requires skills ,tools and close supervision, the cost of production is also very high according to 63.3% of the farmers and this is one of the major challenges of precision feeding in the study area. Results on the hypothesis shows that breed of birds (x2=68.048), types of feed used(x2=346.721,p=0.011),types of housing (x2=30.113,p=0.036) have significant relationship on the challenges of precision feeding among the respondents. In conclusion, it is evident that there is need for training and awareness in the area of precision feeding technology for farmers,particu-

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larly the smallholders and this can be done through the extension agents.

**Keywords:** Precision feeding ; Broiler; Farmers ; Training ; Awareness

### 399P Empowering farmers as advocates: The Manitoba Egg Farmers Ambassador Training program

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Regulated Canadian egg farmers actively engage in proactive training and outreach to build consumer trust and showcase their high standards for animal welfare, food safety, and sustainability. Manitoba Egg Farmers (MEF), representing the province's 170 egg and pullet producers, hosts annual Ambassador Training Workshops designed to build farmers' confidence and equip them to strengthen public trust. Participation is mandatory for any farmer representing MEF at public-facing events. The 2025 workshop was held at the Manitoba Egg Farmers Learning and Research Centre (MEFLRC), a state-of-the-art poultry research, education and outreach facility at the University of Manitoba Glenlea Research Station. Opened in 2023 through a partnership among MEF, the university, and federal and provincial governments, the centre is integrated with the Bruce D. Campbell Farm and Food Discovery Centre (FFDC). This full-day workshop brought together MEF staff and board members, egg farmers, industry representatives, and FFDC personnel to develop practical tools for effective public communication. A total of 36 ambassadors participated in the 2025 workshop, including 26 egg farmers. Most farmers were returning participants, while five attended as first-time ambassadors. The 2025 workshop focused on public trust, utilizing the latest research data from the Canadian Centre for Food Integrity, a not-for-profit organization, to help participants apply research insights to farm tours and public interactions. Participants engaged in media training, practiced responding to challenging questions and developed strategies for sharing complex production data into clear, relatable stories. A highlight of the session was a peer-led panel featuring three farmers who shared their experiences and strategies for authentic engagement. The MEFLRC provides an ideal training environment, featuring a large meeting room, educational displays, and visitor viewing areas for both aviary and enriched-housing laying hen barns. Conducting training in the same setting where ambassadors later lead tours and host visitors helps them become familiar with the facility and build confidence in representing the egg industry. The training emphasized farmer-led storytelling and transparency over promotional messaging, reinforcing the integrity of Canada's supply-managed agriculture and demonstrating a commitment to responsible egg production.

**Keywords:** Ambassador Training Workshop; education; Manitoba Egg Farmers Learning and Research Centre; outreach ; workshops

### 400P First report of whole genome sequencing of *Salmonella* Minnesota isolated from turkey

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Brazil is an important producer of meat-type turkeys and *Salmonella* enterica serovar Minnesota has become increasingly relevant worldwide. It is frequently detected in farms and poultry-derived foods, however, genomic data for this serovar in turkeys remain scarce. Genomic characterization of circulating strains is essential to understand their epidemiology, genetic diversity, and potential adaptation mechanisms, especially given the public health implications of salmonellosis. Therefore, the objective of this study was to perform the complete genome sequencing of a *S.* Minnesota strain from a flock of turkey breeder hens located in the state of Santa Catarina (Brazil). A strain of *S.* Minnesota was isolated in 2023 from a litter drag swab collected in a flock of 12-week-old turkey breeder hens located in the state of Santa Catarina, Brazil. Species and serovar confirmation were performed by the Center for Diagnosis and Research in Avian Pathology (CDPA), Federal University of Rio Grande do Sul (UFRGS). DNA extraction was performed using the PureLink™ Genomic DNA Mini Kit, and DNA quality was assessed by NanoDrop, Qubit, and agarose gel electrophoresis. Library preparation was carried out with the Illumina DNA Prep kit, followed by sequencing on the Illumina MiSeq i100 platform, achieving approximately 30× coverage with Q30 values exceeding 96%. Descriptive statistical analysis was performed in order to better describe the the WGS findings. *De novo* assembly generated a genome of ~4.82 Mb, distributed across ~95 contigs, with an N50 of ~250 kb and a GC content of ~52.1%, consistent with reference genomes of this serovar. A refined search of the NCBI database (December 2025) confirmed the absence of previously published complete genomes of *S.* Minnesota isolated from turkeys. Thus, this sequence represents the first genomic report of this serovar from a turkey host, providing an important reference for comparative genomics, molecular surveillance, and food safety monitoring. The genome will be deposited in NCBI to ensure public access and scientific reproducibility.

**Keywords:** Molecular biology; Next-generation sequencing; Poultry farming; Public health; Salmonellosis

### 401P Climate Adaptation in Poultry: Spatiotemporal Behavioral and Kinematic Responses of Chicken Genotypes to Identical Temperature-Humidity Exposures

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Climate change has intensified the need to understand how different poultry genotypes respond behaviorally to thermal stress. The temperature-humidity index (THI) is a critical metric for assessing heat stress in poultry, influencing welfare and performance. This study investigates the spatiotemporal behavioral responses and kinematic profiles of genetically selected Ross308 broilers and indigenous Ghanaian chickens under similar THI conditions. The objective was to assess genotype-specific behavioral adaptations to thermal stress across different periods of the day (morning, afternoon, and evening). Behavioral time budgets and movement dynamics were recorded and analyzed to evaluate the influence of genetic selection on thermoregulatory behavior. A total of 12 pens per genotype were used, with 20 birds per pen, and 4 repeats per period of the day. The data was analyzed using a two-way ANOVA with genotype and period of the day as the main effects and their interaction. The means were compared using Fisher's Least Square Difference Test. Results revealed significant differences between the two genetic lines. Ross308 chickens exhibited a higher proportion of resting behavior (75.2%) and lower locomotor activity (4.2%) compared to Ghanaian local chickens, which showed increased movement (7.9%) and standing behavior (39.7%). Drinking behavior was more pronounced in Ross308, while eating behavior did not differ significantly between genotypes. Kinematic analysis indicated that Ghanaian local chickens moved faster (2.2 meters per second) and with greater acceleration (1.3 m per second squared) than Ross308 (1.9 m/s and 1.1 m per second squared, respectively). Variation in period of the day also influenced behavior, with morning periods associated with higher movement and standing, while afternoon and evening periods showed increased resting and drinking. Significant genotype by period-of-the-day

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interactions were observed for all behavioral parameters except eating. The results of the current study highlight the role of genetic selection in shaping behavioral thermoregulation and stress coping strategies in poultry. Indigenous chickens demonstrated greater behavioral plasticity and activity under thermal stress, suggesting their potential value in climate-resilient poultry systems. This study contributes to the understanding of genotype-environment interactions and supports the integration of local genetic resources into sustainable poultry breeding programs.

**Keywords:** Genetic Selection; Temperature-Humidity Index; Heat stress; Spatiotemporal analysis; Climate Adaptation

### 402P Evaluating germline specificity of *NANOS* family genes in chicken embryogenesis

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Nanos is a highly conserved gene family across diverse species and plays a critical role in germ cell formation, migration, and differentiation. Nanos functions as a translational repressor that, with Pumilio, regulates mRNAs essential for embryonic development. In Drosophila, nanos is indispensable for abdominal segmentation and gonad formation. Mammalian homologs (Nanos1, Nanos2, and Nanos3) exhibit distinct roles in germline maintenance, particularly, Nanos3 is required for primordial germ cell (PGC) survival, while Nanos2 regulates male germ cell differentiation in mice. Similarly, recent studies in pigs demonstrate that Nanos2 is essential for the maintenance of spermatogonial stem cells, highlighting its conserved role in germline integrity. Despite extensive investigation of nanos genes across phylogenetically distant species, their expression profiles and functional roles remain poorly characterized in avian systems. Current genomic analyses indicate that only NANOS1 and NANOS3 are present in chickens. Therefore, the objective of this study is to characterize the expression patterns and potential roles of NANOS1 and NANOS3 during chicken germ cell development. Spatial expression of NANOS1 and NANOS3 transcripts was assessed by using RNAscope in situ hybridization in male chicken gonads at embryonic days 6, 12, and 18 with one sample analyzed at each time point. Then, the expression levels of known germ cell markers, DAZL and DDX4, as well as NANOS1 were evaluated by using quantitative PCR (qPCR). RNAscope results indicate that NANOS3, but not NANOS1, localizes to germ cells in male gonads across all examined developmental stages as evidenced by co-localization with DAZL. Quantitative PCR analysis revealed increases in abundance of DAZL and DDX4 from embryonic day 6 to embryonic day 12; however, no difference in abundance of NANOS1 was observed across developmental timepoints. Ongoing experiments aim to expand characterization of NANOS1 and NANOS3 abundance by qPCR using 5 samples and validate these findings at later developmental stages of male and female chicken embryos. Overall, these results indicate that NANOS3, but not NANOS1, is associated with germ cells throughout chicken embryonic development, suggesting its potential role in avian germ cell development.

**Keywords:** Genetics; Reproduction

### 403P Genomic comparison of mucoid and non-mucoid Clostridium perfringens isolates from necrotic enteritis–affected broilers

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Pathogenic *Clostridium perfringens* is the primary etiological agent of necrotic enteritis (NE) in poultry, a disease responsible for more than $6 billion in annual global losses in poultry production. Although toxin-mediated virulence has been extensively investigated, less is known about the genomic factors associated with colony morphology and potential biofilm-related traits in field isolates. In the present study, twenty-three *C. perfringens* isolates (13 mucoid and 10 non-mucoid) were obtained from NE-affected commercial broiler farms and subjected to whole-genome sequencing. Mucoid isolates were identified from a commercial broiler house with a high incidence of NE, whereas non-mucoid isolates were recovered from houses with low incidence of NE. Comparative analyses were performed using hierarchical clustering and principal component analysis. The isolates are grouped predominantly according to colony phenotype. Core toxin genes, including plc (alpha toxin) and pfoA (perfringolysin O), were detected in most isolates and did not differ significantly between the two groups ($p$>0.05). In contrast, mucoid isolates demonstrated a higher frequency of genes involved in capsular and exopolysaccharide biosynthesis, including glycosyltransferases and putative polysaccharide transport components (Fisher's exact test, $p$<0.05). Differences were also observed in regulatory elements, particularly sigma factors and transcriptional regulators, as well as in genes encoding surface-associated proteins such as sortase and pilus-related factors (p<0.05). These genomic features are consistent with enhanced extracellular matrix production and suggest increased biofilm-forming potential among mucoid isolates. Overall, the findings indicate that the difference between mucoid and non-mucoid NE isolates is primarily associated with accessory genome content linked to surface structure and regulation, rather than with differences in major toxin genes. These distinctions may influence intestinal colonization and persistence during disease.

**Keywords:** Clostridium perfringens; necrotic enteritis; broilers; Genomics analysis; Mucoid phenotypes

### 404P A Comprehensive Spacio-Temporal Turkey Expression Atlas (TEA)

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Interpretation of omics data in turkey research is constrained by the lack of turkey-specific genetic and genomic resources. Current functional annotation and pathway databases are largely derived from the chicken genome, limiting biologically relevant interpretation of turkey transcriptomic data due to interspecies differences. As RNA sequencing becomes increasingly integrated into poultry research, a centralized, turkey-specific gene expression resource is needed to support data interpretation and hypothesis generation. To address this gap, we developed the Turkey Expression Atlas (TEA), a comprehensive gene atlas that provides spacio-temporal transcriptomic profiles across tissues and developmental stages in both male and female turkeys. The TEA integrates 10 trillion short RNA sequencing reads (580 libraries), encompassing 17 tissues at five defined developmental stages in both sexes. Quality-controlled reads were aligned to the turkey reference genome (Mgal_WUR_HG_1.0) using HISAT2, and transcript assembly and quantification were performed with StringTie. Differential gene expression analysis was conducted using DESeq2 (q < 0.05; |fold change| > 1.5), and weighted gene coexpression network analysis (WGCNA) was performed in R. Together, these analyses generated over 400 comparative datasets. Functional annotation of differentially expressed genes and coexpression modules was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The TEA is a publicly accessible, web-based platform integrating normalized gene expression levels, differential expression results, func-

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tional enrichment outputs, and co-expression network information. Users can query individual genes, evaluate expression patterns across tissues and developmental stages, and explore correlated gene modules to support functional interpretation of transcriptomic datasets. This resource represents the first comprehensive turkey-specific transcriptomic atlas, expanding the genetic toolkit available for turkey research. By providing a turkey-specific framework for transcriptomic sequencing data, the TEA enables functional studies on growth, development, and physiological processes, and lays the foundation for incorporating other omics datasets.

**Keywords:** turkey; expression atlas; transcriptomics; web-based; genetic tool

### 405P Predicting egg quality using computer vision

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Egg quality traits such as egg weight and shell color are very important for the table egg industry. Breeding companies collect egg quality information in specialized laboratories using costly equipment. Such equipment is not available in commercial and field-testing locations spread across the world, or in developing countries where genetic improvement programs for local chicken breeds are being established. The objective of this study was to develop methodology assessing egg weight and shell color using a cell phone camera and computer vision analysis. The reference data was collected using laboratory grade colorimeters and scales on 180 brown and 180 white eggs. Prior to data recording, eggs were placed individually on an egg flat at a defined position and orientation with identifying sticker next to the egg. Two pictures of each egg were taken with a cell phone placed on a tripod with $180^0$ rotation between the pictures. Pictures were processed with GroundingDINO and GroundedSAM2 python packages which automatically detected and segmented individual eggs using a zero-shot learning approach. The egg area was defined as the number of pixels corresponding to the egg, and shell color was characterized by the average red (R), green (G), and blue (B) values of those pixels, calculated using the OpenCV Python package. Quality control eliminated records more than 3 SD from the mean for brown and white eggs separately (718 pictures of 349 eggs were retained). Repeatability was estimated using a mixed model in R (lme4). Measurements of the two sides of the eggs were averaged and merged with the reference data. Regression equations were developed linking egg area to egg weight and shell color read from the pictures to the value from the colorimeter jointly for all eggs and separately for white and brown eggs using the lm function in R. Estimates of repeatability were 0.82, 0.83, 0.93, 0.92 for area, R,G,B. Egg weight and its slope on the area was significantly ($p < 0.01$) different between brown and white eggs. Area explained 89.6% and 73.8% variance of egg weight for white and brown eggs. Across all eggs RGB values explained 99.4% of variation in color, with 84.8% of variance explained for brown and 15.3% of variance explained for white eggs. It must be noted that SD of color for white eggs was 8 times lower than for brown eggs which explains poorer fit of the model due to limited variation.

**Keywords:** computer vision; egg weight; shell color; image analysis; egg quality

### 406P Detection of pathogenicity-associated genes of *Enterococcus cecorum* in mixed fecal samples from turkeys and chickens

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*Enterococcus cecorum* is a major pathogen associated with septicemia, early mortality, and lameness, and differentiation between commensal and pathogenic strains remains challenging. Comparative genomic studies have identified putative virulence factors; however, these genes have been evaluated primarily in pure cultures and chickens, with limited data from mixed samples and other poultry species. The objective of this study was to determine the prevalence of six pathogenicity-associated genes in fecal samples from turkeys and roosters, and to assess their detection and specificity in mixed microbial cultures. To validate species and pathogenicity specificity, pathogenic (3) and commensal (1) strains, as well as *E. faecalis* (1), were included. Pooled fecal samples were collected from three distinct production systems, each containing toms, 14-day-old poults, or roosters, directly after excretion. Pure isolates were cultured on TSA at 37°C under 5% CO2 for 12 h; DNA was extracted using PureLink™ Genomic DNA Kit (pure cultures) and QIAamp PowerFecal Pro Stool Kit (feces). PCR was performed with gene-specific primers, and products were run on 1.5% agarose with SYBR Safe DNA Gel Stain in duplicate. Samples comprised pure cultures, excreta-only controls, and excreta samples separately spiked with commensal or pathogenic strains. Target genes included ECS3_0196, ECS3_0200, ECS3_0662, ECS3_2296, ECS3_2299, and ECS3_2316. Roosters' and toms' spiked samples were serially diluted ($10^0$ to $10^{-6}$) and analyzed, targeting an additional ECS3_2296 147 bp amplicon for LOD determination. Data were analyzed in JMP Pro 18 using Chi-square ($P \leq 0.05$). Poults' sample was negative for all genes except ECS3_0196. Toms' sample was positive for all genes except ECS3_0200 and ECS3_2299. Roosters' sample was negative for all genes, including ECS3_2296. However, all mixed samples were positive when targeting the 147 bp amplicon, indicating that shorter amplicons improve gene detection in mixed fecal cultures, with amplification maintained at the lowest dilution evaluated. No cross-reactivity was observed; the commensal strain and *E. faecalis* were negative for all genes, while the pathogenic spiking strain was positive for all targets. Overall, gene prevalence across host groups was not statistically significant ($P > 0.05$). Although *E. cecorum* is not a major pathogen in turkeys, these findings suggest that they may harbor strains with pathogenic traits similar to those in chickens, highlighting the need to evaluate putative virulence factors across avian hosts. Further studies are required to validate these findings by expanding the sample size and determining whether strains are shared between turkeys and chickens.

**Keywords:** Enterococcus cecorum; pathogenicity factors; turkey; lameness; septicemia

### 407P Transcriptomic evidence of oviduct-specific immune modulation associated with *Salmonella* Enteritidis colonization in laying hens

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*Salmonella* Enteritidis (SE) is a major zoonotic pathogen responsible for foodborne illness worldwide, with contaminated eggs serving as a primary source of human infection. This study aimed to characterize tissue-specific immune response signatures associated with SE colonization in laying hens. A 5-week time-course infection experiment was conducted using 24 specific-pathogen-free laying hens (64 weeks of age), randomly assigned to SE-infected and non-infected control groups. Oral SE challenge over days 1-5 resulted in stable cecal colonization, persistent cloacal shedding, and bacterial dissemination to systemic and reproductive tissues. Transcriptomic profiles of the spleen, ovary, magnum, and isthmus were analyzed by RNA sequencing (n = 4 per tissue per group). Differential gene expression analysis was performed using DESeq2, with significance defined as $|\log2FC| > 1$ and an adjusted P value < 0.05. Limited transcriptomic changes were observed in the spleen and ovary following SE infection. In contrast, the oviduct exhibited significant region-specific immune responses. The magnum showed a regulatory-dominant and low-inflammatory immune microenvironment,

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characterized by upregulation of *IL2RA*, *IL2RG*, and *IL21R,* indicating enhanced lymphocyte signaling capacity. Concurrent increases in *IL10RA*, *TGFB1*, *TGFB2*, and *TGFBR2* suggest activation of immunoregulatory pathways and an anti-inflammatory environment. Elevated *IL16* and *IL34* further indicate immune cell recruitment and support of tissue-resident immune populations. In contrast, downregulation of *IL15* and *IL20RA* suggests reduced cytotoxic and epithelial inflammatory responses. The isthmus demonstrated an activated yet tightly regulated immune microenvironment. Upregulation of *IL-2* receptor subunits (*IL2RA*, *IL2RB*, and *IL2RG*), along with *IL12/IL12RB* and *IL18/IL18R* signaling components, suggests activation of a Th1-skewed, cell-mediated immune response. Concurrent upregulation of IL16 and *TGFB2/TGFBR2* indicates enhanced immune cell recruitment and engagement of immunoregulatory pathways. In contrast, downregulation of *IL6* and *IL6R* suggests that IL6-mediated inflammatory responses, including Th17-associated pathways, may not be the dominant immune mechanism in the isthmus. These findings demonstrate that SE induces compartmentalized immune responses within the oviduct. The immunoregulatory environment in the magnum may create a permissive niche for persistent SE colonization, thereby increasing the risk of vertical transmission through egg contamination.

**Keywords:** Salmonella Enteritidis; immune modulation; RNA sequencing; laying hens

### 408P Rotating coccidiosis control programs: incorporating a live coccidiosis vaccine in conventional broiler production in Canada

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In Canada, coccidiosis vaccines have historically been used primarily in Raised Without Antibiotics (RWA) systems, while conventional broiler production has relied on in-feed anticoccidial medications. The increasing prevalence of anticoccidial resistance has reduced the effectiveness of some programs. Live vaccination with drug-sensitive *Eimeria* strains has been reported to restore anticoccidial sensitivity within broiler house *Eimeria spp.* populations. The objective of this field case report was to evaluate the impact of a rotational program including live-coccidiosis vaccination and anticoccidial medications on broiler performance in conventional production. Two commercial broiler operations in Ontario replaced their in-feed anticoccidial programs with a live coccidiosis vaccine administered by gel-droplet application at the hatchery for a minimum of two consecutive flocks beginning in summer. In-feed antibiotics were maintained to mitigate bacterial enteric challenges. No changes were made to standard management practices, including brooding density, humidity, or litter moisture. Nutritional programs at each farm were unchanged across flocks. Both major broiler genetic strains were represented, and birds were marketed at target weights of approximately 2.3–2.6 kg. Data were evaluated using descriptive performance comparisons between flocks; no inferential statistical analyses were conducted. In the first operation, medicated programs showed notable variability in mortality, feed efficiency, and European Production Efficiency Factor (EPEF), consistent with cyclical coccidial challenge. Following the transition to Immucox 3 vaccination, flocks demonstrated reduced inter-flock variability in livability and efficiency metrics. Mean feed conversion improved by 3–6%, and EPEF increased to ~15–20%. Economic return per kg improved by 18–23% with more consistent profitability. In the second operation, the aggregated performance of vaccinated (n = 10) and medicated (n = 24) flocks was compared. Vaccinated flocks had lower mortality (3.61%) than medicated flocks (4.86%). Growth performance was comparable between programs, with average body weight of 2.305 and 2.330 kg, feed conversion ratio of 1.542 and 1.531, and EPEF of 416 and 415 for vaccinated and medicated flocks, respectively. These field observations suggest that integrating live coccidiosis vaccination into conventional broiler programs can maintain production efficiency while improving livability and reducing inter-flock variability. Rotational use of vaccination and anticoccidial medications may support more sustainable coccidiosis control in commercial broiler systems.

**Keywords:** coccidiosis; anticoccidials; coccidiosis vaccination; rotation; Eimeria

### 409P *In vitro* effects of protease inhibitors on *Histomonas meleagridis* and associated bacteria in xenic culture

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The purpose of this study was to evaluate the activity of protease inhibitors, *in vitro,* against *Histomonas meleagridis* (PHL 2017 strain). Three protease inhibitors, leupeptin, bestatin, and antipain, were evaluated across five assays (n=3 assays for leupeptin and bestatin; n=2 assays for antipain). Treatment groups included: 1) non-treated control, 2) Leupeptin 100 µM, 3) bestatin hydrochloride 100 µM, and 4) antipain dihydrochloride 100 µM. Histomonads were enumerated using a hemocytometer after 48 hours of incubation at 40C in 1.7mL sterile snap cap microcentrifuge tubes (n=4/treatment). Replicate tubes were used for viability tests by transferring the culture into 25cm$^2$ tissue culture flasks (2 flasks/treatment) and were incubated at 40C. Growth was monitored up to three times from d3-7 post-passage. Colony forming units (CFUs) were evaluated (Log10 CFU/mL) by drop plating onto CHROMagar Orientation agar. Agar plates were incubated overnight aerobically at 37C. ANOVA was used to determine significant ($P$<0.05) differences between treatments by assay, with means further separated using Tukey HSD where appropriate. Leupeptin and bestatin significantly reduced *H. meleagridis* (Log10 cells/mL) compared to the non-treated control in only 2/3 assays, whereas this effect was observed with antipain in 2/2 assays. None of the protease inhibitor treatments impacted histomonad viability and no morphological changes were observed. The CFUs were not significantly different between treatments except for bestain, which showed a significant reduction in *Escherichia coli* (7.57±0.03) and *Klebsiella pneumoniae* (7.73±0.44) compared to the non-treated control (9.53±0.12, 9.32±0.16). These results suggest that protease inhibitors could reduce *H. meleagridis* cells, but they may also influence the growth of the gram-negative bacteria present in the xenic culture. More research is needed to investigate protease inhibitors' activity on *H. meleagridis*.

**Keywords:** Histomonas meleagridis; protozoa; protease inhibitor; turkey; histomonosis

### 410P In-feed Supplementation with Metal Amino Acid Complexed Zinc May Improve Early Performance and Bone Deposition in aMPV Vaccinated Turkey Poults

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The objective was to evaluate the effect of dietary Zn at standard and therapeutic levels on performance in turkey poults vaccinated with a commercial aMPV vaccine. A nutritionally adequate corn-soybean meal-based diet was formulated to contain 60 mg/kg of Zn from Zn sulfate (ZnSO4), to which commercially available organic amino acid complexed zinc (Zn-AAC; Zinpro® Propath® LQ Zn) was added at 3 levels: low-therapeutic (+120 mg/kg Zn), mid-therapeutic (+240 mg/kg Zn), and high-therapeutic (+360 mg/kg Zn). There were nine treatment groups. Groups 1

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and 8 received the basal diet from d0-28. Groups 6 and 9 received the high-therapeutic diet from d0-28. Groups 2 and 4 received the low and mid-therapeutic diets from d0-28. Groups 3 and 5 received the basal diet from d0-7 and low and mid-therapeutic diets from d0-28. Group 7 received the basal diet from d0 and the high-therapeutic diet from d7-28. At d0, poults were randomly allocated across 9 treatment groups (n=4 replicate cages/treatment; n=8 poults/cage), individually wing banded, and Groups 1-7 were vaccinated via the ocular route with a 10X dose of Poulvac TRT (aMPV Type A). The aMPV vaccine route and dose were selected based on a preliminary dose titration study evaluating vaccine take efficiency. Groups 8 and 9 were not vaccinated. Body weight gain was recorded at d7, d14, d21, and d28. At d28, the right tibias (n=3-5 replicate cages/group; n=6-8 poults/cage) were collected to evaluate bone ash concentration (%), and content (mg/tibia). Data were analyzed using a 1-way ANOVA and the significance value for analyses was $P < 0.05$. Group 4 (mid-therapeutic, d0-28) had the greatest BWG during all time periods and BWG was significantly greater ($P<0.0001$) than Group 5 (mid-therapeutic, d7-28) from d0-21 (401.38±11.14: 343.33±10.08) and d0-28 (678.25±15.92: 579.3±18.15). Group 2 (low-therapeutic, d0-28) had significantly greater BWG from d0-7 (71.25±2.27) than Group 3 that received the low-therapeutic diet from d7-28 (54.23±3.28) and had numerically greater BWG from d0-14, -21, and -28 compared to Group 3. Poults in Groups 6 and 9 that received high-therapeutic diet from d0-28 had greater numerical BWG at all time points than Group 7 which received high-therapeutic diet from d7-28. A significant difference ($P=0.0291$) was also observed for bone ash content (mg/tibia) between Group 4 (1130) and Group 7 (923). These data suggest that in-feed therapeutic dosing of an amino acid complexed Zn from day-of-hatch may improve bone deposition and performance in both aMPV vaccinated and non-vaccinated turkey poults. The effect of therapeutic levels of Zn on aMPV vaccine take efficiency, seroconversion, and additional gut health parameters are currently being evaluated.

**Keywords:** aMPV; vaccine; zinc; performance; turkeys

### 411P Evaluation of a microbial consortium for control of *Enterococcus cecorum* in broiler chicken

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*Enterococcus cecorum* is an opportunistic commensal in chickens and emerging pathogen causing osteomyelitis, lameness, and increased mortality in broiler flocks. In young birds, overgrowth and translocation from an immature gut to extraintestinal sites increase infection risk. Establishing a stable, diverse, mature chicken derived microbial community early in life may enhance colonization resistance, promote mucosal barrier integrity, and modulate host immunity, thereby altering susceptibility to *E. cecorum* infection later. This study evaluated the ability of a defined microbial consortium to reduce *Enterococcus* abundance and translocation in broiler chickens. Day-old chicks (n = 36) were randomly assigned to two treatment groups, with 6 replicate cages per treatment and 3 birds per cage, recieving either the microbial consortium (MC) or control (sterile PBS). All birds were challenged with a field-derived isolate of *E. cecorum* on Day 2. One bird per cage was sampled at each time point (Day 7, 14 and 21). The MC consisted of 30 commensal bacteria, including short-chain fatty acid producing taxa and representatives from Bacillota, Bacteroidota and Actinobacteriota, mimicking the mature chicken gut microbiome. Birds were housed under controlled conditions. At each time point, body weight was recorded and liver, spleen, and femoral head swabs were collected to quantify *Enterococcus* load and assess bacterial translocation. Quantification was performed using culture-based methods. Data were analyzed using two-tailed t-test or Mann–Whitney U tests, as appropriate, with significance set at $P < 0.05$. Administration of the MC reduced *Enterococcus* burden in liver and femoral head compared to the control, indicating reduced systemic dissemination. No significant difference in weight was observed. These preliminary findings demonstrate that targeted microbial consortia can limit *Enterococcus* translocation in broiler chickens. The observed effects are consistent with enhanced colonization resistance, suggesting that early-life microbial interventions may improve gut barrier function and limit opportunistic pathogen invasion. This approach represents a promising alternative strategy to control *Enterococcus*-associated disease in poultry production systems.

