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ABSTRACTS
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ABSTRACTS
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Milton L. Dendy Keynote Lecture

Integrating artificial intelligence, thermal imaging, and behavioral analytics for early avian disease detection

Guoming Li*¹ *University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Avian infectious diseases such as avian influenza (AI), Newcastle disease (ND), and *Salmonella* infection remain persistent global threats to poultry health and food safety. Their rapid onset and subtle early symptoms demand non-invasive, intelligent monitoring systems capable of identifying physiological and behavioral deviations before clinical signs emerge. Recent advances in artificial intelligence now enable early, on-farm detection through image-based, thermographic, and behavioral analytics. This keynote summarizes several machine learning studies aimed at advancing precision disease diagnostics for poultry. In controlled experimental trials, thermography combined with machine learning achieved rapid detection of AI and ND by analyzing temperature anomalies in targeted body regions. Thermal imaging of broiler chickens' heads and legs, segmented and classified via deep learning models, improved diagnostic accuracy to over 90% within 8 hours post-infection, significantly outperforming models capturing whole body temperatures. The emphasis on non-feathered regions, where vascular heat exchange is highest, proved critical for detecting early pathophysiological changes associated with viral infection. Parallel research applied deep learning and transfer learning to classify fecal images for *Salmonella* risk assessment. A hybrid architecture combining convolutional neural networks with tree-based classifiers achieved up to 85% cross-regional accuracy when validated between American and African datasets of over 5,000 annotated images. The resulting models were integrated into interactive web and mobile applications for real-time field use, enabling caretakers to upload fecal images and receive instant binary predictions ("Positive" or "Negative"). Complementary behavioral analytics introduced the broiler activity index as an early biomarker of infection. By quantifying flock-based movement changes from continuous video recordings, activity index analysis revealed statistically significant differences between *Salmonella*-infected and healthy flocks during the first two weeks of life. Together, while those techniques still require additional efforts in testing and cross-validation, they show great potential in non-invasive early avian disease detection.

Keywords: precision livestock farming; Automatic Disease Diagnosis; Machine learning

*Author presenting paper

GS Denotes Graduate Student Presentation

UG Denotes Undergraduate Presentation

Environment & Management I

M1 Effect of target body weight and the length of the starter phase of broiler breeders on egg weight, geometric traits, and chick weights

Gabriele Jardim Kinchin*^{1UG}, Artur De Freitas Ribeiro¹, Shivi Rao², Jesus Arango², Edgar O. Oviedo-Rondón¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²*Cobb Genetics, Siloam Springs, Arkansas, United States*

Chick weight can be influenced by egg weight (EW) and geometric traits, which are determined by hen size. Pullet target body weight (BW) and development can be affected by feeding during rearing. The length of the starter phase affects nutrient intake, development, and metabolism. This study evaluated the effects of pullet target BW, low (L) and high (H), and the length of the starter phase (4 or 6 wk) on EW, geometrical traits, and uniformity, chick progeny weight, free-yolk BW, and residual yolk. A total of 1,600 Cobb commercial pullets were placed in a dark house with 16 pens (4 reps/treatment) and fed starter, grower, and developer diets. Feed was allocated to achieve target BWs and was adjusted weekly based on observed BW. Pullets were transferred to the laying house at 22 wk with 72 hens and 7 roosters per pen. Eggs were collected three times a day, and 84 eggs/pen ($N=9,888$) were individually weighed and incubated at 27, 29, 31, 34, 36, 38, 40, and 42 wk. Width and length were measured in 30 eggs/pen, and geometric traits were calculated. The EW loss was determined after transfer from the setter to the hatcher at 18 d. Ten chickens per pen were weighed at hatch, sacrificed, and yolk-free BW was determined. Data was analyzed using a completely randomized design with a two-way ANOVA. The EW was affected by an interaction effect ($P<0.01$) and a quadratic effect ($P<0.001$) of breeder age. Eggs from H hens were heavier than those from L hens, but eggs from L hens fed starter for 4 wk were heavier than eggs from hens fed starter for 6 wk. Eggs from L hens were less ($P<0.01$) uniform (CV 6.31%) than those from H hens (CV 6.03%). No effects ($P>0.05$) were observed on egg shape index (76.7); however, the smallest ($P<0.001$) geometric mean diameter, surface area, and volume were observed in eggs from L hens fed starter 6 wk. Chicks from H hens had heavier BW and free-yolk BW ($P<0.001$) than those from L hens, and no effects of starter length ($P>0.05$) were detected. No effects of treatments ($P>0.05$) were observed in EW loss, but the chick yield was higher ($P<0.01$) for hens fed starter for 6 wk than those fed starter for 4 wk. In conclusion, target BW affects EW, egg surface area and volume, with effects on chick BW and free-yolk BW, but no effect on yolk utilization.

Keywords: Egg weight; chick weight; target BW; starter phase; uniformity

M2 Early-age thermal conditioning and its impact on heat stress resilience in pasture-raised broilers

Unishma Ghimire*^{1GS}, Jennifer Timmons¹ ¹*University of Maryland Eastern Shore, Agriculture, Food, and Resource Sciences, Princess Anne, Maryland, United States*

Heat stress (HS) adversely affects the growth performance, physiology, reproduction, health, and immune status of broiler

chickens. The pasture-raised broilers are particularly vulnerable, as they are exposed to high and fluctuating environmental temperatures. This vulnerability makes it crucial to explore mitigation strategies like early-age thermal conditioning (TC). However, studies on the effects of early-age TC in pastured broilers remain limited. The purpose of this study was to evaluate the effects of early-age TC on the growth performance, carcass yield, and immune organs development of acute HS pastured-broilers. A randomized complete block design with a factorial arrangement of 2*2 was used. A total of 336 day of hatch male Ross 308 broilers were assigned to four treatment groups with 7 replicates and 12 birds per replicate: (1) non-TC at day 5 and non-HS at day 28 and 41; (2) non-TC at day 5 and HS at day 28 and 41; (3) TC at day 5 and non-HS at day 28 and 41; and (4) TC at day 5 and HS at day 28 and 41. The broilers were either TC at 37°C or non-TC at 31°C on day 5 for 24 hours. They were placed on pastured at 21 days of age, and either HS (36°C on day 28, 34°C on day 41) or kept at non-HS temperatures (30°C on day 28, 28°C on day 41) under controlled-environmental conditions for 6 hours. The dependent variables measured were weekly feed intake, weekly body weight, cumulative feed conversion ratio, feed intake during HS, mortality, carcass yield, and weight of spleen and bursa of Fabricius. The data were analyzed using ANOVA, and the differences were considered statistically significant at $P<0.05$. No significant interaction between the TC and acute HS was observed for any of the dependent variables. Feed intake during HS was significantly lower in HS birds compared to non-HS group birds, with reductions on both days 28 and day 41 being significant ($P<0.01$). Under the conditions of this study, early-age TC did not induce thermotolerance to acute HS later in life. However, the marked drop in feed intake during HS underscores the severity of HS in pasture-raised broilers.

Keywords: Thermal conditioning; Heat stress; Pastured broilers

M3 Litter performance, yields, and pododermatitis of Ross 708 broilers as affected by litter age

Seth D. Holeyfield*^{1GS}, Mitchell K. Vaught¹, Kenneth B. Nelson¹, Mike Kidd¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Litter quality fluctuates with age, which can influence broiler performance and welfare. Variation in litter management practices has prevented the establishment of a consensus on the optimal litter age for maximizing performance. The objective of this experiment was to evaluate the effects of litter age on broiler live performance and processing traits. Three ages of unamended litter (0-, 1-, and 3-flocks old), composed of pine shavings and rice hulls, were sourced from the same commercial broiler farm and distributed into floor pens. Pens were equipped with hanging feeders and nipple-drinker lines. On placement day, 12 feather-sexed Ross 708 chicks (1,152 total; 576 of each sex) were randomly assigned to each pen. Each sex × litter age combination was replicated 16 times (96 pens total). Pen feed intake and BW were recorded on d0 and d49, and mortality was collected twice daily to determine BW gain, feed intake, percent mortality, and mortality-corrected feed conversion (FCR). On d50, two birds per pen were processed to determine peritoneal fat, chilled parts yields, and the incidence

of pododermatitis and woody breast myopathy. Data was analyzed using a two-way ANOVA, and means were separated using Tukey's HSD. All analyses were performed with SAS 9.4, with significance declared at $P \leq 0.05$ and trends at $P \leq 0.10$. No sex \times litter age interactions ($P > 0.05$) were observed for live performance parameters. A litter age effect was detected, with broilers reared on 3-flock litter tending to have a higher FCR ($P = 0.06$) than those reared on 1-flock litter. For processing traits, sex \times litter age interactions occurred for pododermatitis ($P = 0.03$) and peritoneal fat yield ($P = 0.05$). Male broilers reared on 0-flock litter exhibited higher pododermatitis than females on 0-flock litter. Males reared on 3-flock litter had lower peritoneal fat yield than females reared on 3-flock litter. A litter age effect was observed for carcass yield, with broilers reared on 0-flock litter tending to have higher carcass yield ($P = 0.06$) compared with those reared on 3-flock litter (77.01 vs. 76.29%, respectively). These findings indicate that when new litter is introduced, robust management techniques to minimize pododermatitis should be deployed.

Keywords: Litter; Broiler; Feed conversion; Pododermatitis

M4 Spatial analysis of the influence of environmental features and pen-level differences on chemical composition in poultry litter

Maxim A. Peckenschneider*^{1GS}, Elena G. Olson², Jake K. Byrnes³, Steve Ricke¹ ¹University of Wisconsin-Madison, Animal Science, Madison, Wisconsin, United States; ²University of Wisconsin, Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States; ³Barnwell Bio, San Diego, California, United States

Characterizing nutrient variation in poultry litter is essential for assessing quality and understanding the physical factors that create nutrient distributions within the housing environment. In this study, we examined the spatial patterns of moisture, total Kjeldahl nitrogen (TKN), phosphorus (P), potassium (K), and sulfur (S) across two laying-hen pens using a 32-point grid sampling layout with 1.5-ft intervals ($N = 192$, $n = 3$ per intersection point). Nutrient concentrations, expressed as percentages, were assessed using Pearson's correlation to quantify associations between the attained parameters. Linear mixed-effects (LME) models were used to evaluate the influence of the waterline, feeder, and enrichment-area proximity to the nutrient distributions while controlling for the pen as a random effect ($\alpha = 0.05$). Significance was set at $p < 0.05$. There was strong coordination among the mineral nutrients. K was significantly correlated with P ($r = 0.92$), TKN ($r = 0.31$), S ($r = 0.65$), and moisture content ($r = 0.43$). TKN was only correlated with K, while moisture content significantly affected all minerals except for TKN ($r = 0.01$). There was a clear spatial pattern for P, K, and S. Phosphorus and K decreased with distance from the waterline (estimates: -0.002, -0.001, respectively) and enrichment area (-0.002, -0.003, respectively); and increased with distance from the feeder (0.005, 0.004, respectively) (LME, $P < 0.05$). TKN increased with distances from the feeder (0.012) and decreased with distance from the enrichment area (-0.004) (LME, $P < 0.05$). Moisture content was not significantly impacted by distance from the water source, feeder, or enrichment area. These results demonstrate that mineral

nutrients form predictable spatial zones influenced by pen features. Strong P-K-S coordination indicates shared deposition pathways that generate nutrient "hotspots". In future work, we will integrate microbial abundance data with these mineral gradients and distance metrics to determine how nutrient hotspots influence microbial community structure and pathogen distribution. Together, these controlled studies will help clarify how litter chemistry shapes microbial patterns and inform targeted litter-management strategies.

Keywords: Poultry Litter; Nutrient Composition; Laying Hens; Spatial Variability

M5 Assessing Survival of *Campylobacter jejuni* in Poultry Litter across Different Environmental Conditions

Kassim Sulleyman*^{1GS}, Matthew Bailey¹, Yagya Adhikari², Dianna Bourassa¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States

Campylobacter is a major foodborne pathogen commonly associated with poultry production environments, where litter can serve as a persistent source of contamination for birds. This study evaluated the survival of *Campylobacter jejuni* in poultry litter under varying moisture and temperature conditions. Litter samples ($\leq 8\%$ moisture) were adjusted to three moisture levels (15%, 20%, and 30%) and held at three temperatures (4°C, 42°C, and 50°C). Therefore, the study design had a total of 9 treatments based on moisture-temperature combinations. A ciprofloxacin-resistant *C. jejuni* marker strain was inoculated into 300 g of poultry litter for each treatment to achieve an initial concentration of 8 log CFU/g for a bench-top study. Following inoculation, poultry litter was held under microaerobic conditions using 2-gal zip-top bags at the different temperature-moisture conditions. For each treatment, 10 g of litter was sampled thrice at 8 h, 16 h, 24 h, 36 h, and 48 h. Samples were enumerated on Campy Cefex agar after incubation at 42 °C for 48 h under microaerobic conditions. The results showed that *Campylobacter* survived only in the samples stored at 4°C for 8, 16, and 24 h, regardless of moisture content. However, at 36 and 48 h, counts were below the limit of detection (< 1 CFU/g). *Campylobacter* was not detected at any other temperature or time point. Since *Campylobacter* was not recovered from any samples stored at 42°C or 50°C, these treatments were excluded from statistical modeling, and a generalized linear model was applied to assess differences among the 4°C samples only. We found that for each 1% increase in moisture at 4°C, *Campylobacter* were 1.005 (0.96 - 1.046; 95% CL) times as likely to be detected in litter ($p = 0.80$). These findings demonstrate that *Campylobacter* experiences rapid die-off in litter under all tested conditions, especially at elevated temperatures. Overall, the study highlights how quickly *Campylobacter* loses viability in litter, underscoring its limited ability to survive in the environment outside the host. Findings from this work provide insights into pathogen persistence under diverse on-farm conditions and may support improved litter management strategies to reduce *Campylobacter* prevalence in poultry production systems.

Keywords: Campylobacter; Litter; Poultry Production

Physiology, Endocrinology & Reproduction I

M6 Impact of heat stress on performance, egg quality, and immunity in cage-free laying hens during early laying phase

Shuja Majeed*^{1GS}, Bikas R. Shah¹, Nimra Khalid¹, Mohamad Jaradat¹, Iddris M. Habib¹, Bikash Aryal¹, Lisa Bielke², Qihong Wang¹³, Lingying Zhao⁴, Ali Nazmi¹⁵

¹Ohio State University, Department of Animal Sciences, Wooster, Ohio, United States; ²North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ³Ohio State University, Department of Veterinary Preventive Medicine, Columbus, Ohio, United States; ⁴Ohio State University, Department of Food, Agriculture, and Biological

*Engineering, Columbus, Ohio, United States;*⁵*Ohio State University, Food for Health Discovery Theme, Columbus, Ohio, United States*

Heat stress (HS) remains a major concern for the poultry industry, leading to substantial production and welfare losses. The onset of lay represents a critical physiological phase for hens, during which environmental stressors can further compromise performance. With the egg industry transitioning towards cage-free (CF) housing, it's important to understand how hens in these system respond to HS. Therefore, this study evaluated the effects of HS during early stage of egg production on performance, egg quality, and immune responses in CF-laying hens. At 17 weeks of age, 396 Hyline W-80 pullets were randomly assigned to two environmentally controlled rooms (six pens per room; 33 birds per pen). One room was maintained under thermoneutral (TN) conditions (75°F), while the other was subjected to cyclic HS (95°F for 12 h daily, 8:00 AM–8:00 PM) for 8 weeks. Performance parameters, including body weight, feed intake, egg production, and mortality, were recorded. Egg quality was assessed in weeks 21 and 25 of age. At week 25, blood was collected from two birds per pen for flow cytometric analysis of peripheral blood mononuclear cells (PBMC). Liver, spleen, and oviduct were also sampled for gene expression analysis via qPCR. Data were analyzed using PROC MIXED in SAS, with pen as a random effect and $P < 0.05$ was considered statistically significant. Compared with TN hens, the HS group exhibited reduced feed intake ($P < 0.01$) throughout the trial, less egg weight ($P < 0.01$) from weeks 21–25, and lower egg production at weeks 24 and 25 ($P < 0.05$). Mortality was also 2% higher in the HS group ($P < 0.05$). Egg quality parameters did not differ between treatments except yolk color which was lower for HS at week 25 ($P < 0.05$). Flow cytometry revealed a trend toward lower immune cell populations in HS birds, with cytotoxic T cells ($TCR\alpha\beta^+CD8\alpha\beta^+$) significantly decreased ($P < 0.05$). Gene expression analysis of stress and immune related genes is ongoing. In conclusion, heat stress during the onset of lay negatively impacts performance and may suppress immunity in laying hens housed in CF systems which could increase their disease susceptibility. These findings underscore the need for strategies to mitigate HS stress during this egg production stage.

Keywords: Heat Stress; Layer chicken; immune response; cage free

M7 Evaluation of supplementation with AlphaD3™ on calcium digestibility and ileal regulation of mineral homeostasis in commercial laying hens

R. Alejandra Garcia-Mejia^{*1GS}, Micaela Sinclair-Black¹, Roselina Angel², Bibiana Jaramillo³, Xabier Arbe⁴, David Caver⁴, Laura Ellestad¹ ¹*University of Georgia, Poultry science, Athens, Georgia, United States;* ²*University of Maryland, Animal and Avian Sciences, College Park, Maryland, United States;* ³*Iluma Alliance, Durham, North Carolina, United States;* ⁴*H&N International, Cuxhaven, Germany*

Eggshell and bone mineralization rely on calcium (Ca) availability, and its homeostasis is influenced by the active form of vitamin D₃ (VD₃), 1 α ,25(OH)₂D₃. Older hens exhibit reduced 1 α -hydroxylase activity and ability to form 1 α ,25(OH)₂D₃, impairing intestinal mineral absorption and utilization for maintaining eggshell and skeletal strength. This study evaluated the effects of dietary 1 α -cholecalciferol (AlphaD3™, Iluma Alliance) on ileal Ca digestibility and expression of genes involved in Ca homeostasis. Ileum was collected from Nick Chick hens (H&N International) at 25, 43, 65, 80, and 95w of age during bone mineralization (1.5 hours post-oviposition [HPOP]), eggshell mineralization (15 HPOP), and transitions between them (6 and 21

HPOP). Ileal digesta was collected at 1.5 and 15 HPOP. Hens (n=8/age/HPOP/diet) were fed a control (2000 IU/kg VD₃) or AlphaD3-supplemented (3.5 μ g/kg) control diet and titanium dioxide (0.3%) was used as a marker to determine Ca digestibility. Levels of mRNA were determined by RT-qPCR. All data were analyzed by ANOVA and Fisher's LSD test. Age-by-HPOP and age-by-diet interactions were observed for Ca digestibility. At 1.5 HPOP, Ca digestibility declined as hens aged, whereas at 15 HPOP it was higher, with a slight decrease at 43 and 65w of age ($P \leq 0.05$). AlphaD3 hens exhibited increased Ca digestibility at 65w compared to control hens ($P \leq 0.05$). Parathyroid hormone (PTH) receptor 1 (*PTH1R*) increased up to 80w before decreasing at 95w, while calcitonin receptor progressively increased with age ($P \leq 0.05$). Moreover, levels of Ca transporter sodium-calcium exchanger (*NCX1*) showed a steady decline with age ($P \leq 0.05$). Ileal *PTH1R* and *NCX1* varied across HPOP, where expression decreased between 1.5 and 6 HPOP, but levels recovered at 15 and 21:00 HPOP, respectively ($P \leq 0.05$). Overall, AlphaD3 hens exhibited higher *PTH1R* and lower *NCX1* levels compared to control hens ($P \leq 0.05$). Findings suggest older hens have reduced intestinal Ca uptake during bone mineralization, likely resulting from impaired ileal PTH sensitivity, along with increased calcitonin signaling. Supplementation with AlphaD3 has the potential to improve Ca digestibility by enhancing PTH sensitivity and supporting intestinal mineral homeostasis as hens age.

Keywords: Calcium uptake; calcium homeostasis; parathyroid hormone; vitamin D3; Calcitonin

M8 Effects of mild feed restriction on energy expenditure for reproduction and spatial distribution in cage-free pullets

Olivia McGuire^{*3GS}, Ana Rentsch¹², Gregory Bédécarrats¹, Charlene Hanlon³ ¹*University of Guelph, Department of Animal Biosciences, Guelph, Ontario, Canada;* ²*University of Guelph, Campbell Centre for the Study of Animal Welfare, Guelph, Ontario, Canada;* ³*Auburn University, Department of Poultry Science, Auburn, Alabama, United States*

Cage-free systems offer vertical space, potentially increasing variability in physical activity and feed intake and reducing uniformity in body composition. This study investigated energy expenditure for reproduction and spatial distribution in cage-free pullets. At 12 weeks of age (woa), 600 Lohmann LSL Lite pullets were randomly assigned to 4 rooms, each with 6 aviary-style pens (n=25/pen) under 9L:15D. Pens contained two perches, two elevated platforms, a 45° ramp, and nest boxes. At 13 woa, treatments were initiated: ad libitum (AL) feeding or restricted (R) by 10% body weight. Hourly scan sampling recorded bird locations via pen-mounted cameras between 15 and 20 woa. Egg production was recorded daily. At 14, 16, 18, 19, 20, 21, 22, and 26 woa, one bird per pen (n=6/trt/timepoint) was euthanized for abdominal fat pad measurement and collection of hypothalamus and pituitary tissue for Neuropeptide Y (NPY) mRNA analysis, a key stimulator of feed intake. Data were analyzed using linear mixed models (GLIMMIX; SAS v9.4). R birds spent more time on the litter, while AL birds preferred platforms and high perches ($P < 0.05$). All birds increasingly spent more time on the litter between 18 and 20 woa ($P < 0.001$), indicating a shift in preference during sexual maturation. During the last two hours of light, more R birds were observed on the litter while AL birds in elevated locations ($P < 0.001$). Treatment influenced absolute ($P < 0.001$) and relative ($P < 0.001$) fat pad weight, as well as cumulative egg production to 26 woa ($P < 0.001$), with all parameters lower in R birds than in AL birds. While body composition and early reproductive performance were affected by energetic status, NPY mRNA levels were unaffected. Although relative NPY expression increased with age ($P < 0.001$), this was associated with

reproductive state rather than energetic status. Unchanged NPY expression suggests that the level of restriction in this study may not be sufficient to trigger the metabolic hunger response. Thus, increased litter use by R birds likely reflects unmet behavioral needs rather than energy conservation. These findings demonstrate that changes in body composition and egg production under feed restriction are not attributable to metabolic hunger but may reflect behavioral factors.