**Keywords:** Enterococcus; broiler chicken; bacterial translocation; competitive exclusion ; lameness

### 412P The effects of cyclic heat stress on blood cell profiles and antibody concentrations in broilers selected for low water conversion ratio compared to the modern random-bred broiler

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With the rapid changes in climate conditions, environmental heat stress (HS) and water scarcity are growing concerns for sustainable poultry production. The selection of broiler chickens for low water conversion ratio (LWCR) has shown potential for efficient water usage in poultry farming. While studies on the local and systemic immune response to bacterial components revealed similar innate and adaptive immune response capabilities of LWCR broilers compared to the modern random-bred (MRB) controls, the immune resilience of LWCR under HS conditions has not been investigated. The objective of this study was to examine the effects of cyclic environmental HS on blood cell- and antibody-profiles in LWCR and MRB broilers. LWCR and MRB broilers were reared in 12 environmental chambers (2 pens/chamber) under the same temperature conditions until 28 d, at which time they were either kept at thermoneutral (TN; 24°C) temperatures or subjected to cyclic HS (36°C for 8 h; 16 h TN) conditions (12 pens/treatment) until 42 d. At 42 d, heparinized blood samples were collected from one male and one female broiler per pen (12 broilers per line and treatment) to determine blood cell profiles and the plasma concentrations of IgM, IgG, and IgA antibodies. White blood cell (WBC) population analyses were conducted by flow cytometry after immunofluorescent staining of whole blood cell suspensions, and sandwich ELISAs were used to determine antibody concentration. Three-way ANOVA (sex, line, temperature) revealed no sex differences in any of the aspects examined; hence, data were pooled across sex for 2-way ANOVA to determine the effects of line, temperature, and line-by-temperature interactions. Significance was set at $P < 0.05$. In both LWCR and MRB broilers, cyclic HS reduced ($P < 0.05$) the concentrations of RBCs, total WBCs, lymphocytes, B cells, T cells, and heterophils, as well as the plasma concentrations of IgG, while the concentration of monocytes, IgM and IgA were not affected by HS. Moreover, few line differences ($P < 0.05$) were observed, including lower heterophil concentrations and higher CD4 to CD8 T cell ratios in LWCR than MRB broilers, independent of temperature treatments. Overall, these findings indicate that cyclic HS suppresses aspects of both cellular and humoral immunity and that genetic selection for water-use efficiency did not compromise the ability of LWCR broilers to cope with environmental HS. This work contributes to understanding how genetic selection for resource efficiency influences immune adaptation and highlights potential genetic strategies for improving broiler performance and health in increasingly heat-stressed production environments. Supported by USDA-NIFA SAS # 2019-69012-29905.

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**Keywords:** broilers; heat stress; blood leukocyte profiles; antibody conctrations; water efficiency

### 413P Impact of increasing dietary digestible tryptophan to lysine ratio in broiler breeder pullets on blood serotonin response

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Serotonin is a ubiquitous neurotransmitter responsible for regulating various aspects of mood through its interaction with serotonin receptors (5-HT receptors). The objective of this study was to evaluate the effects of elevating dietary tryptophan (Trp) ratios through inclusion of L-Trp on broiler breeder pullet growth, blood serotonin concentrations, and mortality. A total of 37,600 day-old Ross 708 pullets and 3,000 day-old Yield Plus Males were reared in two commercial pullet rearing houses for 23 weeks. The birds were randomly allocated into each house (440'X60'), which was split equally into two female pens and one male pens. Birds were fed either a control or a treatment diet from 4 weeks-of-age to 20 weeks-of-age. The control feed had 0.16% digestible tryptophan while the treatment feed was supplemented with L-tryptophan, reaching 0.25% digestible tryptophan. Birds were fed using skip-a-day conventional rearing program. Blood samples were collected from both groups at 12 weeks-of age-on two consecutive days, (one on feed day and one off-feed day), to verify whether plasma serotonin levels changed. Blood samples were taken from 15 birds/pen (a total of 180 samples) on both days at the same time (8 am) to reduce time variation. Body weight, livability, coefficient of variation (CV), and uniformity were also measured. Data were analyzed by an independent t-test, and statistical significance was determined at $P \leq 0.05$. There was no significant effect of treatment on body weight, livability, and coefficient of variation. The pullets fed a higher level of Trp expressed a significantly higher level of serotonin in blood samples from both males (P =0.0144) and females (P =0.0087) collected on the off-feed day. On the on-feed day blood sample, only female pullets fed higher Trp exhibited a higher level of serotonin in blood compared to pullets fed control diets (P =0.0414). Male uniformity was improved with higher Trp levels in diets measured at 20 weeks-of-age. Overall, results in this study indicate that an increased level of tryptophan results in higher serotonin levels in the blood that could consequently lead to less aggressive behavior.

**Keywords:** Tryptophan; breeder pullets; serotonin; behavior; uniformity

### 414P Effect of a reduced protein diet and of a proprietary blend of natural compounds on growth performance, litter characteristics, and ammonia emissions in broiler chickens

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Improving the sustainability of poultry production requires the holistic assessment of nutritional strategies to ensure that potential benefits like gut health are not offset by increased pollutant emissions. This study evaluated the effects of a proprietary blend of botanical compounds, an organic acid, and functional fatty acids; (ALT, PROBIOTECH International) on broiler performance, litter characteristics, and ammonia (NH3) emissions, compared with a standard control diet (CT) and a low crude protein diet (LCP). A total of 468 day old male Ross 308 broilers were randomly allocated into 12 environmentally controlled chambers designed to measure NH3 emissions. Three types of diets were tested across starter (1 to 10 d), grower (11 to 25 d), and finisher (26 to 36 d) phases: CTL; CTL supplemented with ALT in all phases (ALT); and an LCP diet containing 24 g/kg and 23 g/kg less crude protein during grower and finisher phases, respectively, with 4 chambers per treatment. The amino acids were balanced with the addition of methionine, threonine, valine, isoleucine and arginine. Measurements included growth performance, water intake, litter composition, and cumulative NH3 emissions. The statistical analysis was performed with MIXED procedure of Minitab with the fixed effect of the treatment and significant difference were assessed using Tukey test with $P \leq 0.05$ as significant difference. Growth performance was high in all treatments, with a production efficiency factor of 590, 622, and 576 for CTL, LCP, and ALT, respectively. Body weight gain and feed intake from 1 to 36 d were not significantly affected by dietary treatment. However, FCR was improved in ALT by 5% compared with LCP and by 4% *vs.* CT (P=0.024). Only the reduction in CP content lowered the water to feed ratio, lowering from 2.0 for the CTL, ALT groups to 1.6 for the LCP (P=0.050). Regarding litter composition, only ammoniacal nitrogen content was affected (P=0.017) showing reduction in LCP. Ammonia emissions were not affected by dietary treatment. Contrary to expectations, the LCP diet did not reduce NH3 emissions compared with CTL, which may be related to a non significant increase in litter pH (8.2 *vs.* 8.5). This study highlights the importance of multicriteria evaluation of nutritional strategies, considering animal responses, litter properties, and gaseous emissions. ALT can be used without negatively affecting NH3 related environmental impacts.

**Keywords:** broiler; nitrogen; ammonia; litter

### 415P Impact of valine to lysine ratio in male turkey poults on growth performance and feather coverage

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Research studies to evaluate valine recommendations for growing turkeys have not been published in the past 3 decades. The objective of this study was to evaluate varying levels of valine to lysine ratios and determine their impact on BW and feather coverage. Five dietary treatments of valine to lysine ratio; 58:100(LLV), 64:100(LV), 70:100(CV), 76:100(HV) and 82:100(HHV) were provided at placement. Poults (1694 Hybrid Converter Male) were randomly allocated across treatments with 6 pens per treatment at the Stanley Balloun Turkey Teaching and Research barn. Feed provided, feed remaining, and mortality were recorded by pen. BW and remaining feed weights were taken every 2 wks. Feather coverage scores were recorded weekly by multiple observers using a published scale on 10 poults per pen. Growth performance data were analyzed using a linear model using the lm package in R with dietary treatment analyzed within each phase (0-14d; 14-28d, 28-42, and 0-42d). Feather coverage data were analyzed using a linear model using the lmer package in R with dietary treatment, age, and dietary treatment by age interaction included in the model as fixed effects. Pen and observer were fit in this model as a random effect. Body weight at placement, 28d, and 42d were similar among dietary treatments (P>0.33), but were significantly altered at 14d (P<0.01) with HHV and HV having lighter BW compared to all other dietary treatments (P<0.05). Feed intake (P>0.42) and livability (P>0.20) were similar across dietary treatment for all phases examined. FCR was altered at d 14 (P<0.01) with HHV and HV having more unfavorable FCR compared to LLV and LV (P<0.05). Poults fed the CV had similar FCR compared to poults on all other dietary treatments (P> 0.05). Feather coverage was impacted by dietary treatment (P<0.01), age of poult (P=0.04), and the interaction of dietary treatment and age of poult (P<0.01). Poults fed the LLV diets had significantly worse feather coverage at wk 2 compared to HV and HHV; at wk 3 compared to LV, CV, and HHV, and wk 4 through 6 for all other dietary treatments (P<0.05). At wk 5, poults on the LV had significantly worse feather coverage compared CV (P<0.05). In conclu-

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sion, reducing the valine to lysine ratio resulted in inconsistent changes in BW and FCR; however, poult feather coverage decreased in poults fed the LLV compared to all other dietary treatments consistently throughout the study. This suggests that poults may divert limited amino acid resources for body weight gain over feather growth and that current commercial recommendations (67:100) should be considered the minimal inclusion.

**Keywords:** poult; valine; titration; feather

### 416P Egg Quality and Nutritional Profiling of Turkey Varieties: A Comparative Study

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This study evaluated egg quality traits, proximate composition, and amino acid profiles of eggs from four turkey varieties (Black, Bronze, White, and Bourbon Red) reared under an intensive production system at Bangladesh Livestock Research Institute, Savar, Dhaka. A total of 240 laying turkeys (60 per variety) were maintained from 24 to 55 weeks of age. At peak production (55 weeks), 120 eggs (30 per variety) were randomly collected, where individual eggs served as the experimental unit for egg quality analysis, and subsamples (n = 5 eggs/variety) were used for proximate composition and amino acid analyses. Amino acid profile was analyzed using pre-column derivatization process by Ultra Performance Liquid Chromatography (UPLC) (Waters, USA). All the statistical procedures were carried out using R Studio (version 4.5.1). Egg weight ranged from 62.6 to 70.4 g and did not differ among varieties (P>0.05). Among egg quality traits, only shape index differed significantly (P<0.001), with the White variety producing more rounded eggs. All eggs were classified as Grade AA, with no differences in Haugh unit (P>0.05). Proximate composition varied significantly among varieties. Moisture content was highest in Bourbon Red (73.37%), whereas crude protein was higher in Black (12.92%) and Bronze (12.80%) varieties (P<0.05). Ash content was also highest in Black eggs (3.22%; P<0.05), while crude fat and fiber were not affected (P>0.05). Amino acid profiling revealed highly significant varietal differences (P<0.0001). The Black variety exhibited higher concentrations of key essential amino acids, including lysine (11.94), leucine (23.46), methionine (5.16), valine (10.30), and arginine (9.25 g/100 g protein), as well as non-essential amino acids such as glutamic acid (22.23), aspartic acid (16.02), alanine (8.14), and serine (12.16). Bronze showed intermediate values, whereas White and Bourbon Red had comparatively lower concentrations. In conclusion, Black and Bronze turkey varieties produced eggs with superior crude protein and enriched amino acid profiles, indicating their potential for functional food development and genetic improvement programs.

**Keywords:** turkey; egg quality; proximate; amino acid profile; UPLC

### 417P Vitamin K as a functional nutrient to support calcium metabolism and egg production in modern hens for robust extended laying period

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Aging process in hens is associated with calcium (Ca) dysregulation, affecting bone integrity and eggshell production. Vitamin K, through dependent proteins, support Ca homeostasis, promoting bone formation and eggshell formation. However, current recommendations are based on studies from the 1980s and may not reflect the requirements of modern hens. Differences among vitamin K forms may also influence its effect on Ca metabolism due to tissue-specific activity. This study evaluated the effect of vitamin K forms and inclusion level on egg production and quality and bone mineralization in laying hens. At 18 weeks, 180 Lohmann White hens (1246 ± 53.3 g) were randomly allocated to 60 cages (n=3/cage) and assigned to 6 treatments: 1) Control (normal VitK3, 3 mg/kg), 2) VitK1 (12 mg/kg of vit K1), 3) VitK2MK-4 (12 mg/kg of vit K2MK-4, [menaquinone]), 4) VitK2MK-7 (12 mg/kg of vit K2MK-7), and 5) VitK3 (12 mg/kg of vit K3). Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were measured every two weeks. Eggs were weighed twice per week, and egg quality was assessed every two weeks using the DET6500. From 20 to 35 weeks, 30 hens were scanned every 5 weeks using dual-energy X-ray absorptiometry to evaluate bone mineral content (BMC). A mixed model ANOVA was used with the treatments set as fixed effects and cage within block as a random, with cage as the experimental unit. Pre-planned orthogonal contrasts assessed the effects of Vit K levels and forms against Control. During the pre-lay phase (week 18), performances were not impacted by vitamin K forms or inclusion level compared to Control. For the production phase (19-36 weeks), ADFI was higher in hens receiving Control compared to all the other treatments (+0.8 %; $P < 0.05$). However, ADG and FCR did not differ between Control and vitamin K treatments. At 35 weeks, BMC was lower in hens receiving VitK1, K2 or K3 compared with Control (-8.4 %; $P < 0.05$). Eggshell strength also tended to be lower in hens fed VitK1, K2 or K3 compared with Control (-0.2 kgf; $P = 0.06$). Total egg production, average egg weight and egg quality were not impacted by vitamin K forms or inclusion levels compared with Control. In conclusion, variations in vitamin K forms and inclusion level influenced slightly feed intake, bone mineralization after 35 weeks, and eggshell strength, suggesting changes in Ca utilization during the early laying period. However, BW, growth performance, and egg production and weight were similar among treatments, indicating that different vitamin K forms and levels supported comparable productive performance in laying hens. These effects should be confirmed over a complete laying cycle.

**Keywords:** Hens; vitamin K; bone; calcium

### 418P Alternative Vitamin K forms as a functional nutrient to support calcium metabolism and egg production in modern hens for robust extended laying period

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Vitamin K is a cofactor involved in bone turnover and eggshell formation, contributing to calcium (Ca) metabolism in laying hens. While commonly supplemented as synthetic K3, other forms such as K1 and K2 (menaquinone, MK-*n*) are also available. Vitamin K forms may differentially affect Ca metabolism via tissue-specific effects. This study evaluated the impact of vitamin K forms and its interaction with vitamin D3 on laying hens' performance, egg production and bone mineralization. At 18 weeks, 180 Lohmann White hens (1241 ± 54.7 g) were randomly allocated to 60 cages (n=3/cage). They were already assigned to 5 treatments as pullets: 1) VitK1 (12 mg/kg of vit K1), 2) VitK2MK-4 (12 mg/kg of vit K2MK-4), 3) VitK2MK-7 (12 mg/kg of vit K2MK-7 and, 4) VitK3 (12 mg/kg of vit K3) all supplemented with 2000 IU of VitD3, and 5) VitK3+D (12 mg/kg of vit K3 + 3000 IU of Vit D3). Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were measured every two weeks. Eggs were weighed twice per week, and egg quality was assessed every two weeks using the DET6500. Bone min-

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eral content (BMC) was evaluated by dual-energy X-ray absorptiometry in 30 hens every 5 weeks, starting at 20 weeks. A mixed model ANOVA was used with the treatments as fixed effect and cage within block as a random effect, with cage as the experimental unit. Pre-planned orthogonal contrasts assessed alternative vitamin K forms (Trt 1,2,3 vs 4), VitK2 vitamers (Trt 2 vs 3) and additive effect of vitamin D (Trt 4 vs 5). During the pre-lay phase (week 18), ADG (-22.9 %) and BW (-2.6 %) were lower in hens fed VitK3 than in those fed VitK1 or VitK2 ($P$<0.05). For the production phase (19-36 weeks), BW was lower in hens fed VitK3 compared to either VitK1 or VitK2 (-2.0 %; $P$< 0.05). Also, ADFI (-2.7 %) and FCR (-3.4 %) were lower in hens fed VitK3 compared to either VitK1 or VitK2 ($P$<0.001) and VitK3+D (-2.0 % and -2.8 %; $P$<0.01). Eggshell percentage was lower in hens fed VitK3 vs VitK1 or VitK2 (-0.4 %; $P$<0.05) or VitK3+D (-0.6 %; $P$<0.05). VitK3 tended to have a lower Haugh unit (-0.6; $P$= 0.07) than VitK1 or VitK2. Average egg weight (+1.2 g; $P$<0.05), yolk diameter (+0.8 mm; $P$<0.01), albumen height (+0.3 mm; $P$=0.08) and Haugh unit (+0.4; $P$=010) was higher in hens fed VitK2MK-4 compared with VitK-2MK-7, but eggshell strength tended to decrease (-0.2 kgf; $P$=010). No differences were observed for total egg production, BMC and BMC/BW. In conclusion, VitK2MK-4 improved internal egg quality compared with VitK2MK-7, while VitK3 reduced ADFI, BW and eggshell percentage. However, overall egg production and bone mineralization were not affected, indicating comparable productive performance among vitamin K forms.

**Keywords:** Hens; vitamin K; bone; calcium

### 419P Dietary supplementation with copper hydroxychloride improves growth performance and footpad health in broilers

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Copper is an essential mineral for poultry, involved in key physiological functions including growth, immune function, antioxidant defence, and footpad health. However, the efficacy of copper depends on its chemical form, with hydroxychloride sources showing greater stability and bioavailability than conventional inorganic salts. This study evaluated the effects of copper hydroxychloride (IntelliBond® C, Trouw Nutrition, The Netherlands) compared with inorganic copper on broiler performance, gizzard erosion and footpad health. A total of 336 one-day-old male Ross 308 broiler chicks were randomly assigned to three dietary treatments in a completely randomized design, with seven replicate pens per treatment and 16 birds per pen: (1) negative control (NC), a basal diet without supplemental copper; (2) inorganic copper (CuSO4), the basal diet supplemented with copper sulfate at 125 mg/kg in the starter-grower phase and 15 mg/kg in the finisher phase; and (3) copper hydroxychloride (IBC), the basal diet supplemented with IntelliBond® C at the same copper inclusion levels. Birds were fed AGP-free wheat-soybean meal-sorghum diets with phytase and xylanase. Performance data (days 0-35) were analysed using one-way ANOVA, while gizzard erosion, hock burn and footpad dermatitis scores (day 35) were analysed using nonparametric methods. Over the entire period (days 0-35), birds supplemented with IBC had a lower feed conversion ratio (1.332) than those in the NC group (1.358; P = 0.002), with birds supplemented with CuSO4 showing intermediate values (1.344). Birds supplemented with IBC tended to have higher body weight gain (2631 g) than those supplemented with CuSO4 (2597 g) or NC (2532 g; P = 0.094). Hock burn scores were lower in IBC-supplemented birds compared with NC and CuSO4 groups (P = 0.007), whereas livability, gizzard erosion and footpad dermatitis scores did not differ among treatments (P > 0.05). These results demonstrate that supplementation with copper hydroxychloride (IBC) improved feed efficiency, tended to increase body weight gain, and reduced hock burn in broilers, indicating that IBC is a more effective source than copper sulfate.

**Keywords:** Broiler; Copper; Performance; Footpad health; Gizzard erosion

### 420P Effect of calcium pidolate on mineral digestibility and intestinal health of layers with reduced dietary levels of calcium and phosphorus

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Calcium pidolate (PID) has been studied in broilers in both conventional field diets and in contexts of reduced levels of calcium (Ca) and phosphorus (P) in feed. These trials have shown that it is possible to reduce the 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 study the impact of PID on mineral nutrients digestibility, but also to study the impact of PID on gut health parameters of layers with reduced dietary levels of Ca and P. 256 Hy-line laying hens of 50 weeks old were distributed in 4 treatments in a completely randomized design (8 replicates of 8 birds per treatment) for 84 days. The experiment was a 2×2 factorial with 2 levels of Ca-P, with the supplementation or not of 300 g/MT of PID (Pidolin®PCa, Dietaxion). Control diet (CTRL) contained 4.508% CaT and 0.377% Pdig. Low Ca and P diet (RED) was formulated to obtain a reduction of 10% reduction in CaT and 30% in Pdig. Parameters studied at the end of the supplementation were digestibility of the mineral matter (MM), digestive organs proportions (proventriculus, gizzard, pancreas, small intestine, large intestine), villi length (VL), crypt depth (CD) and ratio VL/CD in the duodenum, jejunum and ileum, being also measured the level of SCFAs (short chain fatty acids) in the cecal content (acetate, propionate and butyrate) and pH in the different digestive organs. Data were analyzed with ANOVA followed by Tukey tests. RED layers had significantly lower digestive organs proportions (P<0.05), from -8% to -24% depending on the organ, in comparison with CTRL ones. However, there were similar digestive organs proportions between RED-NOPID and RED-PID layers (P>0.05). The digestibility of MM was not different between treatments (P>0.05). Regarding the cecal content, the three SCFAs were improved in the PID layers compared to the NOPID ones (P<0.05). CTRL layers had better results than RED ones only for propionate and butyrate (P<0.05). There was a tendency for PID treated birds to increase the ratio VL/CD in duodenum, jejunum and ileum in the two nutrients levels contexts (P<0.1). These differences remain mostly not significant between RED and CTRL hens for the ratio VL/CD (P>0.05). The pH was similar between all the treatments (P>0.05). No interactions were found for any parameter (P>0.05). Supplementing 50 weeks old laying hens with 300 ppm of PID helps to improve gut health, increasing SCFAs and could stimulate the development of intestinal area to improve nutrients absorption.

**Keywords:** calcium pidolate; laying hens; gut health; SCFAs; digestive organs

### 421P Optimization of Sample Preparation Workflow for Rapid Detection of *Salmonella enterica* in Poultry Using Real-Time qPCR

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**Hypothesis or Objective:** This study hypothesized that optimization of lysis, DNA extraction, and centrifugation steps would improve sensitivity and reproducibility of real-time qPCR detection of *Salmonella enterica* in poultry matrices without enrichment. **Experimental Design:** Chicken rinsate samples were artificially inoculated with *Salmonella enterica* serovar Typhimurium (1.5 × 10^7 to 15 CFU/mL). Three lysis conditions (Proteinase K + PrepMan™ (ThermoFisher Scientific, Waltham, MA, USA) at vary-

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ing volumes and incubation times) were evaluated. Two DNA extraction methods and two centrifugation protocols (single hard spin vs. dual-spin) were compared. Broth-derived and colony-derived inocula were assessed for assay performance. **Materials and Methods:** Samples were homogenized in PBS, lysed using optimized enzymatic and thermal protocols, and DNA was extracted prior to qPCR analysis. Each condition was tested in triplicate across three independent runs. Estimate LOD was ≥1.8 log10 CFU/mL. Mean Cq values, standard deviations, amplification efficiency, and linearity (R^2) were calculated. Data normality was assessed using the Shapiro–Wilk test, followed by two-way ANOVA and Tukey's HSD test (A = 0.05). **Results:** The combination of 20 µL Proteinase K with 400 µL PrepMan™ and a 20-min incubation produced the lowest and most consistent Cq values. Dual-spin centrifugation improved DNA purity and reduced Cq variability across extraction methods. Broth-derived inocula consistently amplified earlier than colony-derived preparations. Although some differences were not statistically significant (p > 0.05), optimized workflows yielded improved amplification efficiency and reproducibility, particularly at low target concentrations. **Conclusions:** Optimization of sample preparation parameters significantly enhanced qPCR performance for *Salmonella* detection in poultry rinsate. The optimized workflow enables sensitive, same-day detection without enrichment, supporting its applicability for poultry surveillance and food safety diagnostics.

**Keywords:** Salmonella enterica; poultry; sample preparation; real-time qPCR; food safety

### 422P Pre-harvest heat stress: connecting the intestinal microbial ecology to the final carcass

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Heat stress is a major concern within the poultry industry as it reduces the integrity of the intestinal walls of broilers, leading to leaky gut syndrome, which promotes bacterial translocation. Pathogenic microorganisms, such as *Salmonella* and *E. coli,* as well as shelf-life associated microorganisms, can pass through the gut walls and infect other organs, potentially resulting in systemic infection. It is hypothesized that heat stress-induced systemic infection alters the carcass microbiome and subsequent shelf-life. Therefore, the objective of this study was to evaluate the impact of heat stress on the bacterial translocation from the gut microbiome to the final carcass after harvest. Over a 42-d period, a heat stress trial was conducted at the University of Arkansas Division of Agriculture Broiler Research Farm (Fayetteville, AR) where birds were housed under three different controlled environmental conditions: Control (CTL): thermoneutral of 23°C, Cyclic HS (HS): 35°C for 8h/d, Pair-fed (PF): thermoneutral conditions, but pair-fed to HS group. Treatments were applied on d29 and harvested on d42. The duodenum, ileum, jejunum, ceca, and liver were collected and frozen upon collection from broilers in each treatment group on d41. During harvest, samples were processed sequentially in the order of TN, PF, then HS. The facility was cleaned between each treatment group, except for the scalding tank. Carcass rinsates were collected following the chilling period of harvest and frozen at -20°C. Carcass rinsates and contents of organs were thawed and collected to be extracted using the Qiagen PowerSoil Pro Kit. According to the Earth Microbiome Project, the library was prepared to target the V4 16S rRNA gene and sequenced on a MiSeq i100 (Illumina). Microbiome data was analyzed by using QIIME2 to analyze taxonomy and the core diversity metrics. Significance was set at P < 0.05. Overall, HS depleted *Streptococcus* compared to TN (P<0.001). Alpha diversity demonstrated a significant treatment x organ interaction (P<0.05). As expected, carcass rinses exhibited greater richness and evenness than intestinal and liver samples (Q<0.05), and cecal samples had greater richness and evenness than other intestinal segments (P<0.05; Q<0.05). Beta diversity analyses showed a significant interaction based on weighted and unweighted Unifrac (P<0.05; Q<0.05), whereas Bray-Curtis and Jaccard metrics were not significant (P>0.05; Q>0.05), suggesting treatment effects were driven by phylogenetic differences. Overall, these findings indicate that heat stress in broilers may be associated with bacterial translocation, which could contribute to systemic infection and have negative effects on the final product quality and safety.

**Keywords:** microbiome; chickens; gut; heat-stress; carcass

### 423P Contrasting functional genomic profiles of *Salmonella enterica* subsp. *enterica* serovars Enteritidis and Heidelberg in Canadian poultry revealed by integrated pangenome-based analysis

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*Salmonella enterica* is among the most prevalent bacterial pathogens impacting poultry production and a persistent concern for public health worldwide. Characterizing its functional diversity enables a deeper understanding of the biological processes underlying its ecological adaptation, persistence, and transmission dynamics, thereby informing the design of targeted and evidence based control strategies. This study aimed to biologically characterize genomic differences between *Salmonella enterica* subsp. *enterica* serovars Enteritidis and Heidelberg circulating in Canadian poultry using an integrated pangenome based framework. A previously established pangenome built from 24 high quality poultry associated genomes from six Canadian provinces Alberta, Ontario, Quebec, New Brunswick, Nova Scotia, British Columbia was used to identify serovar associated genes. Functional interpretation was performed through integrated analyses of metabolic functions, virulence factors, and antimicrobial resistance using eggNOG, the Virulence Factor Database VFDB, and the Comprehensive Antibiotic Resistance Database CARD annotations. Functional categories and pathways were normalized within each serovar to enable biologically meaningful comparisons. Genes associated with *S.* Enteritidis were enriched in metabolic functions, transport systems, and membrane associated components, including ABC transporters and central metabolic pathways, indicating high metabolic flexibility and environmental persistence. In contrast, *S.* Heidelberg showed enrichment in virulence associated functions, including secretion systems, biofilm formation, stress response pathways, and host relevant carbohydrate metabolism. Virulence analysis revealed a conserved adhesion dominated backbone in both serovars, with greater functional diversification in *S.* Heidelberg. Antimicrobial resistance profiling detected no acquired resistance genes in *S.* Enteritidis, whereas *S.* Heidelberg carried the fosA7 gene conferring resistance to fosfomycin. In conclusion, *S.* Enteritidis exhibits a metabolically versatile profile consistent with environmental persistence and broad dissemination in poultry, whereas *S.* Heidelberg displays a more host adapted repertoire enriched in virulence and acquired antimicrobial resistance. Its restricted distribution to Québec and New Brunswick, compared with the wider presence of *S.* Enteritidis, supports epidemiological divergence and highlights the relevance of region specific surveillance and control strategies, with functional differences beyond gene presence contributing to contrasting risk profiles.