Keywords: Reproduction; Cage-free; Energy expenditure; Feed restriction; Body composition

M9 Immune markers reflecting the inflammatory state of laying hens with keel bone damage with or without *Salmonella* challenge

Asgar Sedaghat*^{1GS}, Aden Smith¹, Rami A. Dalloul¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

Keel bone fractures (KBF) and *Salmonella* are two major issues in aged laying hens in cage-free systems and often go unnoticed before they translate into an economic loss or a major public health concern. This study investigated the changes in specific immune markers in laying hens with keel bone damage pre- and 5 days post *Salmonella* challenge. A total of 40 laying hens were randomly selected from a 54-week-old commercial flock raised in a cage-free system, 20 were euthanized and cecal tonsil samples were immediately collected and stored at -80°C . The other 20 birds were challenged with 10^7 CFU of *Salmonella* Enteritidis after 2 weeks of acclimation and sampled 5 days post challenge. The birds were divided into two groups of mild and severe KBF upon euthanasia. RNA was extracted from cecal tonsil samples, cDNA synthesized followed by qPCR to measure the mRNA abundance of selected immune markers. The experimental design was CRD, and data were subjected to one-way ANOVA and significance ($P \leq 0.05$) between mild and severe groups was determined by LSD test. There were no significant differences in the mRNA abundance of interleukin (IL)-1 β , IL-6, IL-8, IL-17A, tumor necrosis factor- α (TNF- α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), IL-23, and cyclooxygenase-2 (COX-2). However, the mRNA abundance of prostaglandin E synthase (PTGEs) was greater in birds with severe bone damage compared to mild KBF birds after the *Salmonella* challenge ($P = 0.04$), but only numerically greater before the challenge. These changes suggest an inflammatory response and immune defense mechanisms prior to clinical signs. The results of PTGEs indicate that wound healing and bone resorption might be occurring at the fracture site, and when the birds are subjected to *Salmonella* infection, this inflammatory

state is more pronounced. This research provides insight into how the severity of KBF might be a contributing factor to inflammation.

Keywords: laying hens; keel bone damage; Salmonella; immunity; mRNA abundance

M10 Corticosteroid binding globulin mRNA expression in hepatic and reproductive tissue of mature chickens

Coleman J. Hatmaker*^{1GS}, Adam Davis¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Corticosterone (CORT) is the principal avian glucocorticoid, and its availability to bind its receptor in target tissues is controlled by its primary transport protein, corticosteroid-binding globulin (CBG). Relative to mammals, CBG in poultry remains understudied. Previously, we reported that in chickens, CBG mRNA is expressed in greatest abundance in hepatic tissue, with lesser amounts detected in the gastrointestinal tract, testes, and mature ovarian follicles. We also reported that hepatic CBG expression decreased while ovarian granulosa cell expression increased during fasting. The goals of the current research were to determine if fasting impacts testicular CBG mRNA expression and if CBG mRNA expression differs between germinal disc (GD) and non-germinal disc (NGD) granulosa cells in the largest (F1) hierarchical follicle of chickens. Total RNA was extracted from testicular tissue samples collected 6, 24, 48, 72, and 96 hours post-feeding from 62-week-old Ross broiler breeder roosters ($n = 6$ per collection time) and 8 GD and NGD replicate granulosa cell samples, with each replicate consisting of either GD or NGD F1 granulosa cells from three 65-week-old Hy-Line W-36 hens. The RNA was then DNase-treated in preparation for two-step real-time RT-PCR analysis. Taqman minor groove binding probes and primers for CBG and GAPDH were created using Primer Express (Applied Biosystems). Relative CBG mRNA expression was analyzed using ANOVA with means for the different fasting time points separated by Tukey's multiple-comparison procedure. Differences were considered significant when $P < 0.05$. Relative to 6 hours post-feeding, testicular CBG mRNA expression was significantly less at 24, 48, 72, and 96 hours post-feeding. Expression of CBG mRNA was significantly greater in GD versus NGD granulosa cells. The results suggest that locally produced CBG at the GD could impact CORT availability, which could be important given that CORT has been shown to influence sex ratio determination just before ovulation in some bird species. Additionally, the results suggest that a decrease in testicular CBG expression may enhance testicular regression in response to elevated plasma CORT levels associated with fasting, which is opposite to what was reported in preovulatory follicles.

Keywords: Stress Response; Broiler Breeders; Laying Hens

Physiology, Endocrinology & Reproduction II

M11 Effects of broiler breeder egg translucency and oviposition time on maternal antibody levels, egg composition, and chick organ weights

Matthew B. Hughes*^{1GS}, Leticia Orellana¹, Kenneth S. Macklin¹, Chrysta N. Beck¹ ¹Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States

Vaccines are administered to broiler breeders to induce pathogen-specific maternal antibody transfer from hen to chick through the yolk. Egg translucency and oviposition can influence egg and chick quality, and oviposition has been reported to affect IgY levels in eggs of wild birds. The objective was to evaluate how translucency and oviposition affect maternal IgY transfer as well as egg and chick composition. Eggs were collected from a 35-wk-old commercial broiler breeder flock at two oviposition times (A-

9am, B-2pm) and sorted into three translucency scores (T1=low, T2=medium, T3=high) creating six experimental groups: T1-A, T1-B, T2-A, T2-B, T3-A, T3-B ($N=30$ eggs/group). Yolk and albumen weight (wt) were recorded, relative egg component (% egg wt) were calculated, yolks were collected and remaining eggs were incubated. At hatch, wt of total chick, liver, and residual yolk (Ryolk) were recorded, relative organ % (% body wt) were calculated, and Ryolk and blood plasma were collected ($N=10$ chicks/group). Total IgY levels in yolk, Ryolk, and plasma were measured by ELISA. Data were analyzed by two-way ANOVA (translucency, oviposition, and their interactions) using GLM Procedure in SAS. Means were separated using Tukey's HSD (significant at $P \leq 0.05$, trends at $0.05 < P \leq 0.10$). There were no differences by translucency level or oviposition time in yolk wt and %, Ryolk wt, liver wt, and total IgY levels in yolk, Ryolk, and

plasma. Liver % ($P=0.002$) was higher in chicks hatched from B eggs (2.6%) compared to those from A eggs (2.4%). For albumen wt ($P=0.054$), T1 and T3 had numerically higher wt (36.9g) than T2 (35.8g). For albumen % ($P=0.057$), T1 was numerically higher (61.7%) while T2 and T3 were lower (61%). For chick wt ($P=0.09$), chicks from A eggs were numerically heavier (41.7g) than those from B eggs (40.3g). There was a trend in Ryolk (oviposition*translucency; $P=0.09$), but this did not translate into differences in IgY levels in Ryolk (in total- or per mL-Ryolk content). Although maternal antibody levels did not differ by translucency score or oviposition time at 35 wk of age, liver wt differences suggest potential impacts on a chick's metabolic and homeostatic processes. Future research will evaluate egg and chick composition as broiler breeder flocks age.

Keywords: Broiler Breeder; Translucency; Oviposition Time; Antibodies; Liver

M12 Effect of group size on estradiol dynamics, follicular development, and egg production in broiler breeder hens

Patricia Quino^{*1GS}, Marcela Quino¹, Kristian J. Almendares¹, Nicole Dundur¹, Madison Berger¹, Olivia McGuire¹, Jeremiah D. Davis², Katie Elliot³, Charlene Hanlon¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States;* ²*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ³*USDA-ARS, Poultry Research Unit, Starkville, Mississippi, United States*

Social environment is a critical factor influencing reproductive performance in broiler breeder hens. This study aimed to determine how group size affects estradiol dynamics, follicular development, and egg production under controlled experimental conditions. A total of 480 Ross 308AP female and 40 Ross 344 male broiler breeders were reared under standard management. At 20 weeks of age (woa), birds were randomly allocated into two treatments ($n = 8$ pens each): small group pens (SG; 4'x6') and large group pens (LG; 15'x6'). SG pens housed 9 females and 1 male per pen, while LG pens housed 36 females and 4 males per pen. Stocking density and female-to-male ratio were consistent across treatments. Birds were photostimulated at 21 woa. Serial blood samples were collected from a representative subset of hens per pen between 18 and 60 woa. Plasma was extracted, and commercial ELISAs were used to measure total circulating 17- β estradiol. Egg production was recorded daily, and weekly hen-day production was calculated through 64 woa. Egg quality was assessed every 5 weeks during lay. Data were analyzed using linear mixed models in SAS v9.4, with treatment and age as fixed effects and pen as the experimental unit. Hens in SG achieved a higher peak production (97.1%) than hens in LG (86.8%; $P<0.001$). This resulted in SG hens exhibiting greater cumulative egg output by 64 woa (200.7 vs. 175.2 eggs; $P<0.001$). SG hens exhibited elevated peak estradiol concentrations (885.3 pg/mL) relative to LG hens (731.1 pg/mL; $P=0.016$), and tended to maintain a greater number of large yellow follicles ($P=0.082$). Although egg weight was not altered by treatment, eggs from SG had greater eggshell breaking strength ($P=0.045$) and a higher proportion of settable eggs due to reduced incidence of dirty eggs ($P<0.001$). These findings indicate that LG environments may contribute to elevated social stress, leading to reduced estradiol secretion, impaired follicular recruitment, and reduced egg production efficiency. Conversely, SG might appear to support a favorable physiological environment, enhancing ovarian function and reproductive performance. Further research is needed to identify the threshold at which group size begins to negatively impact reproductive outcomes under commercial conditions.

Keywords: Broiler Breeder; Estradiol; Follicle Recruitment; Group Size; Egg Quality

M13 Effects of cyclic heat stress on the blood chemistry of broiler breeders selected for water conversion ratio

Allison Weaver^{*1GS}, Karen M. Rivera¹, Rosie Whittle¹, Maricela A. Maqueda¹, Sara K. Orlowski-Workman¹, Shawna Weimer¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Rising global temperatures have put heat stress and water efficiency at the forefront of issues within the poultry industry due to increased water scarcity and mortality risk. We aimed to determine whether selection for water conversion ratio (WCR) affects the ability of broiler breeders to cope with cyclic heat stress. High-water conversion ratio (HW), low-water conversion ratio (LW), and modern random-bred (MRB) broiler breeder lines (36 females/line, 12 males/line) were evenly distributed across 12 environmentally controlled chambers ($N=12$ cages/chamber). Using a 3x2 split-plot design, 6 chambers underwent cyclic heat stress (HS; 32 ± 2 °C for 8 h per day from 31-34 wks), while 6 remained thermoneutral (TN) (22 ± 2 °C). Blood samples were collected for blood gas and chemistry analysis using a portable veterinary blood analyzer at the end of 34 wks (36 females and 35 males sampled over two days) and 1 week post HS in 36 fasted hens ($N=6$ /line/treatment) to analyze HS recovery (R). On sampling days, HS birds were sampled during the midpoint of the 8 h HS period. Sampling data were analyzed using linear regression, with separate models for males, females during the HS period, and R females. Line and treatment were included in each model as fixed effects. In males (M) and females (F) during HS, glucose was lower in LW (M: 233, F: 232 mg/dL) than in HW (M: 245, F: 243 mg/dl; $P\leq 0.03$). In TN males, HW had higher ($P=0.01$) iCa levels than LW (1.41 vs 1.29 mmol/L). In males and R females, LW had higher pCO₂ levels (M:57.2; RF: 42.8 mmHg) than HW (M:44.0; RF: 35.9 mmHg; $P\leq 0.04$). In R females, HW had a higher ($P=0.03$) pH than LW (7.38 vs 7.32) and lower ($P=0.001$) Na levels (145 vs 148 mmol/L). During recovery, TN females had higher ($P=0.005$) K than HS females (4.6 vs 4.1 mmol/L), whereas no difference was observed during the HS period. No significant differences between MRB and the other lines were observed across metrics. These results indicate that selection for WCR influences how broiler breeders respond to HS, and HW breeders may be less susceptible to metabolic and acid-base balance disturbances. Additionally, breeder females recovering from HS may be more susceptible to the effects of fasting.

Keywords: Broiler breeders; Heat stress; Physiology; Water conversion

M14 Effect of target body weight and the length of the starter phase on fertility and hatchability of broiler breeders

Artur De Freitas Ribeiro^{*1UG}, Gabriele Jardim Kinchin¹, Shivi Rao², Jesus Arango², Edgar O. Oviedo-Rondón¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*Cobb Genetics, Siloam Springs, Arkansas, United States*

Target body weight and the length of the starter phase affect feed allocation, cumulative nutrient intake, growth rate, and development, metabolism, and may consequently influence reproductive performance, egg traits, embryo development, 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 commercial 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 22 weeks with 72

hens and 7 roosters per pen. Eggs were collected three times a day, stored at 15 °C for a maximum of 4 days, and 84 eggs per pen were incubated at 25, 27, 29, 31, 34, 36, 38, and 40 weeks 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. Quadratic effects ($P < 0.001$) of breeder age were detected in almost all variables evaluated. Fertility and hatchability were affected by the length of the starter phase ($P < 0.001$) independently of pullet target BW. Hens fed starter for 4 weeks had better average fertility (99.41 ± 0.34 % vs. 98.02 %), average hatchability (93.94 ± 0.55 % vs. 92.20 %), and HOF (95.15 ± 0.43 % vs. 94.13 %) than hens fed starter for 6 weeks. The length of starter feed mainly affected early mortality occurring between 1 and 3 days of embryonic development (ED). Hens fed starter feed for 6 weeks had higher ($P < 0.01$) average 1-3 ED mortality (4.23%) than hens offered starter feed for 4 weeks (3.31%), independent of target BW. No effects of treatments ($P > 0.05$) were detected in other stages of ED, or in eggs contaminated, embryos mispositioned, or malformed. In conclusion, the length of the starter phase during rearing affected hen reproductive performance. Hens fed a starter diet for 6 weeks had lower fertility and hatchability, HOF, and higher early mortality, independent of target BW, up to 40 weeks of age.

Keywords: Fertility; Hatchability; embryo mortality; target BW; starter phase

M15 Testosterone variation in broiler breeder males: Role of group size, female exposure, spiking, and body weight

Kristian J. Almendares^{*1GS}, Marcela Quino¹, Patricia Quino¹, Nicole Dunder¹, Madison Berger¹, Olivia McGuire¹, Jeremiah D. Davis², Katie Elliot³, Charlene Hanlon¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States;* ²*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ³*USDA-ARS, Poultry Research Unit, Starkville, Mississippi, United States*

The global demand for high-quality chicks highlights the need to understand male reproductive performance, including hormonal activity and testicular development. This study evaluated the impact of group size (GS), female exposure, spiking, and body weight (BW) on testosterone profiles and testes size in Ross 344 broiler breeder males. Fifty-eight males were separate-sex reared up to 19 weeks of age (woa). At 20 woa, one week before photostimulation, males were placed into one of three groups: small GS (1 male: 9 females; n = 8 pens), large GS (4 males: 36 females; n = 8 pens), or a spiking group (n = 8 males). BW was recorded weekly to monitor growth. All males were blood sampled at 12, 16, 18 to 30, and every 5 weeks from 35 to 60 woa. Plasma was analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit. At 64 woa, testes and fat pad weights were recorded. Males were also categorized into High and Low BW classes (n=15 males/class) to determine the effect of extreme BW on male performance parameters. Statistical analyses were performed using the MIXED procedure in SAS version 9.4. Fixed effects for all males included age, GS, female exposure, spiking, and their interaction. Fixed effects for extreme BW groups were BW class and age. Age had an effect ($P < 0.001$) on testosterone levels, with levels low in immature birds and increasing between 12 and 21 woa. Testosterone significantly increased between 21 and 24 woa, reaching a maximum at 30 woa, before dropping rapidly at 35 woa and remaining steady thereafter. GS, female exposure, and spiking had no effect on testosterone levels. Regarding BW classes, groups differed in BW by 28 woa. At 64 woa, the High BW group was 5.53 ± 0.09 kg, while the Low BW

group was 4.14 ± 0.09 kg. High BW males had consistently higher testosterone concentration than the low BW males. Fat pad was not affected by BW, GS or age, whereas testes weight was affected by BW ($P = 0.004$), showing that high BW males have heavier testes than low BW. This study has demonstrated that the interaction of roosters with females or other males did not influence the testosterone profile. Therefore, BW was the only factor observed to affect testis weight and, consequently, alter testosterone concentrations.

Keywords: Testosterone; Spiking; Testes Size; Group Sizes; Broiler Breeder Roosters

M16 Ileum barrier integrity in broilers affected with Bacterial Chondronecrosis with osteomyelitis

Lily Luther^{*1UG}, Elizabeth S. Greene¹, Carrie Walk², Sami Dridi¹ ¹*University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States;* ²*AB Vista, Marlborough, United Kingdom*

Although poultry meat provides food security for billions of people worldwide, it is facing several challenges. Lameness, due to bacterial chondronecrosis with osteomyelitis (BCO), is a significant production, welfare, and economic burden to poultry production sustainability. Despite intensive research, it is not well known how the bacteria reaches the animal internally. Possibilities are respiratory, integumentary, and/or digestive. Our hypothesis is that birds infected with BCO are also met with complications in their gut intestinal lining. This would allow ingested bacteria to leak through the gut and enter the bloodstream, ultimately extending to the leg bone. The aim of the present study was, therefore, to assess gut integrity in BCO-affected birds compared to their healthy counterparts. Cobb 500 male broilers (n=1,920) were reared on litter or wire flooring. On day 56, gut integrity was evaluated using FITC-D (n = 10/group). Birds were also scored for lameness, and ileal tissues were collected, rinsed with PBS 1X, and snap frozen in liquid nitrogen. Total RNA was extracted and subjected to reverse transcription and real-time quantitative PCR. The relative expression of target genes was determined by 2- $\Delta\Delta C_t$ method. Data were analyzed by Student "t" test using Graph Pad software. Serum FITC-D levels were significantly higher in BCO compared to healthy birds, indicating a leaky gut syndrome in BCO birds. The expression of the claudin 5 (CLDN5) gene was significantly down regulated in BCO compared to healthy birds. The expressions of CLDN1, 2, 4, 8, 9, and 10 remained unchanged between the two groups. In summary, the expression of tight junction proteins was affected in BCO birds, and further studies to evaluate gap junctions, adherens, and desmosomes are warranted.

Keywords: BCO; tight junctions; claudins; ileum; gut integrity

M17 Replacing soybean meal with black soldier fly larvae meal or fishmeal: Impacts on broiler growth performance and cecal MAO and COMT enzyme activities

Samiru S. Wickramasuriya^{*1}, Joshua M. Lyte¹ ¹*USDA-ARS, Fayetteville, Arkansas, United States*

Despite the numerous studies that have evaluated how protein sources influence broiler growth, far less is known about how these proteins affect neurochemical pathways within the intestinal tract. Considering enteric neurochemicals contribute to a wide range of production-relevant functions including food safety, clarifying how different protein ingredients shape gut neurochemical profiles is essential. Thus, the present study examined whether partial replacement of soybean meal (SBM) with black soldier fly larvae meal (BSFLM) or fishmeal (FM) alters growth performance and the quantitative abundance of cecal monoamine oxidase (MAO-A and MAO-B) and catechol-O-methyltransferase (COMT) in

chickens. Male, day-old chicks were randomly assigned to three dietary treatments containing SBM, BSFLM, or FM. Chicks were housed in floor pens and managed under standard, age-appropriate conditions. Body weights were recorded, and cecal samples were collected from six birds per treatment group at 4 days of age. Tissues were homogenized and protein was quantified using the Bradford assay. The cecal protein lysate samples were analyzed using the Jess capillary immunoassay system, with incorporating a fluorescent standard, as per the manufacturer's instructions. Protein normalization was performed following manufacturer guidelines. All data were analyzed using one-way ANOVA followed by Tukey's post-hoc. Statistical significance was set at $p < 0.05$. At 4 days of age, body weight did not differ among chicks fed the three dietary treatments ($p > 0.05$). Chickens fed with the SBM diet exhibited higher MAO-A levels than those fed the FM

diet ($p < 0.05$), while MAO-A expression did not differ between the SBM and BSFLM treatments ($p > 0.05$). MAO-B levels were similar across all dietary treatments ($p > 0.05$). Membrane-bound COMT expression was higher than soluble COMT activity in all groups; however, no differences in COMT expression were detected among the three protein-source treatments. Partial replacement of SBM with BSFLM did not influence growth or the activity of cecal monoamine oxidase or catechol-O-methyltransferase in broilers by 4 days of age. Additional studies are underway evaluating long term impact of these dietary formulations on performance and intestinal neurochemistry.