**Keywords:** Salmonella enterica; pangenomics; functional genomics; virulence factors; antimicrobial resistance

### 424P Effects of commercially available backyard egg washing solutions on the cuticle, shell permeability, and dye penetration of white table eggs

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There is a movement among backyard flock owners in the United States to clean their shell eggs without the use of chemicals. Enzyme and chemical-based egg washes are available to backyard producers, but there is limited evaluation of how these wash solutions affect the cuticle. The objective was to determine if different enzymatic, chemical, and mechanical egg washing solutions affect the cuticle coverage and shell permeability compared to an unwashed control. Eggs were obtained from a 25-wk-old White Leghorn flock maintained at the MAFES Poultry Research Unit. Eggs were candled to remove any cracked eggs and were divided into 6 treatments (trt): 3 enzyme washes [A, B, C], a chlorinated chemical wash [D], a mechanical wash with only water [E], and unwashed [F]. Ten eggs per trt washed in 3 groups (30 eggs total/trt) following the product instructions. After washing procedures, 10 eggs per trt (randomly selected from each washing group) were used for evaluating cuticle coverage, shell permeability, or dye penetration. Cuticle coverage was evaluated by using a dye method (Tartrazine/Green S) and measuring the ΔE of the shell color (L*a*b*) before and after the dye procedure. Shell permeability was evaluated by measuring water loss (mg $H2O/cm^2$/torr/day) where eggs were stored in a desiccator at room temperature, and eggs weights were measured at 0, 24 and 96 hr post trt. For this, trts were evenly distributed among 5 desiccators and arranged in a randomized complete block design. Dye penetration was evaluated by cracking open dyed eggs and determining if the dye was present in the inner eggshell. Statistical analyses were completed in SAS using a GLM Procedure for cuticle coverage and shell permeability and a Chi-Square for dye penetration with significance at P≤0.05. For cuticle coverage (P=0.0002), unwashed (F; 46.3 ΔE) had the highest coverage followed by C, A, E, D, with B (enzyme wash) resulting in the lowest coverage (30.9 ΔE). For shell permeability (P=0.0315), D (chlorinated chemical wash) had the lowest permeability (0.108 mg $H2O/cm^2$/torr/day) followed by F, C, B, and A, where E (mechanical wash) had the highest (0.138 mg $H2O/cm^2$/torr/day). Dye penetrated the shell for only trt A (enzyme wash) where 40% of eggshells had dye penetration (P=0.0007). In conclusion, the enzymatic egg washes tested maintained higher cuticle coverage but were associated with increased permeability and dye penetration, indicating a potential risk for pathogen entry. The chlorinated wash reduced permeability but also decreased cuticle coverage. These results show potential trade-offs between cuticle preservation and permeability, but conclusions are limited to the specific wash treatments evaluated in this study.

**Keywords:** Egg washing; Enzymatic; Chemical; Mechanical; Cuticle

### 425P Processing Yields and Meat Quality of Broilers from Divergent Water-Efficiency Lines Subject to Short-Term Water Deprivation

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Broilers may experience short periods of water deprivation during the production cycle, such as during mechanical failure, maintenance, or transportation. Broilers selected for water efficiency may respond differently to water deprivation than commercial broiler strains selected for feed efficiency would. Stress is known to have negative effects on growth performance and meat quality. The aim of this study was to assess if short periods of water deprivation affect carcass yields or meat quality of broilers selected for high (HWE) or low (LWE) water efficiency. Broilers were housed by line in 24 pens with 15-17 broilers per pen (n=382), and were assigned water deprivation treatments (0h, 12h, 24h). Water deprivation treatments were applied on days 25-26 and 39-40 in staggered blocks, where each line x treatment combination was represented in each block. On day 42, broilers were processed following 10h feed withdrawal using an inline system using standard processing practices with immersion chilling. At 3h postmortem (4°C), carcasses were cut up and deboned, and part yields were determined. Breast fillets were scored for woody breast (WB) and white striping (WS). Fillet pH, color (L*, a*, b*), % cook loss, and Muellenet Owens Razor Shear Energy (MORS) were assessed. Linear mixed effects models were used with line x treatment as fixed factors. WB and WS were analyzed using multinomial analyses with line x treatment as fixed factors. HWE had higher carcass yield (73.4 vs 71.7%) and leg yield (22.9 vs 21.9%) compared to LWE (P<0.05). LWE had higher live weight (2583 vs 2440g, P<0.05) and fat pad yield (1.7 vs 1.3%, P<0.05). Interestingly, there were no differences in breast or tender yield between lines (HWE: 19.2 and 3.9%, LWE: 18.8 and 3.9%, P>0.05). LWE had higher occurrence of WS than HWE (98 vs 93%, *P*=0.02), but not WB (59.6 vs 45.7%, P=0.37). Water deprivation treatments had no effect on carcass or parts yields (P>0.05). The only difference in meat quality between lines was that LWE had higher a* (redness) values than HWE (3.46 vs 3.12, P<0.05). There was no effect of water deprivation treatment on pH, L*, b*, cook loss % and MORS (P>0.05). Overall, genetics appear to have greater influence on processing yields than environmental factors such as water restriction in this study. Future studies may evaluate water deprivation duration in the 24h prior to slaughter.

**Keywords:** yields; meat quality; water efficiency; water deprivation; broiler

### 426P The Effect of Thermal Environment and Water-Efficiency Genetic Selection Influence on Yield Distribution and Breast Meat Quality in Broilers

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Heat stress is an environmental stressor in broilers that affects body weight, carcass yield, and meat quality. This study evaluated the effects of heat stress and selection for water efficiency on processing yield, myopathy incidence, and breast meat quality traits in broilers. Broilers selected for high (HWE) and low (LWE) water efficiency were assigned to one of three environmental treatments: heat stress (HS), thermal neutral (TN), or thermal neutral–pair fed (TNPF) at a density of 17 birds (9 males, 8 females) per pen. Daily cyclic heat stress of 36C was applied from 28 to 46d of age for 8h daily. TNPF birds received a standardized allocation based on feed consumption of HS treatment (pair-fed) beginning on 28d. Birds were processed at 46d using standard processing practices. After chilling, carcasses were deboned to determine part yields. Breast fillets were scored for woody breast (WB) and white striping (WS). Fillet pH,

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color (L*, a*, b*), % cook loss, and Muellenet Owens Razor Shear Energy (MORS) were assessed. Data was analyzed as a linear effects model using genetic line, sex, and thermal treatment as fixed effects. WB and WS were analyzed using multinomial analyses with line x treatment as fixed factors. TN had higher live weight (2906g) than HS (2615g) with TNPF (2780g) as intermediate (P<0.05*)*. HWE had higher carcass (74.9 vs 72.6%), leg (32.3 vs 30.6%), and breast yield (25.8 vs 25.0%), while LWE had higher fat pad (2.1 vs 1.6%), tender (5.8 vs 5.5%), and rack yield (27.6 vs 25.5%, P<0.05). HS birds had lower breast (24.7%) and tender yield (5.5%) when compared to TN (25.9, 5.7%) and TNPF (25.7, 5.7%, P<0.05), while HS (74.1%) and TN (74.0%) had higher carcass yield than TNPF (73.3%, P<0.05). HS had a higher leg yield (32.3%) than TN (30.8%) and TNPF (31.2%, P<*0.05*). HWE is more likely to have WB (72 vs 39%) and WS (96 vs 79%) (P<0.001). TN had higher WB incidence than HS (P<0.05), while HS had less WS then TN (P<0.001). LWE had lower initial pH (5.96 vs 6.00, P<0.05). LWE had higher a* than HWE (4.1 vs 3.5, *P<0.05*). HS had lower a* (3.7) than TN (4.2) and TNPF (3.9) and TN had higher b* (8.0) than TNPF (7.4) with HS as intermediate (7.5, P<0.05). HWE tended to have higher L* values (P=0.051). No differences were observed in cookloss (P>0.05). HWE had lower MORS than LWE (14.5 vs 15.5, P=0.01) and TN tended to have lower MORS (14.4) than HS and TNPF (15.1 and 15.5, P=0.09). Overall, selection for HWE improved carcass and major part yields but increased WB incidence, whereas LWE birds exhibited greater fat deposition and white striping. Heat stress and pair-feeding altered live performance, yield, and meat quality characteristics compared with thermal neutral conditions in this study.

**Keywords:** yields; meat quality; water efficiency; heat stress; broiler

## Poster Session II

### 427P Anti-Salmonella effects of dietary bacteriophage supplementation on growth performance and pathogen colonization in broiler chickens

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A study was conducted to evaluate whether dietary bacteriophage supplementation reduces *Salmonella* Typhimurium colonization and improves growth performance in broiler chickens. A total of 320 one-day-old Cobb 500 broilers were divided into four treatments with eight replicates (10 birds per cage): non-challenged control (NC), challenged control (CC), CC + bacteriophage at 500 g/MT (BP500), and CC + bacteriophage at 1000 g/MT (BP1000). The bacteriophage product used in this study was ProBe-Bac PE (Pathway Intermediates, USA). Birds in the challenged groups were orally inoculated with 0.5 mL of 1 × $10^9$CFU/mL nalidixic acid-resistant *S.* Typhimurium on day 0. The experiment was conducted for 21 d with ad libitum access to feed and water. Growth performance, including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), was measured throughout the study. Cecal *Salmonella* counts (log10 CFU/g) were measured at 4, 7, 14, and 21 days post-inoculation (dpi). Data were analyzed using one-way ANOVA with Tukey's HSD test, and polynomial contrasts were used to evaluate linear and quadratic responses. No significant overall treatment effects were observed for BW, BWG, or FCR ($P > 0.05$). However, within the challenged groups, BW increased linearly at 7 dpi ($P = 0.023$), and BWG improved during 0–7 dpi ($P = 0.023$) as bacteriophage inclusion level increased. Similarly, FI increased linearly during 0–7 dpi ($P < 0.001$) and 8–14 dpi ($P = 0.027$). Cecal *Salmonella* counts were significantly lower in bacteriophage-supplemented groups at 7 dpi ($P = 0.010$), 14 dpi ($P < 0.001$), and 21 dpi ($P = 0.039$), with reductions of up to 1.32 log10 CFU/g compared with the challenged control. A linear reduction in *Salmonella* counts was observed with increasing bacteriophage inclusion level ($P = 0.037$), and birds fed BP1000 had the lowest counts at all sampling time points. In conclusion, dietary bacteriophage supplementation reduced *S.* Typhimurium colonization in broiler ceca in a dose-dependent manner and improved early growth responses following challenge, whereas feed efficiency was not affected. These results support bacteriophage supplementation as a practical dietary strategy for controlling *Salmonella* in poultry production.

**Keywords:** bacteriophage; Salmonella Typhimurium; broiler; gut health

### 428P Combining postbiotics with essential oil compounds provides greater performance gains than probiotics in male broiler turkeys

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A commercially available multi–strain *Bacillus* probiotic and a combination of *Saccharomyces cerevisiae* fermentation–derived postbiotic with essential oil compounds were evaluated in male broiler turkeys from age day 0 to day 105. Birds were assigned to one of three treatments: a basal wheat–corn–soyabean meal diet control (CONT), a probiotic–supplemented (400g per metric ton of feed) diet (PROB), or a postbiotic–essential oil compounds (Biostrong™ Dual, Cargill Inc., Cedar Rapids, IA, USA) supplemented (400g per metric ton of feed) diet (POEC) provided as crumble and pellet feed. A randomized block design with eight replicate pens per treatment and 15 birds per pen reared under commercial conditions. Body weight, cumulative feed intake, and body weight–adjusted feed conversion ratio (cFCR) were measured at trial completion. Data were analyzed with a mixed model including treatment as a fixed effect and block as a random effect; means were separated by Tukey's test (P<0.05). On day 105, body weights did not differ (P>0.05) among treatments (CONT: 13.71 kg; PROB: 13.57 kg; POEC: 13.80 kg; $P > 0.05$), although POEC birds were numerically heavier. Cumulative feed intake was also not different (CONT 29.79 kg; PROB 28.51 kg; POEC 28.96 kg), with a trend toward reduced cumulative feed intake in supplemented groups (P=0.08). Notably, POEC improved cFCR versus CONT (1.98 vs 2.03; P<0.05), whereas PROB (1.99) was intermediate and not different (P>0.05) from either group. These findings indicate that the postbiotic–essential oil combination provided the most effective nutritional strategy for enhancing feed efficiency in male broiler turkeys, outperforming the commercially available multi–strain *Bacillus* probiotic under the conditions of this study.

**Keywords:** Postbiotic; essential oil compounds; performance; turkeys; probiotics

### 429P Effect of different additives in the drinking water on the relative weight of lymphoid organs of broilers raised on dirty litter

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Immune development and intestinal integrity in the first days of life of broiler chickens are the key to successfully meeting the desired production objectives. The lymphoid organs ensure the immune response capacity in the event of diseases and respond quickly to unhealthy conditions or environmentally challenging conditions. The objective of the study was to evaluate the effect of the use of different additives in drinking water on the relative weights of lymphoid organs of birds raised on dirty litter. For the study, 40 one-day-old chickens were used, which were randomly distributed into four treatments with 10 individually identified birds under dirty floor conditions and fed with a basal diet (corn/soybean) for 12 days. The treatments were: T1, control (normal water); T2, Water + Commercial Biological Mixture (PEC-A APPLY®); T3, Water + Symbiotic (PoultryStar®)

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and T4, water + acidifier (AquaMaster®) (pH: 5.76 – 6.0). The additives were diluted in the drinking water following the recommended doses of the product. At 12 days of age, the chickens were euthanized, the lymphoid organs (thymus, spleen and bursa) were removed from each animal and then weighed. The variables determined were the relative weights of the lymphoid organs. The recorded data were subjected to ANOVA under a Completely Randomized Design and for the comparison of means the Duncan Test was used. The results showed that the live weight of the birds was not influenced (p<0.05) by the treatments. Regarding the measurements of the lymphoid organs, it was found that the relative weights of the spleens were not influenced (p>0.05) by the treatments; However, significant differences (p<0.05) were observed in the relative weights of the bursa and thymus in response to the treatments. Thus, the highest and lowest relative weights of the bursa (0.22+0.04% vs 0.16+0.07%) and thymus (0.59+0.14% vs 0.41+0.15%) corresponded to those of the treatments that received PEC-A APPLY® and PoultryStar®, respectively, while those of the other treatments resulted in intermediate values. In conclusion, under the conditions this experiment was carried out, dirty litter did influence the relative weights of the bursa and thymus; therefore, the addition of the PEC-A APPLY® product to drinking water could be an alternative to stimulate the immune response of birds in situations of environmental stress; however, these results warrant further research.

**Keywords:** Broiler; Bedding; Immunology; Lymphoid organs; Additive

### 430P Dietary inclusion of phytase decreases the severity of bacterial chondronecrosis with osteomyelitis (BCO) in a wire-ramp challenge model in broilers

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Bacterial chondronecrosis with osteomyelitis (BCO) in broilers represents a multi-factorial challenge to the poultry industry, with hefty economic losses due to mortality and reduced growth, as well as significant animal welfare and food safety concerns. As it is derived from a combination of weak bone and bacterial translocation and infection, interventions must target not only apparent lameness but also improve the underlying causes of BCO. To that end, mechanistic understanding of lameness and BCO is necessary to evaluate and design targeted intervention strategies. Here we explored the in-feed supplementation of a phytase and stimbiotic on lameness, BCO, and bone health parameters in broilers. Cobb500 broilers (n=1,536) were reared for 56 days on litter flooring or wire ramps and fed one of 8 diets (6 pens/group, 16 birds/pen): positive control (PC); PC plus phytase (PC+P; 3000 FTU/kg, ABVista), PC plus stimbiotic (PC+S; Signis, ABVista, 100g/tonne), PC plus phytase and stimbiotic (PC+P+S), negative control (NC, Ca and P deficient with 500 FTU phytase/kg); NC plus 3000 FTU/kg phytase (NC+P), NC plus stimbiotic (NC+S), NC plus phytase and stimbiotic (NC+P+S). Birds were monitored twice daily for lameness and mortality, and birds were scored for BCO at sampling or when culled for lameness. Femurs were collected for gene and protein analysis. Data were analyzed by 4-way ANOVA with flooring, basal diet, phytase, and Signis as main effects and pen location in the barn as the blocking factor. JMP Pro 19 was used for analyses with significance of $P \leq 0.05$. When appropriate, means were separated using Tukey's HSD test. All-cause mortality was higher ($P<0.0001$) on wire ramp as compared to litter, regardless of treatment. Similarly, lameness was significantly induced by the wire ramps ($P<0.0001$), though there were no significant differences among the diets. However, BCO severity was reduced by phytase inclusion ($P<0.05$), where the percentage of femurs scored 2+ decreased from 26% to 5.6% in PC+P as compared to PC and 24.9% to 9.1% in NC+P as compared to NC, indicating that phytase super-dosing can impact the onset/progression of lameness in poultry. STAT3, a regulator of osteoblast function and bone formation, was reduced in the femurs with the NC diet, but protein levels were enhanced by the addition of phytase, indicating a potential mechanism by which phytase reduces the severity of BCO. Additional studies are warranted to further delineate the underlying mechanisms by which these products impact lameness and bone health.

**Keywords:** phytase; stimbiotic; broiler; bacterial chondronecrosis with osteomyelitis; lameness

### 431P Effect of dietary inclusion of apple and grape pomace on markers of gut health in broilers (*Gallus gallus*) under DSS-induced chronic inflammation

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The removal of antibiotic growth promoters from the poultry industry has been associated with increased enteric inflammation, compromised gut integrity, and dysbiosis in chickens, negatively affecting health and growth. As the US poultry industry actively seeks antibiotic alternatives, apple pomace (**AP**) or grape pomace (**GP**), an agricultural byproduct rich in bioactive compounds with proven anti-inflammatory, antioxidant, and immunomodulatory properties, may serve as potential alternatives. This study aimed to evaluate the effect of dietary inclusion of AP and/or GP on markers of gut health in broilers under dextran sodium sulfate (**DSS**) induced chronic inflammation. A total of 270 14-day-old male broiler chicks were randomly assigned to one of the five treatments groups: 1) non-challenged positive control (**PC**), 2) DSS-challenged negative control (**NC**), 3) NC + 1% dietary GP (**GP**), 4) NC + 3% dietary AP (**AP**) and 5) NC + dietary 3% AP and 1% GP (**AGP**). To induce chronic inflammation, on days 14, 16, 18, and 20, 1 mL of 2.5% DSS solution was orally inoculated. Growth performance and markers of gut health, including immune response, tight junction proteins, intestinal histomorphology, and fecal quality scores, were measured at d 21 and d 28. Data was analyzed using one-way ANOVA. Significance was set at $P \leq 0.05$ and tendencies at $P<0.10$; means were separated using Fisher's LSD. Results indicated that there were no differences in growth performance parameters at any time points ($P>0.05$). On day 21, the expressions of IL-1B and IL-4 tended to be upregulated in the NC group compared to others ($P<0.1$). The expression of CLDN-1 was downregulated in the AGP ($P=0.011$), whereas MUC-2 was upregulated in the NC ($P=0.080$) compared to others on day 21. However, no differences were observed in gut permeability ($P>0.05$). On day 21, DSS-induced inflammation reduced duodenal villus height compared to PC, whereas dietary pomaces improved villus height compared to the NC ($P=0.040$). Similarly, at d 21, dietary GP improved the villus height to crypt depth ratio and villus surface area compared to NC ($P=0.015$). Additionally, fecal quality and blood score were higher in the DSS-challenged group compared to the non-challenged control; however, dietary pomaces reduced the severity, resulting in lower scores on day 21 ($P<0.05$). These results suggest that fruit pomaces (AP and GP), rich in bioactive compounds, may have the potential to mitigate DSS-induced inflammation and improve gut health without adversely affecting performance in broilers.

**Keywords:** Broilers; chronic inflammation ; pomace; gut health; immune response

### 432P BCO lameness incidence and splenic immune responses in broiler chickens following *in ovo* injections of electron beam-inactivated BCO-causative bacteria

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Bacterial Chondronecrosis with Osteomyelitis (BCO) lameness is a major challenge in broiler production and continues to raise concerns related to animal welfare and food safety worldwide. BCO develops when bacteria enter the circulation from the respiratory or intestinal tract and settle in bone microfractures, eventually causing tissue necrosis and lameness. In our previous study, electron beam (eBeam)-inactivated vaccines significantly reduced the incidence of lameness in broiler chickens compared with the control group. Based on those findings, this study was conducted to improve the eBeam vaccine formulation by modifying the combination of bacterial strains and to evaluate its safety, efficacy, and immunogenicity in an aerosolized BCO challenge model. The eBeam vaccine was prepared using five bacterial strains, including *S. aureus, S. cohnii, E. cecorum, E. faecalis,* and *E. coli*. Eighteen-day-old chicken embryos were immunized and divided into five groups on hatch: negative control (T1), vaccine control (T2), infection source/wire floor (T3), positive control (T4), and vaccinated group (T5). T2 and T5 received the eBeam vaccine while T1, T3, and T4 received a sham (carrier) vaccine. Birds were distributed into two housing units: T1 and T2 were reared in a non-BCO-challenge system and remained unexposed to pathogens, whereas T3, T4, and T5 were reared in an aerosolized BCO challenge system exposed to the pathogen. Daily cumulative lameness was recorded, and spleen samples (n = 4 per treatment per time point) were collected at 7, 21, 35, and 49 days post-treatment and processed for B-cell and macrophage levels via immunohistochemical staining. Lameness data were analyzed using binomial logistic regression in a GLM on R Studio, and the proportion of white blood cells in the spleen across treatment groups and time-points was analyzed using two-way ANOVA followed by Tukey's *post-hoc* tests. The statistical significance was considered at $P<0.05$. BCO lameness incidence was significantly higher in T3 compared to T4 and T5, which were similar to each other within the BCO-challenged system. Hatchability of T2 and T5 was over 25% higher compared to T1, T3, and T4. B cells were significantly higher in T2 and T5 compared to T1 and T4 at Days 7, 21, and 49 ($p < 0.05$), while no significant differences were observed at Day 35. Macrophage proportions were significantly increased in T5 at Day 7 and in T3 and T4 at Day 35 ($p < 0.05$). These findings demonstrate that *in ovo* eBeam vaccination modulates both innate and adaptive immune responses and reduces lameness incidence; however, further optimization is needed to improve hatchability and to better evaluate overall vaccine efficacy.

**Keywords:** eBeam; BCO; vaccines; antibody responses; bacteria

### 433P Influence of Bio-Clay Supplementation on Growth Performance and Intestinal Microbial Dynamics in Broiler Chickens under a Subclinical Necrotic Enteritis Challenge Model.

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Necrotic enteritis, caused by *Clostridium perfringens* (CP), leads to severe intestinal damage and up to $6 billion in global loss. Its rising incidence due to reduced antibiotic use underscores the need for alternative control strategies. This study evaluated the effects of dietary Bio-clay, a sodium-rich montmorillonite conjugated with ZnO, on growth performance, jejunal histomorphology, cecal short-chain fatty acids (SCFAs), jejunal CP load and microbiota in broiler chickens using a subclinical NE model. A total of 240 one-day old broiler chicks were assigned to five treatments with eight replicate cages each: control (basal corn-soybean diet), control + Bio-clay (8 g/kg), NE challenge (basal diet), NE challenge + Bio-clay (8 g/kg), and NE challenge + antibiotics (Bacitracin, 55 mg/kg). NE was induced by co-infection with 5.0 × 10^3oocysts of *Eimeria maxima* on day 13 and 1.0 × 10^8 CFU of CP on days 19–21. Growth performance was monitored and recorded on a weekly basis. There was a tendency for the day 22 jejunal CP load to be higher in NE challenge group than other groups ($P = 0.07$). Growth performance metrics (feed intake (FI), weight gain (WG), final body weight (FBW) and feed conversion ratio (FCR)) were largely unaffected ($P > 0.05$), except for FCR at days 21- 28 period which was greater ($P = 0.01$) in the NE challenge + antibiotics group as against the control. The cecal SCFA concentrations were not significantly different ($P > 0.05$) between treatments for day 22, however, day 28 results showed tendency for acetate ($P = 0.07$), valerate ($P = 0.05$), and total SCFAs ($P = 0.07$) to be elevated in the challenge-only group. The jejunal histomorphology was not significantly ($P > 0.05$) affected on either day 22 or 28. Jejunal bacterial and fungi diversity (alpha and beta) on day 22 were also not significantly ($P > 0.05$) altered. However, differential analysis (Lefse) of the jejunal mycobiome revealed that *Saccharomycetes* (class) and *Saccharomycetales* (order) differed across treatments ($P = 0.05$). Under challenge conditions, Bio-clay increased the relative abundance of *Saccharomycetes* compared to the control, reflecting a shift in specific fungal taxa; however, the functional relevance of this change is uncertain. Taken together, the findings indicate that NE may influence fungal microbiota dynamics, while Bio-clay supplementation had minimal impact on overall microbial diversity and bird performance, suggesting a potential role in maintaining gut stability under challenge conditions.

**Keywords:** Bio-clay; Growth performance; Microbiota; Broiler chickens; Necrotic enteritis

### 434P Botanical-based additives (liquid and powder versions) alter *Eimeria* pathogenicity *in vitro* from anticoccidial-resistant strains obtained from commercial turkey houses

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The aim of this study was to evaluate the *in vitro* effect of both a liquid [Entero-V Poultry® (EV)] and two powder botanicals-based versions of Synbiotec® (SYN#1 for conventional production, SYN#2 for cocci-vaccinated birds) (Probiotech International Inc.) in *Eimeria* infecting turkeys. *E. adenoides*, *E. dispersa*, *E. gallopavonis*, *E. meleagridis*, *E. meleagrimitis* were identified and extracted from fecal samples collected from two commercial flocks treated with conventional anticoccidials. *In vitro* tests (oocyst sporulation, sporozoite viability and host cell invasion capacity) were conducted at concentrations of 12.5, 25, 50 and 100 µg/mL, in three replicates. Host cell invasion data were acquired using qPCR. Data were analyzed by one-way ANOVA. Based on the *in vitro* tests, the *Eimeria* strains showed resistance against zoalene and clopidol but were sensitive to robenidine. EV decreased oocyst sporulation at 100 µg/mL (−13%, $P<0.05$). SYN#1 (−46%, $P<0.05$) and EV (−27%, $P<0.05$) decreased sporozoite viability at 100 µg/mL while SYN#2 showed only a trend at the higher dose (−17%, $P<0.1$). SYN#1 at 100 µg/mL also de-

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creased sporozoite invasion of *E. adenoides* (−4.0 log10, P<0.05), *E. meleagridis* (−1.7 log10, P<0.05), *E. meleagrimitis* (−1.4 log10, P<0.05) and total *Eimeria* (sum of all species, −3.1 log10, P<0.05). Overall, these results support further investigations for *in vivo* studies on these botanical formulations as anticoccidial alternatives in turkeys.

**Keywords:** Botanicals; in vitro; Eimeria pathogenicity; drug resistance; turkeys

### 435P Seasonal Variation and Spatial Distribution of Ammonia, Nitrous Oxide, and Methane Concentrations in Commercial Poultry Farms across Quebec, Canada

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Ammonia (NH3), nitrous oxide (N2O), and methane (CH4) are key gaseous pollutants in poultry housing with direct implications for bird welfare and climate change. This study characterized NH3, N2O, and CH4 concentrations across seasons, detector heights, and barn positions in six commercial poultry farms (Farms A–F) in Quebec, Canada. Measurements spanned a full annual cycle. The farms represented typical broiler production systems with Ross birds fed with corn-based diets containing antibiotics and wood-shaving bedding and controlled environmental management. Four detectors per farm were placed at two heights—human breathing level (1.5 m) and bird level (0.25 m), and two longitudinal positions—front and end—yielding four configurations. In total, 300 NH3, 236 N2O, and 11 CH4 pooled observations were analyzed using Kruskal-Wallis with Mann-Whitney U post-hoc tests (P < 0.05), with data presented as mean ± SEM. NH3 ranged 0–56 ppm (grand mean: 12.7 ± 0.7 ppm). Season significantly influenced NH3 (H = 42.87, P < 0.0001), with Winter markedly elevated (50.5 ± 1.2 ppm) exceeding all other seasons (P < 0.001). Summer was lowest (7.3 ± 0.7 ppm), while Spring (12.0 ± 1.0 ppm) and Fall (12.3 ± 1.0 ppm) were comparable (P = 0.787). Neither height (High: 12.1 ± 1.0 vs. Low: 13.2 ± 1.0 ppm; P = 0.300) nor position (Front: 11.7 ± 0.9 vs. End: 13.6 ± 1.1 ppm; P = 0.302) significantly influenced NH3. N2O ranged 0–1000 ppb (grand mean: 230.1 ± 11.8 ppb) and varied by season (H = 22.96, P < 0.0001): Spring was highest (315.2 ± 32.7 ppb), Fall intermediate (212.6 ± 8.7 ppb), and Summer lowest (140.1 ± 12.4 ppb; P < 0.001 vs. Spring and Fall). N2O showed strong spatial stratification: high-mounted sensors exceeded low-mounted (280.2 ± 20.7 vs. 193.2 ± 12.9 ppb; P < 0.001), front exceeded end positions (282.4 vs. 153.7 ppb; P < 0.001), and high-front sensors recorded the greatest concentrations (304.2 ± 21.0 ppb; H = 79.97, P < 0.001). CH4 was detected only at floor-level sensors during Spring in two farms: Farm E (low-front: 211,404 ± 22,994 ppb; low-end: 35,124 ± 8,719 ppb) and Farm A (low-end: 13,033 ± 6,134 ppb). NH3 was predominantly seasonally driven, peaking in winter due to reduced ventilation. N2O gradients, higher near ceiling and barn inlet, reflect distinct dispersion linked to litter nitrification-denitrification. CH4 at floor level during spring indicates localized anaerobic litter activity. These divergent patterns show that single-point, single-gas monitoring is insufficient, and concurrent multi-gas, multi-location monitoring across all seasons is recommended to accurately assess the environmental and welfare implications of commercial poultry operations.