Keywords: Black soldier fly larvae meal; chicken; gut; poultry; neurochemicals

Physiology, Endocrinology & Reproduction III

M18 Long-term changes in neural plasticity in broiler chickens due to embryonic temperature training: influence of sex and season

Barbara Tzschentke*¹ ¹*Institute for Agricultural and Urban Ecological Projects (IASP) at Humboldt-Universität zu Berlin, Berlin, Germany*

During critical periods of development, body functions can be programmed by environmental influences, with 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 affects neuronal molecular biomarkers related to developmental plasticity and energy metabolism, providing a physiological basis for improved adaptability to a stressful environment in male and female Ross 308 broilers. We investigated how STT (+1°C for 2 hrs per day) affects the basal mRNA expression levels of *Bdnf*, *Npy*, and *Pomc* at 35 days of age in the hypothalamic nucleus infundibular. The experiments were carried out during a winter and a summer trial with consistently higher ambient temperatures. In total, 126 eggs were used, incubated at 37.3°C, of which 62 were temperature trained on embryonic days 17-20. Mean mRNA expression levels at 35 days of age 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 ($N=72$, $p_{\text{adjust}}=0.048$). Multiple linear regression analyses revealed that this effect was mainly driven by differences in the summer trial. For all three target genes descriptively, 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 ambient temperature on the expression levels. For *Bdnf*, the difference was statistically significant. We did not observe any significant sex differences for any of the target genes in both trials. Our findings indicate that STT during a critical developmental period induces changes in mRNA in avian hypothalamic neurons that are associated with developmental plasticity and also correspond to the improved performance and robustness parameters found in numerous previous studies. This STT effect may contribute to a long-term physiological adaptation that can improve resistance to stressful environmental conditions, such as high ambient temperature, in broiler chickens.

Keywords: thermoregulation; gene expression; energy metabolism; brain-derived neurotrophic factor; broiler chicken

M19 Stress responses to water deprivation in the liver of broilers selected for low- and high-water efficiency

Seong W. Kang*¹, Chloe H. Sanchez², Rosie Whittle¹, Angela Perretti¹, Jaelen M. Cherry¹, Maricela A. Maqueda¹, Sara K. Orłowski-Workman¹, Shawna Weimer¹ ¹*University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States;* ²*University of Arkansas, Department of Biological Sciences, Fayetteville, Arkansas, United States*

Water is a fundamental resource for various physiological functions, and consequently, water shortages may negatively affect the productivity and welfare of chickens. Two broiler chicken lines were selected for high (HW) or low (LW) water efficiency, but their hepatic stress responses to water deprivation (WD) remain unclear. This study aimed to evaluate the impacts of WD on stress-responsive gene expression in the liver. HW and LW birds were subjected to 0, 12, and 24 hrs of WD, and liver samples were collected on D 38-40 ($n=8-12$ male and female/ WD treatment). Hepatic expression levels of glucocorticoid receptor (GR), 11 beta-hydroxysteroid dehydrogenase-1 (11b-HSD1), and brain-derived neurotrophic factor (BDNF) genes were determined by qPCR. Differences among treatment groups were analyzed using a one-way ANOVA within WD treatment and sex, followed by Tukey's HSD post hoc test (JMP Pro). Significance level was $p < 0.05$. Expressions of GR and 11b-HSD1 were increased by 50% and 28% in LW females compared to HW females, respectively ($p < 0.05$), with no differences in males, indicating a sex-dependent effect of water-efficiency selection on the hepatic stress response. WD increased GR and 11b-HSD1 expression 3.2- and 2.3-fold, respectively, in HW males, but not in the HW females ($p < 0.05$). LW females showed the downregulation of GR expression by WD, and 11b-HSD1 expression was reduced by 24 hrs of WD stress in both sexes ($p < 0.05$). BDNF expression in HW males was threefold higher compared to HW females ($p < 0.05$), but there was no sex difference in LW birds. WD caused a significant time-dependent decrease in BDNF expression in both lines, suggesting that the effect of selection for water efficiency in broilers on the BDNF expression was not sex-dependent. Taken together, these results suggest that divergent selection for water efficiency in broilers significantly reduced hepatic stress response to WD in female birds but not in males. Moreover, the pronounced stress responses of male HW broilers to WD stress, compared with females, indicate sex-specific, favorable WD stress responses in HW than in LW broilers. The downregulation of BDNF by WD may indicate a functional role for BDNF in the WD stress response in the livers of broilers selected for water efficiency.

Keywords: Broilers; Water deprivation; Liver; Stress responses; BDNF

M20 Bardoxolone methyl reduces oxidative stress in turkey uterovaginal junction organoids by activating Nrf2 and modulating NF- κ B signaling

Pitchaya Santativongchai^{*1}, Sunantha Kosonsiriluk¹, Scott O'Grady¹, David Brown², Ben Wileman³, Marissa Studniski³, Kahina Boukherroub¹ ¹University of Minnesota, Animal Science, Saint Paul, Minnesota, United States; ²University of Minnesota, Veterinary and Biomedical Sciences, Saint Paul, Minnesota, United States; ³Select Genetics, Willmar, Minnesota, United States

Turkey production faces significant challenges in maintaining breeder flock fertility, particularly after 10 weeks of lay, restricting commercial poult supply. Our previous studies revealed increased inflammation and accelerated aging in the distal reproductive tract, coinciding with fertility decline. Both processes are known to elevate oxidative stress. Bardoxolone methyl (BAR), a semi-synthetic plant derivative of oleanolic acid, reduces inflammation and oxidative stress in mammals by activating the antioxidant Nrf2 pathway and inhibiting the pro-inflammatory NF- κ B pathway. This study aimed to determine the effect of BAR on the Nrf2 and NF- κ B signaling in turkey oviductal organoids derived from the distal reproductive tract. Organoids were generated from uterovaginal junction (UVJ) tissues of four peak-laying hens (6th week of lay) and treated with 200 μ M hydrogen peroxide (H₂O₂) and increasing concentrations of BAR (0.25, 0.5, and 1 μ M). Data

were analyzed by one-way ANOVA followed by Bonferroni's post-hoc test for multiple comparisons and unpaired t-test for two-group comparisons. Treatment with 0.5 μ M BAR increased organoid viability ($p < 0.05$) at 3 h post-treatment. BAR also reduced NF- κ B nuclear translocation at 6 h and significantly decreased reactive oxygen species production ($p < 0.05$) at 24 h in H₂O₂-treated organoids, indicating lower oxidative stress and potential inflammation. Furthermore, BAR (0.5 μ M) enhanced Nrf2 nuclear translocation and upregulated mRNA expression of Nrf2-downstream antioxidant genes (i.e. *HMOX1* and *TXNRD1*) and the Nrf2-responsive genes (i.e. *OSGIN1* and *MAFG*) in both treatments with and without H₂O₂. Preliminary *in vivo* data (2-week BAR treatment, n = 20) showed no negative effect on body weight or egg production, but a significant spleen weight reduction ($p < 0.05$) and upregulation of the phosphatidylinositol signaling pathway and the related genes in vaginal tissues, suggesting dampened oxidative-stress and inflammatory signaling, although further confirmation is needed. These findings suggest that BAR activates the Nrf2 pathway and mitigates oxidative stress in turkey oviductal organoids. Long-term *in vivo* studies are required to assess its potential for alleviating inflammation and improving fertility in breeder hens.

Keywords: Fertility; Inflammation; Oviduct; Oxidative stress; Turkey hen

Welfare & Behavior I

M21 Impact of phytase and azomite supplementation on the radiographic and ultrasonographic findings in laying hens

Mackenzie A. Ripper^{*1GS}, Ishab Poudel¹, Rebecca Wysocky¹, Alba Frias-De-Diego¹, Kenneth E. Anderson¹, Rocio Crespo¹ ¹North Carolina State University, Raleigh, North Carolina, United States

This study evaluated bone density and dimensions, and keel fractures by radiography and ultrasound in post-peak production laying hen. Nine hundred and sixty, 50-wk-old hens were randomly divided into two dietary groups supplemented with phytase at 0 or 1000 FYT/g. Each group was further subdivided into 3 groups receiving Azomite® supplementation at 0%, 0.25% and 0.5%. Each experimental unit consisted of 30 hens, with 5 replicates per treatment. At 75 weeks of age, one hen from each replicate was randomly sampled for radiographs and ultrasound images of the keel and the left tibiotarsus. Radiographs were obtained in lateral and ventrodorsal projections. Ultrasound was performed using a handheld portable ultrasound, while the bird was in lateral recumbency. Mean grayscale values were measured for the entire cross section, cortex and medulla of the tibiotarsus. Ultrasound images of the keel were evaluated for their reliability in identifying bone fractures compared to radiographs. Data were analyzed using a linear regression model in JMP® Student Edition 18.2.1, with a significance level of $P < 0.05$. Results showed no difference in the diameter of the tibiotarsus measured by radiograph and ultrasound. No differences in the diameter of the tibiotarsus were observed between the phytase or Azomite® treatment groups either. However, phytase supplementation significantly increased total and cortical bone density but not medullary bone density or incidence of keel fractures. Azomite® had no significant effect on the bone density or the incidence of keel fractures. Imaging modality had a significant effect in the detection of keel fractures. In summary, 1) phytase but not Azomite® supplementation enhanced bone density in post peak production laying hens; 2) both radiography and ultrasound are comparable for measuring the diameter of the tibiotarsus; 3) no significant differences were observed in the number of keel fractures by dietary treatment or imaging technique. Radiographs provided clear visualization of long bone density and their cortical

margins. Ultrasound offered real-time, dynamic imaging but was limited in the ability to evaluate bone density of long bones. Overall, radiography provided superior bone structure and density evaluation compared to ultrasound.

Keywords: Ultrasound; Radiograph; Layers; Azomite; Phosphorous

M22 Effects of housing design on musculoskeletal health in Hy-Line Brown laying hens

Alexis Millspaugh^{*1GS}, Cerano Harrison¹, Janice Siegford², Ahmed Ali¹ ¹Clemson University, Animal and Veterinary Sciences, Walhalla, South Carolina, United States; ²Michigan State University, Department of Animal Science, East Lansing, Michigan, United States

Osteoporotic implications on skeletal health, such as increased bone fragility and susceptibility to fractures, are major welfare concerns in laying hens, causing pain, limiting mobility, and reducing productivity. Exercise opportunities support bone mineral deposition and muscle hypertrophy by stimulating mechanical loading and adaptive remodeling. Housing configurations shape activity by influencing resource layout and movement paths, ultimately affecting musculoskeletal outcomes. This study evaluated the effects of two aviary configurations and their associated exercise opportunities on musculoskeletal health in laying hens. The objective was to compare hens housed in two commercially available aviaries [Big Dutchman NATURA STEP (STEP) and NATURA 60 (N60)] to generate recommendations for housing and management in commercial settings. Hy-Line Brown pullets (n=3,696) at 16 WOA were randomly allocated to the two configurations, each subdivided into three rooms with four sections per room. At 60 WOA, 5% of hens per aviary were euthanized for assessment of bone mineral density, keel bone damage, muscle weights, biomechanical properties, and ash percentage. Differences across systems were analyzed using GLMM with Tukey's post-hoc test applied to significant effects ($\alpha=0.05$) in R 3.3.1. Cortical and medullary bone mineral densities were significantly higher in STEP hens ($p=0.029$ and 0.036). Keel damage rates differed between systems for both new and old

fractures (cranial incomplete $p = 0.036$; caudal complete $p=0.042$; caudal incomplete $p = 0.029$; cranial callus $p=0.039$; caudal callus $p=0.026$). The proportion of keel deviation was lower in STEP hens ($p=0.031$). Muscle weights, including biceps brachii, pectoralis major and minor, and the leg-muscle group, were higher in STEP hens ($p=0.026$; 0.031; 0.039; 0.042). Biomechanical testing indicated greater tibial and humeral breaking strength and humeral stiffness in STEP ($p=0.033$; 0.048; 0.043). Tibial and humeral ash percentages were likewise higher ($p=0.032$; 0.039). In conclusion, aviary systems that enable flight-related movement patterns are associated with improved musculoskeletal health but also higher rates of collision-associated fractures, highlighting the need for targeted design to mitigate injury risk.

Keywords: Musculoskeletal Health; Aviary Systems; Keel Bone Damage; Bone Mineral Density; Laying Hen

M23 Effects of delayed nest box placement on nest box utilization in laying hens aged 41 to 53 weeks

Xavia A. Harris^{*1GS}, Patrick Tamatey¹, Stephanie A. Ansah¹, Dervan Bryan¹ ¹*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States*

The global egg industry is undergoing a significant transformation as it shifts from conventional caged systems to cage-free production, driven by consumer demand for improved animal welfare, retailer commitments, and evolving legislative pressures. Cage-free environments allow hens more natural behaviors like perching and dust-bathing, which aligns with public expectations for humane treatment. However, this transition introduces complex management challenges, particularly the issue of floor eggs. Eggs laid outside designated nesting areas pose hygiene concerns, increase labor demands, and can reduce overall egg quality and marketability. These issues highlight the need for refined facility design and equipment usage, early hen training, and nest accessibility strategies to reduce floor eggs. In the current study, we hypothesized that delaying nest box placement early in the life of laying hens would increase the incidence of floor eggs beyond 40 weeks of age. A total of 1200 Lohmann LSL-Lite pullets, 16 weeks of age, were randomly distributed to 24 floor pens measuring 10ft x 8ft with 50 birds per pen. Each pen was equipped with two-line nipple drinkers, each with 3 nipples and two feeders. A 10-hole nest box was introduced to 12 replicate pens at 17 weeks of age (On-Time) and at 23 weeks of age (delayed). All other management practices were based on the breeder's recommendation. The number of floor eggs was recorded from 41 to 53 weeks of age. The data was analyzed using one-way ANOVA. Differences were considered significant at ($p \leq 0.05$). Delayed nest box placement significantly reduced the number of eggs laid in the nest box by hens between 41 and 52 weeks ($P < 0.05$), with the on-time group consistently showing higher weekly egg numbers in the nest box. The greatest difference occurred at week 45 (174.8 vs. 81.9 eggs; $P < 0.001$). Although the delayed group exhibited gradual improvement over time, a non-significant difference between treatment groups was only observed at week 53 ($P = 0.173$). These findings highlight the importance of timely nest box access for maintaining optimal nest box utilization during the laying period.

Keywords: hen; nest box; cage-free; floor eggs

M24 Creatures of habit? Impact of chick feeder tray location on feed consumption at 0-7 days of age

Emily K. Green^{*1GS}, Zac Williams¹, Shawna Weimer¹, Benjamin Parsons¹, Samantha Robinson² ¹*University of Arkansas, Poultry Science, Farmington, Arkansas, United States*; ²*University of Arkansas, Center for Agricultural Data Analytics, Fayetteville, Arkansas, United States*

Early chick nutrition is critical for establishing long term performance in broilers. This study evaluated spatial factors within commercial broiler houses that influence feed consumption during days 0-7. Observing behavior and eating preferences can help prepare producers to create critically planned and effectively designed areas for maximum feed intake. Two controlled trials were conducted in a 12.2 x 122 m broiler house. In Trial 1, a one-way experimental design was used in which the house was divided into four sections using migratory fences at every 30.5 m, house sections were included as the fixed factor. A total of 300 chick trays were distributed throughout the house and 18 trays per section were randomly selected for feed intake measurements. Feed consumption differed significantly across sections, increasing from 0.135 kg in section 1 to 0.188 kg in section 4 ($p < 0.05$). In Trial 2, a 3 x 4 (treatment by location) factorial design was implemented to evaluate the effects of spatial barriers and feedline position on feed intake. All feed was provided using standardized chick trays, placed directly under chickmats to mimic commercial operations and eliminate feeder-type effects. The statistical model included treatments of wall (W), migratory fence (MF), and no fence (NF) and feedline location (north vs. south) as fixed factors. A total of 293 trays were monitored for daily feed additions and final feed weight on day 7. ANOVA with post-hoc pairwise comparisons demonstrated significant differences among treatments (W: 3.69 kg; MF: 2.69 kg; and NF: 2.25 kg; $p < 0.0001$) and between feedline locations (north: 2.85 kg; south: 2.18 kg; $p < 0.0001$). Across both trials, the highest feed consumption was observed near walls and along the north feedline, indicating these spatial locations provide more favorable conditions for early feed intake in broiler houses. Optimizing house layouts to account for chicks' preferred areas might enhance early feed intake and promote better overall flock performance for 0-to-7-day old broilers.

Keywords: Broilers; Chick nutrition; Behavior; Brooding; Location

M25 The regulation of the hepatic angiotensinogen and renin expression by water deprivation in low- and high-water efficient broilers

Chloe H. Sanchez^{*1UG}, Seong W. Kang², Rosie Whittle², Angela Perretti², Jaelen M. Cherry², Maricela A. Maqueda², Sara K. Orłowski-Workman², Shawna Weimer² ¹*University of Arkansas, Department of Biological Sciences, Fayetteville, Arkansas, United States*; ²*University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States*

Water is crucial for the sustainability of broiler production and welfare. Two broiler lines selected for high (HW) and low (LW) water efficiency were used to investigate the mechanisms underlying water intake and osmotic regulation. This study aimed to evaluate the effects of water deprivation (WD) on hepatic gene expression of angiotensinogen (AGT), a component of the renin-angiotensin system that regulates osmotic fluid balance, and renin (REN), a component that regulates fluid and salt balance. HW and LW broilers were subjected to either 0h (control), 12h, or 24h of WD on D25-27 and D38-40. Liver samples were collected on D39 and D40 ($n=8-12$ males and females/treatment). Hepatic expressions of the AGT and REN genes were determined by qPCR. Differences among treatment groups were analyzed using one-way ANOVA within WD treatment and sex, followed by mean separation using Tukey's HSD test using JMP Pro. Significance level was $p < 0.05$. AGT expression was higher in males compared to females in both selected lines ($p < 0.05$), and the effects of 12h and 24h of WD were more pronounced in HW birds compared to LW, in both sexes. AGT expression was 43% and 62% higher in HW and LW males compared to females ($p < 0.05$).

indicating sex-dependent differences in AGT expression within both lines. REN expression was higher in HW males compared to HW females (38%, $p < 0.05$), and expression was considerably lower in LW males compared to HW males (23%, $p < 0.05$), suggesting sex-specific levels of REN expression in both lines. REN expression in HW males was increased by 12h and 24h of WD compared to control males ($p < 0.05$), but there were no differences in REN between control and 12h of WD HW females. REN expression in LW females and males was not altered by 24h of WD. Taken together, these results suggest WD influenced hepatic AGT and REN expression levels in broilers, with responses varying by genetic line and sex. HW male broilers exhibited greater activation of these genes under WD, indicating improved physiological mechanisms for maintaining osmotic balance and sex-specific control of water homeostasis.

Keywords: broilers; liver; angiotensinogen; renin; water deprivation

M26 Quantitative and biological evidence for health-dependent space-use dynamics in broilers

Oluwadamilola M. Oso^{*1GS}, Guoming Li¹²³, Chongxiao Chen¹³, Prafulla Regmi¹, Samuel E. Aggrey¹, Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States;* ²*University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States;* ³*University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States*

Poor gait impairs broiler mobility; however, the effect on spatial behavior remains unquantified. This study evaluated the effect of gait health on broiler spatial behavioral diversity and space-use patterns. Ten healthy (gait score 0) or unhealthy (gait score 1-2) broilers were tracked for 1 hour in the morning, afternoon, and evening for 3 consecutive days a week at weeks 4-7. The pen was divided into zones of unequal areas: corners, open spaces, and feeding and drinking areas. Data was normalized by zone's area to account for size disparity. The Shannon Index (H') measured how evenly individuals in each group distributed their time across zones. A higher H' shows greater diversity or more even use of space. Cluster-bootstrap resampling method with 5,000 iterations was used to calculate 95% confidence intervals for group differences. Space-use patterns were assessed via Shannon decomposition (ΔH ; healthy minus unhealthy), centered log-ratio transformation (CLR) and principal component analysis (PCA). Spatial behavioral diversity was significantly higher in unhealthy birds than healthy birds (at weeks 4 and 5, at week 6, and at week 7). In weeks 4-5, CLR-PCA revealed drinking-area use as the main driver of variation against feeding areas/open spaces (PC1: 65.6% and 57.1%), with corner use as secondary (PC2: 34.0% and 37.1%). By weeks 6-7, corner use became the dominant factor (PC1: 68.0%, 59.9%), with the drinking area as secondary (PC2: 22.5%, 35.5%), indicating an age-related shift in spatial behavior linked to gait health. The ΔH showed differences (-0.004 and -0.005 nats) in weeks 4 and 5 due to intense use of the drinking area by unhealthy birds (zone contributions of ≈ -0.007 and -0.006 nats). By week 6, ΔH was 0.000 nats due to opposing behavioral tendencies. In week 7, unhealthy birds contributed more to diversity differences ($\Delta H \approx -0.001$ nats) as they increasingly confined themselves to pen corners (a contribution of -0.003 nats). In summary, unhealthy birds transitioned from resource-driven patterns (weeks 4-5) to corner-based spatial segregation (weeks 6-7). These results indicate that more even space-use patterns do not always mean better welfare, since spatial restriction may cause apparent "evenness" while signaling underlying health issues.

Keywords: Broiler welfare; Gait score; Spatial behavior; Precision poultry; Poultry management

M27 Impact of rearing environment and social rank on the reproductive behaviors in broiler breeder roosters

Anusha Gautam^{*1GS}, Adam Davis¹, Prafulla Regmi¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

Early-life social factors and male hierarchical status can influence reproductive outcomes in birds, and these dynamics may partly explain the fertility decline observed in broiler breeders. This study evaluated 1) differences in dominance-related behaviors as influenced by visual access to females and social rank during rearing, and 2) differences in reproductive behaviors between the highest- and lowest-ranked roosters during early production. A total of 152 1-d-old male Ross 308 parent stock chicks were randomly assigned to 4 floor pens (38 birds/pen), with two pens having visual access to females. At 20 weeks, males were mixed with females (4 roosters:38 females) in 12 production pens equipped with nestboxes and with the floor space consisting of one-third litter and two-thirds elevated slats. Behavioral data were collected from 1-d of video observation during rearing at 16 and 20 weeks using scan sampling. During early production (21 weeks), the social rank of individual roosters ($n=24$) was determined using a Win-Loss ratio from observed encounters. Aggressive, submissive, and reproductive behaviors were recorded using a predefined ethogram and compared using the Kruskal-Wallis test. No significant differences were observed in dominant or submissive behaviors between roosters with visual access ($6.875\% \pm 1.064$) and no visual access ($6.625\% \pm 0.749$) to females during rearing or early production. However, prior social rank influenced male-to-female aggression, with dominant males spending more time exhibiting aggression ($2.8s \pm 0.820$) than subordinate males ($2.0s \pm 0.698$) during early production. Roosters that were submissive during rearing tended to remain submissive during early production ($P = 0.05$). At 21 weeks, dominant males tended to spend more time performing reproductive behaviors ($1.83s \pm 0.792$) than subordinate males ($0.167s \pm 0.167$) ($P = 0.06$). Overall, visual exposure to females during rearing had a limited impact on male-male aggression and reproductive behaviors during the early laying period, whereas early social rank predicted some reproductive outcomes in broiler breeder roosters.

Keywords: social hierarchy; aggressive behaviors; dominance; mating

M28 The effects of dietary fiber and feed form on feather pecking, feeding, and foraging behaviors of turkey toms

Juan F. Castro Baltodano^{*1GS}, Adam C. Fahrenholz¹, Jesse Grimes¹, Lisa Bielke¹, Allison Pullin¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Feather pecking in turkeys can lead to injuries, culls, and mortality. Dietary interventions have been successful in mitigating this behavior in laying hens. Including higher levels of fiber promoted gut fill, while providing fines particles required birds to spend more time eating and less time pecking flock mates. However, these strategies have not been studied in turkeys. We aimed to evaluate the effects of dietary fiber and feed form on feeding, foraging, and feather pecking. On day of hatch, 740 Large White male poults were provided with one of two dietary fiber levels in a crumble feed form: High fiber (3% CF) and Low fiber (2% CF; 16 pens/treatment, 22 birds/pen). At 5 wk, feed form changed from crumbles to either fines or pellets to create four treatments ($n=8$ pens/treatment): High fiber, pelleted (HFP; 4% CF), High fiber, fines (HFF; 4% CF), Low fiber, pelleted (LFP; 2% CF), and Low fiber, fines (LFF; 2% CF). By 9 wk, the CF increased to 5% for both HF treatments while LF treatments remained at 2% CF. Eight focal birds/pen were video recorded for 30 min at 4 and 9 wk to analyze the frequency of back, wing, and tail pecking (BWT),

foraging bouts, and feeding bouts. Data were analyzed in R software using generalized linear mixed models to assess the effect of fiber at 4 wk and feed form, fiber, and their interaction at 9 wk. At 4 wk, there was no effect of fiber on BWT pecking ($p=0.77$), feeding ($p=0.44$), or foraging ($p=0.16$). At 9 wk, there was a main effect of feed form on BWT pecking ($p=0.05$) and feeding bouts ($p=0.003$). Fines-fed birds displayed more BWT pecking than pellet-fed birds (40.3 [32.9, 49.3] and 25.1 [20.0, 31.6] BWT pecks/bird, respectively; mean [95% CI]). Fines-fed birds also displayed more feeding bouts than pellet-fed birds (8.7 [6.1, 12.4] and 3 **Keywords**: 8 [2.6, 5.4] feeding bouts/bird, respectively). Fiber did not affect BWT pecking ($p=0.26$) or feeding bouts ($p=0.19$) at 9 wk. Foraging was not affected at 9 wk by feed form, fiber, or their interaction ($p>0.05$). The fines feed was likely more difficult and time consuming to eat than pellets. Inefficient feeding may have caused frustration and redirected pecks onto flock mates. The relatively small differences in CF between treatments likely limited the effects of fiber on behavior.

Keywords: turkey; feather pecking; fiber; pellet; fines

M29 Exploring fear in Japanese quail selected for high and low corticosterone response to immobilization

Rosie Whittle^{*1}, Gregory S. Fraley², Maricela A. Maqueda¹, Sara K. Orłowski-Workman¹, Shawna Weimer¹ ¹University of Arkansas, Fayetteville, Arkansas, United States; ²Purdue University, West Lafayette, Indiana, United States

Corticosterone (CORT) is a key hormone in avian stress physiology, and the selective breeding for its responsiveness offers a model for studying behavioral and physiological coping mechanisms. This study investigated how genetic selection for CORT responsiveness influences behavioral and physiological

stress responses of Japanese quail. Three genetic lines of quail, selected for high or low CORT response to immobilization stress and a random bred line (RB), were raised in mixed sex floor pens (48 pens, 16 per line). At 5 and 10 weeks, tonic immobility (TI) and open field (OF) tests were conducted on 3 quail per pen (N=144). Videos of OF tests were analyzed using EthoVision to measure distance, velocity, and transitions. At 10 weeks, an immobilization handling test was conducted for 15 mins on 63 quail. Before (baseline) and after handling, blood samples were collected for CORT measures with an ELISA, and infrared thermal images of the eye and beak were collected. Using a split-plot design with Line, Sex, and Age as fixed factors, data were analyzed in R using LMM, Cox proportional hazard, and multinomial analyses. Line did not affect TI duration, and the duration was shorter at 10 (167s) than 5 weeks (285s, $p<0.001$). High were more likely (0.82) to have TI induced on the first attempt than Low (0.53, $p=0.004$) and RB (0.65, $p=0.098$). Low quail had greater distance, velocity, transitions, and activity during the OF (3114cm, 10.39cm/s, 64.2, 0.56%) than RB (1814cm, 6.04cm/s, 35.3, 0.33%, $p<0.001$), but did not differ from High (2428cm, 8.09cm/s, 48.5, 0.44%). There was no difference in baseline CORT levels for High (4526pg/mL), Low (4461pg/mL), or RB (4456pg/mL). After handling, High quail tended to have higher CORT (46196pg/mL) than Low (25187pg/mL, $p=0.08$) but not RB (41977pg/mL). Eye temperature increased after handling for High (34.9 to 36.0C, $p=0.003$) and RB (35.1 to 35.8C, $p=0.03$), but not for Low (35.6 to 35.4C). Beak temperature was not affected by Line ($p=0.22$) but generally increased post-handling (30.9 to 36.4C, $p<0.001$). Line-specific variation in physiological stress and behavior suggests that corticosterone selection may not be directly linked to fear responses.

Keywords: Japanese quail; Corticosterone; Selection; Stress; Fear

Pathology

M30 Effect of two phytogetic extracts on coccidiosis and growth performance in broiler chickens

Manuela Parini^{*1}, Noel Lumbo², Krystalene Decena², Vanessa Mulimbayan⁴, Filippo Gasparri³, Rommel Sulabo² ¹A.W.P. S.p.A., R&D, Castelnuovo Rangone, Modena, Italy; ²College of Agriculture and Food Science, Institute of Animal Science, Laguna, Los Baños, Philippines; ³A.W.P. S.p.A., Commercial and R&D, Castelnuovo Rangone, Modena, Italy; ⁴Biostar, Commercial and R&D, San Pablo City, Laguna, Philippines

Coccidiosis remains a major challenge for the poultry industry, particularly in antibiotic-free production systems. Moreover, it is a well-recognized predisposing factor for necrotic enteritis, a disease responsible for substantial global economic losses in poultry production. The objective of the present study was to evaluate the efficacy of two phytogetic extract compositions - CoxxOil (formulated for coccidiosis control) and MixOil++ (formulated for necrotic enteritis control) - on broiler performance under a coccidiosis challenge induced by the application of used litter containing coccidia from previous production cycles. A total of 600 day-old, straight-run Cobb 500 broiler chicks were randomly assigned to six dietary treatments following a randomized complete block design, with 10 replicate cages per treatment and 10 birds per cage. The group T1, negative control (NC), did not receive any antibiotic or coccidiostat; the group T2 received 1,330ppm of zinc bacitracin (ZB) and 500ppm of maduramicin (MAD), both from 0 to 24 dd; the group T3 received 1,330ppm of ZB (dd 0-24); the group T4 received 1,330ppm of ZB and 100ppm of CoxxOil, both from 0 to 24 dd; the group T5 received 100ppm of CoxxOil (dd 0-24) and MixOil++ dosed at 1000ppm (dd 0-11), 700ppm (dd 12-24) 500ppm (dd 25-33); the group T6 received 500ppm of MAD (dd 0-24) and MixOil++

dosed at 1000ppm (dd 0-11), 700ppm (dd 12-24), 500ppm (dd 25-33). Data were analyzed using the MIXED procedure of SAS, with pen considered the experimental unit. At day 33, the average daily gain (ADG) was significantly higher ($P < 0.05$) in treatments T4, T5, and T6, all of which received phytogetic extracts, compared with T1 (NC), T2, and T3 (ZB or ZB + MAD). The feed conversion ratio (FCR) was significantly lower ($P < 0.05$) in T4 and T5 compared with T1, T2, and T3. In T6, the FCR was significantly lower ($P < 0.05$) than in T1 and T3, and did not differ from T2, T4, or T5. The production efficiency index (PEI) was significantly higher ($P < 0.05$) in T4, T5, and T6 relative to the remaining groups. In conclusion, the results demonstrate that phytogetic extracts represent a promising alternative to ionophore coccidiostats and zinc bacitracin as performance enhancers under coccidiosis challenge conditions in broilers.

Keywords: Coccidiosis; Phytogetics; Essential oils; Antibiotic-free; Natural coccidiostats

M31 Cracking the *Eimeria* code: A survey of commercial US turkey operations

Vijay Durairaj^{*1}, Steven Clark², Emily Barber¹, Ryan Vander Veen¹ ¹Huvepharma Inc, Lincoln, Nebraska, United States; ²Huvepharma Inc, Peachtree City, Georgia, United States

Turkey coccidiosis causes significant economic losses to the poultry industry. Understanding the prevalence of *Eimeria* species helps to identify appropriate strategies to combat coccidiosis. During May – October 2025, 51 sample submissions were received from different U.S. commercial turkey operations. Among the samples, 78.4% were from vaccinated flocks and 21.6% were from non-vaccinated flocks. The submitted samples included feces,

litter and intestines. The samples were processed, and PCR was performed for a panel of six *Eimeria* species: *E. meleagridis*, *E. adenoides*, *E. gallopavonis*, *E. meleagridis*, *E. dispersa* and *E. innocua*. The vaccinated flocks were positive for the *Eimeria* species included in the vaccines. Among the non-vaccinated flocks, the prevalence of *Eimeria* species in decreasing order is reported as *E. meleagridis* / *E. meleagridis* > *E. adenoides* > *E. gallopavonis* > *E. dispersa* > *E. innocua*.

Keywords: Coccidiosis; Turkeys; *Eimeria* species; *Eimeria* surveillance; Vaccination

M32 A synopsis of avian metapneumovirus outbreak in broilers and broiler breeders in Alabama

Pankaj P. Gaonkar*^{1,2,3,4,5}, Carey Laster¹, Arthur Cheng^{1,2}, Larsen Bryan¹, Brigetta Hughes³, Sam Christenberry⁴, Terry Slaten⁵, Margaret Handley¹, Kellye Joiner², Heather Walz¹, Mohammadreza Ehsan¹, Erfan Chowdhury¹ ¹Thompson Bishop Sparks State Diagnostic Laboratory System, Auburn, Alabama, United States; ²College of Veterinary Medicine, Auburn University, Pathobiology, Auburn, Alabama, United States; ³Mitchem Sparks State Diagnostic Laboratory System, Boaz, Alabama, United States; ⁴J.B. Taylor State Diagnostic Laboratory System, Elba, Alabama, United States; ⁵Hinton Mitchem State Diagnostic Laboratory System, Hanceville, Alabama, United States

Avian metapneumovirus (aMPV) causes highly contagious upper respiratory and occasionally reproductive infections in poultry. It has recently emerged in the United States: type A in 2023 and type B in 2024. It can lead to increased morbidity and mortality in commercial flocks, resulting in significant economic losses. This study summarizes diagnostic findings from commercial poultry submissions to the Alabama Veterinary Diagnostic Laboratory System (AVDLS) between January and October 2025. A total of 511 oropharyngeal, tracheal or nasal swabs from 221 commercial broiler breeder and broiler cases submitted at AVDLS for necropsy were tested for aMPV-A and B, and occasionally -C by PCR. Overall, 30.77% cases (n=68/221; 30 breeder, 38 broiler) were aMPV positive. Serotype A was detected in 33.96% (n=54/159) of samples, serotype B in 5.85% (n=11/188), and both in 0.86% (n=3/347). There was no detection of serotype C (n=0/11). The age of affected breeder flocks ranged from 168-420 days, and broilers from 34-53 days. Flock histories provided with necropsy submission included high mortality, swollen heads, and lameness. Gross lesions included swollen head, fibrinous and/or caseous exudates in the nares, skull, lung, heart, liver, and joints. Histologically, fibrinoheterophilic, caseous-granulomatous, and necrotizing lesions with intralésional bacteria were observed in these tissues. Lymphocytic meningitis (n=3), encephalitis (n=1), and tracheitis (n=36) were also observed. The major bacterial pathogens isolated were *Escherichia coli* (n = 47), *Pasteurella multocida* (n = 3), and *Staphylococcus* spp. (n=14). Viral co-detections included infectious bronchitis virus (n=38), avian paramyxovirus (n=1), and vaccinal infectious laryngotracheitis virus (n=3). ELISA- aMPV (A, B, C) on 3138 sera from 90 broiler breeder and 4 broiler farms showed 86.63% (n=2690/3105) of breeders and 78.79% (n=26/33) of broilers were positive for aMPV antibodies. Among 20 farms tested by ELISA and PCR, 50% were positive only by ELISA, and 50% were positive by both assays. These results highlight the ongoing presence of aMPV in Alabama commercial poultry and underscore the need for continued diagnostic surveillance through both PCR and ELISA to better understand and mitigate its impact.

Keywords: Avian metapneumovirus; Surveillance; Pathology; PCR; ELISA

M33 Molecular and pathological insights into Fowl adenovirus-E (FAdV-8b) in southeastern U.S. poultry

Md Saidul Haque*^{1,2}, Saiada Farjana¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States

Fowl adenoviruses (FAdVs) are globally distributed poultry pathogens causing economically significant diseases, including Inclusion Body Hepatitis (IBH). In the United States, the incidence of IBH and the detection of FAdV, particularly FAdV species E serotypes such as 8b are increasing, highlighting a growing regional and national concern. Despite endemic circulation, molecular and pathological characterization of U.S. field strains remains limited. This study aims to characterize the molecular and pathogenic features of five FAdV isolates (four from Alabama, one from Georgia) collected from clinical IBH cases between 2024-2025. Isolates were propagated in specific-pathogen-free chicken embryos, DNA was extracted, and the hexon gene was amplified by PCR and sequenced via Sanger methods. Sequences were quality-filtered, aligned, and analyzed using MEGA 12; phylogenetic tree was constructed using the Neighbor-Joining method with 1,000 bootstrap replications. Partial hexon gene sequencing identified all isolates as FAdV-E serotype 8b. Phylogenetic analysis revealed a monophyletic clade with two internal sub-lineages, suggesting multiple co-circulating lineages. Comparative analysis indicated high nucleotide identity with global FAdV-E/8b strains from Turkey, Israel, Bangladesh, Indonesia, Pakistan, Uganda, and Peru. In vivo embryo inoculations revealed variable lesions severity, including multifocal hepatic necrosis, hemorrhage, renal necrosis, chorioallantoic membrane thickening, edema, and stunted growth. In vitro, primary chicken embryo kidney and liver cells exhibited consistent cytopathic effects. Together, these findings confirm FAdV-E 8b as the predominant serotype involved in recent southeastern U.S. IBH cases and demonstrate significant pathogenic variation among closely related isolates. This underscores that genetic similarity in the hexon gene does not uniformly predict virulence, highlighting the need to integrate molecular data with phenotypic assessments. This study emphasizes the importance of continuous surveillance, expanded genomic characterization via WGS, improved understanding of genotype-phenotype relationships, vaccine development, and enhanced biosecurity to mitigate the growing threat of FAdV to U.S. poultry production.

Keywords: Fowl adenovirus (FAdV); Hexon gene; Phylogenetics; Inclusion Body Hepatitis (IBH); Chicken embryo pathology

M34 Innovative serological assays for poultry vector vaccines monitoring and DIVA testing of Newcastle disease, Infectious Laryngotracheitis and Infectious Gumboro Disease

Stephanie Lesceu¹, Chloe Redal¹, Jean-Emmanuel Drus¹, Catherine Lefebvre¹, Marianne Zorbas¹, Philippe Pourquier¹, Marina Gaimard², Rafael Forero*² ¹Innovative Diagnostics, Grabels, France; ²IDvet INC, Hampton, New Hampshire, United States

Recombinant vector vaccines based on FPV or HVT enable broader protection against major poultry pathogens and support DIVA (Differentiation of Infected and Vaccinated Animals) strategies. Conventional serological assays often fail to detect seroconversion to recombinant constructs. This study evaluated newly developed ELISA assays for monitoring vaccination responses to FP-LT-gB, rHVT-ND-LT and rHVT-IBD-LT vaccines and assessed their suitability for DIVA applications. Three evaluations were conducted. In Study 1, layers from Jordan (n=15) vaccinated with an FP-LT-gB recombinant vaccine were sampled 5 weeks post-vaccination and tested using ILTGBS and

ILTgI kits. In Study 2, broilers from Poland (n=21) vaccinated at day-old with rHVT-ND-LT were tested with ILTGIS, ILTGDS, ILTGBS, NDVS and NDVNP. In Study 3, two broiler flocks from the USA vaccinated in-ovo with rHVT-IBD-LT were evaluated: Flock 1 (n=15) non-challenged and Flock 2 (n=12) challenged with a virulent ILTV strain at 30 days of age. Samples were analyzed using ILTGIS, ILTGDS, ILTGBS, IBDVP2 and IBDS. Statistical analysis included mean titers, standard deviations and positivity rates. In Study 1, ILTGBS detected strong vaccine seroconversion (mean titer 2545; 100% positivity), while ILTgI remained negative, confirming absence of ILTV field infection. In Study 2, ILTGIS and ILTGDS confirmed ILT seroconversion, and ILTGBS negativity indicated no ILTV challenge. NDVS detected robust NDV seroconversion (maximum titer 21,538), and NDVNP positivity evidence a likely recent NDV challenge. In Study 3, non-challenged broilers showed expected ILT and IBD vaccine seroconversion with negative challenge-detection assays. Challenged broilers showed elevated ILT titers and 100% ILTGBS positivity at 11 days post-challenge. These results demonstrate that ILTGIS, ILTGDS, ILTGBS, IBDVP2 and IBDS ELISAs reliably monitor recombinant vaccine seroconversion and accurately differentiate vaccine-induced antibodies from field infection, supporting robust DIVA implementation.

Keywords: Recombinant vaccines; ELISA; DIVA; ILT; IBD

M35 Microbiome shifts in turkey poult associated with age and *Cochlosoma anatis* infection

Justin Lowery*^{1GS}, Caleb M. Marshall¹, Christina S. Sigmon¹, Benjamin Callahan², Lin Walker¹ ¹North Carolina State University, *Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²North Carolina State University, *College of Veterinary Medicine, Raleigh, North Carolina, United States*

Cochlosoma anatis is a flagellated protozoan parasite of the turkey small intestine that causes diarrhea, enteric lesions, stunting, and coinfections, yet its disease mechanisms are poorly understood. This preliminary study evaluated the effects of *C. anatis* infection on developing poult gut microbiota. Sixteen samples were analyzed, including ileal and jejunal content from naïve d14 poults and ileal and jejunal content and jejunal mucosa from naïve and infected d28 poults (inoculated at d14). Microbiota were sequenced with Illumina whole-genome shotgun sequencing. Taxonomic profiling, α - and β -diversity, and differential abundance analyses were performed in CLC Genomics Workbench using richness, Bray-Curtis PCoA, and heat maps. Metadata associations were evaluated using MaAsLin2 in R. Diversity analyses were conducted independently for ileal and jejunal contents and jejunal mucosa, with comparisons by age and infection status within sample types to account for sequencing depth variation. Naïve poults at d14 showed higher taxonomic diversity in ileal content than naïve poults at d28, with no difference in jejunal content taxonomic diversity among naïve poults. Infected poults differed from naïve poults at d28 by taxonomic diversity only in the jejunal content, with richer microbiota in infected poults. No distinct β -diversity clustering was observed. Lactobacillaceae were associated with relative abundance differences across age and infection, accounting for 19-23 % of the differences in naïve poults and 23-46 % of the infection-associated differences at d28. They also accounted for three of the four taxa with $q < 0.05$, indicating statistically significant differences in addition to observed qualitative taxonomic differences. Bacterial richness decreased with host maturation, as previously reported. Infection appeared to impair jejunal mucosal microbiota development, leading to reduced community stability. Lactobacillaceae play key roles in

fermentation and nutrient absorption and may be a target for gut microbiota modulation during *C. anatis* infection. Future research should incorporate analyses of bacterial taxa and manipulation of the poult gut microbiota to resist changes associated with disease.