**Keywords:** ammonia; nitrous oxide; methane; poultry housing; seasonal variation

### 436P Productive performance of Shuvra layer in saline and non-saline agro ecological regions of Bangladesh

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Bangladesh Livestock Research Institute (BLRI) has developed a layer strain named Shuvra. The present study evaluated the productive performance of Shuvra layer under two contrasting agro-ecological environments of Bangladesh. The study was conducted in Rangpur (non-saline area) and Barishal (saline-prone coastal area) from 21 to 40 weeks of age. A total of 1000 birds were used with 500 birds in each region arranged in five replicates of 100 birds. All the birds were fed with same commercial feed in both regions. Overall management condition was almost similar in both areas. Individual bird was reared in individual pen. Weekly data on mortality, feed intake, body weight, egg production (EP%), egg weight (EW), egg mass, and feed conversion ratio (FCR) were recorded and analyzed using IBM SPSS Statistics. Results are presented as mean ± standard error. Mortality remained very low in both regions (<0.05%) throughout the experimental period. Average feed intake was slightly higher in Rangpur (87.5 ± 1.8g/bird/day) compared with Barishal (82.7 ± 2.1g/bird/day). Birds reared in Rangpur maintained a higher body weight (1708 ± 21g) than those in Barishal (1665 ± 24g). Egg production increased gradually with age in both regions and reached peak production at 40 weeks. Mean egg production was significantly higher in Rangpur (73.6 ± 3.2%) than in Barishal (69.4 ± 3.6%) (p < 0.05). Egg weight was comparable between regions averaging 57.9 ± 0.9g in Rangpur and 57.3 ± 1.0g in Barishal. Egg mass was also higher in Rangpur (42.6 ± 2.4g/hen/day) compared with Barishal (39.8 ± 2.7g/hen/day). Consequently, feed conversion ratio was slightly better in Rangpur (2.02 ± 0.08) than in Barishal (2.18 ± 0.09). Overall, the results indicate that the Shuvra layer can perform well in both saline and non-saline environments although birds reared in the non-saline region showed slightly better production efficiency. These findings suggest that Shuvra layers possess good adaptability to diverse agro-ecological conditions and can be successfully reared in coastal as well as inland regions of Bangladesh.

**Keywords:** Shuvra; Layer; Strain; Saline ; Region

### 437P PRODUCTION PERFORMANCE OF FIVE PHILIPPINE NATIVE CHICKENS UNDER IMPROVED MANAGEMENT

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The Philippines possesses diverse native poultry genetic resources that require systematic characterization and evaluation to support strategic breed development. This study evaluates the growth performance and production traits of selected Philippine native chicken breeds, namely *Darag (n=600)*, *Banaba (n=60), Banabang Kalabaw (n=794), Joloano (n=580),* and *Paraoakan (n=628)*, using key indicators such as day-old weight (BWT0), body weight at 16 weeks (BWT16), dressing percentage (DP), and egg number at 40 weeks (EN40). Data were obtained from three native chicken research centers. Descriptive analysis indicate notable variation across breeds. *Paraoakan* and *Joloano* exhibited the highest growth performance, with BWT16 of 1338.50 g and 1270.10 g, respectively, suggesting their suitability for meat production. *Banabang Kalabaw* also demonstrated relatively high growth (1163.80 g), while *Darag* and *Banaba* showed comparatively lower body weights at 16 weeks (915.00 g and 873.73 g, respectively). In terms of dressing percentage, Darag recorded the highest value (73.60%), followed by Banabang Kalabaw (70.87%) and Banaba (70.00%), indicating better carcass yield efficiency in these breeds. Egg production varied considerably, with Banaba achieving the highest EN40 (58 eggs), followed closely by *Darag* and

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*Banabang Kalabaw* (56 eggs). *Paraoakan* showed moderate egg production (51.00), whereas *Joloano* had the lowest (35 eggs). These findings highlight the trade-offs between growth and reproductive performance among native chicken breeds. Overall, the results provide valuable baseline data for breed selection and genetic improvement programs aimed at enhancing meat and egg production in Philippine native chickens

**Keywords:** Darag; Banaba; Paraoakan; Joloano; indigenous chickens

### 438P Time-of-day effects on enrichment use and proximity in commercial broiler chickens

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As welfare concerns rise among producers and consumers, environmental enrichment is increasingly incorporated into commercial broiler facilities to promote natural behaviors and enhance bird welfare. Structural enrichments, such as platforms, provide elevated resting opportunities and may influence how birds distribute themselves within the house. Broiler activity patterns vary throughout the day, potentially affecting enrichment interactions. Birds may also cluster near enrichments without directly using them, which could affect space utilization and localized conditions within the house. Understanding how the time of day influences patterns in enrichment proximity may provide insight into bird distribution and inform management strategies in commercial settings. Thus, the objective of this study is to evaluate whether enrichment proximity in broilers varies across different times of day in a commercial setting. On day 0, three wooden structural platforms were placed within a commercial broiler house. Three replicates were conducted, with one platform per replicate. Video data were collected using 5-second recordings and analyzed via scan sampling from day 0 to day 44. Platforms were positioned at equal distances from feed and water lines. Bird distribution was collected by counting the number of birds on the platform (# using) and the number of birds surrounding the perimeter of the platform. Observations were collected at five time points throughout the day (6:30, 9:30, 13:30, 17:30, and 21:30). A single flock was used, and data were pooled across replicates for analysis. Data were evaluated using Tukey's HSD in JMP ($P < 0.05$). Platform use varied across time of day ($P < 0.05$), with the highest use observed at 9:30 in the morning (6.95±0.36) and the lowest at 17:30 in the late afternoon (5.24±0.40). Birds using the platform differed significantly from one another at 9:30 and 17:30; meanwhile, usage at 6:30, 13:30, and 21:30 did not differ significantly between groups. Bird counts around the perimeter did not differ across time of day ($P > 0.05$), with mean values ranging from 8.07±0.41 to 9.49±0.36 birds. These findings indicate that while platform use varies with time of day, enrichment proximity remains consistent across daily periods. Incorporating time-of-day considerations into enrichment evaluation may support more strategic scheduling of house walkthroughs, improve monitoring of bird distribution, and enhance the effectiveness of enrichment implementation in commercial broiler systems.

**Keywords:** enrichment; broiler; platform; commercial

### 439P Effects of drinking water sanitizer classes on intestinal morphology, respiratory immune status, and early broiler performance

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Studies evaluating the effects of different water sanitizers on growth performance, immune status, and intestinal morphology in broilers are limited. This study was conducted to determine the effects of drinking water sanitation using sodium hypochlorite- based, hydrogen peroxide (HP)-based, and quaternary ammonium compounds (QAC)-based products on growth performance, intestinal morphology, and respiratory immune status in early-age Cobb 500 broilers (d0-10). In Experiment 1, there were three treatment groups, each consisting of 20 birds. The drinking water treatments were untreated water (control), water treated with hypochlorite-based (3 ppm), and water treated with peroxide-based (~25 ppm). In Experiment 2, a total of 100 broilers were randomly divided into five treatment groups, each consisting of 20 birds. The treatments were: CON (untreated water), QAC1 (~50 ppm QAC-treated water), QAC2 (~100 ppm QAC-treated water), QAC3 (~150 ppm QAC-treated water), and QAC4 (~200 ppm QAC-treated water). Growth performance, intestinal morphology and respiratory mucosal gene expression of IgA, IL8 and TLR3 were observed. For both experiment, each bird was considered as experimental unit and data from each experiment were analyzed using one way ANOVA followed by Tukey's post-hoc test and significance level was considered as $P<0.05$. In Experiment 1, the HP group showed higher final body weight and body weight gain compared with the control group. Additionally, the HP group exhibited higher respiratory mucosal expression of the TLR3 gene. No significant differences were observed in intestinal morphology among the treatments. In Experiment 2, all QAC treatment groups showed greater villus height and a higher villus height-to-crypt depth ratio compared with the control group. The 50 ppm QAC treatment showed higher gene expression of TLR3 in the respiratory mucosa of broilers. However, none of the QAC doses had a significant effect on growth performance. In conclusion, hypochlorite-based and peroxide-based water sanitation showed positive effects on broiler growth performance and respiratory immune defense while drinking water sanitized with QAC improved intestinal morphology and enhanced respiratory immune status.

**Keywords:** broiler; chlorine; hydrogen peroxide; quaternary ammonium compound; TLR3

### 440P Evaluating the relationship between stocking density and platform usage in broiler chickens

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Environmental enrichment can enhance the welfare of broiler chickens. Well-designed and properly placed platforms can improve environmental complexity, encourage physical activity, and promote positive natural behavior. In order to understand the relationship between stocking density and environmental enrichment usage, we designed an experiment to determine the effect of four different stocking densities (1.68 birds / m2, 3.37 birds / m2, 5.05 / birds / m2 and 6.73 birds / m2 ) on platform usage level in broilers. Eight mini-pens measuring 32 ft2 were constructed within a 1,486 m2 tunnel-ventilated house, each with a water line and two tunnel feeders. Pens were assigned stocking densities of 5, 10, 15, and 20 Ross 708 broilers (P5, P10, P15, P20), with two replicates per treatment. Each mini-pen contained one rectangular platform with a usable surface area of 0.27 m2. Pens were assigned to treatments in a spatially balanced manner to account for environmental gradients within the house (e.g., proximity to heaters, fans, and inlets), ensuring each treatment was distributed across different microclimates. Platform usage was recorded in all pens and used as data for this experiment from d6 to d43 of growout at times 3:30, 9:30, 13:30, 17:30, and 21:30 using a ceiling-mounted cc-TV system with the pen acting as the experimental unit. Total bird count and bird proportion usage (number of birds on the platform / number of birds in the pen) were collected and used for a statistical analysis, a binomial GLMM with Tukey's Post hoc test applied to significant results in Rstudio (A set at 0.05) was used. No significant effect of stocking density was detected on bird platform usage; percent usage over the entire flock for P5,

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P10, P15, and P20 was 14.3, 16.3, 14.5, and 15.1 percent, respectively. Platform usage increased with age ($P < 0.05$), with usage rising from 9% in younger birds (day 18) to 24% in older birds (day 43). Time of day also influenced platform usage, with the highest usage observed in the late evening at 21:30 (17.7%) and the lowest in the morning at 9:30 (13.3%) ($P < 0.05$). Variation among pens and cameras was minimal, indicating consistent behavior across replicates. While this study aimed to determine the relationship between stocking density and environmental enrichment, we found that stocking density did not significantly affect bird platform usage. While replication was limited, results indicate that platform usage in broilers increases with age. It also suggests that birds use platforms at a higher rate in the late evening compared to the morning.

**Keywords:** Enrichments; Broilers

### 441P Effects of nitrate levels in drinking water supplies on early broiler performance

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Three controlled trials were conducted to evaluate the impact of nitrate-contaminated drinking water on early broiler performance. In Experiment I, 240 broilers were assigned to 0, 10, 30, or 50 ppm nitrate for 14 days, Experiment II involved 180 broilers assigned to 0, 100, or 200 ppm nitrate for 14 days, and Experiment III included 240 broilers assigned to 0, 200, 400, or 600 ppm nitrate for 21 days to assess responses to higher exposure levels. Each treatment group in experiment I-III consists of six replicates/treatment group with 10 birds/replicate. Measured variable included body weight, weight gain, feed intake, feed conversion ratio, water intake, mortality, relative organ weights (liver, heart, spleen, and bursa), and blood methemoglobin concentration, histological changes (liver, kidney, and intestine), and gene expression analysis of selected stress-related and hypoxia-related markers. Data were analyzed using one-way ANOVA followed by Tukey's HSD test. In the first two experiments, no significant differences were observed among treatments in growth performance, water intake, mortality, or organ weights. However, blood methemoglobin concentration (expressed as ng/mL based on ELISA quantification of absolute MetHb levels) increased significantly ($P < 0.05$) with increasing nitrate levels in both experiments, with the highest values recorded at 50 ppm in Experiment I (0.429 ng/mL) and at 200 ppm in Experiment II (0.682 ng/mL). In Experiment III, growth variables were similar during the first two weeks; however, birds receiving 400 and 600 ppm nitrate exhibited reduced final body weight, weight gain, and feed intake during the later growth phase. Blood methemoglobin concentration was also significantly higher ($P < 0.05$) at higher nitrate doses, indicating increased physiological stress at elevated nitrate exposure. In addition, relative spleen and bursa weights were reduced at higher nitrate concentrations, suggesting potential effects on immune organ development, whereas liver weight remained unaffected. Histological evaluation revealed mild hepatic steatosis at 600 ppm, with no observable changes in kidney or intestinal tissues. Gene expression analysis is ongoing to further characterize molecular responses to nitrate-induced stress. Overall, these findings indicated that broilers tolerated nitrate concentrations of up to approximately 200 ppm in drinking water without negative performance, but prolonged exposure to higher concentrations (>200 ppm) may impair performance, increase methemoglobin formation, and alter immune-related organ.

**Keywords:** Nitrate; Broilers; Methemoglobin; Drinking water quality; Performances

### 442P Nonlinear Growth Models of Creole Pullets from the Dry Tropics of Mexico

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Creole birds are an important genetic resource that adapts to unfavorable environmental conditions; they provide animal protein (meat or eggs) and economic income to rural communities in Mexico. The objective of this study was to estimate the parameters and selection criteria for the Gompertz-Laird, Logistic, and Richards nonlinear growth models in creole pullets from the Lower Mixteca region of Puebla. Thirty-eight-day-old pullets were used and individually identified until day 112. The corn-soybean meal diets were starter (1 to 63 days) with 21% crude protein (CP) and 3000 kcal/kg of metabolizable energy (ME), and finisher (64 to 112 days of age) with 19% CP and 3000 kcal/kg of ME. Water and feed were provided ad libitum during the experimental period. Individual live weight (*LW*, g) was recorded every week from 0 to 112 d of age. Three criteria were used to select the best model: adjusted regression coefficient ($AR^2$), Akaike information criterion (*AIC*), and Bayesian information criterion (*BIC*). The estimated parameters were hatching weight (*W0*, g), asymptotic weight (*WA*, g), age of maximum growth (*ti*, d), relative initial growth rate (*L*, $d^{-1}$) and relative rate of decay (*K*, $d^{-1}$). The number of observations in the database was n= 646. Data were analyzed using the NLIN procedure of SAS (*Marquardt algorithm*). The Gompertz-Laird model yielded an *L* value of 0.06430 and underestimated *W0* by 15.98 g compared to the actual weight of 34.38 g. The Logistic model yielded the highest *K* value (0.04133), while the Gompertz-Laird model yielded the lowest (0.01160). Regarding the *ti* parameter, the Gompertz-Laird model yielded a value of 147.7, the Logistic model 95.7, and the Richards model 92.9 days. Regarding *WA*, the Gompertz-Laird model produced 4711.4 g, the Logistic model 1541.2 g, and the Richards model 1364.5 g. The Logistic model had the highest $AR^2$ value (0.90214) and the lowest *AIC* (7851.03) and *BIC* (7868.91) values compared to the other models. Therefore, the Logistic model provided the best fit for describing the growth curve of pullets in the dry tropics of Mexico.

**Keywords:** Growth curve; Gallus gallus domesticus; Creole Chickens; Nonlinear models

### 443P From Heat Gain to Heat Loss: A Dynamic Mechanistic Heat Balance Model to Predict Core and Surface Body Temperature in Poultry

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Global poultry meat production exceeds 130 million tonnes annually. Rising environmental temperatures increase the risk of chronic heat stress in intensive poultry systems, which has been associated with reduced feed intake and body weight gain, and increased feed conversion ratio. Mechanistic models can examine, in silico, how nutrition, genetics, and environmental conditions interact under heat stress. Existing models do not dynamically solve core and surface temperatures as state variables, limiting representation of heat load, heat dissipation, and thermoregulatory responses. The objective of this study was to develop and

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evaluate a dynamic mechanistic heat-balance model that predicts core body temperature, surface temperature, and heat flux partitioning under thermoneutral and elevated temperatures. Model inputs included metabolizable energy intake, body weight, protein and lipid deposition rates, and environmental factors (ambient temperature, relative humidity, air velocity, and litter properties). The model consists of two coupled differential equations representing core and surface thermal compartments, where heat balance is converted to temperature using body mass and heat capacity. Core and surface temperatures are dynamic state variables. Core temperature is raised by metabolic heat production and reduced via heat transfer to the surface and respiratory evaporation, while surface temperature is raised by internal heat transfer and reduced via environmental heat losses through convection, radiation, conduction to the litter, and cutaneous evaporation. Internal heat transfer includes tissue conduction and temperature-dependent blood flow conductance, and respiratory evaporation is regulated by a sigmoid panting response to core and air temperature. Heat transfer processes (convection, radiation, evaporation, and conduction) were calculated using established thermophysical relationships. Model evaluation used literature data reporting core body temperatures under thermoneutral and heat stress conditions. Simulations under increasing environmental heat load showed declining sensible heat loss and increasing reliance on evaporative heat dissipation, accompanied by progressive increases in predicted core body temperature, consistent with heat stress onset. This study presents a dynamic, poultry heat-balance model integrating metabolic heat production, internal heat transfer, and environmental exchange to predict core and surface temperatures as outcomes that can be evaluated using experimental data, providing a quantitative tool to evaluate heat load and support integration into mechanistic growth models.

**Keywords:** poultry; heat stress; heat balance; core body temperature; surface temperature

### 444P Effect of Light Stimulation Age on Keel Bone Integrity and Egg Quality Traits in Chickens

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It is widely accepted today that most laying hens receive damage or fractures to their keel bone for various reasons throughout their laying cycle. This study was done to find an economical and simple way to improve keel bone health in laying hens without sacrificing production or egg quality traits. It was hypothesized that manipulating the age of light stimulation of commercial laying hens in rearing could have an impact on long term skeletal health, specifically the keel bone. To address this, we performed a 79-week study to determine the effects of age of light stimulation on keel bone health, egg quality traits, and production throughout lifetime of the flock. This study used 4 groups of birds under two different lighting schedules. White Leghorns (WL) and Rhode Island Reds (RIR) were included in the study. Each breed had one control group which received an industry standard lighting program, and one treatment group which received an early stimulation lighting program. Each group had two replicates totaling 280 birds per treatment group. The differences in the lighting programs started at 14 weeks of age. The birds were reared on the floor in different rooms. At the lay farm, birds were housed in group cages by treatment group; five birds per cage for WL and four per cage for RIR. Throughout the trial, production was recorded daily by treatment group. Egg quality characteristics were recorded at four different ages (22, 39, 55, and 76 weeks) by treatment group. Keel bones were palpated twice for fractures and deviations, once at 22 weeks of age and once at 79 weeks of age. Birds were also necropsied and scored for keel bone fractures and deviations at 79 weeks of age. While the birds in the early stimulation group started laying sooner, both groups reached peak lay at the same time. A two-tailed two-sample equal variance *t*-test was used to determine significant differences between the two treatments within a breed. RIR at week 22 was the only age which showed significant differences ($p<0.05$); the treatment group had higher egg weight (+2g) and breaking strength (+279g/F). A *t* test was used to compare the palpation and necropsy scores of the keel bones for both groups at 22 and 79 weeks of age. While palpation showed no statistical differences at either age, necropsy scores were significantly different with the control group having more damage than the treatment group for both breeds. Our results indicate that early stimulation can improve skeletal integrity later in life without negatively impacting other traits of economic value.

**Keywords:** Light Stimulation; Managment; Skeletal Integrity; Productiuon; Rearing

### 445P Dose–response meta-analysis of phytase supplementation on ileal phosphorus and calcium digestibility in turkeys

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Phytase supplementation is widely used in turkey nutrition to enhance phosphorus utilization and growth performance; however, variability among studies complicates interpretation of its overall efficacy and dose–response relationship. A systematic review and meta analysis were conducted to quantify the effects of dietary phytase on ileal mineral digestibility in turkeys. Literature searches were performed in Google Scholar and PubMed August 2025, and studies were screened using predefined criteria including commercial diets, measurable phytase activity, appropriate control treatments, and reported variance measures. Four studies met the inclusion criteria for quantitative synthesis of apparent ileal phosphorus AID P and calcium AID Ca digestibility, yielding 10 phytase versus control contrasts. Effect sizes were calculated as standardized mean differences Hedges' g and analyzed using inverse variance weighted random effects models PROC MIXED, incorporating study as a random intercept. Heterogeneity was assessed using Cochran's Q, $I^2$, $\tau^2$, and prediction intervals. Dose–response relationships were evaluated using nonlinear mixed models PROC NLMIXED with broken line specifications to estimate potential saturation points. Statistical significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a trend. AID P was significantly improved Hedges' g = 4.42; 95% CI: 1.601–7.240; $P$ = 0.002, although heterogeneity was substantial $I^2$ = 92.0%; $\tau^2$ = 7.406, with a wide prediction interval −8.824 to 17.665. AID Ca was also significantly improved Hedges' g = 1.015; 95% CI: 0.067–1.963; $P$ = 0.036, with moderate heterogeneity $I^2$ = 72.2%; $\tau^2$ = 0.673. Back transformation of Hedges' g to grams indicated that phytase increased AID P by approximately 15 g per kg 95% CI: 5–25 g per kg and AID Ca by approximately 4.8 g per kg 95% CI: 0.3–9.2 g per kg. The broken line model estimated a population average breakpoint at 1130 FTU per kg SE = 270.1; $t$ = 4.18; DF = 3; $P$ = 0.025, consistent with a saturating response; however, the wide 95% confidence interval 270–1989 FTU per kg reflects limited study level information. The pre plateau slope was positive b1 = 0.005 and showed a tendency toward significance $P$ = 0.084, indicating diminishing marginal returns with increasing phytase dose. In summary, phytase supplementation markedly improved AID P and AID Ca in turkeys and demonstrated a biologically coherent saturating dose–response pattern. However, the precise inclusion level required to achieve maximal digestibility remains uncertain given the limited number of independent studies and residual heterogeneity. Additional controlled studies are warranted to refine dose recommendations.

**Keywords:** Phytase; Turkeys; Ileal digestibility; Phosphorus; Meta-analysis

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**446P A multi-component protease maintains production performance, improves economic benefits, and reduces pathogenic bacteria populations in laying hens fed a low-density diet under commercial conditions.**

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The use of exogenous enzymes is a key strategy to enhance productivity and sustainability in commercial poultry production. This study evaluated the effects of a multi-component protease (PRO) on laying performance, intestinal pathogenic bacteria, and economic outcomes in hens during peak production under commercial conditions. A total of 72,960 Shaver White laying hens (20 weeks of age) were used in a 16-week trial. Birds were assigned to two dietary treatments: a control diet based on corn–soybean meal and a treatment diet formulated with reduced metabolizable energy (−10 kcal/kg) and digestible amino acids (−2.5%), supplemented with PRO at 125 g/t (Jefo Nutrition Inc). Hens were housed in cages (8 birds/cage), with 4,560 cages per treatment in the same facility. For performance measurements, a representative subset of 100 cages per treatment was randomly selected and monitored weekly. Parameters included egg production, eggs per hen housed, feed conversion ratio (FCR; kg feed/kg egg), and mortality. At week 8, jejunal samples were collected from 20 birds per treatment to quantify *Escherichia coli* populations. Data were analyzed using t-test procedures, with significance declared at P < 0.05. Despite the reduced nutrient density of the PRO diet compared to the control, there was no difference (P > 0.05) in the average weekly egg production (91.8 vs 91.6%), and average weekly number of eggs per hen housed (6.34 vs 6.32) among treatments. Birds fed PRO diets had improved (P < 0.05) average weekly FCR per kg egg produced compared to the control (1.85 vs 2.10). At the end of the trial, birds fed the PRO diets had lower (P < 0.05) weekly mortality than birds in the control group (0.11 vs 0.12%). There was a reduction (P < 0.05) in the *Escherichia coli* populations in the jejunum of birds fed PRO diets as compared to the control (1.46 vs. 2.21 log10 CFU/g). Birds fed the PRO diets had lower (P < 0.05) feed costs per kg egg produced when compared to the control group ($0.53 vs. $0.56). In conclusion, these findings indicate that the use of a multi-component protease based on its nutritional matrix supports egg production efficiency while reducing feed costs, with potential benefits related to improve gut health through a reduction in pathogenic bacterial populations.

**Keywords:** egg production performance; Escherichia coli; layers; mortality; protease

**447P Investigation of interactions between phytase, protease, and anti-nutritional factors in soybean meal**

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The objective was to evaluate the effect of phytase and protease on anti-nutritional factors in soybeans. In Experiment 1, growth performance and pancreatic weight (expressed as % of BW) were evaluated in broiler chickens using a 3 d bioassay. Birds were provided a common nutritionally complete starter diet from 0 to 7 d-of-age. From 7 to 10 d-of-age, corn-soybean meal-based experimental diets were provided ad libitum. Diet 1 was a corn-soybean meal-based diet. Diet 2 was similar to diet 1, except raw soybeans (15%) were added. Diet 3 was the same as diet 2, except phytase (2000 FTU/kg) was added and phytase received a matrix value for energy, calcium, non-phytate P, and sodium. Diets 4 and 5 were the same as diet 3, except a 1x and 2x dose of protease (IFF) were added, respectively. All diets were formulated to be nutritionally adequate excluding any reductions in nutrient availability caused by anti-nutritional factors. In Experiment 2, growth performance and pancreatic weight were evaluated in broiler chickens using a 14 d bioassay. Birds were provided a common nutritionally complete starter diet from 0 to 7 d-of-age. From 7 to 21 d-of-age, corn-soybean meal-based experimental diets were provided ad libitum. Dietary treatments were the same as Experiment 1, except one additional diet was evaluated which was the same as diet 3, except that phytase received no matrix value for energy (diet 6). On the final day of each experiment, BW gain, feed intake, and pancreatic weights were recorded. Experiments 1 and 2 contained 8 replicate pens of 5 broiler chickens per treatment. All data were analyzed using a 1-way ANOVA and significance was determined at $P < 0.05$. In Experiment 1 (3 d bioassay), feed efficiency and weight gain were reduced and pancreatic weight was increased by raw soybeans (diet 1 vs. 2; $P < 0.05$). The addition of phytase (diet 3) caused further reductions in weight gain compared with diet 2 ($P < 0.05$). Protease (diets 4 and 5) had no effect ($P > 0.05$) on weight gain or feed efficiency compared with diet 3; however, pancreatic weight was reduced ($P < 0.05$) by protease. In Experiment 2, similar results were observed for phytase, where phytase (diet 3) reduced BW gain compared with diet 2 ($P < 0.05$). The addition of phytase without a matrix value for energy (diet 6) improved weight gain compared with diet 3 ($P < 0.05$). The addition of protease (diets 4 and 5) improved feed efficiency compared with diet 3 ($P < 0.05$). In conclusion, the addition of phytase to diets with raw soybeans reduced BW gain, which may be due to phytase-anti-nutritional factor interactions and phytase matrix values. These effects, however, may be partially ameliorated through protease supplementation.

**Keywords:** trypsin inhibitors ; phytase; protease; soybeans; broilers

**448P Multi-component protease improves gut morphology, crude protein digestibility, and caecal microflora in broilers fed under- and over-processed soybean meal**

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Soybean meal (SBM) processing quality is a critical determinant of broiler gut health, as both under- and over-processing can compromise intestinal integrity, nutrient utilization, and microbial balance. This study evaluated whether multi-component protease (PRO) supplementation could mitigate these adverse consequences across varying SBM processing qualities. A total of 960 one-day-old male Ross 308 broilers were randomly assigned to 6 treatments (10 replicates, 16 birds/pen): Control (standard SBM), Negative Control U (NCU; under-processed SBM), Negative Control O (NCO; over-processed SBM), each supplemented with PRO at 125 g/t. SBM quality was defined by potassium hydroxide protein solubility: under-processed (93-95%), standard (81-84%), over-processed (63-67%). Corn-SBM diets were fed in three phases (starter: d 0-10, grower: d 11-21, finisher: d 22-41) with phytase (1,000 FTU/kg) and xylanase (100 g/t). On d42, jejunal histomorphology, ileal crude protein (CP) digestibility, and caecal microflora were assessed (3 birds/replicate). Data were analyzed by ANOVA and Tukey's HSD (P<0.05). NCU and NCO reduced villus height (VH; 1313 and 1337 µm) and increased crypt depth (CD; 207 and 192 µm) versus Control (1422 and 157 µm), lowering VH:CD (6.4 and 7.1 vs. 9.3; P<0.05). PRO improved VH (NCU+PRO: 1385; NCO+PRO: 1404 µm), reduced CD (179 and 165 µm), and improved VH:CD ratio (8.0 and 8.7) versus NCU and NCO to levels comparable to Control (P<0.05). Control+PRO (VH: 1449 µm; CD: 145 µm; VH:CD: 10.2) did not differ from Control. Ileal CP digestibility was reduced in NCU (67.7%) and NCO (68.4%) versus Control (73.6%; P<0.05). PRO improved CP digestibility in NCU+PRO (71.9%) and NCO+PRO (71.5%) versus NCU and NCO to levels comparable to Control, while Control+PRO (75.9%) did not differ from Control. Caecal coliforms were elevated in NCU (8.1 log CFU/g) and NCO (8.0 log CFU/g) versus Control (6.8 log CFU/g; P<0.05); PRO did not reduce coliforms in NCU+PRO (7.4) or NCO+PRO (7.1 log CFU/g) versus NCU and NCO (P>0.05), though both remained comparable to Control, and Control+PRO (6.3 log CFU/g) did not differ from Control (P>0.05). Lactic acid bacteria

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(LAB) were reduced in NCU (7.1 log CFU/g) but not in NCO (7.7 log CFU/g) versus Control (8.5 log CFU/g; P<0.05 and P>0.05, respectively); PRO restored LAB in NCU+PRO (8.0) and NCO+PRO (8.2 log CFU/g) to levels comparable to Control (P>0.05), while Control+PRO elevated LAB (9.7 log CFU/g) above all treatments (P<0.05). Improperly processed SBM compromises gut morphology, CP digestibility, and microbial balance, and PRO effectively mitigated these effects across all SBM processing qualities.

**Keywords:** Soybean meal; Protease; Gut morphology; Crude protein digestibility; Caecal microflora

### 449P Effects of multienzyme supplementation on production performance, egg quality, and nutrient digestibility in laying hens

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This study evaluated the effects of the supplementation of a multienzyme complex containing xylanase, phytase, B-glucanase, and protease on laying performance, internal and external egg quality traits, and nutrient digestibility in 59-week-old Lohmann laying hens. 96 hens were randomly allocated to conventional cages and assigned to two dietary treatments: a control diet (CON) and a diet supplemented with 0.02% multienzyme (SSF) for 6 weeks. Each treatment had 8 replicates of 6 hens per cage. Hens were weighed at the start and end of the experiment (week 0 and 6). Body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for the entire experimental period, while the hen-day egg production (HEDP) and the average egg weight (EW) were calculated in each period (3 weeks per period). Egg quality parameters were analyzed at the end of each period. Excreta samples for nutrient digestibility were collected on week 6 and were analyzed for dry matter (DM), gross energy (GE), crude protein (CP), calcium (Ca), phosphorus (P), and neutral detergent fiber (NDF) digestibility. Data were analyzed for statistical significance at $P < 0.05$, and tendencies were considered at $0.05 < P < 0.10$ using mixed-model procedures in SAS (PROC GLIMMIX) with Tukey's adjustment for means separation. Compared with CON, the final body weight and ADFI were significantly lower in SSF ($P < 0.05$). FCR tended to decrease in SSF ($P < 0.10$), whereas no notable differences in BWG were observed among treatments ($P > 0.05$). Hens fed CON tended to have higher HEDP and EW during period 2 ($P < 0.10$), with a tendency of a treatment × period interaction in EW ($P < 0.10$). CON also showed a marginally higher egg specific gravity ($P < 0.10$) compared with the SSF. However, no significant differences were observed among treatments for Haugh unit, eggshell breaking strength, or thick albumen height at any period ($P > 0.05$). Yolk color tended to be greater in SSF than in CON ($P < 0.10$). Eggshell thickness and egg shell weight were significantly higher in CON compared with the SSF ($P < 0.05$) during period 1. A significant treatment × period interaction was observed for eggshell thickness ($P < 0.05$), with a tendency for interaction in egg shell weight ($P < 0.10$). The apparent digestibility of DM, GE, CP, P, and NDF was significantly higher in CON compared with SSF ($P < 0.05$). In conclusion, apart from a modest increase in yolk color, 0.02% SSF provided no measurable benefits in 59 week-old laying hens under the conditions of this study. Instead, hens receiving SSF showed reduced BW, ADFI, weaker eggshell traits, and decreased nutrient digestibility compared with the control.

**Keywords:** egg quality; laying hen ; multienzyme supplementation; nutrient digestibility; production performance

### 450P Effects of increasing doses of a novel *E. coli* phytase SuperPhy on growth performance of broilers fed diets reduced in metabolizable energy, calcium, and phosphorus

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The objective of this study was to evaluate the effects of increasing doses of a novel thermostable 6-phytase derived from *E. coli* and expressed in *Pichia pastoris* (SuperPhy®; Hanley International, LLC) on the growth performance of broilers fed diets reduced in metabolizable energy (ME), calcium (Ca), and available phosphorus (aP). A total of 3240 one-day-old male Ross 308 broilers were allocated to 54 floor pens (60 birds per pen) in a randomized complete block design. Birds were fed 1 of 4 dietary treatments over a 40-d, three-phase feeding program: a positive control (PC; nutrient-adequate diet formulated according to Aviagen recommendations and supplemented with 500 FTU/kg SuperPhy) and three nutrient-reduced diets (−90 kcal/kg ME, −0.15% Ca and aP) supplemented with increasing SuperPhy levels (500, 1500, or 3000 FTU/kg). Body weight gain (BWG) and mortality-corrected feed intake (FI) and feed conversion ratio (FCR) were determined by phase. Data were analyzed using one-way ANOVA to evaluate treatment effects, and linear and quadratic effects of SuperPhy inclusion were assessed among the three nutrient-reduced diets. During the starter (d 0–14) and grower (d 15–28) phases, FI and BWG were not affected by dietary treatment ($P > 0.05$), whereas FCR differed among treatments ($P = 0.0462$ and $P = 0.0056$, respectively). In the starter phase, birds fed the PC had numerically better FCR ($P > 0.05$), while in the grower phase, birds receiving 1500 FTU/kg SuperPhy showed improved FCR ($P < 0.05$) compared with the PC and the 500 FTU/kg nutrient-reduced group, with the 3000 FTU/kg group intermediate. Increasing SuperPhy inclusion linearly reduced FI during the grower phase ($P = 0.0506$). In the finisher phase (d 29–40), FI differed among treatments ($P = 0.0199$) without impacting BWG ($P > 0.05$). FCR was affected ($P < 0.0001$) by treatments, with the PC achieving the optimal FCR, followed by the 3000 FTU/kg SuperPhy group. Increasing SuperPhy inclusion resulted in a linear reduction in FI ($P = 0.0266$) and a corresponding linear improvement in FCR ($P = 0.0048$). Across the entire trial (d 0–40), FI was affected by treatment ($P = 0.0393$), whereas BWG was not ($P > 0.05$). FCR differed among treatments ($P < 0.0001$), with the PC performing the best, but it did not differ from the 3000 FTU/kg SuperPhy group ($P > 0.05$). Increasing SuperPhy levels led to a linear decrease in FI ($P = 0.0104$), which was associated with a linear improvement in FCR ($P = 0.0001$). In conclusion, these results indicate that superdosing SuperPhy can effectively offset nutrient reductions, maintaining growth performance comparable to a nutrient-adequate diet. This further demonstrates SuperPhy's effectiveness in improving nutrient utilization.

**Keywords:** phytase; growth performance; metabolizable energy; phosphorus; broiler

### 451P Evaluation of agZyme supplementation on the performance of broiler chickens fed a corn–soybean meal–based diet

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agZyme is an enzymatic *blend* developed by Agroceres Multimix, composed of a combination of xylanase, amylase, proteases, and phytase, and is used to enhance nutrient digestibility, thereby improving animal performance and contributing to reduced feed costs. The objective of this study was to evaluate the effects of agZyme supplementation on the performance of broiler chickens fed corn–soybean meal–based diets. The trial was conducted at Agroceres Multimix Technology and Innovation Center. A total of 4,800 one-day-old male Ross 308 broiler chicks

were randomly allocated to one of three dietary treatments with 20 replicates of 80 birds each. All experimental diets were corn–soybean meal–based and were provided *ad libitum* throughout the experimental period. The dietary treatments were as follows: 1) Positive control (PC), consisting of a basal diet formulated to meet the nutritional requirements of broiler chickens; 2) Negative control (NC), consisting of a diet with nutrient reductions based on the nutrient matrix values proposed for agZyme, including ME, protein, AAs, phosphorus and calcium reductions; and 3) NC + agZyme, consisting of the negative control diet supplemented with agZyme, aiming to achieve nutritional levels similar to those of the PC diet. Performance data were collected on day 42, and were analyzed using a one-way ANOVA test followed by Tukey's test for multiple comparisons of means, with statistical significance set a P<0.05. Supplementing agZyme in the NC diet resulted in a 5.9% increase in final body weight (BW) or weight gain (P<0.001) compared with the NC group, with BW similar to the PC group. Birds in the NC+agZyme showed the highest feed intake (P<0.001) among the treatments, approximately 4.4% higher than that of the PC group. The PC group had the best feed conversion ratio (FCR) (P<0.001) among the treatments. Although the NC + agZyme treatment showed a FCR similar to the NC group, the supplementation of agZyme in the NC diet resulted in a 8.4% increase of the European Production Efficiency Factor compared with the NC group, with value comparable to the PC group (P<0.001). The utilization of agZyme improves broiler performance fed corn–soybean meal–based diet.

**Keywords:** amylase; broiler; phytase; protease; xylanase

### 452P Effect of egg storage duration on fertility, hatchability and day old chick quality in Suborno strain

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This study evaluated the effect of egg storage duration on fertility, hatchability and day-old chick quality in Suborno strain, a meat type chicken strain developed at Bangladesh Livestock Research Institute. A total of 600 eggs were collected from 60 hens (10 eggs per hen) following artificial insemination. From each hen, 10 eggs were divided equally into two storage groups: fresh eggs stored for 3 days and old eggs stored for 12 days at 16 degree celsius before incubation. Eggs were then set as a single batch in a PETERSIME VISION incubator at 99.5°F with 55% relative humidity. Candling was performed at 10 days of incubation and fertile eggs were transferred to the hatcher at 18 days. Data were analyzed using SPSS with hen as a random effect to account for replication. Fertility and hatchability were analyzed using chi-square tests while egg weight and day-old chick weight were analyzed using mixed-model ANOVA. Descriptive statistics are presented as mean ± standard deviation. Average egg weight was slightly higher in fresh eggs (54.60 ± 1.18 g) than in old eggs (53.65 ± 1.14 g). Fertility significantly decreased with prolonged storage (90.0% vs. 78.0%; $p < 0.05$). Hatchability remained high in both groups but was marginally lower in old eggs (91.45% vs. 95.55%). Day old chick weight was also declined slightly with extended storage (37.42 ± 0.88 g vs. 36.58 ± 0.85 g; $p < 0.05$). These results indicate that minimizing egg storage duration prior to incubation enhances reproductive performance and improves chick quality in the Suborno strain. Moreover, replication across 60 hens strengthen the reliability of the findings and provides practical guidance for optimizing hatchery management.

**Keywords:** Suborno ; Storage; Incubation; Fertility; Hatchability

### 453P Comparison of white LED lighting to poultry specific lighting with moon phase on production

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Artificial lighting used in poultry production is typical white light and lacks parts of the spectrum that may be more beneficial to poultry; furthermore, the effect of simulating moonlight has not beeninvestigated. To determine if adding "moon" lighting to more poultry specific lighting spectrums wouldaffect stress and fear response of laying hens, we reared laying hens from 18 to 72 weeks of age undereither a commonly used LED fixture (Control) or a poultry specific lighting program, which included amoon phase (DYNM), consisting of dim blue light at night. The moonphase would simulate a natural environment during the night, as the birds would never be in complete darkness. Birds were housed in the same barn but separated by a tarp and were subjected to the same environmental conditions with the exception of the lighting program. For each treatment, 30 cages were used with 3 birds per cage. Egg weights and percent hen in lay were recorded daily from 18 to 72 weeks of age. Egg quality (egg shell thickness (ESTh), egg shellbreaking strength (ESBS), egg shell puncture strength (ESPS), albumin height (AH), and Haugh score(HS)were recorded every two weeks from 18 to 72 weeks of age. Data was analyzed using GLM: a significantdifference was considered P < 0.05. There were no differences between the treatments in any measureat the start of the study (P > 0.05). No differences (P > 0.05) were observed between treatments duringearly lay (18-35 weeks of age, EL) or mid lay (36-53 weeks of age, ML) in ESPS or ESBS (5.72 ± 0.01mm;5.81 ± 0.01mm; 4428 ± 43 g; 4423 ± 39 g). There was also no difference (P > 0.05) observed in ESBSduring late lay (54-72 weeks of age, LL) or overall (4077 ± 39g; 4309 ± 25g); However, the ESPS washigher in DYNM during LL and overall (5.96 ± 0.03 mm, P = 0.004; 5.83 ± 0.01 mm, P = 0.01) comparedto CON (5.84 ± 0.02; 5.79 ± 0.01). Both AH and HS were higher in the DYNM during EL, ML and overall(9.44 ± 0.11 mm, P = 0.007; 9.23 ± 0.08 mm, P = 0.005; 9.31 ± 0.09 mm, P = 0.001; 97.2 ± 0.5, P =0.01;95.6 ± 0.4, P =0.004; 96.0 ± 0.4, P = 0.001) and showed a trend for being higher during LL (9.27 ± 0.23mm, P = 0.07; 94.9 ± 1.0, P = 0.07) compared to CON (9.06 ± 0.08 mm; 8.88 ± 0.09 mm; 8.78 ± 0.11 mm;8.91 ± 0.06 mm; 95.6 ± 0.4; 93.8 ± 0.05; 92.7 ± 0.6; 94.1 ± 0.3). There was no difference (P > 0.05)observed in EW or percent hen in lay throughout the study; however, the DYNM hens did lay on average sixmore eggs. While the total eggs laid per bird was not statistically significantly different, this is aneconomically significant finding at scale. These results demonstrate that rearing laying hens with DYNM canimprove egg quality and increase egg numbers where it may have a significant economic impact.

**Keywords:** Layer; Light; Fear; Stress; Moonphase

### 454P Age-Associated Hepatic Proteomic Across the Laying Cycle in Commercial Hens

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Sustaining egg production during extended laying cycles is a major priority in modern poultry systems; however, productivity declines during mid-to-late lay remain a persistent challenge with important economic consequences for commercial egg production. Age-associated stress and metabolic adaptation may impair hepatic function, a central regulator of yolk precursor synthesis, lipid transport, endocrine signaling, and oxidative balance. Characterizing these changes may guide targeted strategies to sustain extended laying performance. This study investigated age-related hepatic proteomic remodeling across peak (37 wk), mid-decline (67 wk), and late-decline (87 wk) production phases in Hy-Line W-36 laying hens (n = 5/group). Mass spectrometry data were processed using MaxQuant (v1.6.3.3) against the UniProt *Gallus gallus*

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database, followed by statistical analysis in R. LC–MS/MS quantification revealed significant differential protein expression (FDR q < 0.05), with the most pronounced changes between 67 and 87 weeks. Principal component analysis demonstrated clear separation by age, indicating distinct stage-specific proteomic profiles. At mid-lay (67 vs 37 wk), pathway enrichment revealed suppression of cholesterol, sterol, and bile acid biosynthesis, supported by downregulation of key enzymes including HMGCR, MVD, CYP7A1, and AKR1D1, alongside increased fatty acid metabolism and amino acid biosynthesis, suggesting a metabolically adaptive response. In contrast, late-lay (87 vs 37 wk) was characterized by a shift toward stress and structural remodeling, with upregulated proteins such as CYP3A5, BST1, NUP35, and CWF19L1 associated with detoxification, immune signaling, and gene regulation, along with activation of MDA-5–mediated immune pathways. Concurrently, extracellular matrix organization, integrin signaling, and cell adhesion pathways were downregulated, indicating structural and functional deterioration of hepatic tissue. Overlap analysis showed that most differentially expressed proteins were stage-specific, with limited overlap across transitions. Collectively, these findings demonstrate that hepatic aging in laying hens follows a stage-dependent trajectory rather than a uniform decline, marked by a transition from adaptive metabolic reprogramming to structural and functional deterioration that constrains sustained egg production.

**Keywords:** Liver; Ovary; Fatty acid; Amino acid; Proteomics

### 455P *In ovo* vaccination with chitosan nanoparticle–based subunit vaccine enhances resistance to *Campylobacter jejuni* colonization in broiler chickens

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*Campylobacter jejuni* infection in poultry presents a significant public health concern due to the consumption of contaminated chicken meat and related products. Vaccination has been proposed as a potential mitigation approach to control infections in chickens and reduce the risk of zoonotic transmission. Yet, no commercial vaccines are currently available for controlling *Campylobacter* in chickens. This study aimed to investigate the efficacy of mucosal delivery (*in ovo* and oral) of multi-antigen chitosan nanoparticle (CNP)-encapsulated vaccines, including *C. jejuni* lysate and outer membrane proteins (OMPs), administered alone or in combination with the Toll-like receptor ligand CpG ODN as an adjuvant for controlling *C. jejuni* infection in broilers. On embryonic day 18, 225 viable eggs were randomly assigned to nine groups receiving *in ovo* amniotic vaccination with CNP-encapsulated *C. jejuni* OMPs, OMPs + CpG ODN, lysate (low or high dose), CpG ODN alone, CNP only, soluble lysate (low or high dose), or PBS control, followed by an oral booster with the corresponding formulation at 14 days of age. All birds were orally challenged with *C. jejuni* at 21 days of age, with blood and cloacal sampling and necropsy-based tissue collection at 15–16 and 35 days of age. Statistical analyses were conducted using JMP® Pro 17.1.0. Parametric data were analyzed by one-way ANOVA followed by Tukey's post hoc test, while non-parametric data were analyzed using the Kruskal–Wallis test with Dunn's multiple comparisons. Data are presented as mean ± SEM, with statistical significance set at $p < 0.05$. The results revealed that administration of CNP-encapsulated OMPs alone or in combination with CpG ODN significantly reduced *C. jejuni* colonization by 1.2 and 1.24 log10, respectively. In contrast, the groups vaccinated with low or high doses of CNP-encapsulated *C. jejuni* lysate reduced it by 1.3 log10. The observed reductions in *C. jejuni* colonization in vaccinated birds were associated immunologically with a significant elevation of specific IgA antibody levels in bile and the alteration of cytokine gene expression, particularly interleukin (IL)-1B, IL-12, IL-13, interferon (IFN)-Γ, and transforming growth factor (TGF)-B in the cecal tonsils of these vaccinated groups. However, no such effects were observed for the groups vaccinated with CpG ODN alone or the soluble form of the *C. jejuni* lysate. In conclusion, CNP-encapsulated *C. jejuni* OMPs or lysate represent a potential vaccine candidate to curb *C. jejuni* infection, with a feasible mucosal delivery approach in broiler chickens.

**Keywords:** Chicken; Campylobacter ; Vaccine; Chitosan; CpG ODN

## Poster Session III

### 456P Effects of red osier dogwood (*Cornus stolonifera)* extract on growth performance, protein digestibility, immune response, and gut health of broiler chickens in a coccidiosis vaccine challenge model

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As the poultry industry moves away from using antibiotics as growth promoters due to concerns about antibiotic resistance, challenges such as coccidiosis, heat stress, and Salmonella, which were previously controlled, have become more common. Polyphenol-rich phytogenic feed additives improve intestinal development, exhibit antimicrobial and antioxidant effects, and boost growth performance. Red osier dogwood extract (RDE) is rich in polyphenols and enhances chicken growth, gut microbiota, and intestinal health, and has mitigated the effects of Salmonella and heat stress in broilers. However, no study has demonstrated the effect of RDE on chickens challenged with coccidiosis. This study examined RDE's effects on growth, digestibility, meat and organ yields, bone strength, immune response, intestinal morphology, and gut microbiota in coccidiosis-challenged broilers. A total of 336 one-day-old mixed-sex Ross 308 broiler chicks were randomly assigned to 3 dietary treatments: Control corn-soybean meal diet (CON), CON + 0.15% RDE, and CON + 0.30% RDE. On d14, half of the birds were challenged with a 12x label dose of Immucox 3®, and the other half received the same volume of saline solution. Each dietary x challenge treatment had 8 replicate bioassay cages with 7 birds/cage. One male bird/pen was euthanized on d21 and d28. Jejunum tissue, ileal content (for microbiome analysis), and blood samples were collected on d21. Organ relative weight and ileal content (for digestibility) were collected on d28. Data were subjected to analysis of variance using PROC MIXED of SAS. Coccidiosis challenge increased bursa weight, decreased crypt depth ($P$=0.0048), villus height to crypt depth ratio (VH:CD) ($P$=0.0004), liver weight ($P$=0.0001), ileal ($P$=0.0531) and cecal ($P$=0.0174) alpha-diversity, and the relative abundance of beneficial bacteria ($P$<0.05). RDE increased ($P$=0.0179) the weight of spleen linearly, without affecting bursa and liver weight, suggesting a beneficial immunostimulant effect, given that there was a quadratic increase in IgM titers ($P$=0.0340) and crude protein digestibility ($P$=0.0429), with 0.15% RDE yielding the highest values, and 0.30% RDE improved feed intake and body weight compared to CON. In the challenged group, 0.15% yielded higher ($P$<0.05) villus height and VH:CD than 0.30%. Therefore, given the evidence of RDE's benefits to growth performance, gut microbiota, and intestinal integrity of chickens with coccidiosis, the inclusion of 0.15% RDE could be beneficial in promoting health and growth performance of broiler chickens.

**Keywords:** Coccidiosis; Gut health; Performance; Phytogenic feed additive; Red osier dogwood extract

### 457P DOSE response effect of a lysolecithin product on broiler performance, blood profile and gene expression in the jejunum, liver and muscle

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The current study assessed the effect of two doses of a lysolecithin product (Rovabio LeciMax Dry, Adisseo NL B.V., Netherlands, abbreviated as Lec) on broiler performance, blood profile and gene expression (GE) in the jejunum, liver and muscle. It was hypothesized that lysolecithin addition yields a dose-dependent improvement of performance by modulating metabolic pathways in key metabolic tissues. A randomized block design was used consisting of three treatment groups, each with 12 replicates of 16 birds. In total, 576 male one-day-old Ross 308 birds were randomly assigned to one of three treatments: control group (basal diet), Lec-250 (basal diet + 250 g/t Lec) and Lec-500 (basal diet + 500 g/t Lec). Feed and water were provided *ad libitum*, and the study lasted 35 days. Body weight, body weight gain, feed intake and FCR were determined at day 0, 14, 28 and 35. At day 28, footpad dermatitis score (FPD) and breast burn score were assessed and blood was analyzed for lipid profile, liver damage factors, beta-hydroxybutyrate (BHB), glucose and non-esterified fatty acids (NEFA). For GE by mRNA quantification, 16 birds per treatment were randomly selected and jejunal, hepatic and muscle samples were collected. Data were subjected to ANOVA and Tukey's test with the significance level set at P<0.05. Compared to the control, Lec-250 and Lec-500 decreased FCR (P=0.001) while Lec-500 also improved FPD (P=0.011). Lec-500 decreased NEFA (P=0.014) and increased BHB (P=0.009). The GE results in jejunal samples showed activation (P<0.05) of nutrient transport pathways while decreasing carbohydrate absorption and energy metabolism with Lec-250. In Lec-500, inhibition of digestive pathways and protein fermentation was observed while improving (P<0.05) fatty acid and vitamin transport. The lower neuropeptide Y expression and increased glucagon-like peptide-1 expression explained the lower feed intake in both groups. In the liver, Lec-250 showed a clear pathway activation (P<0.05) of lipid redistribution while catabolic activity was decreased. Lec-500 shifted to organelle turnover and stress responsive protein production while maintaining amino acid, vitamin and fatty acid metabolism but inhibiting muscle growth pathways. Similarly, in muscle tissue, both Lec-250 and Lec-500 shifted (P<0.05) to membrane maintenance and carbohydrate utilization while muscle growth pathways were inhibited (P<0.05) leading to no effect on growth performance. In conclusion, lysolecithin improves nutrient utilization in a dose-dependent way wherein a low dose creates an optimized system for energy conservation and nutrient efficiency, and a high dose reflects a state of maintenance and reduced digestive processes due to oxidative stress.

**Keywords:** Emulsifier; Lysolecithin; Transcriptomics; Performance; Broilers

### 458P Effects of phytogenic supplements containing butyrate on broiler performance

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Phytogenic feed additives containing butyrate, organic acids or plant-derived compounds are increasingly used in broiler nutrition to support performance and feed efficiency. The objective of this study was to evaluate two phytogenic feed supplements combining butyrate with phytogenic compounds in broilers compared with an unsupplemented control and a Bacillus-based probiotic. A total of 600 straight-run Cobb 500 broiler chicks were placed in 40 pens (15 birds/pen; 5 pens/treatment) arranged in 5 blocks of 8 pens, and assigned to 4 treatments in a randomized complete block design on d 0: CON (unsupplemented control); PHY1 (calcium butyrate, hydrolysable tannins, phytogenic compounds) at 150 ppm; PHY2 (calcium butyrate, buffered organic acids, phytogenic blend) at 250 ppm; PRO (Novela EC probiotic, UAH, USA) at 100 ppm. Birds were reared on wood shavings under standard management conditions. Birds were provided feed and water *ad libitum*. Birds received a common corn–soybean meal basal diet fed as starter (0–14 d), grower (15–28 d), and finisher (29–42 d). Bodyweight and feed intake (FI) were measured by pen at d 0, 14, 28, and 42; mortality was recorded daily. Bodyweight gain (BWG) was calculated and FCR adjusted for mortality (MFCR). Data were analysed using ANOVA in a randomized complete block model with treatment as a fixed effect and block as a random effect. Residual normality was evaluated and data were transformed when required; when assumptions were not satisfied, non-parametric tests were applied. Pairwise comparisons were adjusted using the Holm procedure. Significance was declared at $p < 0.05$ and trends at $p < 0.10$.BWG and FI were not affected by treatment at d 14, 28, or 42 ($p > 0.10$). On d 42, PRO (3124 g) and PHY2 (3107 g) were 1.4–1.9% heavier than CON (3065 g). On d 14, FCR and MFCR tended to be higher in CON than supplemented treatments ($p = 0.056$). On d 28, FCR ($p = 0.016$) and MFCR ($p = 0.016$) were lower in PHY1 and PHY2 than CON, while PRO was intermediate. On d 42, MFCR ($p = 0.028$) was lower in PHY1 (1.571) and PHY2 (1.570) than CON (1.628), while PRO (1.596) was intermediate. Mortality was low and unaffected by treatment. Mortality on d 42 was 4.0, 0.0, 1.3, and 5.3% in CON, PHY1, PHY2, and PRO, respectively. Low-dose phytogenic supplements combining butyrate with phytogenic compounds reduced MFCR by up to 3.5% relative to CON without affecting final body weight. Comparable responses between PHY1 (butyrate–phytogenics–tannin blend) and PHY2 (butyrate combined with organic acids and phytogenics) suggest that different formulation strategies may support improved feed utilization in broiler production.

**Keywords:** broiler chickens; butyrate; phytogenics; organic acids; feed efficiency

### 459P Field Report: Yeast derived components improved growth performance, cecal microbiota composition, and intestinal integrity in 0-to-33-day old broilers.

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Proverde Liquid (Natural Biologics, Inc., Newfield, NY) is an all-natural blend of autolyzed yeast and highly concentrated yeast cell wall. This blend of complex carbohydrates, nucleotides and peptides reduces enteric pathogen challenges, improves digestive efficiency, supports the immune system and lowers gut inflammation. This study was conducted to evaluate the effect of Proverde Liquid on broiler growth performance, cecal microbiota, and intestinal integrity of 0-to-33-day-old broilers. Sixty-eight thousand Arbor Acres, straight run, day-of-hatch broilers were used in this trial. Broilers were reared in two commercial broiler houses with 34,000 broilers per house. Both houses were on the same farm, with identical nutrition and environmental conditions. One house served as the Control group, while the birds in the second house were given Proverde Liquid. Proverde Liquid was administered at a rate of 0.50 mL/L of water from day 1 to 3 of age, 0.25 mL/L of water from day 21 to 23 of age, and 0.50 mL/L of water from day 30 to 33 of age. Throughout the trial, all broilers were fed the same corn-soybean meal-based starter, grower, and finisher diet. Weekly (d1, 7, 14, 21, 28, and 33) body weights were taken for 150 randomly selected broilers per house. Average daily gain and feed intake were measured for each feeding phase. Feed conversion ratio was calculated for each feeding phase. Mortality was recorded daily, and mortality rate was calculated for each week. At the end of the trial, the small intestine was collected from 30 randomly selected broilers per house to determine villus height and crypt depth ratio in the duodenum, jejunum, and ileum. Ceca also were collected to determine counts of *Lactobacillus* spp., *Enterobacteriaceae,* and *Clostridium spp*. Data were analyzed by one-way ANOVA. Broilers given Proverde Liquid tended to have heavier BW (P=0.10) and higher ADG (P<0.05) from d 22 to 33 than control broilers. Broilers given Proverde Liquid had lower mortality from d 1 to 7 and the entire trial (P<0.05). Additionally, broilers given Proverde Liquid had

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higher (P<0.01) *Lactobacillus* spp. counts and lower (P<0.01) *Enterobacteriaceae*, and *Clostridium* spp. Intestinal villus height to crypt depth ratio was numerically increased in the duodenum and ileum when broilers were administered Proverde Liquid. These results suggest that Proverde Liquid can support the modulation of cecal microbiota in broilers, as well as prevent intestinal disturbances associated with feed phase changes. Proverde Liquid can be administered, at times of stress or challenge, to improve growth performance, the microbiome balance, and intestinal health.