Keywords: *Cochlosoma anatis*; Microbiome; Turkeys; Poults; Enteritis

M36 Development of an *in-vivo* model to evaluate pathogenesis and bacterial translocation of *Enterococcus cecorum* in broilers

Thaciane L. Amaral*^{1GS}, Demilade I. Ibiwoye¹, Philip Yeboah², Tracy Omasanuwa³, Muhammad Wajeeh Ul Hassan⁶, Alexandra Ulans¹, Allison Pullin⁴, Hugo Romero⁵, Lisa Bielke¹ ¹North Carolina State University, *Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²North Carolina State University, *Prestage Poultry Science Department, Raleigh, North Carolina, United States*; ³North Carolina State University, *Prestage Poultry Science Department, Raleigh, North Carolina, United States*; ⁴North Carolina State University, *Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ⁵Novus International, *Wake Forest, North Carolina, United States*; ⁶North Carolina State University, *Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Enterococcus cecorum (EC) is associated with septicemia in young broilers and with degenerative lesions of the femoral head (FH), tibial head (TH), and free thoracic vertebrae (FTV), which leads to lameness later in production. Despite increasing reports, *in-vivo* models that capture EC progression across the full broiler cycle remain limited. This study aimed to develop an EC challenge model to assess early systemic translocation, skeletal lesion development, and late mobility impairment. Male Cobb 500 broilers were assigned to a positive (PC) and negative (NC) groups, with 12 pens per treatment and 14 birds per pen (336 birds). PC were infected with 1×10^4 CFU of an EC lameness-associated isolate on d1 and 14. On d14, 21, 28, 35, and 42, 2 birds/pen were assessed for latency-to-lie (LTL), spleen recovery (Log₁₀ CFU/mL), and FH and TH lesion scores; FTV samples were collected on d42. Data were analyzed using one-way ANOVA for Log₁₀ CFU/mL and LTL and ordinal logistic regression for lesion scores ($p < 0.05$). Results indicated that systemic bacterial translocation differed between treatments, with PC birds showing higher spleen Log₁₀ CFU/mL on d14 (4.39 vs. 3.79; $P = 0.0042$). Thereafter, both groups showed similar bacterial loads (3–5 Log₁₀ CFU/mL) until d28, followed by a decline to less than 3 Log₁₀ CFU/mL from d35–42. FH and TH lesions increased progressively in both groups, but differed on d42. LTL values began to separate on d28–35 (NC 60.0 s; PC 31.6 s) and differed on d42 ($P = 0.0413$), when NC birds remained standing longer than PC birds (63.0 s vs. 26.4 s; $P = 0.046$). Early mortalities happened between d0–13 (PC=8; NC=2) and lameness-related deaths peaked on d28–34 (PC=6; NC=5). Total mortality was higher in PC (20) than NC (12). On d42, FTV cultures were predominantly positive in both groups (PC=10/10; NC=8/10). Overall, the model reproduced the expected progression of EC infection. A shift from systemic to skeletal infection was evident, as higher early bacterial loads aligned with higher lesion scores while spleen recovery declined as lesions advanced. Further refinement is needed to maintain true negative controls and strengthen the model for evaluating preventive or therapeutic strategies targeting bacterial translocation, lameness, and performance losses.

Keywords: *Enterococcus cecorum*; *in vivo*; pathology; lameness

M37 Immuno-metabolic consequences of IL-6 modulation on performance and energy utilization of *Salmonella* Infantis

challenged chickens raised with/without probiotics at early growth phase

Oluwatomide Ariyo*^{1GS}, Josephine Kwakye¹, Adelumola Oladeinde², Michael Rothrock², Tomohiro Hamaoka³, Walid Al Hakeem¹, Anthony Pokoo-Aikins², Jodie Plumblee Lawrence², Marie Milfort¹, Alberta Fuller¹, Samuel E. Aggrey¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²USDA, ARS, Athens, Georgia, United States; ³ASAHI Biosciences, Peachtree, Georgia, United States

Salmonella Infantis (*S. Infantis*) is an emerging cause of foodborne illness and has been shown to induce inflammation in chickens. IL6 is a proinflammatory cytokine that stimulates immune responses and modulates metabolic pathways, linking inflammation to altered nutrient use and energy balance that may affect chicken performance. We inoculated broilers with *S. Infantis* and explored the efficacy of probiotics on performance and *Salmonella* reduction in flock using 520 one-day-old Cobb 500 broiler chickens. Birds were randomly allotted to 4 treatment groups: Negative Control without probiotic nor *S. Infantis* (NeC), *S. Infantis*; –without probiotic (PoC), –with commercial probiotic in feed (Pro1), and –with ARS probiotic in litter (Pro2), each replicated 4–6 times. Each replicate had 13 males and 13 females of similar body weight (BW) across all pens. On day 0, 6 broilers/replicate were inoculated with both antibiotic-susceptible and antibiotic-resistant strain of *S. Infantis* in the infected groups while the NeC group received PBS. The BW of birds were measured by pen to determine body weight gain (BWG), and the feed conversion ratio (FCR) was calculated by dividing feed intake by BWG. On day 7, ileum (mRNA expression of nutrient transporters, IL6, PPAR α , ACO and CPT1 α) and ceca (*Salmonella* abundance) were collected from eight random broiler chickens per treatment. Data collected were subjected to one way ANOVA and means were separated by Tukey test. Pro1 and Pro2 had a lower ceca *Salmonella* load compared to PoC but PoC had a significantly higher BWG and lower FCR compared to Pro1 and Pro2 had a higher ($P < 0.0001$). The mRNA expressions of SGLT1, FABP1, FABP2 and FABP3 were downwardly expressed in the Pro1 chickens compared to the PoC, NeC and Pro2 groups. IL6 was downwardly expressed with probiotic intervention. Evaluating IL6 as a metabolic agent revealed a downward expression of PPAR α and ACO in the probiotics group while PPAR γ was not influenced when compared to the PoC and NeC groups. In sum, our results indicate that the ability of probiotics to reduce *S. Infantis* load and suppress inflammation in broilers comes at a metabolic cost which can impair performance via IL6 mediated glucose and fatty acid metabolism.

Keywords: Salmonella; Probiotics; Interleukin 6; immunometabolism; Performance

M38 Whole genome sequencing characterization of broiler breeder to chick transmitted bacteria

Muhammad Wajeeh Ul Hassan*^{1GS}, Lisa Bielke¹, Nivedita Pareek² ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, North Carolina, United States

The gut microbiome is essential to poultry health, influencing digestion, immunity, and disease resistance. As artificial incubation limits maternal microbial transfer, chicks often develop microbiomes shaped by their environment rather than their hens. This study characterized the microbiome of broiler breeder hens and day-of-hatch chicks and assesses the potential for bacterial transmission between them. Samples from five 69-week-old hens including ceca, crop, small intestine, cloaca, and eggs, day-of-hatch chicks, and hatchery fluff were processed for whole-genome

shotgun sequencing. DNA was processed for whole genome sequencing. Reads were quality-filtered, trimmed, and taxonomically classified with Kraken2/Bracken. Results showed that alpha diversity, measured by Pielou's evenness, ranged from 0.72 to 0.89, indicating generally balanced microbial communities across sample types. The ceca and small intestine were the most stable, while the crop and day-of-hatch GIT showed greater variability. Although some site pairs showed minor differences ($p < 0.05$), none remained significant after correction ($q > 0.05$), indicating overall similar evenness across groups. Bray–Curtis PERMANOVA showed that the cloaca and small intestine were the hen sites most similar to the day-of-hatch GIT, with only marginal or non-significant differences ($q = 0.05–0.17$), suggesting limited maternal contribution and some continuity along the intestinal axis. Eggs and hatchery fluff showed similar microbial profiles ($q = 0.21$) and were not significantly different from the small intestine or day-of-hatch GIT ($q = 0.05–0.21$). In contrast, the ceca and crop formed distinct communities ($q \leq 0.03$) and are unlikely contributors to initial chick microbiota. Our findings suggest that the cloaca and small intestine are the most likely maternal contributors to early chick microbiota, while the hatchery environment also exerting a significant influence. The cloaca and small intestine were most comparable to day-of-hatch GIT communities, while eggs and fluff only moderately and non-significantly overlapped. Overall, these patterns imply that rather than direct egg-based transmission, initial colonization is a result of both hatchery exposure and limited maternal cloacal input.

Keywords: gut microbiome; whole genome sequencing; broiler breeder; transmission; hatchery

M39 Impact of probiotics on the prevalence of antimicrobial resistant *Escherichia coli* population in broiler chickens challenged with *Salmonella* Infantis

Josephine Kwakye*^{1GS}, Oluwatomide Ariyo¹, Adelumola Oladeinde², Michael Rothrock², Tomohiro Hamaoka³, Walid Al Hakeem¹, Anthony Pokoo-Aikins², Jodie Plumblee Lawrence², Marie Milfort¹, Alberta Fuller¹, Samuel E. Aggrey¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²USDA-ARS, U.S. National Poultry Research Center, Athens, Georgia, United States; ³ASAHI Biosciences, Peachtree, Georgia, United States

The gut microbiome of poultry serves as a reservoir for antimicrobial resistance (AMR) posing a significant threat to food safety. *Escherichia coli* and *Salmonella* co-exist in the gut of poultry and may exchange AMR genes. To evaluate the impact of probiotics on the prevalence of drug-resistant *E. coli*, 480 Cobb 500 broiler chicks (1:1 sex ratio) were randomly assigned to four treatment groups (T1-*Bacillus velezensis* added to feed (10^9 colony forming unit (CFU)/g); T2-ARS probiotic on litter; T3-positive control; T4-negative control). Birds were housed in floor pens (6 replicates for T1/T2, 4 for T3/T4). On day 0, six birds from T1–T3 were orally inoculated with two *S. Infantis* strains (10^6 CFU/chick), one resistant to nalidixic acid (Nal) and one resistant to six antibiotics including Nal. Ceca samples were collected at random on days 7, 14, 28 (8 birds/treatment) and day 42 (12 birds/treatment); fecal droppings were collected after feed withdrawal on day 49 (4 replicate/treatment). All isolates recovered were confirmed as *E. coli* by quantitative PCR, and AMR was assessed via broth microdilution to determine the minimum inhibitory concentrations of *E. coli* isolates against 13 antibiotics (24 isolates/treatment; $n=480$). Pearson's chi-squared with Bonferroni correction (chi square= 20.42, d.f. = 3, $p = 0.00014$) revealed significant differences in resistance profiles. Birds in T2 had the highest proportion of susceptible isolates (98.5%) and the lowest resistance (1.5%), followed by T1 (97.9%

susceptible, 2.1% resistance), and T3 (96.9% susceptible, 3.1% resistance). Surprisingly, the negative control (T4) had the highest resistance (3.9%) and lowest susceptibility rate (96.1%) across treatment groups. The proportion of resistant isolates in T2 was significantly lower than T4 (adjusted $p = 0.00024$) and T3 (adjusted $p = 0.034$). Similarly, resistant isolates in T1 group were significantly lower than T4 (adjusted $p = 0.013$) but not T3 (adjusted $p = 0.53$). We did not observe any significant difference (adjusted $p > 0.05$) between the two probiotic groups (T1 vs. T2) or the two control groups (T3 vs. T4). These findings imply that probiotics can reduce the prevalence of AMR in broiler chickens.

Keywords: Probiotics; Antibiotic susceptibility; *Escherichia coli*; *Salmonella*; Broiler chickens

M40 Restricted ovulator hens as a novel experimental model of fatty liver disease

Daniel Lee*^{1,2,GS}, Venkata Prathap Reddy Keshavareddy¹, Doyun Goo¹, Hemanth Reddy Katha¹, Hamid Reza Rafeian Naeini¹, Ishwari Gyawali¹, Sai Kumar Reddy Ragyari¹, Indira Neupane¹, Seshidhar Reddy Gudidoddi¹, Deependra Paneru¹, Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*; ²*Seoul National University, Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul, Korea (the Republic of)*

Fatty liver syndrome (FLS) is a condition in laying hens characterized by excessive hepatic lipid accumulation, which often leads to reduced egg production and can result in substantial economic losses in poultry production. Therefore, establishing reliable experimental models is essential for advancing research on the prevention and mitigation of FLS. Among current induction methods, a low-choline and high-cholesterol diet is widely recognized as a rapid and effective approach for producing fatty liver. Restricted ovulator (RO) hens possess a point mutation in the oocyte VLDL receptor that limits ovulation and leads to marked hyperlipidemia and hypercholesterolemia. Given these metabolic features, we hypothesized that RO hens may serve as a valuable model for studying FLS. In this study, a total of 72 Hy-Line hens (24 weeks old), consisting of 36 wild-type (WT) and 36 RO hens, were assigned to one of three diets: a normal diet, a low-choline diet, or a low-choline and high-cholesterol diet. The experiment was conducted for 4 weeks. Data were analyzed using two-way ANOVA with genotype and diet as main effects. RO hens exhibited significantly higher liver-to-body weight ratios than WT hens, with the greatest increases observed under the low-choline and high-cholesterol diet. Hepatic total lipid, cholesterol, and triglyceride levels showed the same pattern, showing significant elevations in RO hens compared to WT hens, and reached the highest levels under low-choline and high-cholesterol diet. Lipogenic gene expression did not differ between the genotypes but was significantly upregulated by the low-choline and high-cholesterol diet. These findings indicate that RO hens develop

hepatic lipid accumulation more effectively than WT hens, making them a valuable model for inducing FLS.

Keywords: Fatty liver disease; Fatty liver syndrome; Fatty liver hemorrhagic syndrome; Restricted ovulator hens

M41 Hematologic patterns associated with blepharitis severity in turkey breeder hens

Carlie Caron*¹, Sunantha Kosonsiriluk¹, Paul F. Cotter², Pitchaya Santativongchai¹, Rosemary Marusak³, Sally L. Noll¹, Kahina Boukherroub¹ ¹*University of Minnesota, Department of Animal Science, St. Paul, Minnesota, United States*; ²*Cotter Laboratory, Arlington, Massachusetts, United States*; ³*University of Minnesota, Department of Veterinary Medicine, St. Paul, Minnesota, United States*

Blepharitis is an inflammatory eyelid condition in turkey breeder hens that poses important welfare and economic concerns. The condition begins with mild crusting and progresses to severe swelling and eye closure, leading to anorexia, welfare culls, and reduced reproductive performance. Its poorly understood, multifactorial etiology likely includes environmental irritation, host factors, and secondary inflammatory responses that may extend beyond the local lesion. This study aimed to determine if blepharitis progression is associated with systemic immune changes using $\Delta H/L$ ratios and differential leukocyte counts. Blood samples were collected from a turkey breeder flock near the end of production (60.5 wks of age; $n = 26$). Birds were assigned to 3 lesion-severity scores: 0 (unaffected, $n = 7$), 1 (mild/moderate swelling/crusting, $n = 5$), and 2 (severe swelling/crusting, complete eye closure, $n = 14$). Blood smears were stained with Wright's stain, scanned using a Huron TissueScope LE, and annotated in QuPath. Quantified parameters included $\Delta H/L$ ratio, lymphocyte subsets, monocytes, eosinophils, and basophils. $\Delta H/L$ was calculated as the difference between the traditional heterophil-to-resting-lymphocyte ratio and an expanded ratio incorporating active lymphocytes. Data were analyzed using Kruskal-Wallis tests with Dunnett's T3 post-hoc comparisons. Basophils followed a low-high-low pattern, with a trend toward elevation in mildly affected hens versus unaffected hens ($p = 0.078$) and a significant decrease from mild to severe lesions ($p = 0.011$). This aligns with their role in acute inflammation via vasodilation activity. Plasmacytes, rare in scores 0 and 1 but increased in severe cases, indicated a humoral response. $\Delta H/L$ did not differ significantly across scores; however, values > 5 occurred only in severely affected hens and were absent in scores 0 and 1. This supports a systemic B-cell response during severe progression. These hematological changes suggest that the progression of blepharitis involves a systemic immunological shift from acute inflammation to a chronic humoral response and support the concept that this condition extends beyond a localized ocular lesion.

Keywords: Blepharitis; Hematology; Inflammation; Leukocyte profile; Turkey breeder hens

SCAD I

M42 A dual-window approach reveals local and systemic inflammatory responses triggered by intradermal injections of lipopolysaccharide in turkeys

Jossie M. Santamaria*¹, Chrysta N. Beck², Aaron Forga¹, Kasey A. Matusik¹, Delaney Roberts¹, Adeola A. Abe¹, Diego Cortes¹, Melissa Martinez¹, James Higuaita¹, Karen Echevarria¹, Danielle Graham¹, Gisela Erf¹ ¹*University of Arkansas System Division of Agriculture, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States*; ²*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States*

The information about the immunostimulatory effects of bacterial components in turkeys is limited. In this study, local and systemic inflammatory responses to intradermal (i.d.) growing feather (GF)-pulp injections of lipopolysaccharide (LPS; *Salmonella* Typhimurium), a cell wall component of gram-negative bacteria, were evaluated. Two treatment groups, each consisting of 5-wk-old turkey poults, received i.d. GF-pulp injections (10 μ L/GF; 16 GFs/poult) with either endotoxin-free PBS ($n=5$) or LPS (1 μ g/GF; $n=8$). GF and blood were sampled before (0h) and at 3, 6, 24, 48, and 72h p.i. to assess GF-pulp and blood leukocyte profiles using FACS. A 2-way ANOVA and a 2-way repeated

measures ANOVA were used to analyze GF and blood data, respectively. Student's t-test means comparison was applied when appropriate, and significance was set at $P \leq 0.05$. GF-pulp injection of LPS triggered a gradual influx of heterophils and MHCII⁺ cells, reaching peak levels (% pulp cells) at 6h, and declining to near baseline levels at 72h p.i. ($P < 0.001$). Levels of heterophils and MHCII⁺ cells were higher with LPS than with PBS at all time points p.i. ($P < 0.001$). Following injections, CD4⁺ T cells increased to highest levels at 6h, and remained elevated thereafter ($P = 0.016$), whereby levels were higher with LPS than with PBS ($P < 0.001$). Independent of time, levels of CD28⁺ T cells were higher with LPS than with PBS ($P < 0.001$), and levels of CD8⁺ cells did not change. Independent of treatment, B cell levels were highest at 48h p.i. and remained elevated up to 72h p.i. ($P = 0.003$). In the blood, independent of treatment, concentrations (cells/ μ L) of heterophils peaked at 3h and returned to baseline levels by 24h p.i. ($P = 0.002$), while those of MHCII⁺ cells peaked at 48 h, and returned to near baseline at 72h p.i. ($P < 0.001$). LPS resulted in a drop in the concentrations of CD28⁺ ($P = 0.015$), CD4⁺ ($P = 0.003$), CD8⁺ ($P = 0.007$), and B cells ($P = 0.002$) at 3h and 6h p.i., returning to near baseline levels thereafter. The results from the dual-window approach in turkeys offer temporal, qualitative, and quantitative insights into the immunostimulatory effects of LPS, revealing similar patterns of leukocyte infiltration at the injection site and changes in blood leukocyte concentrations to those observed in chickens.

Keywords: Turkeys; Lipopolysaccharide; GF bioassay; Inflammatory response; Leukocytes

M43 Development and *in vitro* characterization of a circular RNA vaccine platform against avian reovirus

Zubair Khalid*^{1,2}, Suman Bhattacharai¹, Lok Raj Joshi¹ ¹*University of Georgia, Precision One-Health, Athens, Georgia, United States;* ²*University of Maryland, Animal and Avian Sciences, College Park, Maryland, United States*

Contemporary immunization strategies against avian reovirus (ARV), an economically significant pathogen in the poultry industry, rely primarily on live-attenuated and autogenous vaccine formulations. These approaches remain resource-intensive and time-consuming, underscoring the need for adaptable and rapidly deployable antigen delivery systems. Circular RNAs offer a promising next-generation platform for efficient antigen expression, as their covalently closed structure confers resistance to exonucleolytic degradation and supports sustained translational activity. Building on this concept, we developed and characterized a circular RNA expression system for the ARV Sigma-C (σ C) protein, which mediates host-cell attachment and serves as the principal target of virus-neutralizing antibodies. First, a pilot construct encoding green fluorescent protein was employed to optimize circularization and compare expression from circular and linear RNA templates. The ARV S1133 σ C gene was subsequently cloned into a plasmid engineered to produce circular RNA transcripts through a permuted intron-exon splicing mechanism. *In vitro* transcription of the plasmid yielded RNA of the expected size, verified by denaturing agarose gel electrophoresis, and RNA quality was assessed using a Bioanalyzer. Circularization was confirmed by RNase R digestion, which selectively degraded linear RNA species, and by Sanger sequencing of the back-splice junction. Expression of circular and non-circular control plasmids was demonstrated in T7 polymerase-expressing BHK cells, validating the design. Furthermore, *in vitro*-transcribed circular RNAs were transfected into DF-1 and HEK293T cells, where σ C expression was confirmed by immunofluorescence using a rabbit polyclonal antibody specific to ARV σ C. Collectively, these findings demonstrate that the permuted intron-exon configuration

enables efficient RNA circularization and robust protein translation in avian and mammalian cells. This work establishes a foundational molecular framework for future evaluations of circular RNA-based vaccines against emerging ARV field strains.

Keywords: circular RNA; avian reovirus; sigma C; expression; vaccine

M44 Molecular characterization of virulent *Enterococcus faecalis* strains associated with a recurring outbreak of amyloid arthropathy in laying chickens

Grayson K. Walker*¹, Mitsuo Suyemoto¹, Mike Petrik², Rocio Crespo¹ ¹*North Carolina State University College of Veterinary Medicine, Population Health and Pathobiology, Raleigh, North Carolina, United States;* ²*McKinley Hatchery, St. Marys, Ontario, Canada*

Enterococcus faecalis (EF) normally colonizes the intestines and rearing environments of poultry, but virulent strains have been implicated as the etiology of lameness due to amyloid arthropathy (AA) in laying chickens. A recent outbreak of AA in brown layer breeder pullet flocks has been reported in Canada. The goal of the present study was to characterize EF strains isolated from a recurring outbreak reared at the same production facility despite cleaning and disinfection between flocks. Five EF isolates were cultured from hock and stifle joints of brown layer breeder pullets that were culled due to lameness and exhibited gross evidence of AA. Isolates were obtained from a single flock of approximately 3,200 pullets reared in cages. Whole-genome sequencing was performed for the five isolates using Oxford Nanopore Technology with subsequent genome assembly and annotation. EF genome sizes were each 3.3 Mb and assembled coverage and annotated genes ranged from 101 to 104 X and 3,241 to 3,256 genes, respectively. Phylogenetic analysis was used to compare these EF to those isolated from a previous AA outbreak, which revealed the five isolates were clonally related to one another but had distinct genotypes from earlier strains. Strains were further characterized by *in silico* virulence genotyping, multi-locus sequence typing, and human pathogen probability calculation. Similar to a historic virulent EF strain associated with AA, the five strains were determined to be sequence type 82 and contained cytolysin (*cylA,B,L,M*) and the *gelE* protease virulence genes. The EF strains exhibited 0.896 probability of being a human pathogen based on genotype, highlighting public health risk and supporting phenotypic virulence in poultry. In conclusion, virulent EF strains associated with AA may be an emerging disease in North American layer production and further investigation, including determining the origin of these strains in poultry production, is warranted.

Keywords: *Enterococcus faecalis*; amyloid arthropathy; layers; epidemiology

M45 Field experiences with a live immune-complex IBD vaccine in broilers in the Southeast USA

Eric Shepherd*¹, Brian Jordan¹, Kalen Cookson², Tim Cummings¹, Po-Hsin Yu³ ¹*Zoetis, Aberdeen, North Carolina, United States;* ²*Zoetis, Hoschton, Georgia, United States;* ³*Zoetis, Durham, North Carolina, United States*

Infectious Bursal Disease Virus (IBDV) is a constantly evolving challenge in dense areas of commercial broiler production in the USA. Since the USA primarily is confronted with variant IBDV, the manifestations of this disease are secondary to the resulting immune suppression which can be quite dramatic. One of the most important causes of broiler mortality has been associated with Inclusion Body Hepatitis (IBH) where adenovirus can be either a primary or secondary pathogen. Internal data has shown that

complexes that have over relied on HVT vectored IBDV vaccines have seen their variant challenge window shift to progressively earlier over time. The purpose of this field trial was to evaluate the effectiveness of a live immune-complex vaccine, Poulvac® Bursaplex®, in a commercial broiler complex to combat a high variant IBDV challenge. Data assessed included: Bursal surveys, Livability, Body Weight (BW), Adjusted Feed Conversion (Adj. FCR), Percent Condemns, and Total Cost Per Pound Body Weight. Post Bursaplex flocks (vs Pre Bursaplex flocks) improved in every performance category: 15pts of BW, 6pts of adj. FCR, 2.4% livability, 0.13% condemns and cost of 1.7 cents/lb BW. This integrator showed an immediate improvement in bursal health, bird health and performance, and a reduction in IBH mortality once Bursaplex was introduced into their vaccine program. The employment of a rotational IBD vaccine program between immune-complex and HVT vectored IBD vaccines has shown benefits in multiple locations by protecting against and reducing stubborn variant challenge in the field. Ongoing field trials are in progress and are showing the continued benefit of both the introduction and rotational use of Bursaplex for IBDV control in commercial broiler operations.

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Keywords: Bursaplex; Bursal Disease; IBDV; immune-complex; immunosuppression

M46 A study measuring spleen HVT takes over time in commercial broilers *in ovo* vaccinated with a recombinant HVT-IBD vaccine

Kalen Cookson^{*1}, Benjamin N. Alig¹, Brian Jordan¹, Po-Hsin Yu¹ ¹Zoetis, Durham, North Carolina, United States

Recombinant HVT vaccines have been a mainstay of the broiler industry for well over a decade. Because the HVT virus does not horizontally transmit, it is critical that every broiler chick is properly administered the vaccine in order to acquire both Marek's protection and an immune response to the gene(s) the recombinant HVT is carrying. Using an HVT PCR assay, our goal was to determine what might be considered a "normal" flock profile at different ages of testing for our rHVT-IBD vaccine. Study Design: 200 commercial broiler day-of-age chicks were placed in a pen with pine shavings after being vaccinated *in ovo* with a full dose of recombinant HVT-IBD vaccine. On days 3, 7, 10, 14, 21 and 28, twenty birds were randomly selected, sacrificed and their spleens tested for the presence of HVT using real time PCR of a fragment of the SORF1 gene. Results: By day 3, 50% of samples were already PCR positive (Ct<40) and at each subsequent sampling (days 7 through 28) 95% of samples were positive for HVT at each test interval. The mean Ct value of positives was 35.9 at day 3 with more light positives (35% above Ct-35) than "strong" (15% below Ct-35). Between 7-21 days the mean Ct ranged between 32.1 (day 7) to 33.4 (day 14) with only 10-20% being light positives. While there were still 95% positives at the study's termination at 28 days of age, the mean Ct value rose to 34.2 with 35% light positives. The Ct values of the positives rarely dipped below Ct-30 at each interval from 1 to 4 weeks of age—so 75-85%

of the samples had Ct values between 30-35. Conclusion: Sampling spleens using real time PCR for HVT has the potential to establish what are "normal" replication dynamics for the different HVT vaccines. Knowing this can help set the expectations for onset of insert expression and immunity as well as help troubleshoot various critical control points in the hatchery such as proper vaccine handling/application and even embryo staging at time of injection.

Keywords: IBD; recombinant; PCR; HVT; spleens

M47 Understanding recombinant HVT-ILT-IBD vaccine seroconversion using indirect ELISA with recombinant proteins as coated antigens

Daniel Maekawa^{*1}, Ana Zamora², Roshan Paudel², Stephanie Lesceu³, Maricarmen Garcia² ¹Merck Animal Health, Rahway, New Jersey, United States; ²University of Georgia, Population Health, Athens, Georgia, United States; ³IDvet, Grabels, France

Recombinant vaccines that utilize the Herpesvirus of Turkey (HVT) as a vector have become widely available in the poultry industry. HVT viral vectors have large double-stranded DNA genomes that can accommodate expression cassettes with foreign genes encoding immunogenic viral proteins such as VP2, F protein, and glycoprotein D (gD) and I (gI) for Infectious bursal disease virus (IBDV), Newcastle disease virus (NDV), and Infectious laryngotracheitis virus (ILTV), respectively. With the widespread use of recombinant vaccines, new ELISA tools for monitoring recombinant vaccine seroconversion have emerged, utilizing heterologous recombinant proteins as the ELISA antigen. The objective of this study was to evaluate the serological response to two recombinant (r) HVT-ILT-IBD vaccines using conventional and recombinant indirect ELISA kits (ID Screen®, IDvet, Grabels, France). The rHVT-ILT-IBD (gD-gI) and the rHVT-IBD-ILT (gD) vaccines were administered to commercial broilers at 18 days of embryonation, and blood samples were collected at 4, 6, and 8 weeks of age to evaluate seroconversion with ELISA kits with recombinant antigens (gD, gI, gB, VP2, VP3) and viral antigens (ILTV, IBDV). Antibody titers against gI and gD for rHVT-ILT-IBD (gD-gI) vaccinated chickens were comparable at 4, 6, and 8 weeks of age. However, a higher percentage of serum positive samples was observed for gI (100%) than for gD (83%). For the rHVT-IBD-ILT (gD) vaccinated group of chickens, as expected, seroconversion to gD but not gI was detected at 4, 6, and 8 weeks, with positive samples ranging from 83% to 100%. Assessment of IBDV antibody responses with the VP2 ELISA at 4, 6, and 8 weeks of age after rHVT-ILT-IBD (gD-gI) or rHVT-IBD-ILT (gD) vaccination showed that both vaccines induced comparable seroconversion titers against VP2. Additionally, assessment of IBDV and ILTV antibody responses using recombinant ELISA (VP2, gD, and gI) was more effective in detecting seroconversion, as evidenced by higher titers and a higher percentage of positive samples, compared to the conventional IBD and ILT ELISA. Overall, this study demonstrated that indirect ELISA based on recombinant proteins (VP2, gD, gI) are an effective tool to detect circulating antibody responses generated by recombinant vaccines.

Keywords: ELISA; recombinant; seroconversion; titers; vaccine

SCAD II

M48 *Megasphaera stantonii*, a commensal bacterium, confers colonization resistance against necrotic enteritis in chickens

Isabel Tobin^{*1GS}, Jing Liu¹, Jiaqing Guo¹, Melanie Whitmore¹, Markus Kim¹, Joy Scaria², Glenn Zhang¹ ¹Oklahoma State University, Animal Science, Stillwater, Oklahoma, United

States; ²Oklahoma State University, Veterinary Pathobiology, Stillwater, Oklahoma, United States

Necrotic enteritis (NE), caused by *Clostridium perfringens*, costs the global poultry industry approximately \$6 billion annually. Since the withdrawal of in-feed antibiotics in the United States in

2017, NE incidence has increased, highlighting the need for alternative mitigation strategies. This study aimed to investigate the potential of microbiome-mediated colonization resistance to NE in chickens. Twelve phylogenetically diverse commensal bacterial strains isolated from the ceca of healthy feral chickens were screened for their ability to inhibit *C. perfringens* growth using an *in vitro* co-culture assay. Using a novel high-throughput macrophage reporter assay, the bacterial cell-free supernatants were further tested for their capacity to induce the synthesis of host defense peptides (HDPs), key effector molecules of innate immunity. A chicken NE model was used to evaluate the protective efficacy of the top-performing bacterium. A total of 160 day-of-hatch broilers were divided equally to four treatments in a 2x2 factorial design (bacterium vs. no bacterium; NE vs. no NE). Chicks received the bacterium or culture medium on days 0 and 1, followed by mock infection or *Eimeria maxima* and *C. perfringens* challenges on days 10 and 14, respectively. Cecal and ileal digesta were collected for 16S rRNA sequencing and intestinal lesions were scored post-necropsy on day 17. Survival and lesion data were analyzed by log-rank or Kruskal-Wallis tests, while microbial differential abundance was determined using LEfSe and ANCOM-BC2. Seven of 12 commensal strains inhibited *C. perfringens* growth *in vitro*, with *Megasphaera stantonii* showing the greatest inhibition (~62%). Its supernatant also most effectively induced HDP gene expression. *M. stantonii* administration significantly improved ($P < 0.01$) NE survival (98% vs. 48%) and reduced intestinal lesion severity ($P < 0.05$). Microbiome analysis revealed that *M. stantonii* promoted recovery of the intestinal microbiota post-infection by enriching short-chain fatty acid- and lactic acid-producing taxa while suppressing pathobionts. These findings clearly demonstrate the potential of *M. stantonii* to mitigate NE through direct pathogen inhibition, enhancement of host innate immunity, and modulation of the gut microbiome.

Keywords: necrotic enteritis; Probiotic; Antibiotic Alternative

M49 Exploring the relationship between *Clostridium perfringens* load, toxin profiles, and fecal MRP-126 in necrotic enteritis of broiler chickens

Mahmoud A. Mahmoud*^{1GS}, Vinicius Buiatte¹, Craig Baumrucker¹, Dervan Bryan¹, Mark Jenkins², Christopher Chadwick³, Katarzyna Miska⁴, Alberto Gino Lorenzoni¹
¹Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States; ²Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Service, Beltsville, Maryland, United States; ³Life Diagnostics Inc., West Chester, Pennsylvania, United States; ⁴Animal Biosciences & Biotechnology Laboratory, Beltsville Agricultural Research Service, Beltsville, Maryland, United States

Necrotic enteritis (NE), a major enteric disease in broiler chickens caused by *Clostridium perfringens* and predisposed by *Eimeria* co-infection, poses significant health and economic challenges in poultry production. While prior studies have independently examined *C. perfringens* bacterial load, toxin profiles, or the myeloid-related protein 126 (MRP-126) as a biomarker for NE severity, integrated analyses of these factors to understand disease pathogenesis and biomarker reliability remain limited. This study aims to quantify the relationships among *C. perfringens* load, NetB and Alpha toxin profiles, and fecal MRP-126 levels in a controlled NE challenge model to elucidate their contributions to disease pathogenesis and evaluate MRP-126 as a non-invasive biomarker of disease progression. Broilers were divided into two treatments: a negative control (NC; uninfected) or a Necrotic Enteritis (NE; co-infected with *E. maxima* and *C. perfringens*), both fed a pro-inflammatory diet. Jejunal *C.*

perfringens loads and virulence gene copies (CP-16S rRNA, *plc*, *netB*) were quantified via qPCR in groups categorized as negative control (NC), NE no lesion (NL, score 0), NE moderate lesion (ML, scores 2–3), and NE severe lesion (SL, scores 4–5). Concentrations of NetB, Alpha toxin, and MRP-126 were measured using ELISA in these groups, with the NL group further subdivided into NE no-lesion low *C. perfringens* count (NLLC) and NE no-lesion high *C. perfringens* count (NLHC) subgroups. Bacterial load and *netB* gene copies increased significantly with lesion severity, with NetB toxin levels markedly elevated in ML and SL groups compared to NC and NL groups. Conversely, Alpha toxin levels were highest in NC and lowest in SL, suggesting a limited role in disease pathogenesis. Fecal MRP-126 concentrations strongly correlated with NetB levels and were significantly elevated in the ML and SL groups, with higher levels in the NL groups compared to the NC. These findings confirm NetB as the primary driver of NE pathogenesis, highlight the non-essential role of Alpha toxin, and validate fecal MRP-126 as a sensitive biomarker for early detection and severity assessment of NE, offering a valuable tool for flock health monitoring.

Keywords: NetB toxin; Alpha toxin; MRP-126

M50 Effects of yeast-derived dietary additives on performance and immune responses of broilers during a subclinical necrotic enteritis challenge

Gabriella C. Ribeiro*^{2GS}, Davis A. Fenster², Bingqi Dong², Abhisek Niraula², Paniz Zarghami Dastjerdi², Maria A. Rodriguez¹, Rami A. Dalloul² *Olmix SA, Brehán, Bretagne, France*; ²University of Georgia, Avian Immunobiology Laboratory, Department of Poultry Science, Athens, Georgia, United States

As antibiotic use declines, necrotic enteritis (NE) continues to threaten poultry production, driving growing interest in yeast-derived additives rich in β -glucans and mannan oligosaccharides for improving performance and immune response. A 42-day (d) study evaluated the effects of two yeast-derived additives on performance, gross pathology and immune responses of broilers subjected to a NE challenge. Day-of-hatch male broiler chicks (n=600) were randomly assigned to four treatments (six replicate floor 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 d 14, all birds were gavaged with 2,000 sporulated oocysts of *Eimeria maxima*, followed by *Clostridium perfringens* inoculations (~10⁸ CFU/mL) on d 19 and d 20. Birds and feeders were weighted on d 0, 14, 28 and 42 to calculate average daily gain (ADG) and feed intake (ADFI), as well as feed conversion ratio (FCR), all adjusted for mortality. At d 21, the small intestine of four birds/pen was scored for NE lesions. At d 14, d 21 and d 28, one bird per pen from NC and GF-GM groups was euthanized and cecal tonsils and jejunum were collected to measure mRNA abundance of immune response genes (IFN γ , IL10, IL12B, IL1 β , IL22 and TNF α). Performance and mortality were analyzed with one-way ANOVA and Fisher's test, respectively. As for NE lesion scores, Kruskal-Wallis test was used, while mRNA abundance was compared with unpaired Student *t*-test. Differences in all comparisons were considered significant when $P \leq 0.05$. At most intervals (d 0-14, d 0-28, d 0-42, d 14-28), PC had significantly higher ADG and lower FCR compared to NC, GM and GF-GM, except during d 14-42 when it was similar to GF-GM. Also, PC had statistically higher ADFI during d 0-14. No differences between groups were seen in NE lesion scores and mortality. Compared to NC, GF-GM had greater mRNA abundance of TNF α in the cecal tonsils at d 14 and d 28, and greater IL10 in the jejunum at d 14. In conclusion, yeast-

derived additives did not impair growth performance and showed potential to enhance early immune activation and improve intestinal readiness under NE challenge.

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

M51 Is *Castellaniella* a primary pathogen? Establishing an *in vivo* animal model to investigate pathogenicity in chickens

Yi Chen Luo*^{1GS}, Jenny Nicholds², Daniel A. Sebastian Pineda¹, Yu-Yang Tsai¹, Grazieli Maboni¹ ¹University of Georgia, Athens, Georgia, United States; ²Prairie Livestock, Red Deer, Alberta, Canada

Increasing evidence suggests that *Castellaniella* causes fatal disease in chickens and other animals. We have recently reported over 20 cases associated with *Castellaniella ginsengisoli* in commercial broiler breeders, presenting with increased mortality, lameness and swollen wattles. Since all cases involved commercial broiler breeders, we hypothesized their specific genetic background and raising conditions are critical for development. Therefore, the aim of this study was to validate the pathogenicity of *C. ginsengisoli* by fulfilling Koch's postulates in commercial broiler breeders raised under conditions mimicking a commercial set up. To establish a reliable infection model, three potential inoculation routes were tested: oral, subcutaneous, and intramuscular over a two-week trial with groups of six 60-week-old healthy commercial broiler breeders. Clinical signs varied among individuals, with the most severely affected birds died within the first week. Gross examination of these birds showed splenomegaly and hepatomegaly, both with diffuse white foci and hemorrhages, as well as atretic or coagulative changes in ovarian follicles. Birds that survived the acute phase developed a chronic form of the disease, characterized by lameness in the second week. In addition to the lesions described above, these chronically affected birds also presented with femoral head necrosis and swollen knee and hock joints with tenosynovitis. The six birds from the uninoculated group (control) remained healthy throughout the course of the experiment. *C. ginsengisoli* was reisolated on blood agar and it was detected by quantitative PCR from the liver, spleen, follicles, hock joints and blood samples of sick birds, thus fulfilling the Koch's postulates. This study is the first to show that *C. ginsengisoli* has potential to cause disease as a primary pathogen.

Keywords: Castellaniella; Koch's postulates; Animal model; Pathogenicity; Broiler Breeders

M52 Local and systemic leukocyte responses following vaccinations with electron beam or formalin inactivated *Staphylococcus aureus* in broiler chickens

Ruvindu Perera*^{2GS}, Jossie M. Santamaria¹, Chrysta N. Beck³, Gisela Erf¹, Adnan Alrubaye²¹, Palmy Jesudhasan⁴ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ²University of Arkansas, Cell and Molecular Biology, Fayetteville, Arkansas, United States; ³Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States; ⁴University of Arkansas, USDA-ARS, Fayetteville, Arkansas, United States

Optimized electron beam (eBeam) treatment inactivates bacteria by disintegrating nucleic acids while preserving surface epitopes that endow immunogenicity in hosts. Using eBeam, we developed an inactivated vaccine against *Staphylococcus aureus* (SAu), a common foodborne pathogen that causes bacteremia and infective endocarditis in humans. SAu poses severe economic losses in the

broiler industry via diseases (septicemia, arthritis, lameness), resulting meat condemnation. This study compared local and systemic leukocyte responses in broilers vaccinated with eBeam inactivated (eB)- or formalin-killed (FK)-SAu. Endotoxin-free PBS was the vehicle/sham control. This study had 6 treatments (trt) with 5 chickens/trt, where each vaccine trt was divided into two *in ovo* vaccination groups (Group A and B; i.e., A-eB, A-FK, A-sham, B-eB, B-FK, B-sham). Groups A received eB/FK and B received the sham vaccines *in ovo*. At 34d of age, intradermal (i.d.) injections of respective trt into growing-feather (GF) pulps were conducted to elicit recall- or primary-immune responses in group A and B, respectively. Blood was collected at specific times post-*in ovo* vaccination (Phase 1) for immunofluorescent (IF) staining and flow cytometry. Following i.d. GF injections (Phase 2), blood and GFs were collected at various times p.i. to assess systemic and local, primary and recall leukocyte profiles by IF staining of blood and pulp cells, respectively, followed by flow cytometry. Two-way ANOVA was conducted to test effects of trt, time, and their interactions followed by Tukey's HSD tests. Statistical significance was set at $P < 0.05$. In Phase 1, eB vaccine triggered higher total lymphocyte concentrations compared to FK. In GF-pulps, In Phase 2, the FK and eB vaccines induced higher infiltration (% pulp cells) of total lymphocytes, and T cells into GF pulps after primary or recall vaccinations. In blood, the recall eB vaccine induced high concentrations of monocytes, CD8+ T cells and total lymphocytes, while the recall FK vaccine triggered higher heterophil concentrations ($P < 0.05$). In conclusion, both the eB- and Fk-SAu vaccines stimulated robust local inflammatory responses in the GF-pulp, however, the systemic blood leukocyte mobilization was significantly impacted by the eB-SAu vaccine.