**Keywords:** Autolyzed yeast; Yeast cell wall; Broilers; Growth performance; Microbiota

### 460P Alleviation of carbon-tetrachloride induced liver damage supplemented by a blend of artichoke extract, vitamin B complex, amino acids, lemon essential oil and silymarin in chickens

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Vitamins, essential amino acids and Phytogenic feed additives (PFA) improve the body weight and feed conversion ratio (FCR), especially in antibiotic-free poultry production. This study evaluated the hepatoprotective effect of a blend of artichoke extract, vitamin B complex, amino acids, lemon essential oil and silymarin in chickens with liver damage induced by Carbon-tetrachloride (CCl4-). Artichoke extract, lemon essential oil and silymarin are PFA that show antioxidant and liver protectant effect. Also, amino acids like betaine, lysine, methionine and Vitamin B complex reduce the fatty liver, lipoxidation, and promotes fat transport. However, the effective concentration and combinations of amino acids, vitamins and PFA against liver injury is still unclear. The experiment was conducted with three groups, six replicates and thirty birds per replicate. The groups were as follows: Treatment (CCL4- /basal feed blend of 7.4% vitamin B complex + 5.7 amino acids + 2.5% Artichoke extract + 0.5% lemon essential oil and 4% silymarin - 0.5 g/kg), Positive control (CCL4-/basal feed) and Negative control (basal feed). Chickens of treatment and positive control group were inoculated with CCl4- in olive oil (2 ml/kg BW) at 14d, 17d, 21d and 24d of age. Body weight, feed conversion ratio (FCR), gross lesions, histology analysis, biochemical analysis and liver mRNA expressions of fibrogenesis were evaluated. One way- ANOVA was used to analyze the data, and the means showing significant differences were then compared using the Post-Hoc Tukey test ($p \leq 0.05$). The supplementation with a blend of artichoke extract, vitamin B complex, amino acids, lemon essential oil and silymarin reduced FCR in chickens and increased the body weight ($p < 0.05$) compared with positive and negative control group. About biochemical markers, both treatment and negative control group showed not significantly different values of cholesterol, total protein and albumin compare with positive control group. Moreover, positive control group showed higher percent of fatty liver and hydropic degeneration in histology compare with treatment and negative control group ($p \leq 0.05$). Regard to liver mRNA expressions of fibrogenesis (TGF-B1, and ACTA2), chickens of treatment group under expressed these compared with both control groups ($p \leq 0.05$). The results showed that the blend of artichoke extract, vitamin B complex, amino acids, lemon essential oil and silymarin improved the growth parameters and showed hepatoprotective effect, reducing the damage and fibrosis in chickens with CCl4- induced liver injury when compared to positive control group.

**Keywords:** Poultry; Phytogenic feed additive; Toxic; Liver

### 461P Double buffered sodium butyrate improves eggshell and bone quality of laying hens in conventional or low energy and protein diets

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Lower levels of energy and protein in layers' feed could help the egg production sector to save resources and reduce nitrogen contamination. Sodium butyrate associated with buffer salts (DBSB) has been studied in broilers in both conventional field diets and in contexts of reduced energy and protein diets. These trials have shown an improvement in growth and feed conversion ratio thanks to DBSB. However, this ingredient has been little studied in laying hens. The objective of this trial was to assess whether DBSB also improves production performance in this species and other parameters in different contexts of energy and protein levels in the diets. 256 Hy-line laying hens of 50 weeks old were distributed in four treatments in a completely randomized design (8 replicates of 8 birds per treatment) for 84 days. The first group was fed a standard field diet (CTRL) formulated with 2900 kcal/kg of Apparent Metabolizable Energy (AME) and 16.4% of Crude Protein (CP). The feed distributed to the DBSB group was the CTRL diet supplemented with 600g/MT of DBSB (Butylin®54, Dietaxion). The LEP groups were fed CTRL diet reduced (-1.5%) in AME, CP and main digestible amino acids (Lys, M+C, Thr). LEP-DBSB group was supplemented with 600g/T of DBSB. Parameters studied at the end of the supplementation were feed intake (FI), egg production, feed conversion ratio (FCR), rate of fragile eggs (FE), Haugh unit (HU), eggshell thickness (ETH), specific gravity of egg (SG) and tibia and femur resistance. Data were analyzed with ANOVA followed by Tukey tests. There were no significant differences between groups for FI, egg production and FCR: the improvement of the number and the mass of eggs produced with DBSB was just numerical ($P>0.05$). DBSB significantly improved SG in DBSB and LEP-DBSB group by +2.4% (1.101 vs 1.075g/cm3; $P<0.05$) and +1.4% respectively (1.072 vs 1.057g/cm3; $P<0.05$). FE, ETH and HU were only numerically improved by DBSB ($P>0.05$). Tibia resistance was significantly improved in LEP-DBSB by +11% compared to LEP (14.7 vs 13.2kgf/cm$^2$; $P<0.05$), but only numerically in a common diet ($P>0.05$). For femur resistance, it was improved by + 10% in DBSB compared to CTRL (15.2 vs 13.8 kgf/cm$^2$; $P<0.05$), but only numerically in a LEP diet ($P>0.05$). Supplementing 50 weeks old laying hens with 600ppm of DBSB preserves eggshell quality and improves bone resistance in both common or reduced energy and protein diets. DBSB is then a good strategy to save raw materials resources in the poultry sector while maintaining layer performance.

**Keywords:** sodium butyrate; laying hens; eggshell quality; bone strength

### 462P Comparative effects of sodium gluconate and sodium butyrate on growth performance and intestinal health of broiler chickens

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**ABSTRACT** - Sodium gluconate (SG) is known to act as a prebiotic that promotes gut microbial fermentation and is associated with increased butyrate production. This study compared the effects of dietary SG and sodium butyrate (SB) supplementation on growth performance, carcass yield, and intestinal health of broiler chickens. A total of 1,440-day-old male Cobb® 500 chicks were assigned to a completely randomized design with six treatments, eight replicates per treatment, and 30 birds per replicate as the experimental unit. Dietary treatments included a basal diet

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without additives as the Control (CON); a basal diet supplemented with SB at 1,000 ppm from 1 to 42 d (SB); and basal diets supplemented with SG at 500 (LSG), 1,000 (MSG), or 1,500 ppm (HSG) from 1 to 42 d. An additional treatment consisted of SG supplementation at 1,000 ppm from 1 to 21 d followed by 500 ppm from 22 to 42 d (M&LSG). Canthaxanthin (40 g/ton) was included in the finisher diet as an indirect indicator of intestinal absorptive function. Growth performance variables such as body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated. At 42 d of age, two birds per pen were selected for carcass evaluation, serum color measurement, intestinal morphology, and cecal microbiological analyses (CFU of Gram-positive and Gram-negative bacteria, respectively). Data were analyzed using the MIXED procedure of SAS®, with means compared by Tukey's test ($P < 0.05$). At 42 d of age, both BWG and FCR were significantly improved ($P < 0.05$) in all SB- and SG-supplemented groups compared with the CON. Among the supplemented groups, BWG of MSG was significantly greater than LSG ($P < 0.05$) while HSG, M&LSG and SB were intermediate. Compared with the Control, both SB and SG supplementation increased villus height and reduced crypt depth throughout the small intestine, resulting in higher villus-to-crypt ratios ($P < 0.05$). Among the supplemented groups, MSG exhibited a greater villus-to-crypt ratio than the other treatments ($P < 0.05$) in ileum. Serum color values as an indication of Canthaxanthin absorption were significantly increased, and populations of both Gram-positive and Gram-negative bacteria in the cecum were significantly reduced by SB and all SG supplementation groups ($P < 0.05$) compared to the CON. These results suggest that both SB and SG potentially improve broiler performance, which is associated with enhanced intestinal morphology in the small intestine, modulation of the cecal microbial population, and improved nutrient absorption, indicating SG can work as a butyrate source in broilers as expected. The most pronounced effects were observed with 1,000 ppm of SG.

**Keywords:** broiler performance; carcass; intestinal quality; sodium gluconate; sodium butyrate

### 463P Evaluation of the effects of a combination of *Bacillus subtilis* 29784 and a multi-carbohydrase complex on broiler performance and nutrient digestibility

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Nutritional strategies combining complementary feed additives are increasingly explored to improve growth performance and nutrient utilization in poultry. This study evaluated the effects of a combined supplementation of a Bacillus subtilis and a multi carbohydrase enzyme on broiler performance and nutrient digestibility. Three experiments were conducted to assess growth performance and energy utilization in broilers fed diets supplemented with the probiotic–enzyme combination compared with a non-supplemented control. Two performance trials were carried out from 0 to 42 days of age under a three-phase feeding program (starter, grower, finisher). A total of 960 day old chicks were allocated to 48 pens (24 pens/trt; 20 bird/pen). For performance trial wheat soybean meal and corn soybean meal diets were reduced in energy and digestible amino acids by 5% relative breed nutrition guide. Measured parameters included feed intake, body weight gain, and feed conversion ratio (FCR), with FCR adjusted for similar body weight. Digestibility trial was conducted on 60 broilers (30 individual caged bird/trt) from 13 to 22 days of age, including a 7-day adaptation period followed by 3 days of total excreta collection, to determine apparent metabolizable energy corrected for nitrogen balance (AMEn). For digestibility trial wheat soybean meal diet were formulated to meet the animal requirement. Data were analyzed by variance analysis using trial (n=2), treatment (n=2) and block (n=24) for performance trails and using treatment (n=2) and block (n=30) for digestibility trial The combined supplementation significantly improved broiler performance, resulting in increased body weight gain, reduced feed intake, and an overall improvement in feed efficiency. The greatest response was observed for adjusted FCR, with an improvement of 3.2% ($P<0.001$) compared with the control diet. In the digestibility trial, the probiotic–enzyme combination significantly increased dietary AMEn, with an improvement of 141 kcal/kg dry matter ($P<001$). Mechanisms beyond the improvements in growth efficiency involve both digestibility and health improvement. In conclusion, supplementation with a combination of Bacillus subtilis and multi carbohydrase consistently improved broiler performance and energy utilization. These results support the use of complementary probiotic–enzyme strategies to enhance feed efficiency, although further research is needed to better elucidate the underlying biological mechanisms.

**Keywords:** carbohydrase; enzyme; probiotic

### 464P A blend mainly based on oregano essential oil and spices increased apparent metabolizable energy and improved growth performance in broilers

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Essential oils and spices are known for their effects on gut health and growth performance. This improvement in performance may stem from an increase in the feed's apparent metabolizable energy (AME). This would be particularly beneficial in challenging rearing conditions (poor litter quality, high stocking density, etc.). The aim of this study is to investigate whether an essential oils and spices blend (EOB, provided by Phodé) can improve the AME of a corn- and soybean-based diet under conditions of overcrowding and low litter quantity. Three hundred thirty-six male Ross 308 broiler chicks were distributed across 24 pens (14 birds/pen; 14 birds/m2) in a randomized complete block design with 2 treatments and 12 replications/treatment. A challenge was maintained throughout the 35-day trial by combining high stocking density with reduced litter quantity (3.5 kg/m2 instead of 6) in a closed house with tunnel ventilation in Thailand. T1 was a corn-soybean meal diet formulated to Ross 308 (2019) specifications across starter (0–10 d), grower (10–24 d), and finisher (24–35 d) phases. T2 included EOB at 125 g/t replacing 125 g/t of corn in the same diet formulations compared to T1. AME was determined using TiO2 (0.3%) as an inert marker. Diets containing TiO2 were provided from d 30 and excreta were collected on d 34. Feed and excreta were analyzed for gross energy (bomb calorimetry) and TiO2 concentration; AME was derived by inert marker ratio. Body weight and feed intake were recorded at each phase transition. Data were subjected to one-way ANOVA in a randomized complete block design using SAS, $p < 0.05$ was considered as significant. EOB significantly increased AME (3,209 vs. 3,127 kcal/kg; $p < 0.001$) and energy retention (79.39 vs. 77.94%; $P = 0.003$), representing a gain of 82 kcal/kg (+2.6%). Crude protein retention was not affected (71.92 vs. 71.66%; $P = 0.720$). Over 0–35 d, supplemented birds exhibited significantly higher feed intake (3,684 vs. 3,584 g/bird; $P = 0.036$) and body weight gain (2,545 vs. 2,467 g/bird; $P = 0.004$). Feed conversion ratio was not significantly different (1.447 vs. 1.453; $P = 0.457$). EOB at 125 g/t significantly increased dietary AME by 82 kcal/kg, without affecting protein retention, suggesting a selective enhancement of energy utilization. This improvement in energy availability was associated with greater body weight gain over 35 days but surprisingly without significant effect on feed conversion ratio. This could be explained by a difference in carcass yield, likely due to a shift in the distribution of muscle versus fat on the carcass.

**Keywords:** Broilers; essential oils; apparent metabolizable energy; carvacrol; growth performance

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**465P Dose-response effects of *Macleaya cordata* extract on performance, immune response, and ileal microbiota of broilers under *Clostridium perfringens* challenge**

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Globally, the poultry sector incurs severe financial losses due to necrotic enteritis (NE). *Clostridium perfringens* (*C. perfringens*) is a spore-forming bacterium that can cause intestinal NE, which is considered one of the most prevalent bacterial infections in the poultry industry. The current study aimed to evaluate the effects of different doses of *Macleaya cordata* extract (MCE), a natural feed additive, on maintaining performance and intestinal health in broilers recovering from NE. Three doses of MCE supplementation (60, 90, and 120 ppm) were compared to the AGP group in broilers challenged with *C. Perfringens*. In a CRB, a total of 360 0-day-old Ross-308 chicks were randomly allocated to six treatments (10 replicates per treatment; six birds per replicate) as follow: T1, control, no additive or challenge (-ve control); T2, control + *C. Perfringens* (+ve control), T3, T2 + 10 ppm AGP, T4, T5, and T6 were T2 + 60, 90, and 120 ppm MCE, respectively. On day 7, all birds except T1 were orally inoculated with *C. perfringens* at the rate of 4× $10^8$ CFU/ml. Data were analyzed using one-way ANOVA followed by Tukey's test at a significance level of P<0.05. The data revealed that during the cumulative period (0-35 d), birds that received T1, T3, T4, T5, and T6 had higher average daily gain (ADG), FCR, and performance efficiency factors than those that received T2 (P<0.001). Villous height VH, crypt depth (CD), and VH: CD ratio improved in MCE-supplemented groups like the -ve control group, regardless of the dose. Moreover, the data revealed a significant increase in IgY (P<0.01) and IgA (P<0.001) for T4, T5, and T6 compared with T2, with no significant difference compared with T1. IL-1B, IL-2, IL-6, and IFN-Γ decreased significantly for T4, T5, and T6 (P<0.001) compared to T2. The MCE doses did not affect levels of the various cytokines, except for IL1, which was significantly lower at T6 than at T4 and T5. The ileal *C. Perfringens* count was significantly lower at T1 compared to all other treatments (P<0.001), while T3, T4, T5, and T6 were intermediate and had significantly lower counts compared to T2. Based on all previous results, it can be concluded that the different doses of MCE used in this trial equally restored performance, intestinal histological parameters, antibodies, immune responses, and inflammation in broilers challenged with *C. perfringens*.

**Keywords:** Broilers performance; Clostridium Perfringens; phytobiotic feed additive; microbial count; immune responses

**466P *In-ovo* Administration of Phytogenic MyoS2 Promotes Embryonic Skeletal Muscle Development via the Suppression of Myostatin and Activation of Myogenesis in Broilers**

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Embryonic muscular development is a significant factor of production efficiency in broilers. Phytogenic compounds contain multifunctional and complex bioactivities, which some of bioactive compounds represent ability to promote muscle growth and health, such as against sarcopenia. However, few studies have identified effective agents that enhance this process. This study aimed to identify a phytogenic that can inhibit myostatin (*Mstn*) transcriptional activity with *in vitro* L8 model and evaluate its effect on embryonic muscular development in broilers via *in-ovo* administration. The promoter of *Mstn* was cloned upstream of a luciferase reporter gene, and the construct was transfected into rat L8 cells to assess MyoS2-mediated regulation of *Mstn* transcriptional activity. A total of forty Arbor Acre broiler eggs were randomly assigned into four groups, including sham-control (PBS) at embryo day 10 (ED10) or embryo day 18 (ED18) and 25 mg/mL MyoS2 treatment at ED10 or ED18, followed by euthanasia on embryo day 21 (ED21). Thigh muscle weight and myofiber size based on histological analysis were used for representing muscle growth. For identification of muscular development, gene expression of myogenesis, DNA methyltransferase, energy metabolism-related genes were quantified. Comparisons between MyoS2 and PBS treatments were performed using Student's t test whereas one-way ANOVA with Tukey's post hoc analysis was used for comparisons among multiple treatments. The results demonstrated that MyoS2 significantly inhibited ($p < 0.05$) luciferase activity in L8 cells which indicated the decreasing of *Mstn* transcriptional activity. In chicken embryos, MyoS2 treatment at both administration times significantly increased ($p < 0.05$) thigh muscle weight, myofiber size, and expression of myogenic markers (*MyoD*, *Myf6*) in thigh muscle of ED21 chicks. Further, MyoS2 treatment at ED18 showed stronger effects on increase of DNA methyltransferase (*DNMT1*, *DNMT3a*, *DMNT3b*), other myogenic markers (*PCNA*, *MyoG*, *MEF2C*, *IGF1*, and *Myf6*) gene expression and reprogramming energy metabolism by modulating gene expression of *METTL21C* and *SGLT1*. These findings suggest that MyoS2 acts as a potential enhancer for embryonic skeletal muscle development in broilers and stimulates myogenic and epigenetic programming. Overall, *in-ovo* administration of MyoS2 represents a promising early-life intervention strategy to maximize muscle growth in commercial broilers.

**Keywords:** Broilers; In-ovo feeding; Myogenesis; Gut health; Phytogenic

**467P Evaluation of postbiotic, phytogenic, and essential oil combination products on broiler performance**

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As the industry continues to evaluate novel technologies that support bird performance and health, interest has grown in additive solutions containing postbiotics and phytogenics, which are generally recognized for their ability to support gut health and provide antimicrobial and antioxidant effects. The objective of this trial was to evaluate the effects of a postbiotic and essential oil compound product (PEOC; 0.8 lb/t), a quillaja saponin-based product (QS; 0.6 lb/t), a postbiotic and phytogenic (saponin-based) combination product (PPC; 1.2 lb/t), and a postbiotic and phytogenic (saponin-based) nucleus combination product (PPNC; 1.2 lb/t) on broiler performance and health in a commercial production setting. Products were evaluated as formulated, and inclusion rates were based on prior internal company trial data. A total of 864 day-old male broiler chicks (sourced according to standard commercial production practices) were placed in 4 × 4 ft floor pens at a commercial broiler integrator facility and were randomly assigned to one of four dietary treatments (12 replicates/treatment, 18 birds/replicate): PEOC, QS, PPC, or PPNC. Birds received a standard three-phase feeding program consisting of a starter phase (d 0-17), grower phase (d 17-28), and finisher phase (d 28-33). Diets were corn-soybean meal-based, provided ad libitum throughout the trial, met nutrient requirements, and contained a coccidiostat. Performance data were collected on d 0, 17, 28, and 33. Data were analyzed in R using mixed models with treatment as the fixed effect, pen as the experimental unit, and location block and house as random effects. Performance variables were analyzed using linear mixed models, and mortality was analyzed using a binomial generalized mixed model. Significance was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$. No treatment differences were detected ($P > 0.05$) during the starter or grower phases. During the finisher phase, PPC and PPNC had ($P < 0.002$) the lowest FCR (1.565 and 1.513, respectively), PEOC had the highest FCR (1.664), and QS was

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intermediate (1.587). Final BW tended ($P < 0.090$) to differ, with PPNC showing the heaviest final BW (5.42 lb) at d 33. For the overall trial, PPNC had ($P < 0.019$) the lowest FCR (1.484), QS had the highest FCR (1.532), and PEOC and PPC were intermediate (1.522 and 1.492, respectively). Mortality was not impacted ($P > 0.05$) by treatment for the overall trial. Overall, PPNC improved FCR, while early-phase performance was similar across treatments. In practice, this advantage positions PPNC as a valuable nutritional strategy for improving feed utilization, supporting its potential to enhance broiler performance under commercial conditions.

**Keywords:** broiler; phytogenic; postbiotic; saponin; gut health

### 468P The effect of feeding a supplemental sodium gluconate on performance and gut health parameters of commercial male broilers

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Our previous study (2026) indicated that a supplemental sodium gluconate could improve the growth performance of male broilers. The objective of the present study was to evaluate the sodium gluconate (SG) effects on the gut microflora, production of short chain fatty acids (SCFAs) in the gut, and biological parameters associated with gut health. Four dietary treatments were tested, each with 16 pens containing 26 day-old Ross 308 males: (1) Standard 3-phase diet with no gut health feed additives (NC), (2) NC diet + Deccox at 0.5kg/MT (PC), (3) NC diet + SG at 1.0 kg/MT in all dietary phases (SG1), and (4) NC + SG at 2.0 kg/MT in all dietary phases (SG2). All treatments were reared on previously used litter and vaccinated at hatch with a 2x dose of COCCIVAC®-B52. On D21 and D42, 1 bird from each pen was randomly selected for collection of cecal and ileal digesta as well as ileal epithelium for the subsequent analysis of organic acid concentrations, microbiome, and gene expressions of cytokines (IL-10 and IL-12) and tight junction-related proteins such as Claudin-3 (CLA3), Occuldin (OCLN) and Zonula Occludens 2 (ZO2). Data were analyzed using the MIXED procedures of SAS®. On D21, the cecal propionic acid concentration was significantly lower ($P < 0.01$) for PC, SG1 and SG2 than NC. The concentrations of organic acids in ileum were not affected by dietary treatments on D21 or D42. The microbial population did not differ ($P > 0.10$) by treatments in ileum either on D21 or D42. In cecum, the population of Genus *Blautia* on D42 was significantly higher ($P < 0.05$) for NC than the other three treatments while dietary treatments did not affect total bacterial population. The gene expression of IL-10 or IL-12 in ileal epithelium was not affected by dietary treatments ($P > 0.10$) either on D21 or D42. On D21, the gene expression of CLA3 in ileum was higher for PC and SG1 than NC ($P < 0.05$) while SG2 was intermediate. On D42, the ileal gene expression of CLA3 and OCLN was significantly higher for SG1 and SG2 compared to NC ($P < 0.05$). These results indicate that SG supplementation could improve the growth performances of broilers as previously reported through optimizing the gut barrier function by upregulating the gene expressions of proteins associated with tight junction in immunologically challenged broilers. Additional investigations will be required to determine the optimal feeding rate of sodium gluconate and to further scrutinize the mode of action.

**Keywords:** sodium gluconate; broiler; tight junction

### 469P Production of value-added chicken egg through dietary manipulation of selected herbal plants

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There is a possibility to modify the eggs nutrients, especially omega-3 fatty acids and lower the cholesterol. This study aimed to investigate the effects of incorporating *Moringa oleifera* leaf (MOL) meal, *Linum usitatissimum* (flaxseed), and *Trigonella foenum-graecum* (fenugreek) into layer diets to improve egg quality, yolk fatty acid composition, and cholesterol levels. A total of 168 laying hens were randomly assigned to seven dietary treatment groups: T1 (Control), T2 (0.5% Flaxseed + 1% MOL), T3 (1% Flaxseed + 1% MOL), T4 (1.5% Flaxseed + 1% MOL), T5 (0.5% Fenugreek + 1% MOL), T6 (1% Fenugreek + 1% MOL), and T7 (1.5% Fenugreek + 1% MOL). The birds are distributed to 7 treatments with 4 replications following CRD. The feeding trial was conducted on 25 weeks old of Fayoumi laying chicken for 16 weeks. Egg quality parameters were assessed, and yolk cholesterol and fatty acid profiles were analyzed using gas chromatography (GC-8890, Agilent, USA). The results indicated no significant differences in general egg quality traits (weight, length, width, shell thickness, and shape index) among treatment groups. However, birds supplemented with 0.5% flaxseed or fenugreek alongside 1% MOL for a longer period of time ($16^{th}$ weeks) produced eggs with significantly improved weight, increased omega-3 fatty acid content, and reduced yolk cholesterol compared to the control group. The eggs were collected for analysis after the trial. Fatty acids were analyzed to know the changed in fatty acids especially Poly Unsaturated Fatty Acids, PUFA (Linoleic Acid, A Linolenic Acid, Eicosapentaenoic Acid, EPA and Docosahexaenoic Acid, DHA) after addition of dietary feed additives. The extraction procedure of egg samples were done at the lab and the fatty acids profile of egg yolk were determined using Gas Chromatography, GC Agilent 8890, PRC, BLRI, Savar, Dhaka. The chromatograms were compared with the fatty acids standard from animal sources containing 37 components. Addition of flaxseed and fenugreek in the diet (0.50 to 1.00%) with 1.00% MOL can increase PUFA up to 15 to 20% and the cost of an egg can be saved 3 to 4 taka compared to the commercial value added eggs are in the market.

**Keywords:** Value added chicken egg; Omega 3 fatty acids; Flax seed; Moringa ; Fenugreek

### 470P Effects of agProFito on the quality of fresh and stored eggs

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Agroceres Multimix developed agProFito, a *blend* of essential oils, botanical extract, and prebiotics with antimicrobial and antioxidant effects that improves the quality of fresh and stored eggs. The aim of this study was to assess the effects of agProFito on fresh and stored eggs quality produced by laying hens. The trial was conducted at Agroceres Multimix Technology and Innovation Center. A total of 672 Hy-Line W-80 laying hens, 18 weeks of age, were randomly allocated to two dietary treatments, with 24 replicates of 14 birds each. The experimental period lasts for 20 weeks. The experimental diets included whole wheat grain, rice bran, and poultry viscera meal, in an attempt to experimentally reproduce a condition of intestinal dysbiosis. Both diets were formulated to maintain similar nutrient levels, varying only by the inclusion of agProFito. The dietary treatments were defined as follows: 1) Negative control (NC), consisting of a basal diet formulated to meet the nutritional requirements of laying hens and without agProFito; and 2) agProFito supplementation, consisting of the NC diet added with 1.0 kg/t of the *blend*. A total of 48 eggs per treatment or two eggs per replicate of every cycle (4 weeks)

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were randomly collected and analyzed in the same day using a *Digital Egg Tester* to assess fresh egg quality. For the evaluation of stored egg quality, six eggs per treatment were collected and stored for 30 days prior to lipidic oxidation analysis (TBARS analysis). In addition, 30 eggs per treatment were collected and stored for 30 days (*shelf-life* analysis) for egg quality assessment using the *Digital Egg Tester*. A split-plot ANOVA was performed to analyze the fresh egg quality data, whereas a one-way ANOVA was used to evaluate the TBARS and *shelf-life* parameters of the stored eggs data. Mean comparisons were performed using t-test, with statistical significance set at $P < 0.05$ and tendency set at ($P<0.10$). Regarding fresh egg quality, supplementation of agProFito in the NC diet resulted in a 1.6% reduction in yolk diameter ($P<0.001$), a 1.9% increase in yolk index ($P<0.05$), and tendency toward a 2.7% increase in shell strength ($P<0.10$). With respect to stored egg quality, there was a tendency toward lower lipid oxidation ($P < 0.10$) in the agProFito group (20.6% reduction), along with an 8.9% increase in the yolk index of stored eggs ($P<0.05$). Additionally, agProFito markedly lowered yolk membrane rupture (30% in NC vs. 3.3% with agProFito). These findings demonstrate beneficial effects of agProFito on improving the quality of both fresh and stored eggs.

**Keywords:** Egg quality; fresh; oxidation; shelf-life; stored

### 471P The effect of feeding *Lactobacillus reuteri* and *Escherichia coli Nissle* on growth performance of broiler chickens

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There is a pressing need for innovative strategies to improve feed efficiency, reduce reproduction costs, and limit the therapeutic use of antimicrobials, which contribute to antimicrobial resistance and pose significant public health concerns. Probiotics have emerged as promising alternatives to antibiotics by enhancing broiler chicken health and performance. This study aimed to evaluate the antimicrobial activity and effects on poultry performance of two probiotic strains, *Lactobacillus reuteri* (LR) and *Escherichia coli* Nissle (EcN). The antimicrobial activity of LR and EcN was assessed based on their ability to inhibit *Salmonella Typhimurium*. The probiotic bacteria were encapsulated using a wall material composed of citrus peel pectin, maltodextrin, and gum Arabic in a ratio of 1:1:2.5, then freeze-dried before incorporation into experimental diets. A total of 300 one-day-old broiler chicks were assigned to a completely randomized design with four dietary treatments: a standard corn-soy (SCS) negative control, SCS plus wall material (positive control), SCS + 100 ppm LR, and SCS + 100 ppm EcN. The feeding trial lasted six weeks, with feed and water provided *ad libitum*. Birds were weighed weekly, and body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) were recorded. Data were analyzed using one-way ANOVA with the General Linear Model procedure of SAS, and means were separated using the least squares means method. Both EcN and LR exhibited strong antimicrobial activity against *S. Typhimurium*, as evidenced by substantial inhibition zones. During weeks 1–3, cumulative feed consumption was higher in birds fed EcN or LR compared to other treatments. Birds receiving EcN showed greater BWG from hatch to three weeks of age than those in other groups. However, neither EcN nor LR significantly affected FCR. Additionally, birds fed LR and those receiving the wall material had significantly higher drumstick and abdominal fat weights compared to other treatments. In conclusion, both EcN and LR demonstrated significant antimicrobial activity against *S. Typhimurium*. While both probiotics increased feed consumption, only EcN improved body weight gain in broiler chickens.