Keywords: Staphylococcus; eBeam; vaccine; leukocytes; chicken

M53 Using chicken embryo challenge models and histopathological analysis of yolk sacs to elucidate the pathogenicity of novel Avian pathogenic *E. coli* (APEC) serogroups

Klao Runcharoon*^{2GS}, Alexandra Whitfield¹, Maurice Raccousier¹, Vicente Antonio Reyes³, Catherine M. Logue² ¹Poultry Diagnostic Research Center, College of Veterinary Medicine, University of Georgia, Population Health, Athens, Georgia, United States; ²College of Veterinary Medicine, Population Health, Athens, Georgia, United States; ³College of Veterinary Medicine, Pathology, Athens, Georgia, United States

Colibacillosis, caused by Avian Pathogenic *Escherichia coli* (APEC), causes high morbidity and mortality in poultry. Among APEC, serogroups O1, O2, and O78 are often associated with disease. However, our research has identified several novel APEC serogroups. This study evaluated the pathogenicity of 21 APEC strains, representing 13 novel serogroups, using the embryo lethality assay (ELA), and histopathological analysis. We hypothesized some strains would exhibit higher virulence than others. In the ELA, each group consisted of 10-15, 12-day-old SPF eggs inoculated with APEC strains (300-500 CFU/0.1 ml) via the allantoic fluid. A negative control (PBS), a positive control (APEC WT O18), and control (Avian fecal *E. coli*) were included. Eggs were candled daily, and deaths recorded for 6 days. The body weight (B.W.) of surviving embryos at 18 embryonation age (E) were measured. The yolk sac (YS) of those E18 surviving embryos were collected for histopathological analysis. Isolates causing embryo death of >29%, 10-29% and <10% were classified as, virulent, moderately virulent, and avirulent isolates. Results showed all 21 APEC strains were virulent, with highest mortality (100%) for O86:H2, O149:H4 and O45:H19, while lowest mortality (50%) was noted for O88:H25. Using Log-rank (Mantel-Cox) test to compare survival against a control strain (AFEC),

found 18 strains caused significantly higher mortality ($P < 0.05$). The mean B.W. of all APEC-challenged E18 groups were lower than the negative control group (B.W. = 21.98 g) with O17:H8 (13.175 g), O25:H4 (13.38 g), O86:H51 (15.09 g), O45:H19 (17.1g), O88:H8 (15.25 g), O91:H34 (14.04 g), Onovel12:H4 (14.8 g), and O115:H9 (13.8 g) being noted. The YS histopathological results found destruction of YS structure compared to the control group. Using Periodic-acid Schiff (PAS) stain, found a reduction of glycogen/glycoprotein granules suggesting these novel APEC strains significantly damage embryo YS. Novel APEC serogroups exhibit high pathogenicity in embryos, affecting the embryo development process, leading to poor body weight gain. Further investigation of novel APEC serogroups is warranted as they can significantly impact poultry health, especially in younger birds.

Keywords: Avian pathogenic *E. coli*; Poultry; histopathological; Colibacillosis; Embryo

M54 Evaluation of virulence genotypes of avian pathogenic *Escherichia coli* using a subcutaneous infection model in layer pullet chicks

Carlos A. Guardado*^{1GS}, Daniel Wilson², Chris Magee³, Kelsy Robinson³, Jeffrey D. Evans³, Nickki Tillman⁴, Li Zhang¹, Jia Linan¹, Kenneth S. Macklin¹, Pratima Adhikari¹ ¹Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States; ²Wilson Vet Co., Franklin, Indiana, United States; ³USDA ARS Poultry Research Unit, Starkville, Mississippi, United States; ⁴Nutritional Statistics LLC, Buford, Georgia, United States

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, leading to mortality, reduced growth, and economic losses in layers. This study compared APEC isolates of varying virulence using a subcutaneous infection model. Four hundred unvaccinated 0-day-old Hy-Line W-36 pullets were randomly distributed into 72 cages across four rooms, with all four treatments evenly represented: virulent MS5033, avirulent MS1130, positive control MS1657, and sterile PBS as negative control. These isolates were categorized into avirulent and virulent based on the presence of five virulence-associated genes (VAGs) by previous research in our lab. Birds were inoculated at 7 days with 1×10^7 CFU in 0.1 mL and monitored for clinical signs, mortality, lesions, and performance through 14 days post-inoculation (dpi). Data was analyzed using the PROC GLM procedure of SAS 9.4. The virulent and positive control isolates caused moderate mortality (10.4 %; $P=0.005$), while the avirulent and negative controls showed minimal or no mortality (4.2%; $P=0.048$). Lesion scores reflected distinct virulence patterns: perihepatitis was most severe in the positive control group (0.33; $P=0.0001$), although airsacculitis and peritonitis remained low. The virulent strain reduced body weight gain by 8-12 % and increased FCR compared to avirulent and control ($P=0.002$ and 0.003 , respectively). Feed intake differences were minor ($P > 0.05$), reflecting transient anorexia during acute infection. At 7 dpi, bacterial enumeration confirmed 1 log higher in the liver ($P=0.008$) and spleen ($P=0.0347$) of the virulent group in contrast to the avirulent and control group, demonstrating systemic dissemination, but declined by 14 dpi, indicating partial clearance. In conclusion, the virulent (MS5033) isolate induced measurable lesions and reduced growth, in comparison to the positive control (MS1657) that caused the most severe perihepatitis and moderate mortality. Avirulent and negative control isolates showed minimal lesions and average performance parameters. Thus, we conclude that *E. coli* isolates of different combinations of VAGs exhibit distinct colonization patterns when introduced via subcutaneous infection in layer pullet chicks.

Keywords: Avian Pathogenic *E. coli*; subcutaneous challenge; Hy-Line W-36 pullets; bacterial colonization; poultry health

M55 Flow Cytometric characterization of dietary onion peel-induced changes in lymphocyte populations in *Salmonella*-infected broiler chicks

Odinaka C. Iwuzo*^{1GS}, Oluwabunmi Apalowo¹, Oluferu Orimaye¹, Safiu A. Suberu¹, Samuel T. Quarmyne¹, Lekan Elegbede¹, Olanrewaju A. Binuyo¹, Deji A. Ekunseitan¹, Yewande Fasina¹ ¹North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States

Salmonella Enteritidis (SE) remains a significant foodborne pathogen, posing a major threat to poultry health and public safety. Onion peel powder (OPP) is rich in polyphenolic compounds, particularly quercetin which are considered to possess antimicrobial and immunomodulatory properties. This study evaluated the effect of OPP on lymphocyte subpopulations in SE-infected broiler chicks. Ross 708 (day-old) broiler chicks ($n=500$) were randomly allocated to five treatments (5 replicates of 20 chicks each) in completely randomized design. Treatments included CON, CONSE (0 g/kg OPP), BMDSE (0.055 g/kg bacitracin methylene disalicylate), OPPXSE (3 g/kg OPP), and OPPYSE (6 g/kg OPP), all fed corn-soybean meal basal diets. All groups, except CON, were challenged with 1 mL SE (4.80×10^8 CFU/mL) at 2 days of age. On d 5 and 19 post-challenge (PC), ceca SE was enumerated on xylose Lysine Deoxycholate agar, and spleen samples were analyzed by flow cytometry to quantify key lymphocyte populations. Data were analyzed by one-way ANOVA using SAS software ($P < 0.05$). The CON treatment was free of SE throughout the experiment, while CONSE, BMDSE, OPPXSE, and OPPYSE treatments harbored SE (0.11 - 4.66 Log₁₀ CFU/g), thereby confirming infection. On d 5 PC, chicks in OPPYSE had lower SE (3.18 Log₁₀ CFU/g; $P < 0.05$) compared to those in CONSE (4.66 Log₁₀ CFU/g). By d 19 PC, chicks in OPPXSE (0.28 Log₁₀ CFU/g) and OPPYSE (0.11 Log₁₀ CFU/g) had lower ($P < 0.05$) ceca SE compared to CONSE chicks (1.98 Log₁₀ CFU/g). The proportion of CD45⁺ leukocytes (T cells) was higher ($P < 0.05$) in OPP-fed groups than in CONSE on d 5 PC, but by d 19 PC, it was lower than in BMDSE, indicating early T-cell priming. Activated helper T cells (CD44⁺CD4⁺) were reduced ($P < 0.05$) in OPP groups compared with CONSE at d 5PC, while cytotoxic T cells (CD8⁺CD4⁺) were lowest ($P < 0.05$) in OPPXSE on d 19 PC. On d 5 PC, differentiated B cells (Bu1a⁺IgM⁺) were higher ($P < 0.05$) in all SE-infected chicks, whereas immature B cells (Bu1⁺aIgM⁻) were lower ($P < 0.05$) in OPP groups compared with CON. Overall, OPP supplementation reduced SE colonization by enhancing an early immune activation that was characterized by rapid T-cell priming and B-cell differentiation, and a controlled immune regulation.

Keywords: *Salmonella* Enteritidis; onion peel powder; broiler chicks; flow cytometry; lymphocyte populations

M56 Development and validation of a long-read sequencing-based method for *Escherichia coli* O-serogroup typing

Sunita Shrestha*^{1GS}, Jia Linan¹, Chuan-yu Hsu², Mark A Arick II², Xue Zhang³, Kenneth S. Macklin¹, Kelsy Robinson⁴, Li Zhang¹ ¹Mississippi State University, Department of Poultry Science, Starkville, Mississippi, United States; ²Institute for Genomics, Biocomputing and Biotechnology, Starkville, Mississippi, United States; ³Mississippi State University, Department of Animal and Dairy Sciences, Starkville, Mississippi, United States; ⁴Poultry Research Unit, USDA, Agriculture Research Service, Starkville, Mississippi, United States

Rapid and accurate typing of *Escherichia coli* O-serogroups is essential for surveillance and control of avian pathogenic *E.*

coli (APEC), a major cause of colibacillosis and economic loss in the poultry industry. Traditional methods such as agglutination assays and multiplex PCR are limited in speed, scalability, and accuracy, while whole-genome sequencing (WGS), although comprehensive, remains too costly for routine diagnostics. Long-read sequencing offers a promising alternative by enabling the capture of complete O-antigen gene clusters (O-AGCs) in single reads, providing a rapid, scalable, and cost-effective approach for O-serogroup identification. We developed and evaluated a workflow combining long-range PCR for full-length of O-AGCs amplification with Oxford Nanopore sequencing on the MinION platform. The resulting long reads were processed through a bioinformatics pipeline to identify complete clusters, cluster sequences, and assign O-serogroups using the SerotypeFinder database. The method was tested on 137 isolates, including 72 ECOR strains and 65 field isolates, all of which had WGS-based serogroup assignments. Typing success and concordance were reported with 95% confidence intervals calculated using the Wilson method for binomial proportions. The ONT-based method identified O-serogroups in 122 of 137 isolates (89.1%; 95% CI 82.7–93.3). Six isolates showed discrepancies between ONT and WGS, and primer-specific PCR supported the ONT serogroups for all six cases, suggesting possible sample mix-ups, mislabeling, or biological variation. Two isolates failed long-range PCR, likely due to exceptionally large O-AGCs (>30 kb). Thirteen isolates (9.5%) remained untyped by both methods, indicating that their O-antigen loci may be absent, highly divergent, or unrepresented in current databases. The method also detected multiple serogroups in four isolates, demonstrating its ability to identify co-existing O-serogroups within a single sample. These results support long-amplicon ONT sequencing as a rapid, accurate, and scalable platform for *E. coli* O-serogroup typing. Its strong performance across a diverse isolate set highlights its potential value for routine APEC surveillance and diagnostics in the poultry industry.

Keywords: *Escherichia coli*; O-serogroup typing; Avian pathogenic *E. coli*; long-range PCR; Nanopore sequencing

M57 Bacterial diversity in avian metapneumovirus cases

Autumn Gregg^{*1GS}, Isabella Hannay¹, Natalie Armour Manginsay¹, Sofia Egana-Labrin¹, Heidi Huffman Rose², Martha Pulido-Landinez¹ ¹Mississippi State University Poultry Research and Diagnostic Laboratory, Pearl, Mississippi, United States; ²Mississippi Veterinary Research and Diagnostic Laboratory, Pearl, Mississippi, United States

Avian metapneumovirus (aMPV) is a respiratory virus infecting turkeys and chickens. Since late 2023, aMPV subtypes A and B have quickly spread across most of the United States, predominantly affecting the eastern states with negative impacts on bird health and productivity. Several authors have reported high bacterial diversity and the isolation of bacteria from uncommon sites in infections occurring secondary to aMPV infection. This study aimed to analyze the diversity of secondary bacterial infections identified in 68 aMPV cases submitted to the Mississippi State University's Poultry Research and Diagnostic Laboratory over the eight-month period following the first detection of aMPV subtype A in Mississippi in late February 2025. Secondary bacterial coinfections were identified in 58 out of 68 aMPV cases. In most cases (n=42), *Escherichia coli* (*E. coli*) was the predominant secondary bacterial infection associated with aMPV. *Enterococcus cecorum* (*E. cecorum*), *Pasteurella multocida*, *Staphylococcus aureus*, *Staphylococcus agnetis*, and *Staphylococcus chromogenes* were isolated as the only aMPV bacterial coinfections in 12 cases. There were 177 individual isolations from various sampling sites. *E. coli* accounted for approximately 65.5% (n=116) of total bacterial

isolations, commonly from the liver, heart, brain, and bone marrow. The second most common agent identified was *E. cecorum* (n=15), predominantly from the heart, spleen, and free thoracic vertebrae. Other bacteria isolated from these cases included *Gallibacterium anatis* (n=8), *Staphylococcus aureus* (n=12), *Staphylococcus agnetis* (n=11), *Staphylococcus chromogenes* (n=3), *Salmonella* spp. (n=7), and *Pasteurella multocida* (n=12). Among 23 accessions with neurological signs, bacterial isolates of suspected or potential significance were recovered from all but two cases. The results of this study provide an overview of the range of bacterial coinfections that could occur in broiler breeders and broilers infected with aMPV, as well as the role of secondary bacterial infections in exacerbating disease severity during natural field outbreaks. This report of common and emerging bacterial infections associated with aMPV infection will contribute to the understanding of the impact of the aMPV in the poultry industry.

Keywords: aMPV; Secondary bacterial infections; Emerging bacteria

M58 Mycoplasma synoviae macroscopic and microscopic lesions in chicken embryos

Daniel A. Sebastian Pineda^{*12GS}, Eniope Oluwayinka¹, Naole Ferguson-Noel¹ ¹University of Georgia, Poultry Diagnostic & Research Center, Population Health Department, Athens, Georgia, United States; ²University of Georgia, Department of Pathology, College of Veterinary Medicine, Athens, Georgia, United States

Mycoplasma synoviae (MS) is an important poultry pathogen that most typically causes subclinical infections but can also result in respiratory disease, synovitis, and eggshell abnormalities. These conditions lead to considerable economic losses due to reduced flock productivity and increased costs associated with prevention and control. MS is transmitted both horizontally, from bird to bird, and vertically through transovarian transmission, and infection can reduce hatchability and increase embryo mortality. The aim of this study was to characterize the gross and histopathological lesions in chicken embryos infected with seven MS strains during 19 days of incubation. Seven MS isolates (WVU1853, K6646, K6654, K6639, K6931, K6932B, and K6677) were inoculated into 6-day-old SPF embryonated eggs via the yolk sac. Embryos from each group were collected at the end of the incubation period in formalin for macroscopic evaluation, and multiple tissues were processed and examined histologically. Infected embryos exhibited more frequent and severe gross lesions, including skin hemorrhages, hemorrhagic and cloudy yolk sacs, and reduced embryonic size compared with controls. Ongoing analyses will assess tissue colonization patterns and potential mechanisms of MS-induced embryonic damage. These findings will improve understanding of MS pathogenesis in embryos and support the development of more effective strategies for controlling MS infections in poultry populations.

Keywords: *Mycoplasma synoviae*; chicken embryo; macroscopic lesions; microscopic lesions

M59 Experimental reproduction of clostridial dermatitis in turkeys and evaluation of immune responses against pathogenic *Clostridium septicum* bacteria

Raveendra Kulkarni^{*1}, Valeria Criollo¹, Oscar Fletcher¹, Rocio Crespo¹ ¹North Carolina State University, Raleigh, North Carolina, United States

Clostridial dermatitis (CD) is an emerging disease of increasing economic importance in turkeys characterized by sudden deaths and necrotic dermatitis. *Clostridium septicum* is one of the two

major clostridial causative agents of CD. Despite its spiking incidences and economic burden, immune responses during CD are poorly understood and part of the problem is lack of a well characterized disease model. Here, we used three strains of *C. septicum*, namely Str. A1, Str. B1 and Str. C1, isolated from field outbreaks, to experimentally infect turkeys to evaluate local (skin and muscle) and systemic (spleen) immune responses. Results showed that while all three strains produced an acute disease, Str. A1 and B1 caused higher ($P \leq 0.05$) mortality when compared to Str. C1. Gross and histopathology evaluation showed that birds infected with Str. A1 and B1 had severe inflammatory, edematous, granulomatous and necrotic lesions in the skin, muscle and spleen, while these lesions produced by Str. C1 were relatively less severe and mostly, confined to skin and/or muscle. Immune gene expression in these tissues showed that Str. B1-infected birds had higher ($P \leq 0.05$) expression of interleukin (IL)-1 β , IL-6 and interferon (IFN) γ genes compared to uninfected control,

suggesting a robust inflammatory response both locally as well as systemically. The transcription of IL-1 β and IFN γ in the muscle or spleen of Str. A1-infected birds and IL-1 β in the skin of Str. C1-infected group was also higher ($P \leq 0.05$) than control. Additionally, Str. A1 or B1-infected groups had significantly higher ($P \leq 0.05$) IL-4 transcription in these tissues, while birds infected with all three strains developed *C. septicum*-specific serum antibodies. Furthermore, splenic cellular immunophenotyping in the infected turkeys showed a marked reduction ($P \leq 0.05$) in CD4+ cells. Collectively, it can be inferred that host responses against *C. septicum* involve an acute inflammatory response along with antibody production and that the disease severity seem to depend on the strain of *C. septicum* involved in CD in turkeys.

Keywords: Clostridial dermatitis; Clostridium septicum; Turkey; Immune response; Immunopathology

Processing & Products I

M60 Rheological and intermolecular interaction of woody breast meat proteins during thermal processing as influenced by phosphate concentration

Novotná Tereza², Rudro Deb Sardar¹, Harleen Kaur¹, Chinelo S. Onah-Kachiside¹, Thiago Belem¹, Pavel Mokrejš², Elavarasan Krishnamoorthy*¹, Amit Morey¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Tomas Bata University in Zlin, Department of Food Technology, Vavreckova, Zlin, Czechia

Woody Breast (WB) myopathy negatively affects the functional properties of chicken breast meat, particularly its ability to form stable protein gels during thermal processing. Phosphates are commonly used functional additives that improve protein solubility and gelation. Rheological measurements are used to better understand changes in meat proteins during thermally induced gelation. However, the influence of different phosphate concentrations on the heat-induced rheological behavior of WB meat batter compared with normal breast meat has not been fully clarified. The objective of this study was to evaluate the effects of three phosphate concentrations (0%, 0.2%, and 0.5%) on the rheological and intermolecular properties of WB meat batter during thermal gelation. Meat batters were prepared from WB and NORM breast meat containing 78–78.5% meat, 1.5% salt, 20% water, and phosphate at 0%, 0.2%, or 0.5%, and each formulation was produced in three independent replications. Rheological measurements were performed using a HAAKE MARS iQ Air rheometer equipped with a 35 mm serrated parallel plate under strain-controlled mode. Dynamic rheological measurements included a temperature ramp test (25–90 °C, 3 °C/min, 1 Hz, 0.001 strain) and isothermal gelation at 50, 60, 70, and 80 °C for 900 s. Intermolecular interaction and protein participation was assessed through solubility and SDS-PAGE profiling of proteins extracted from raw and cooked meat batters. Statistical analysis was performed using one-way ANOVA with significance set at $p < 0.05$. NORM batters exhibited an early onset of gelation at 55–60 °C with a pronounced increase in storage modulus (G'), whereas WB batters showed delayed gelation (65–80 °C) and lower viscoelastic strength. Phosphate addition improved gelation in both meat types, increasing G' and shifting the onset of gelation to higher temperatures, with a more pronounced effect in WB samples. Intermolecular interaction analysis indicated stronger ionic, hydrogen, and hydrophobic interactions in NORM, while WB showed weaker interactions consistent with its delayed gelation behavior. Phosphate addition significantly improves heat-induced gelation and rheological properties of WB meat batter and can partially compensate for its reduced functional performance.