**Keywords:** broiler chickens; Lactobacillus reuteri; Escherichia coli strain nissle; growth performance; antimicrobial activity

### 472P Cecal Colonization by Salmonella Enteritidis in Commercial Layer Pullets Fed With a Mannan-Rich Fraction

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Non-typhoidal Salmonella, including serovar Enteritidis, remains a leading cause of bacterial foodborne illness globally and continues to represent an important food safety concern associated with egg production systems. Nutritional interventions such as yeast-derived prebiotics have been investigated as dietary strategies to influence gastrointestinal microbial ecology and host–pathogen interactions in poultry. The objective of this study was to evaluate cecal colonization by Salmonella Enteritidis (SE) in developing commercial layer pullets supplemented with a mannan-rich yeast cell wall product. A total of 156 Salmonella-negative day-old W-36 pullets were reared under BSL-2 isolation and randomly assigned to one of two dietary treatments: (1) unsupplemented corn-soy basal diet (CON) or (2) corn–soy basal diet supplemented with 400 g/ton mannan rich fraction (MRF: Actigen®, Alltech, Inc). Birds were fed experimental diets from placement and orally challenged at 21 days of age with a nalidixic acid-resistant SE strain at a dose of $1.8 \times 10^9$ CFU per bird. Cecal contents were collected from 26 birds per treatment at 6, 10, and 14 days post-challenge for isolation and enumeration of SE using selective plate counting methods. Data were subjected to one-way ANOVA (GraphPad Prism), and treatment means were separated using Tukey's multiple comparisons test at $P \leq 0.05$. Cecal SE colonization was detected in both groups at 6 days post-challenge with comparable infection intensities. Salmonella concentrations declined over time in both groups; however, birds receiving MRF exhibited significantly ($P < 0.05$) lower cecal SE counts at 14 days post-challenge compared with control. Mean SE concentrations at 14 days were 2.67 log10 CFU/g in the MRF group compared with 4.37 log10 CFU/g in the control. Additionally, the rate of decline in infection intensity was 50% greater in birds supplemented with MRF compared to the control. Under the experimental conditions of this study lower cecal colonization of SE were found in experimentally challenged layer pullets supplemented with MRF at 400 g/ton. These results suggest that the inclusion of MRF may mitigate intestinal SE colonization in layer pullets.

**Keywords:** Salmonella enteritidis; Mannan-rich fraction; Layers; Pullets

### 473P Antibiotic resistance, biocide susceptibility, and genomic characterization of *Klebsiella* spp. from broiler chicken farms

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The poultry industry uses antimicrobial interventions to reduce bacterial contamination, but *Klebsiella* susceptibility to these compounds remains poorly understood. This study compared antibiotic resistance, biocide susceptibility, and genomic determinants of *Klebsiella* spp. from broiler farms, bovine milk, and surface water to determine their relationships. *Klebsiella* spp. were isolated from litter and wild bird droppings on broiler farms (n=17), surface water from dairy farms (n=24), and bovine milk (n=9). Their susceptibility to 14 antibiotics and to 15 biocides were determined, and isolates underwent whole genome sequencing (WGS) and analyses. As expected, all isolates showed intrinsic resistance to ampicillin. Multidrug resistance occurred in poultry and surface water *K. pneumoniae*, including resistance to B-lactams, tetracycline, sulfonamides, tri-

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methoprim-sulfamethoxazole, chloramphenicol, and quinolones. Biocide susceptibility among broiler isolates (n=17) varied widely. Halogen compounds showed inhibition at high concentrations (1,3 dibromo 5,5 dimethylhydantoin inhibited 100% at 2496 µg/mL; sodium hypochlorite inhibited 71% at 1576 µg/mL and 29% at 3152 µg/mL). Alkaline/phosphate compounds showed defined thresholds (sodium metasilicate inhibited 47% at 30,160–60,320 µg/mL; trisodium phosphate inhibited 100% at 18,856 µg/mL). The arsenical compound arsenite displayed a broad inhibition range (12–29% across 28–112 µg/mL). Quaternary ammonium compounds were most active. Cetylpyridinium chloride and hexadecyltrimethylammonium chloride inhibited 100% at 20–40 µg/mL; hexadecyltrimethylammonium bromide inhibited 94% at 40 µg/mL; dodecyltrimethylammonium chloride and benzalkonium chloride inhibited 29–59% within narrow ranges. Organic acids showed compound-specific effects. Acetic acid, citric acid, and acidified sodium chlorite inhibited 100% at 1640, 6312, and 640 µg/mL, respectively while lactic acid inhibited 71% at 3776 µg/mL. Peroxyacetic acid inhibited 82% at 440 µg/mL. WGS identified diverse resistance genes including *blaSHV*, *blaLEN-16*, *blaOKP-A5*, *CMY-190*, *fosA5*, *aadA2*, *qacEΔ1*, *sul1*, and *tetA*. Phylogenetic analysis revealed clustering among isolates from the same origins, while *Klebsiella* isolates from broiler farms showed dissimilarity from those of other sources. No carbapenemase or extended spectrum beta-lactamase producing strains were detected among the studied isolates; however, our findings indicate that due to potential public health concerns, large-scale investigations of *Klebsiella* susceptibility to antibiotics and biocides are warranted in broiler production.

**Keywords:** Broiler chicken; Klebsiella; Antibiotics; Biocides; Genomic comparison

### 474P Food Safety Risks and Antimicrobial Resistance of *Salmonella*, *E. coli*, and *S. aureus* in Broilers, Layers, and Water Sources

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Foodborne pathogens in broilers, layers, and associated water sources represent an important food safety and public health concern. This study evaluated the occurrence of *Salmonella* spp. and *Staphylococcus aureus* in pasture-raised broilers and caged layers. It also characterized AMR profiles of *Salmonella* spp., *Escherichia coli*, and *S. aureus* recovered from cloacal swabs and egg-rinse samples from birds supplied with well or municipal water. A cross-sectional comparative study was conducted using 2 poultry housing systems, pasture-raised broilers, and caged layers. Cloacal swabs (n = 80) were collected from 40 pasture-raised broilers and 40 caged layers. Additional samples included well-water cloacal swabs (WCS), municipal-water cloacal swabs (MCS), well-water egg rinses (WE), and municipal-water egg rinses (ME). Bacteria were isolated using tryptic soy broth and CHROMagar *Salmonella*, CHROMagar ECC for *E. coli*, and CHROMagar *Staph aureus*. Eggs were rinsed in phosphate-buffered saline before plating. Presumptive isolates from WCS, MCS, WE, and ME were tested by Kirby-Bauer disk diffusion. Prevalence data were analyzed by chi-square test, and resistance data were summarized as percentages of resistant isolates. *S. aureus* was detected in 22.5% of pasture-raised broilers and 12.5% of caged layers, whereas *Salmonella* spp. were detected in 17.5% and 25.0%, respectively. *S. aureus* prevalence differed between housing systems (P = 0.04), whereas *Salmonella* prevalence did not (P = 0.18). *Salmonella* spp. showed high resistance to ampicillin (88.2%) and amoxicillin-clavulanic acid (88.2%), followed by chloramphenicol (55.9%). Resistance to tetracycline was low (5.9%), and no resistance was observed to ceftazidime, meropenem, gentamicin, ciprofloxacin, or trimethoprim-sulfamethoxazole. *E. coli* isolates were fully susceptible to all antibiotics tested. *S. aureus* showed the highest resistance burden, with resistance to penicillin (94.1%), clindamycin (76.5%), erythromycin (35.3%), oxacillin (29.4%), linezolid (29.4%), tetracycline (11.8%), and gentamicin (5.9%). No resistance was detected to doxycycline, ciprofloxacin, or trimethoprim-sulfamethoxazole. Poultry housing system influenced *S. aureus* occurrence, whereas *Salmonella* occurrence was comparable between systems. *S. aureus* carried the greatest resistance burden, *Salmonella* showed marked resistance to beta-lactams, and *E. coli* remained pan-susceptible. These findings identify poultry and associated water sources as potential reservoirs of foodborne pathogens and AMR and support targeted biosecurity, water quality management, sanitation, and antimicrobial stewardship to reduce transmission and food safety risks.

**Keywords:** Poultry; Salmonella; Escherichia coli; Staphylococcus aureus; Antimicrobial resistance.

### 475P Comparing drug-resistant strains of *Salmonella* Infantis and *Salmonella* Schwarzengrund for their efficacy to colonize ceca and disseminate internal organs in growing turkeys

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Multidrug-resistant (MDR) strains of *Salmonella* Infantis and *S.* Schwarzengrund are circulating in turkey production, posing a potential risk of foodborne outbreaks. Controlling these emerging serovars requires understanding their cecal colonization and systemic dissemination patterns. Developing short-duration turkey infection models is essential for testing colonization patterns and evaluating effective intervention strategies. The objective of the study was to compare the cecal colonization and early dissemination of *Salmonella* Infantis and *Salmonella* Schwarzengrund inoculated individually or in combination in growing turkeys. Two separate experiments were conducted using a total of forty-eight 5-week-old growing turkeys. In each experiment, 24 turkeys were assigned to 4 treatments (Negative Control (NC) - unchallenged; S1, *S*. Infantis; S2, *S*. Schwarzengrund; S3, *S*. Infantis and *S*. Schwarzengrund in combination) with n= 6 birds/treatment. Turkeys were challenged with the respective inoculum (5 log10 CFU/bird directly to crop) and euthanized 48 h later for collection of ceca, liver, spleen, crop, and breast muscle (Pectoralis major). Cecal samples were homogenized, serially diluted, and plated onto Xylose Lysine Deoxycholate (XLD) plates for quantitative recovery of strains. To detect dissemination of strains to internal organs, samples were also enriched in selenite cysteine broth. Cecal *Salmonella* counts (log10 CFU/g) were analyzed by one-way ANOVA, and organ positivity was analyzed by Fisher's exact test (A=0.05). Cecal colonization did not differ among S1, S2, and S3 treatments ($P$>0.05, mean ± SD log10 CFU/g: S1, 4.94 ± 0.87; S2, 4.93 ± 0.55; S3, 4.79 ± 0.81). Systemic dissemination did not differ statistically across strains in any organs ($P$>0.05). Crop positivity of strains in S1, S2, and S3 were 75%; 83% and 83%, respectively ($P$>0.05). Likewise, liver recovery rates of strains were 67%, 83%, and 50% in the S1, S2, and S3 treatments, respectively ($P$>0.05). Spleen recovery of strains was 42%, 58%, and 58% for S1, S2, and S3, respectively ($P$>0.05). Breast muscle recovery was low in all treatments (0–17%) and did not differ from NC ($P$>0.05), indicating limited rapid migration to edible tissues within 48 h. Results reveal that *S.* Infantis and *S.* Schwarzengrund exhibited comparable early colonization and dissemination patterns in 5-week-old growing turkeys. A combination challenge can be used for short-duration intervention screening when there is no evidence of single-serovar dominance.

**Keywords:** Salmonella Infantis; Salmonella Schwarzengrund; turkeys; cecal colonization; organ dissemination

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## Poster Session IV

### 476P Specialty protein sources in starter feeding program have transient effects on cecal metabolomics in broiler chickens

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Limitations of soybean meal (**SBM**) in starter diets have driven the development of specialized programs incorporating functional specialty protein ingredients (**SPI**), including processed vegetable and animal proteins. This study evaluated the effects of SPI on cecal metabolomic profiles in broilers. Day-old male Ross 708 chicks were allocated to 48 floor pens (35 birds per pen), with 8 replicate pens per dietary treatment. Diets were formulated with partial replacement of SBM using enzymatically treated SBM (**ETSBM**), soy protein concentrate (**SPC**), pork meal (**PM**), or black soldier fly larvae meal (**BSFLM**). A corn–SBM diet served as the negative control (**NC**), while a positive control (**PC**) included an antibiotic growth promoter. One bird per pen was sampled for metabolomic analysis. Cecal digesta were collected at d 10 and 28 (one bird per pen) and analyzed by C18 reversed phase and hydrophilic interaction chromatography (**HILIC**). Untargeted profiling was performed using Compound Discoverer 2.1 (Thermo Scientific) with a 5-ppm mass tolerance for raw signal processing and peak detection. Processed data were normalized and log transformed in MetaboAnalyst 6.0 prior to statistical analysis. Principal component analysis, multivariate tests, and pathway enrichment analysis were applied to determine treatment effects relative to NC. Permutational multivariate analysis revealed marked dietary effects at d 10 (C18: $F = 71.7$, $R^2 = 0.895$, $P = 0.001$; HILIC: $F = 138.4$, $R^2 = 0.943$, $P = 0.001$). PM and BSFLM altered the greatest number of pathways (30 and 31, respectively), followed by SPC (26), ETSBM (16), and PC (4). The strongest individual enrichments (FDR < 0.05) included arginine–proline, porphyrin, and nicotinate/nicotinamide metabolism in C18, and vitamin B6, phenylalanine, and histidine metabolism in HILIC. By d 28, permutational multivariate analysis did not detect any treatment effects ($P > 0.05$). These findings demonstrate that specialty protein sources induce pronounced, diet-specific remodeling of the cecal metabolome during the starter phase, with PM and BSFLM producing the broadest effects. The disappearance of differences and pathway enrichment by d 28 indicates that these metabolic shifts are transient and age dependent.

**Keywords:** soybean meal replacement; pathway enrichment; pork meal; black soldier fly larvae meal

### 477P Linoleic acid:alpha-linolenic acid ratios associated with vitamin E levels on bone quality of quail breeder hens

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Nutritional factors, such as the balance between polyunsaturated fatty acids and antioxidants, may directly influence bone metabolism and mineral deposition. The ratio between linoleic acid (LA) and alpha-linolenic acid (LNA) may modulate inflammatory processes and mineral absorption, while vitamin E protects against oxidative stress. Therefore, this study aimed to evaluate the effects of vitamin E in diets containing two LA:LNA ratios on bone quality parameters in quail breeder hens through the analysis of the Seedor index, length, relative weight, ash content, calcium (Ca), and phosphorus (P) of the tibia and femur. A completely randomized design was used in a 2 × 3 factorial arrangement, with two LA:LNA ratios (9.29:1 and 13.75:1) and three vitamin E levels (25, 200, and 250 mg), with 10 replicates. Data were subjected to analysis of variance in a factorial scheme, and means were compared using Tukey's test. In the femur, the 9.9:1 ratio increased relative weight ($P = 0.011$) and ash content ($P < 0.001$), whereas the 13.75:1 ratio showed a higher phosphorus content ($P = 0.045$). Vitamin E levels influenced relative weight ($P = 0.013$), ash content ($P < 0.001$), and phosphorus ($P = 0.031$), with greater mineral deposition observed at 200 mg. A significant interaction between factors was observed for relative weight ($P = 0.029$), Seedor index ($P = 0.017$), ash content ($P = 0.001$), and phosphorus ($P = 0.043$), indicating that bone response depends on the combination of fatty acid ratio and vitamin E level. The combination of 9.29:1 and 200 mg vitamin E promoted the highest Seedor index and ash content. For the tibia, the LA:LNA ratio affected relative weight ($P = 0.006$), ash content ($P < 0.001$), and phosphorus ($P = 0.045$), with better results for the 9.29:1 ratio regarding relative weight and ash content; however, this same ratio showed lower phosphorus content. Vitamin E levels significantly influenced ash content ($P < 0.001$) and phosphorus ($P = 0.031$), with 200 mg associated with greater mineral deposition. There was a significant interaction for ash content ($P = 0.001$) and phosphorus ($P = 0.043$) in the tibia. The interaction analysis showed that within the 9.29:1 ratio, 200 mg of vitamin E resulted in the highest ash content (18.08). For phosphorus, the 13.75:1 ratio combined with 250 mg increased the content to 2.01; however, this increase was not consistently accompanied by greater mineral deposition. It is concluded that the 9.29:1 LA:LNA ratio associated with 200 mg of vitamin E improves bone mineral deposition and increases the Seedor index in quail breeder hens.

**Keywords:** Bone mineralization; Antioxidant nutrition; Seedor index

### 478P Effect of *In-ovo* Feeding on the Market-age Growth Performance and Muscle Development of Broilers: A Systematic Review and Meta-analysis

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*In-ovo* feeding technology with various nutrients or bioactive compounds has emerged as a promising strategy to stimulate early embryonic intestinal and muscular development in poultry. However, results regarding the long-term efficacy of this early intervention remain inconsistent. Since most *in-ovo* feeding studies focus on immediate post-hatch parameters, there is lack of consensus on whether these early developmental advantages translate into economic benefits at market age. This study aimed to quantitatively synthesize available evidence to determine if *in-ovo* feeding significantly improves market-age growth performance and meat yield in commercial broilers. A systematic literature search was conducted across Web of Science, PubMed, Scopus, and Centre for Agriculture and Bioscience International databases without date restrictions. Inclusion criteria required studies to be randomized controlled trials that evaluate *in-ovo* administration of nutrients or bioactive compounds in commercial broilers with a sham-injected control or non-injected control. Furthermore, studies had to report growth performance or carcass characteristics at market age (day 35 or day 42). Data extraction and risk of bias assessment were performed independently. Statistical analysis was conducted using a random-effects model to address methodological heterogeneity which was assessed using the I-squared statistics. Preliminary screening of literature yielded 1,451 initial records across the selected databases. Current data synthesis, which includes 27 articles, indicates a positive overall trend regarding the long-term efficacy of *in-ovo* feeding. Specifically, pooled analysis shows early interventions significantly ($p < 0.05$) improve market-age body weight and optimize the overall feed conversion ratio compared to the sham-control groups. Although a smaller number (7 articles) of studies regarding carcass characteristics were observed, pooled analysis shows early interventions significantly ($p < 0.05$) enhanced meat yield, reinforcing the link between embryonic ma-

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nipulation and late-stage muscle accretion. Overall, the present results suggest that the early physiological programming induced by *in-ovo* feeding can translate into significant late-stage economic benefits. Understanding these long-term dynamics would shed light on developing more precise early-nutrition strategies for commercial hatchery applications.

**Keywords:** Broilers; In-ovo feeding; Growth performance; Meta-analysis; Early-nutrition

### 479P Comparison of Metabolizable and Net Energy Systems Using Different Metabolic Weight Exponents ($W^{0.75}$ vs $W^{0.70}$ ) in Modern Broilers: Effects on Growth Performance and Energy Partitioning Under Different Dietary Profiles

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The objective of this study was to compare the predictive ability of Metabolizable Energy (ME) and Net Energy (NE) systems, using two metabolic weight exponents ($W^{0.75}$ and $W^{0.70}$), on growth performance and energy partitioning in modern broilers under different dietary profiles (high protein, HP; low protein, LP; and high fat, HF). It was hypothesized that the NE system, particularly with $W^{0.70}$, would provide a more biologically accurate estimation of energy utilization. A total of 360 one-day-old Ross 308 male broilers were allocated to a 3×3 factorial arrangement in a completely randomized design, with three energy systems (ME, NE75, NE70) and three diet types, resulting in 9 treatments with 8 replicates each. Diets were formulated to be isocaloric within each system and offered ad libitum for 21 days. ME diets were based on AMEn values following NRC (1994) and Aviagen recommendations, while NE diets were formulated using efficiency coefficients (k) derived from Moscoso (2020). Energy partitioning was assessed using the comparative slaughter technique with initial and final body energy determined at days 7 and 21, respectively, and energy retention calculated accordingly. Data were analyzed using two-way ANOVA to evaluate main effects of energy system (ES), diet type (DT), and their interaction. In general, no significant ($p>0.05$) ES × DT interactions were observed. On the other hand, significant effects ($p<0.05$) of ES on average daily gain (ADG) and feed conversion ratio (FCR), with ME achieving a lower FCR (1.295) compared to NE75 (1.362) and NE70 (1.357) were observed. Diet type also significantly ($p<0.05$) influenced performance, with HP diets yielding the highest ADG (57.94 g/d). Overall, the ME system resulted in superior productive performance over the NE system, either expressed as NE75 or NE70; the proposed NE system with $W^{0.70}$ did not demonstrate practical advantage over the traditional $W^{0.75}$. Additionally, diet composition had a greater impact on performance than the energy evaluation system. These findings suggest that, despite the theoretical benefits of NE in representing biological energy utilization, it did not translate into improved growth performance under the conditions of this study.

**Keywords:** Net Energy; Broiler; Metabolizable Energy; Feed Eficency; Metabolic Weight

### 480P Crude protein level determines the impact of raw potato starch on amino acid digestibility during acute phase *Eimeria acervulina* challenge

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We previously reported that raw potato starch (RPS) inclusion increased FCR in reduced crude protein (RCP) diet but had opposite effect in standard CP (SCP) diets in *Eimeria acervulina*-challenged (E) broilers. The objective of this 35-day study is to determine how dietary crude protein (CP) level, raw potato starch, and *E. acervulina* challenge interact to influence nutrient utilization, and gut health in broiler chickens. A total of 1,056 male broiler chicks were allocated to eight treatments (2×2×2 factorial) with six replicates per treatment: CP (standard vs. reduced level), RPS (0 vs. 40g/kg), and E (no or yes). The resulting treatments, in order are: SCP, SCP-RPS, RCP, RCP-RPS, SCP+E, SCP-RPS+E, RCP+E, and RCP-RPS+E. Challenged birds were orally gavaged with a solution of 50,000/ml *E. acervulina* on d 12. On d 18, five birds per pen were euthanized and digesta samples from the ileum were pooled for amino acid (AA) digestibility and jejunal digesta for pH analysis. Spleen and bursa were collected for inflammatory genes expression analysis. Data were analyzed as a three-factor mixed model. There was no three-way interaction observed for AA digestibility. There was a CP × RPS ($P < 0.05$) for all AA except Cys, Gly, and Ser, whereby inclusion of RPS in RCP diets significantly reduced the digestibility of AA compared SCP. In addition, a CP × E ($P < 0.05$) was observed for Ala, Arg, Glu, His, Ile, Leu, Phe, Pro, and Val, where AA digestibility declined in RCP+E treatment. Digesta pH in the jejunum was significantly reduced by *E. acervulina* challenge ($P < 0.01$) but increased in the RPS treatments ($P < 0.05$). There was a significant CP × RPS interaction in the relative expression of pro-inflammatory cytokine gene IFN-Γ ($P = 0.05$), being upwardly expressed in the SCP-RPS+E treatment and downwardly expressed in the RCP-RPS+E treatment. In conclusion, RPS inclusion and RCP diets may create a starch-protein mismatch that reduces AA digestibility, and pro-inflammatory gene regulation during acute phase following *E. acervulina* challenge.

**Keywords:** Raw potato starch; reduced crude protein; E. acervulina; amino acid digestibility; pro-inflammatory genes

### 481P Stepwise replacement of soybean meal with canola meal in nutrient-adequate diets reduce growth performance, dependent on environmental stress

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We have reported effects of partial replacement of soybean meal (SBM) with canola meal (CM) in broiler chicken diets in various settings. Although both are oilseed meals with similar nutrient profiles, CM contains higher fiber and lower crude protein (CP), which may influence nutrient utilization, especially under environmental stress. So, we hypothesized that partially replacing SBM with CM in a nutrient-adequate diet would depress broilers' growth performance and increase gut permeability dependent on cyclic heat (CH) challenge. A total of 1,200 male broiler chicks were randomly assigned to three diets in either a thermoneutral (TN) or a CH room, with eight replicates per treatment. Diets consisted of 1) SBM control, 2) SBM partially replaced with 50 g/kg CM, and 3) SBM partially replaced with 100 g/kg CM. Birds in the CH room were subjected to seven hours of elevated temperature of 93°F (34°C) to imitate a hot summer day between 10 am – 5 pm from d 14 to 42, whereas the birds in the TN room were kept in breeder-recommended temperature curve. Starter d0-10, grower d10-29, and finisher d29-42 diets were fed. Body weight (BW) and feed intake (FI) were measured on d 10, 14, 22, 29, and 42. Serum FITC-d assays were done on d 21 and 40 for gut permeability. The data collected were analyzed using one-way ANOVA. Before cyclic heat, FI and WG decreased linearly as CM increased ($P < 0.05$) at d10 and d14, respectively. For the grower phase, WG and FI decreased quadratically and linearly, respectively ($P < 0.05$) as dietary CM increased for birds in the TN room. For birds in the CH room, FI and FCR tended ($P < 0.10$) to decrease as dietary CM increased. For the finisher phase, WG tended to decrease ($P = 0.073$) linearly, but decreased ($P < 0.01$) quadratically as dietary CM level increased for birds in the TN and CH rooms, respectively. The FI decreased, and FCR increased ($P <$

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0.05) linearly as dietary CM increased for birds in the CH room, but no diet effects for birds in the TN room. On d 42, BW tended (P = 0.056) to decline linearly as CM increased for birds in the TN room, whereas BW and FI decreased, and FCR increased linearly (P < 0.01) as dietary CM increased for birds in the CH room. There was no treatment effect on the serum FITC-d level in either room. In conclusion, a stepwise replacement of SBM with CM in the TN room marginally affected growth performance but had a more pronounced effect in the CH room during the finisher phase. Because the effect was more marked in the later growth phase, this may be attributed to prolonged heat exposure, higher metabolic heat challenge in older birds, or both. Possible outcomes of the treatments on nutrient utilization and immune response will be further examined.

**Keywords:** Soybean meal; Canola meal; cyclic heat stress; thermoneutral; gut leakage

### 482P Impact of reducing crude protein in diets of broiler chickens on nitrogen excretion and emissions: Insights from a meta-analysis

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Regulating nitrogen excretion and emissions is critical in poultry industry, causing damage in animals, humans and environment. Despite several individual studies, there is lack of clarity whether reducing crude protein (RCP) influence directly nitrogen excretion (NE) and emissions in the form of nitrous oxide (N2O), ammonia (NH3) and CH4 (methane). The aim of the investigation was to evaluate the impact of reducing crude protein in diets of broiler chickens on nitrogen excretion and emissions using a meta-analysis. Studies published between 2000 and 2020 were systematically searched using PRISMA guidelines from Scopus, Pubmed and Web of Science databases. In the current study, fifty-seven of the 845 studies were extracted according to inclusion criteria process. Standardized mean difference (SMD) and corresponding 95% confidence interval (CI) were estimated using a random effects model. Inconsistency index was used to analyze heterogeneity. Every evaluation was performed with R program language. The results indicated that RCP reduced NE (SMD = − 0.23; 95% CI = -0.33 to -0.12), N2O (SMD = − 0.37; 95% CI = -0.45 to -0.31), and NH3 (SMD = − 0.35; 95% CI = -0.53 to -0.16) while RCP no showed an impact to CH4 (SMD = 0.05; 95% CI = -0.15 to 0.24). Overall, the reduction of crude protein below the requirements may diminish the pollution in conventional broiler chicken production systems, possibly due to the amount of nitrogen that broiler chickens cannot metabolize efficiently. In conclusion, reducing crude protein in broiler chicken diets is a good nitrogen emissions mitigation alternative without affecting broiler chicken productive performance.

**Keywords:** Meta-analysis; Crude protein; Broiler chickens; Nitrogen excretion; Nitrogen emission

### 483P An updated dynamic mechanistic model of male turkey growth and metabolism

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Mechanistic models have the potential to describe poultry growth based on biological processes while accounting for interactions among genotype, nutrition, and management. While several effective mechanistic poultry growth models exist, many are behind paywalls, exist mainly in the commercial space, or represent quasi-mechanistic approaches. The most recent fully reported mechanistic model for turkeys uses exponential kinetics to simulate fractional synthesis and degradation rates of body protein and fat, which may generate instabilities under a high nutrient supply. In this study, we updated the mechanistic turkey growth model by replacing exponential kinetics with Michaelis-Menten kinetics within the previously published framework, aiming to improve its stability. Similar to the original model, the updated model contained state variables representing eleven body composition pools (protein, water, and ash in carcass, viscera, and feather, and lipid in carcass and viscera), four circulating metabolite pools (amino acids, fatty acids, glucose, and acetyl-CoA), and a newly added zero-pool representing ATP. Driving input variables for the turkey mechanistic model consisted of key daily dietary nutrients, including protein, triglycerides, and carbohydrates. Twenty-two parameters related to basal fractional synthesis/degradation rate and Michaelis-Menten kinetics were estimated. A *Differential Evolution* algorithm was used to minimize the squared difference between standardized predicted body compositions and standardized observed values from dietary treatments reported in the previously published framework. Behaviour and sensitivity analysis were conducted to examine the influence of driving variables and parameters on the model outputs. Dietary protein and parameters related to basal fractional synthesis rates for carcass and visceral protein were more influential in behaviour analysis and sensitivity analysis, respectively. Model performance was evaluated using treatment means from three independent published datasets. The model captured overall growth trajectories in two of the datasets but underpredicted feed-to-gain ratio in the third. When simulating growth of modern turkey genetic lines using commercial farm data, the model overpredicted final body weight by 2.9 to 5.4 kg across three genetic lines, and required further reparameterization to represent modern genetics. Overall, the updated model in this study could simulate the trajectories of metabolism and growth in male turkeys. Future improvements could be achieved by incorporating environmental factors to account for interactions between the bird and its environment, and genetic factors to capture variation among turkey breeds and genetic lines.

**Keywords:** Male turkey; Mechanistic model; Parameter optimization; Turkey growth; Turkey metabolism

### 484P Nutritional value of dried extruded-expelled soybean meal and the effects of multi-carbohydrases on its utilization in broilers and laying hens

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Although soybean production is steadily increasing in Western Canada, the limited availability of local crushing facilities means soybeans are either exported or processed by extrusion to produce soybean expellers. Characterizing the nutritional value of these products and identifying strategies to enhance their utilization is essential for their effective inclusion in poultry diets. This research evaluated the chemical composition of dried extruded-expelled soybean meal (DESM) and investigated the potential of exogenous enzymes to improve its nutritional value for broiler chickens and laying hens. On a dry matter (DM) basis, DESM contained 44.0% crude protein, 2.7% lysine, 0.6% methionine, 8.1% crude fat, 17.0% non-starch polysaccharides (NSP), 10.3% neutral detergent fibre, and 0.37% non-phytate phosphorus. In vitro evaluation of two enzyme blends: EA (multi-carbohydrase) and EB (pectinase) demonstrated significant NSP depolymerization (P<0.05), suggesting the release of potentially bioactive glycobolites. In Study 1, the effects of EA and EB supplementation on the growth performance of broiler chickens fed DESM-based diets were evaluated using 300 one-day-old, as-hatched Ross 308 AP broilers. Birds were allocated to five dietary treatments with twelve replicate pens each, and five birds per pen. Diets included positive and negative controls (the latter formulated with reduced crude protein and metabolizable energy), and the negative control with or without EA, EB, or a double dose of EB. Overall, enzyme supplementation did not affect growth performance (P > 0.05). In Study 2, 48 Dekalb laying hens (22 weeks of age) were ran-

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domly assigned to four treatments, with 12 replicate pens per treatment and one hen per pen. Following a one-week adaptation period on a commercial basal diet (BD), hens were either maintained on the BD or fed test diets in which 30% of the BD was replaced with DESM, with or without a multi-enzyme (EA). Excreta were collected on days 6 and 7 to determine the apparent metabolizable energy (AMEn) of DESM. The AMEn of DESM was 2,801 kcal/kg DM, while 2,860 kcal/kg DM when supplemented with enzyme (P>0.05). Data were analyzed using the MIXED procedure of SAS. Overall, DESM has considerable potential for inclusion in poultry diets as a valuable source of protein and energy. While both enzymes effectively depolymerize NSP in vitro, enhancements in energy utilization in laying hens and growth performance in broiler chickens were not significant.

**Keywords:** dried extruded-expelled soybean meal; apparent metabolizable energy; enzyme; non-starch polysaccharides

### 485P Cecal microbiota profiles in broiler breeder hens on three feeding regimens

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Fast-growing broiler breeders need feed restriction to maintain steady growth curves, flock uniformity, and reproductive performance. Skip-a-day is a popular feeding method, but long fasting periods are a welfare concern. A high-fiber, high-volume diet allows for daily feeding without added nutritional value. Dietary fiber in the avian digestive tract is largely digested by cecal fermentation. 4-week-old Cobb broiler breeder hens were divided into three feeding regimens (FRs): daily (Control), skip-a-day (Skip), and daily high-fiber alternative diet (Alt). Cecal contents were collected and snap frozen at 6, 12, 20, 22, 23, 30, and 50 weeks of age (woa) (n = 4-6 hens/FR/age) for microbiota analysis using 16S rRNA amplicon sequencing. Microbial DNA was extracted with Qiagen DNeasy PowerSoil Pro kits, and the V3-V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq. Raw sequences were processed using DADA2 with Quantitative Insight into Microbial Ecology 2 software (Qiime2, 2025.7). Data were analyzed by non-parametric tests and significance was set at p<0.05. Alpha diversity was tested on Shannon Index (SI) using Kruskal-Wallis pairwise comparisons between each successive age group within each FR. Non-parametric permutational analysis of variance (PERMANOVA) based on the Weighted UniFrac distance matrix was employed for beta diversity and principal coordinates analysis (PCoA). Predicted function of the microbiota was determined by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), and the MaAsLin3 R package was used to analyze differential abundance via a linear model with FR and age as main effects including interactions. All hens reached target body weight from the Cobb broiler breeder management guidelines by 26 woa; however, the growth trajectory was steepest in Alt, most shallow in Skip, and intermediate in Control. SI decreased from 22-23 woa within the Skip group (p=0.028). Microbiota of the FRs across all ages were distinct (p=0.001) based on weighted UniFrac distances. Control vs Skip pairwise comparisons within age groups showed microbiota were distinct at 12 woa (p=0.047) and 22 woa (p=0.018), whereas Control vs Alt pairwise comparisons showed distinct microbiota at 50 woa only (p=0.024). L-alanine biosynthesis and adenine/adenosine salvage pathways were differentially abundant in Control and Skip groups overall, respectively. Hexitol and homolactic fermentation pathways were less abundant in Skip at 12 woa and 50 woa (p<0.05, q<0.1), respectively. Feeding regimen can have significant long-term effects on cecal microbiota, which ultimately determine bird health, growth, and reproductive performance.

**Keywords:** microbiota; feed restriction; skip-a-day; cecal contents; broiler breeder

### 486P Evaluation of the net energy system to predict the performance response in laying hens

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Energy is the first component in feed cost and is considered the primary limiting factor regulating feed intake. In this sense, it is essential to consider a more accurate system to express the energy feed values for laying hens. This study aims to evaluate the net energy system (NE) in feed formulation versus classical apparent metabolizable energy (AME) in practical diet formulation, and to assess the predictability of productive response. A study was conducted at the Poultry Science Laboratory at São Paulo State University (Jaboticabal, Brazil). A total of 672 Bovans White laying hens at 51 weeks old were randomly assigned to 12 treatments, with 7 replications and 8 birds per experimental unit. The birds were allocated in cages with environmentally controlled conditions. Experimental diets were formulated based on two criteria: (i) formulation based on traditional AMEn with levels of 2755, 2900, and 3045 kcal/kg, and (ii) formulation based on the NE system with three levels (1881, 1980, and 2074 kcal/kg. For each level in both groups, two diets were formulated based on two utilization efficiencies (80% and 70%). The experimental design consists of two energy systems to feed formulation, three levels of energy, and two efficiencies. The experimental period consisted of two 28-day periods (from 51 to 63 weeks). The measurements consisted of metabolic determination of the energy values by energy balance and indirect calorimetry to determine AMEn and NE values, respectively. Additionally, the performance responses of feed intake and egg production, and the calculation of egg mass and feed conversion ratio, were assessed. The data were analyzed separately in both groups (NE and AMEn) using a two-way ANOVA (3 levels of energy × 2 efficiencies), and linear regressions were performed to evaluate the predictive sensitivity. Increasing energy levels significantly improved FCR in both energy systems (P < 0.001). In the ME system, FCR was also affected by efficiency level (P < 0.001), whereas no effect of efficiency was observed under NE. No significant effects of energy levels on EM were detected in either system. Linear regressions showed a negative relationship between energy levels and FCR for both systems, with a higher coefficient of determination for NE (Rsq = 0.7971) than for ME (Rsq = 0.7631), indicating superior predictive accuracy for NE. In conclusion, the NE system achieved greater accuracy in predicting feed conversion ratio in laying hens than the ME system. Increasing dietary energy density improved feed efficiency without affecting egg mass output, supporting the potential of NE-based formulation to enhance precision in layer nutrition.

**Keywords:** Energy metabolism; Energy system; Prediction; Layers

### 487P Influence of neutral detergent fiber on the nitrogen–corrected apparent metabolizable energy of canola meal in broilers: a meta–analysis

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The transition from fossil–derived to plant–based products fuel has increased oilseed production and, consequently, the availability of oilseed by–products. In Canada, canola meal (CM) is a major co–product of oil extraction and an important protein source for livestock. Optimizing its nutritional value is essential for both the canola production chain and the livestock industry. However, variability in CM chemical composition, particularly in neutral detergent fiber (NDF), can influence its energy content especially in broilers. This study aimed to determine the relationship

between NDF and the nitrogen–corrected apparent metabolizable energy (AMEn) of solvent–extracted CM using a meta–analysis approach. A dataset comprising 5 studies (29 experimental treatments) that reported AMEn values of solvent–extracted CM, along with analyzed chemical composition data of crude protein (CP), NDF, acid detergent fiber (ADF), ether extract (EE), and ash, was compiled. This dataset was extracted from an initial collection of publications addressing nutrient and energy digestibility of canola, rapeseed, or double–zero rapeseed meals for poultry. Studies lacking ingredient composition data as well as the chemical analysis of the component listed above were excluded. All variables were adjusted to a dry matter ether extract free basis (DMEEf). Meta–regression was conducted in JMP using AMEn (kcal/kg DMEEf) as the dependent variable and NDF (% DMEEf) as the predictor. The random study effect was not significant. Other composition variables such as CP did not explain additional variation. The average AMEn (kcal/kg DM) was 1743 ± 228, and average NDF was 30.5 % DM ± 4.0. The model explained 53.6% of total variation and showed a significant negative effect of NDF on AMEn (−33.5 kcal per 1% point of NDF; P < 0.001). These findings confirm the well established negative relationship between NDF and AMEn and highlight NDF as a key determinant of CM energy value. The resulting predictive model provides a practical tool for estimating AMEn from routine chemical analyses, supporting more accurate formulation of CM based poultry diets. It also supports CM selection toward reducing NDF content.

**Keywords:** Energy; Canola; NDF; Broiler; Meta-regression

### 488P Optimizing growth and profitability of native Bangladeshi geese through Oat fodder supplementation and controlled feed restriction

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Geese are widely recognized in the poultry industry for their strong adaptability to diverse feeding and production systems, resilience under adverse environmental conditions, and relatively high feed intake capacity compared to other poultry species. This study evaluated the effects of partial replacement of concentrate feed with oat (*Avena sativa*) fodder on growth performance, lipid oxidation value, and economic efficiency of native Bangladeshi geese under semi-intensive conditions. A total of 120 White and Grey geese were randomly allocated to four dietary treatments-30 geese/treatment: T0 (200 g concentrate), T1 (180 g concentrate + 138 g oat fodder), T2 (160 g concentrate + 276 g oat fodder), and T3 (140 g concentrate + 415 g oat fodder) per bird per day. Growth was monitored from 5$^{th}$ week to 12th week; birds were slaughtered at 13$^{th}$ weeks for carcass and meat quality evaluation. All data were subjected to one-way ANOVA using R-Studio (version 2025.05.01). Growth performance was significantly improved (P < 0.05) at moderate inclusion levels, with T2 showing the highest final body weight and better muscle yield. However, no significant differences (P > 0.05) were observed in breast and thigh parameters during storage, indicating stable meat quality across treatments. Economic analysis revealed reduced feed cost per unit live weight with oat fodder inclusion. Overall, moderate inclusion of oat fodder can be considered a cost-effective and sustainable feeding strategy for native Bangladeshi geese without compromising productivity or product quality.

**Keywords:** Geese; Oats fodder; growth performance; Restricted feeding; economic efficiency

### 489P The density of energy: protein ratio in the diet affects the metabolizability of nutrients in Japanese quail (*Coturnix japonica*)

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The density of metabolizable energy (ME) and crude protein (CP), when the ME:CP ratio is constant, determines the metabolizability of nutrients, which in turn indicates potential nutritional excesses or deficiencies that affect production performance. In this study, four treatments were evaluated with a constant ME:CP ratio of 145 (T1: 3190/22, T2: 3045/21, T3: 2900/20, T4: 2758/19). The treatments were randomly assigned to sixty-four experimental units, each containing eight mixed Japanese quails of 7.31 ± 0.16 g per bird. The birds were housed in electric batteries with free access to water and feed, following the institutional guidelines for care and use of experimental animals. At 2 and 6 weeks of age, the dry matter, gross energy, and nitrogen metabolizabilities were assessed. Titanium dioxide was used as an inert marker (3 g/kg) in the experimental diets for eight days (five days of adaptation and three days of excreta collection). A completely randomized design was used, analyzed by the SAS GLM procedure, and means were compared using the Tukey test (P < 0.05). At 2 weeks of age, no significant differences (P > 0.05) were observed in the metabolizability of dry matter and gross energy among treatments; however, nitrogen metabolizability was higher (P<0.001) in T3 (62.51%) compared to T1 (56.12%), T2 (57.69%), and T4 (61.65%). At 6 weeks, the highest gross energy metabolizabilities (P < 0.05) were observed in T1 and T2 (77.43% and 77.64%, respectively), while the lowest values were recorded in T3 and T4 (76.12% and 74.63%, respectively). In conclusion, with an ME:CP ratio of 145, the 2900/20 ratio favors the metabolism of nitrogen during the initial phase (2 weeks), while the 3190/22 ratio favors the metabolism of dry matter and energy at later stages (6 weeks) of Japanese quail performance.

**Keywords:** Japanese quail; energy and protein densities; gross energy; nitrogen metabolizabilities

### 490P Effects of digestible lysine content and oat hull inclusion in the diet on growth performance and gastrointestinal tract development of two pullet strains from 0 to 35 days of age

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Two experiments at the same time were conducted to study the effects of dietary standardized ileal digestible lysine (DLys) content and oat hull (OH) inclusion on growth performance and gastrointestinal tract (GIT) traits from hatch to 35 d of age in Lohmann LSL Ultra Lite (White) and Lohmann Brown Classic (Brown) pullets. The experimental design was completely randomized with 4 treatments arranged as a 2 × 2 factorial with DLys (0.96 vs. 1.00%) content and OH (0 vs. 3%) inclusion as main effects. All other AA were formulated according to the ideal protein concept, and OH was included on top. Each treatment was replicated 10 times and the experimental unit was a cage with 14 pullets. Growth performance [ADG, ADFI, energy intake (EI), and feed (FCR) and energy (ECR) conversion ratios] was determined weekly from 0 to 35 d of age, and GIT traits were evaluated at 35 d of age. Data were analyzed using the MIXED procedure of SAS with repeated measures. The model included the main effects, age as the repeated factor, and their interactions. No interactions between main effects were detected. From 0 to 35 d of age, increasing the DLys content improved (P < 0.001) the performance of White pullets, resulting in higher ADG (9.88 vs. 10.2 g), and better FCR (2.044 vs. 1.985 g/g) and ECR (6.10 vs. 5.93 kcal/g). In Brown pullets, higher DLys also en-

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hanced ADG (10.8 vs. 11.2 g), ADFI (21.3 vs. 21.9 g), and EI (63.5 vs. 65.5 kcal/d). The inclusion of OH increased ADFI in both strains ($P < 0.001$), ECR ($P < 0.05$) in White pullets, and ADG ($P < 0.05$) in Brown pullets. At 35 d of age, OH inclusion increased ($P < 0.001$), in both strains, the absolute and relative weight of the gizzard, with and without the contents, but no effect of DLys content was detected for GIT traits. Numerous interactions between age and OH inclusion, and between age and DLys content in the diet were found for White and Brown pullets, respectively. For White pullets, OH inclusion increased ADFI for the third and fourth wk ($P < 0.05$), improved FCR for the first wk ($P < 0.01$), but impaired it for the last wk ($P < 0.05$), and improved ECR for the first and fourth wk of age ($P < 0.05$). For Brown pullets, an increase in DLys content increased ADG for the first ($P < 0.05$), third ($P < 0.001$), and fourth ($P < 0.001$) wk. In addition, an increase in DLys content improved FCR and ECR for third ($P < 0.05$) and fourth ($P < 0.01$) wk. In summary, in both strains, an increase in DLys content improved growth performance whereas inclusion of OH consistently enhanced gizzard development. However, the response to dietary DLys and OH differed between strains over time, indicating that nutrient requirements may vary among strains and warrant further investigation.

**Keywords:** Digestible lysine; gastrointestinal tract trait; oat hulls; pullet performance; pullet strain

### 491P Regulation of phosphorus metabolism to mitigate osteoporosis and extend the laying cycle in aged laying hens

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Aging hens may develop osteoporosis due to disruption of calcium (Ca) homeostasis. In old hens, bone resorption releases phosphorus (P), potentially affecting Ca metabolism. This study evaluated the impact of dietary P level and limestone particle size (PS) on performance, eggshell quality and bone mineralization in laying hens between 70 and 100 weeks of age. At 70 weeks of age, 216 Lohmann White hens were balanced by body weigh (BW; 1.69 ± 0.02 kg) and randomly allocated in 72 cages (n=3/cage) and assigned to one of six treatments: Fine particle size (0-0.5 mm, FPS) or coarse particle size (2–4 mm, CPS) with either a control P (0.32%, NP), medium P (0.24%, MP) or low P level (0.16%, LP). Body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were measured every two weeks. Eggs were weighted twice per week, and egg quality was assessed every two weeks using the DET6500. Bone mineral content (BMC) was evaluated by dual-energy X-ray absorptiometry in 42 hens every 5 weeks starting at 70 weeks. A repeated measures mixed model ANOVA was used for the factorial design 2 × 3 (Particle size × P level). The treatments, time, and their interactions were included as a fixed effects. Block and cage (nested within the block) were included as a random effect, with the cage as the experimental unit. Results presented correspond to the period from 70 weeks to 84 weeks, as the study is ongoing. During this period, ADFI tended to be reduced in hens receiving NP with FPS compared to MP with FPS (-3.9 g/d; Interaction; $P = 0.07$). In contrast, FCR, calculated as feed intake divided by egg weight, tended to be higher in hens fed NP with CPS compared with NP with FPS (+3.7%, Interaction; $P = 0.07$). Subsequently, BW tended to be higher in hens fed FPS compared with CPS (+14.8 g; $P = 0.08$). However, ADG was not significantly different between treatments. Regarding egg quality, albumen height (+0.3 mm; $P < 0.001$), Haugh unit (+2.3; $P < 0.01$), yolk height (+0.2 mm; $P < 0.05$) and diameter (+0.5 mm; $P < 0.05$) were higher in hens receiving CPS. In contrast, yolk color score (+0.2; $P < 0.001$) was higher in hens fed FPS. Additionally, egg weight was increased with CPS compared to FPS, specifically at week 84 (+1.3 g; Interaction PS × Week; $P < 0.05$). No differences were observed in eggshell strength and thickness, BMC or BMC/BW. Overall, CPS mainly improved egg quality, but performance results suggest that NP level together with PS should be considered in diets for aging laying hens. As the study is ongoing, metabolic implications of dietary P levels on Ca homeostasis remains to be elucidated.

**Keywords:** Hens; phosphorus; bone; limestone size

### 492P Effects of Dietary Nutrient Density and Probiotic Supplementation on Feed Efficiency, Processing Yield, and Myopathy Incidence in Broilers

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Feed conversion ratio (FCR) and processing yield are critical indicators of production efficiency and economic return in modern broiler systems and are strongly influenced by dietary nutrient density and feed additives. Probiotic supplementation has been proposed as a strategy to improve nutrient utilization and growth performance, potentially enhancing both feed efficiency and carcass yield characteristics. This study was conducted to assess body weight gain (BWG), FCR, and processing yields in broilers that were fed differing diet densities and probiotics. A total of 1,280 male Cobb 500 birds were assigned to dietary treatments (8 replication pens per treatment per processing age; n=20 birds) in a 2x2 factorial design with nutrient densities high (H) or low (L) and treatment control (C) or probiotic (P). Dietary treatments were corn and soybean meal-based diets. High density diets had 100 kcal/kg more than low density diet during each phase. L contained 90% and H 108% of recommended amino acid (AA) levels resulting in an 18% greater AA in H throughout. Probiotic diets used "Agris" (Kent Nutrition), a *bacillus*-based probiotic (24 kcal/kg). Processing yield was assessed at D42 and D56 of age after birds were processed using an inline system using standard practices including immersion chilling. At 3h postmortem (4C), carcasses were cut up and deboned to determine part yields. Breast fillets were scored for woody breast (WB) and white striping (WS). Data was analyzed using linear effects model with density and probiotic treatment as fixed effects. Birds fed H diet had lower FCR than L diets from starter to finisher1 (1.19-1.28 vs 1.28-1.36, $P<0.05$), but in finisher2, H diets had higher FCR than L diets (2.65 vs 2.39, $P<0.05$). During the grower phase, P diets had higher FCR than C diets (1.25 vs 1.20, $P<0.05$). Birds fed H diets had greater carcass yield, breast yield, and overall part yield than L diets ($P<0.05$). L diets resulted in higher fat pat yield and rack yield than H diets ($P<0.05$). Birds fed H diets had increased likelihood of inducing woody breast compared to low density diets on D42 (85% vs 72%, $P<0.001$) but was a trend on D56 (95% vs 98%, $P=0.09$). HP had lower tender yield than LP on D42 (5.10 vs 5.23%, $P<0.05$). Probiotic diets tended to have higher part yield than control on D42 (76.0 vs 75.8%, $P=0.05$). Overall, high-density diets improved FCR and processing yields but increased the incidence of woody breast, whereas probiotic supplementation had limited and inconsistent effects on performance and yield characteristics.

**Keywords:** nutrient density; feed efficiency; broiler; yields; probiotic

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### 493P Organ-and Strain-Specific Response of Commercial and Native Chicken Strains to Heat Stress

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In recent years, broiler production has been steadily rising alongside population and income levels. Genetic selection and advancement in livestock breeding techniques have improved the growth rate of broilers. While these performance-driven improvements have been a major industrial achievement, they have also led to reduced resistance to environmental stress. Meanwhile, indigenous chickens show resistance to environmental stress, making them a valuable genetic resource. This study was conducted to determine differential heat-stress responses in two chicken strains: Commercial broilers, such as Arbor Acre (CBR), and Korean Native Chicken (KNC). A total of 96 chicks from each breed were randomly assigned to control (CON) or heat-stress (HS) rooms (8 cages each, 6 chicks/cage). For 4 weeks, the chicks were reared as per standard rearing protocol. At day 29, the birds in the HS room were subjected to an acute heat stress for 8 hours. The temperature was gradually elevated from 21 ± 1°C to 37 ± 1°C (4°C/h), maintained at the highest temperature for 3 hours, and then returned to baseline within 1 hour. The CON birds remained at 21 ± 1°C. After HS, 8 birds per treatment were slaughtered to collect liver and jejunum samples for gene expression analysis. Data was analyzed using two-way ANOVA with strain and temperature as main factors. Hepatic expression of NADPH oxidase (NOX)1, NOX4, heat shock protein (HSP)70, HSP90, and glutathione peroxidase (GPX)1 were significantly higher ($p < 0.05$) in CBR-HS compared to KNC-HS. Similarly, ten-eleven translocases (TET) 1, TET2, and TET3 were upregulated in the CBR-HS group ($p < 0.05$) compared with the KNC-HS group. While the jejunal expression of catalase (CAT) and occludin was significantly lower, NOX4, HSP70, HSP90, and GPX1 were upregulated in CBR-HS compared to KNC-HS. Consistently elevated expression of NOX4 and GPX1 in both liver and intestinal tissues suggests that CBR exhibit more pronounced oxidative responses under heat stress, likely attributed to their rapid growth rates and higher metabolic demands compared to indigenous breeds. The marked activation in fast-growing commercial broilers may indicate greater physiological load, while slow-growing native birds may achieve heat tolerance with reduced stress-response activation. The findings provide important insights into how broilers with different genetic backgrounds respond to environmental stress, thereby offering a scientific basis for developing targeted management strategies.

**Keywords:** Heat stress; Fast growing; Slow growing; Antioxidant; Gene expression

### 494P Characterization of proteomics in hypothalamus of male broiler breeders divergently selected for low and high water efficiency

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Seven generations of genetic selection has resulted in the establishment of Low (LWE) and High (HWE) Water Efficiency broiler lines. The purpose of this study is to begin to characterize proteomic profiles of the hypothalamus obtained from LWE and HWE male broiler breeders. At 62 wk, male (n=5/line) birds were euthanized and the hypothalamus isolated, snap-frozen in liquid nitrogen, and stored at -80°C. For proteomics analysis, total protein was extracted from the hypothalamus and sent to the IDeA National Resource for Quantitative Proteomics (University of Arkansas for Medical Sciences) for analysis. The identified proteins were further analyzed using IPA (Ingenuity Pathway Analysis) software. A total of 6983 proteins were identified in male HWE hypothalamus. Using cutoffs of -0.5 to 0.5 experimental log ratio [ExpLog] (corresponding to <-1.4 fold to > 1.4 fold difference) and $P < 0.05$, there were 157 proteins down-regulated and 92 proteins up-regulated in the HWE male hypothalamic tissue. The 3 highest up-regulated proteins in HWE hypothalamus were; 1) NmrA like redox sensor 1 (NMRAL1), 2) Oxytocin (oxytocin/neurophysin I prepropeptide, OXT), and 3) glycoprotein M6B (GPM6B). OXT has been reported to exhibit antidiuretic activity and may be involved in drinking behavior. The 3 proteins with the greatest down-regulation in HWE hypothalamus were; 1) tubulin gamma complex component 4 (TUBGCP4), 2) high mobility group nucleosome binding domain (HMGN3), and 3) RNA polymerase I and III subunit D (POLR1D). Upstream analysis revealed that estrogen receptor 1 (ESR1) and peroxisome proliferator activator receptor alpha (PPARA), were predicted to be activated and HRas proto-oncogene GRPase (HRAS) was predicted to be inhibited in the HWE group. Predictions in IPA are considered significant when absolute Z-scores are greater than 2.0 with $P < 0.05$ and are based on expression of downstream target molecules in the dataset. Three pathways that were predicted to be inhibited in the HWE hypothalamus were G-protein beta gamma signaling, extra nuclear receptor signaling, and eicosanoid signaling. This information begins to paint a picture of hypothalamic proteomic landscape of water efficiency in these unique broiler lines. Supported by USDA-NIFA SAS # 2019-69012-29905.

**Keywords:** water efficiency; broiler breeders; proteomics; hypothalamus; expression analysis

### 495P Characterization of proteomics in hypothalamus of female broiler breeders divergently selected for low and high water efficiency

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Recent genetic selection has resulted in the establishment of Low (LWE) and High (HWE) Water Efficiency broiler lines. The purpose of this study is to begin to characterize proteomic profiles of the hypothalamus obtained from LWE and HWE female broiler breeders. At 62 wk, female (n=5/line) birds were euthanized and the hypothalamus isolated, snap-frozen and stored at -80°C. For proteomics analysis, total protein was extracted and sent to the IDeA National Resource for Quantitative Proteomics (University of Arkansas for Medical Sciences) for analysis. The identified proteins were further analyzed using IPA (Ingenuity Pathway Analysis, Qiagen) software. A total of 6958 proteins were identified in female HWE hypothalamus. Using cutoffs of -0.5 to 0.5 experimental log ratio [ExpLog] (corresponding to <-1.4 fold to > 1.4 fold difference) and $P \leq 0.05$, there were 200 proteins down-regulated and 120 proteins up-regulated in the HWE compared to LWE female hypothalamus. Binomial distribution analysis revealed a highly significant skew of down-regulated differentially expressed proteins ($P<0.000002$) in HWE compared to LWE hypothalamus. Antidiuretic hormone (arginine vasotocin, AVT) was up-regulated in the hypothalamus of HWE females (ExpLog 1.19, $P = 0.040$) that would contribute to enhanced water reabsorption in the HWE phenotype. Surprisingly, a large number of electron transport chain proteins were down-regulated in HWE female hypothalamus. Down-regulation of these mitochondrial proteins likely contributed to the prediction of impairment of respiratory electron transport, oxidative phosphorylation, and complex 1 biogenesis, and prediction of activation of mitochondrial dysfunction in hypothalamus of HWE females. Predictions of activation and inhibition of these canonical pathways is based on an absolute Z-score of 2.0 or more and a P-value less than 0.05 by the IPA software. These findings indicate that there is a possible role of mitochondrial function/

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biochemistry in the phenotypic expression of water efficiency in female broiler breeders. Supported by USDA-NIFA SAS # 2019-69012-29905.

**Keywords:** water efficiency; broiler breeders; proteomics; hypothalamus; expression analysis

### 496P Estimating the ossification ratio of developing keel bones in laying hen pullets using deep learning and computer vision

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The study of keel bone development, while important in the pursuit of optimizing keel bone health, often requires the time-consuming process of manually annotating the ossified bone and cartilage area. Our aim was to develop an automated means of estimating bone and cartilage area and ossification ratio from photos of dissected keel bones using deep learning and computer vision. The dataset consisted of 99 keel bone photos derived from laying hen pullets (Hy-Line Brown) that were euthanize and dissected at 14 (n=9), 16 (n=36), 18 (n=27) and 20 (n=27) weeks of age. The dissected bones were frozen before being photographed next to a reference object for size calibration (a 5-eurocent coin). The keel bones images for all birds were manually annotated using the Labelme software to mark the area of the bone, the cartilage and the reference object. A panoptic segmentation model was trained on 87 of these images using Detectron2 in Python v3.11. The model was then tested on the remaining 12 slices (n= 3/age), yielding preliminary bone and cartilage area values of 2139.3 ± 120.72 $mm^2$ and 365.3 ± 63.33 $mm^2$, with confidence levels of 98.1 ± 0.42 % and 93.0 ± 2.71 % and intersection over union (IoU) scores of 0.9 ± 0.12 and 0.8 ± 0.06, respectively. For numerical validation, the 12 images of the testing set were annotated using the Fiji version of the ImageJ software. Area measures of bone, cartilage, and reference object (in $pixel^2$) from the manual annotation and the model were statistically compared by calculating the root mean squared percentage error (RMSPE). The accuracy of the model predicted area values was acceptable for bone (8.11 %), with noticeable deviations for cartilage (12.8 %), and excellent for the reference object (2.24 %). Next steps include adding a second set of keel bones from pullets of another genetic strain to the training and testing images to improve generalisability, confidence and numerical accuracy. Overall, the model shows strong potential as a rapid, automated tool for assessing keel bone development, with further improvements expected as the dataset expands.

**Keywords:** Laying hen pullets; Keel bone ossification; Deep learning and computer vision; Bone; Cartilage

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