Keywords: Woody Breast meat; Myopathy meat; Thermal gelation of proteins; Rheological properties; Intermolecular interaction of proteins

M61 Modeling of processing yields and breast myopathies to maximize broiler economic value

Hudson Thames*¹, Haley Nabors¹, Jonathan Moon¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States

Woody breast (WB) myopathy continues to reduce value in commercial broiler processing. The purpose of this study was to evaluate differences in carcass yield, WB incidence, and retained economic value (EV) between two commercial genetic lines and to identify line-specific market-weight optima. A split-plot design was used with genetic line applied at the commercial house level and sex as the subplot factor within house sections. Two commercial houses were used, and lines were crossed over between houses across two consecutive runs. Each house contained four sections, and section-by-sex means served as the experimental unit with birds as subsamples. Per run, 384 birds were selected (768 total). Yield traits and WB scores (0–3) were collected at debone. Data were analyzed using PROC GLIMMIX (SAS v9.4) with line and sex as fixed effects and house(run), and section(house \times run) as random effects. Pearson correlations and linear regressions related chilled carcass weight to breast weight and WB. An EV model integrated regressions with price multipliers (WB0–1 = 1.0, WB2 = 0.5, WB3 = 0.0). Line 1 exhibited higher breast yield than Line 2 (30.88% vs. 29.91%, $P = 0.001$). WB incidence least-squares means differed by line (WB3: 24.4% vs. 5.8%, $P < 0.001$; WB0: 18.3% vs. 54.6%, $P < 0.001$). Breast weight scaled strongly with carcass weight in Line 1 (slope ≈ 0.31 lb/lb; $R^2 \approx 0.97$; $P < 0.0001$) but weaker in Line 2 (slope ≈ 0.15 lb/lb; $R^2 \approx 0.28$; $P = 0.034$). EV peaked for Line 1 at approximately 7.70 lb carcass weight with 2.38 lb breast (EV index ≈ 0.64) and for Line 2 at approximately 6.72 lb with 2.14 lb breast (EV index ≈ 0.89). These results show that Line 1 produces larger breasts but suffers steep WB penalties at heavier weights, whereas Line 2 retains higher EV at lighter weights due to lower WB prevalence. Future work should focus on improving alignment of breast weight with carcass weight, increasing WB0 yield at heavier weights, decoupling breast size from WB severity, enhancing the value of WB2–3 meat, and identifying non-size factors influencing WB development across lines.

Keywords: woody breast; myopathy; economics; yield; processing

M62 A molecular chemistry framework for assessing degree of processing in commercial poultry products using force-selective protein solubilization and buffering behavior under influence of different reconstitution methods

Harleen Kaur^{*1}, Thiago Belem¹, Rudro Deb Sardar¹, Chinelo S. Onah-Kachiside¹, Elavarasan Krishnamoorthy¹, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Further processing of poultry meat involves addition of approved ingredients, emulsification, and thermal processing, that re-establishes the intermolecular interaction of proteins. As a result, various types of intermolecular forces (IMF) such as ionic, hydrogen, hydrophobic, and disulfide bonds are reformed. These chemical forces govern the fate of protein consumed and potentially influence the associated health outcomes. Estimation of IMF and alterations in it can serve as a tool to assess the degree of processing but rarely has been considered in accepted food classification systems. This study attempts to explore fundamental IMF and the associated changes. Commercially produced, fully cooked, frozen whole-muscle (WM) and emulsified (EM) chicken nuggets were used throughout the study. Chicken nuggets were reconstituted in air-frying (AF) (8 min, 350-360 F) and microwave (MW) (2-3 min, High power). Breeding was removed and the nugget meat was used for further analysis. Textural parameters of nugget meat was analyzed by razor-blade shear force (SF) and shear energy (SE). Gastric buffering capacity (GBC) was assessed by titrating homogenized tissue with 0.5 M HCl to pH 2.0. The insoluble meat pellets obtained were neutralized to pH 5.7 and corresponding weight gain was recorded. Data for IMF destabilization mediated proteins extractability was collected and proteins were quantified by Bradford assay and SDS-PAGE. Data were analyzed using JMP with Tukey's HSD ($P < 0.05$). Results indicated that EM nuggets had lower GBC than WM nuggets in the baseline and both re-heating methods (e.g., 35-50 mEq/100g vs 60-80 mEq/100 g for EM and WM respectively). IMF profiling showed greater force-resistant fractions in WM (20-26 mg/g of sample protein in disulfide-targeting MW extracts) than EM (<17 mg). Neutralized MW pellets partially restored solubility but retained more IMF-stabilized protein in WM. Texture patterns matched these trends, with WM showing higher SF than EM (5.8-6.6 N WM AF; 2.5-3.2 N EM) and 2 fold greater SE. Collectively, analyses indicated that WM nuggets maintain stronger structural interactions than EM products and MW reconstitution intensifies these features more than AF providing a molecular basis for defining the degree of processing.

Keywords: degree of processing; intermolecular forces; Gastric buffering capacity; protein structure; chicken nuggets reconstitution

M63 Evaluation of growth performance and determination of optimum marketable weight, and age of combined crossbred dual-purpose chickens, in Addis Ababa

Abraha N. Gebrehiwot^{*1,2GS}, Wondemench Esta¹, Mckonnen Girma¹, Tadelle Dessie², Ashenafi Mengistu¹ ¹*Addis Ababa University College of Veterinary Medicine and Agriculture, Animal Production Studies, Addis Ababa, Addis Ababa, Ethiopia*; ²*International Livestock Research Institute/ ILRI - Ethiopia, Live Gene, Addis Ababa, Addis Ababa, Ethiopia*

Crossbreeding plays a critical role in determining the production performance of chickens in filling demand-driven chicken meat with minimum production cost and in a short period of time, instead of investing high cost in the fragile commercial breeds. The study aimed to identify high-performing dual-purpose chicken strains, using crossbreeding lines developed under the ILRI AI breeding program and supported by broiler ration to enhance fast

growth. A total of 291 chickens, composed of 136 cockerels and 155 pullets, representing five strains. R studio analysis results showed that Sasso T451A outperformed in growth, FI, FCR, and livability, followed by *BILWIL* and *WILBIL* compared to *NHILLIL* and *LILNHIL*. The overall feed consumption was 2.75 kg/pullet/90 days, ranging FI from 84 to 110 g/pullet/day for the crossbreds and Sasso T451A respectively. At 8, 10, and 12 weeks of age, the chickens' growth performance was as follows: Pullets: 715.12±18.36g, 991.06±27.25g, 1290.58±31.81g and Cockerels: 861.35±19.44g, 1237.28±26.62g, 1620.32±34.78 g. ADBG was 20.10±9.3 for pullets and 17.65±9.3 for cockerels, FCR was 2.89±1.26 for pullets and 3.23±1.26 for cockerels. The optimal marketable weights at week 12 were 1290.6g and 1620.3 g for pullets and cockerels, respectively. Regression analysis confirmed that strain, age, and sex have affected performance significantly ($p < 0.001$). The finding revealed that integrating broiler ration feeding, complemented by improved crossbred strains, has enhanced early growth and marketability of dual-purpose chickens in Addis Ababa. Future research should focus on cost-effective feeding practices and advanced crossbreeding strategies to further improve productivity and economic returns.

Keywords: Broiler feeding strategies; Crossbreeding, Dual-purpose chicken; Growth performance; Marketable age

M64 Impact of two vitamin E levels in different feeding phases in Cobb broilers

Virginia G. Slick^{*1GS}, Kenneth B. Nelson¹, Savannah Wells¹, Mike Kidd¹ ¹*University of Arkansas, Fayetteville, Arkansas, United States*

Seven experiments evaluating the impact of two vitamin E levels (25 and 125 IU with 5 replicate pens each) in Cobb broilers were conducted. The experiments varied in Cobb broiler strain, sex, processing age, feeding phase lengths, and phase in which the diets were administered in order to establish a reference base for future vitamin E work in modern broilers. All experiments used an RCB design and were analyzed using one-way ANOVA in JMP pro17. Experiment (Exp) 1 (0-18 d experimental feed; 18-35 d common feed) used Cobb 500 female broilers and resulted in no effects ($P > 0.05$) on live performance, d 36 processing traits, or meat quality. Exp 2 (0-14 d experimental feed; 14-42 d common feed) used Cobb 500 males and broilers fed increased vitamin E had improved ($P \leq 0.05$) feed conversion (FCR) at d 14 and decreased ($P \leq 0.05$) leg yield on d 43. Exp 3 (0-14 d experimental feed; 14-42 d common feed) with female Cobb 500 broilers resulted no effects ($P > 0.05$) on live performance, d 43 processing traits, or meat quality. In Exp 4 (0-18 d experimental feed; 18-36 d common feed), Cobb CDP5 male broilers were used and increased vitamin E improved ($P \leq 0.05$) BW gain and FCR at d 18, and final BW at d 37 processing. However, improved ($P \leq 0.05$) leg yields, myopathies, and drip loss were observed in birds fed 25IU from d 0 to 18 (Exp 4). Exp 5 (0-18 d experimental feed; 18-36 d common feed) used female Cobb CMXM broilers and improved ($P \leq 0.05$) FCR was observed in the birds fed 125IU, as well as improved ($P \leq 0.05$) carcass, breast, and total breast meat yields at d 37. In Exp 6, however, (0-32 d common feed, 32-52 d experimental feed) no effects ($P > 0.05$) on live performance, d 53 processing traits, or meat quality were observed in Cobb CDP5 male broilers. In Exp 7 (0-32 d common feed, 32-52 d experimental feed), although d 52 live performance of female Cobb CMXM broilers was not affected by vitamin E ($P > 0.05$), 125 IU vitamin E did improve ($P \leq 0.05$) d 53 carcass yield. Feeding 125IU vitamin E in the starter phase for male broilers showed some improvement in FCR, but processing traits and meat quality responses are inconclusive. Moreover, feeding 125IU vitamin E in the finisher phase did not

impact male broilers, but did increase carcass yields in female broilers.

Keywords: Vitamin E; fat soluble vitamin; Broiler; feed conversion; meat quality

M65 The impact of a peanut skin supplemented corn-soy diet on broiler meat quality

Afsana Rahaman Munmun*^{1,2GS}, Jean C. Caceres², Christina S. Sigmon², Yabaiz Tahir², Thien Vu^{2,3}, Ondulla Toomer^{2,3}, Lin Walker², Yan Campbell² ¹*North Carolina State University, Food, Bioprocessing & Nutrition Sciences Department, Raleigh, North Carolina, United States;* ²*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ³*U.S. Department of Agriculture, Food Science & Market Quality and Handling Research Unit, Agricultural Research Service, Raleigh, North Carolina, United States*

Peanut (PN) skins, a peanut processing by-product, contain polyphenols and nutrients that may enhance sustainability and oxidative stability in poultry diets. This study evaluated the impact of dietary PN skin inclusion on broiler growth performance and meat quality. 90 broilers (45 birds per treatment; 3 pens per treatment; pens = experimental units) were randomly assigned to either a control diet or a diet supplemented with 5% ground PN skins (w/w). Diets were formulated as a 3-phase corn-soy mash regimen and were isoenergetic and isonitrogenous across all phases: starter (3,000 kcal/kg, 22% crude protein), grower (3,100 kcal/kg, 21% crude protein), and finisher (3,200 kcal/kg, 19% crude protein). The feeding trial lasted 6 wks, after which 5 birds per pen (15 per treatment) were processed for carcass evaluation and meat quality assessment. Measurements included live weight, carcass yield, breast weight, pH (15 min, 2 hr, 24 hr, and 2.5 mo postmortem), color, texture, composition, water-holding capacity (thaw and cook loss), lipid oxidation, sarcomere length, and sensory attributes. Data were analyzed using one-way ANOVA and GLM procedures in SAS 9.4, with mean separation by Duncan's New Multiple Range Test ($P < 0.05$). Broilers fed the PN skin diet had lower live body weight carcass, and breast weights compared with controls ($P < 0.05$). No differences were observed for pH at 15 min and 24 hr, color at 24 hr and 2.5 mo, texture, proximate composition, lipid oxidation after 7 mo of storage, or sarcomere length after 7 mo of storage ($P > 0.05$). However, pH at 2 hr and 2.5 mo postmortem was lower in the PN skin group ($P < 0.05$). Cook and thaw losses were reduced in the PN skin group as compared to the conventional diet ($P < 0.05$), indicating greater water-holding capacity. Sensory evaluation revealed no adverse effects on flavor, odor, or texture, with no evidence of rancidity or off-flavor development ($P > 0.05$). In conclusion, inclusion of 5% PN skin in the diet did not adversely impact overall meat quality with the potential to enhance water holding capacity. While additional feeding trials are needed, these findings suggest that PN skins may serve as a suitable poultry feed additive without negatively impacting meat quality traits.

Keywords: broiler; meat quality; peanut skin; alternative poultry feed ingredients

M66 Comparative evaluation of MORS and BMORS texture analysis methods in characterizing spaghetti meat

Zahidul Tushar*^{1GS}, Matthew Bailey¹, Rishav Kumar¹, Jinquan Wang¹, Rabin Raut¹, Dianna Bourassa¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Spaghetti meat (SM) is an emerging and challenging myopathy of the broiler *Pectoralis major* muscle in the global poultry industry. It is characterized by overall impaired muscle integrity with separation of the fibers. Texture evaluation is essential for

assessing meat quality; however, most studies have focused on cooked meat, leaving limited knowledge of texture measurement in raw broiler breast. This study aimed to compare two mechanical texture analysis methods, Meullenet-Owens Razor Shear (MORS) and Blunt Meullenet-Owens Razor Shear (BMORS), in assessing the texture characteristics of raw broiler breast fillet. For this, 60 (Ross 708) breast fillets were collected from a commercial processing plant, scored 0, 1, and 2 according to their spaghetti breast severity. After 24 h, drip loss, pH, color, shear force, and proximate analysis were measured. For texture, breast fillets were sheared perpendicularly to the muscle fibers with 5 shears per method on the cranial region. Data was analyzed using a two-way ANOVA model by the PROC GLM procedure in SAS OnDemand for Academics with significance at $P < 0.05$, and means were separated by Tukey's HSD. There was no significant ($P > 0.05$) difference in L*, a*, b*, or pH between the severity score categories. However, SM2 (16.43%) showed a difference ($P < 0.05$) in drip loss compared to SM0 (11.01%) and SM1 (14.12%). There was a significant ($P < 0.001$) difference in shear force and shear energy. BMORS had a higher shear force (10.52 N) and shear energy (95.05 N.mm) than MORS (6.50 N and 62.19 N.mm). The BMORS method significantly ($P < 0.0001$) distinguished all three meat types based on texture, with shear force values decreasing from SM0 (9.99 N) to SM1 (8.19 N) and SM2 (7.13 N). In contrast, there was no significance in shear force and energy value from the MORS. For proximate analysis, the SM2 breast fillet showed a greater ($P < 0.05$) value in moisture, soluble, and insoluble collagen. Whereas SM1 showed a greater value in protein, and SM0 showed a greater ($P < 0.05$) value in fat and salt. This study found that BMORS delivers higher and more discriminative texture readings than MORS and can be utilized as an identifier in small samples, especially throughout spaghetti meat severity levels in raw breast fillet.

Keywords: spaghetti meat; texture; meat quality

M67 Use monofilament needle's buckling indentation depth to differentiate moderate woody breast from normal chicken breast

Ziyuan Zhao², Yu Wang², Morgan Farnell¹, Gregory Archer¹, Giri Athrey¹, Kiju Lee², Dan Feng³, Ziteng Xu*^{1GS} ¹*Texas A&M University, Poultry Science, Bryan, Texas, United States;* ²*Texas A&M University, College Station, Texas, United States;* ³*Howard University, Washington, District of Columbia, United States*

This study aimed to develop an objective and low-cost method to differentiate normal and moderate woody breast (WB) fillets using a monofilament needle based on the contact mechanics and beam buckling theory. The approach evaluates contact stiffness through indentation and uses the indentation depth of needle's first-buckling response to evaluate stiffness of meat samples. A total of 27 normal fillets and 18 moderate WB fillets were selected by a trained evaluator. Six monofilament needle types were tested. A programmable indentation system compressed the cranial region in 0.15 mm increments. Needles were positioned to lightly contact the surface, and buckling occurrence was visually confirmed during each incremental indentation. The first-buckling indentation depth was recorded for each sample and repeated 3 times to assess the method's repeatability. One-way ANOVA test was performed for each needle type to compare buckling indentation depth between normal and moderate WB fillets, and replication effects were also tested. Support vector machine (SVM) analysis was used to determine the optimal indentation depth threshold for moderate WB identification. All six needle types showed significant differences in the buckling indentation depth between the two groups ($p < 0.05$), and no significant differences among replications ($p > 0.05$). Two needle types

demonstrated complete separation of the two fillet groups. For Needle A (Energy Flo J type 0.14mm x 30mm), buckling depth was 2.21 ± 0.18 mm for normal and 1.24 ± 0.18 mm for moderate WB, and SVM identified 1.72 mm as the optimal classification depth. For Needle B (J type 0.10 mm x 15 mm), depths were 2.67 ± 0.24 mm (normal) and 1.84 ± 0.14 mm (moderate WB), with an optimal depth of 2.22 mm. The samples were indented by the needles at the optimal classification depth, and the method achieved over 94.1% classification accuracy. In conclusion, moderate WB showed significantly higher contact stiffness than normal breast meat, which can be quantified via buckling indentation depth. Based on this characteristic, moderate WB can be accurately, objectively, and inexpensively detected using the presented method.

Keywords: Woody Breast; Poultry Processing; Contact Pressure; Beam Buckling; Monofilament Needles

M68 Effect of reduced metabolizable energy and amino acid density on breast meat quality and myopathies incidence in Ross 708 broilers

Chinelo S. Onah-Kachiside*^{1GS}, Telah Black¹, Maria Brizuela Medina¹, Katherine Sierra¹, Vianca Tashiguan¹, Jose Isaac Vargas¹, Wilmer Pacheco¹, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Breast muscle myopathies such as woody breast (WB), white striping (WS), and spaghetti meat (SM) remain a major challenge to the broiler industry due to negative effects on meat quality and consumer acceptance. Adjusting apparent metabolizable energy (AME) and amino acid (AA) density may reduce myopathy severity. This study evaluated the effects of AME and AA density on carcass traits of broilers on d 50. Diets were formulated using two AME levels: standard (SE) and reduced (RE; -100 kcal/kg) combined with two AA densities: (100 and 90%). The positive control (PC) diet contained SE and 100% AA, while the three negative control treatments included reductions in energy, AA, or both nutrients: negative control-energy (NC-E), negative control-amino acids (NC-AA), and negative control-energy plus amino acids (NC-EAA). A total of 1,200 male YPM-Ross 708 broilers were sorted in 48 pens and assigned to four treatments in a complete randomized block design. At deboning, breast fillets were evaluated for WB (score 0-3), WS (score 0-3), and SM (present/absent). Measurements for breast weight, drip loss, cook loss, and color attributes (CIE L*, a*, and b*) were also collected. Data were analyzed using one-way ANOVA in R, followed by LSD post-hoc comparisons ($\alpha=0.05$). The diets did not influence ($P < 0.05$) WS and SM. However, the diets produced significant effects on WB severity for scores 0 and 2 ($P < 0.05$). Birds fed the NC-EAA diet exhibited the highest proportion of WB with score 0 compared with other treatments (50.0 vs. 23.7, 10.5, and 15.5%). Additionally, the NC-EAA treatment showed a lower ($P < 0.05$) incidence of moderate WB (score 2) at 4.7%, compared with 25.6% (PC), 41.9% (NC-E), and 27.9% (NC-AA). Broilers fed the PC and NC-E diets had the heaviest breast weights (544 and 550 g), while those fed the NC-AA and NC-EAA diets showed reduced weights (486 and 463 g). Despite differences in breast weight, drip loss and cook loss were not significantly affected by dietary treatments. Reducing both dietary AME and AA density lowered WB severity in male broilers while maintaining meat quality traits (drip loss, cook loss and color). These results suggest that combined nutrient reduction is a practical strategy to mitigate woody breast without compromising meat quality.

Keywords: Myopathy; woody breast; Apparent Metabolizable Energy; Amino acid; Meat quality

M69 The effects of silvopastoral vs. conventional production systems and broiler breed on meat quality

Jean C. Caceres*^{1GS}, Camila Hammel Sobreira¹, Afsana Rahaman Munmun²¹, Christina S. Sigmon¹, Athena He-DeMontaron¹, Yabaiz Tahir¹, Allison Pullin³, Jesse Grimes¹, Lin Walker¹, M. W. Schilling⁴, Lisa Dean², Yan Campbell¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; 2*North Carolina State University, Food, Bioprocessing & Nutrition Sciences Department, Raleigh, North Carolina, United States; ³North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ⁴Mississippi State University, Food Science, Starkville, Mississippi, United States

As consumer interest in broiler management practices increases, it becomes essential to understand how these practices impact meat quality to enhance product appeal and meet market demands. This study investigated the effects of rearing system (silvopastoral system; SP and conventional system; CV) and broiler breeds (fast-growing; Ross 708 and slow-growing; SASSO) on meat quality traits, meat yield, myopathy (woody breast = WB, spaghetti meat = SM, white striping = WS), fatty acid profile, oxidative stability, and sensory attributes. A 2x2 factorial in CRD design was evaluated in PROC GLM in SAS 9.4. Pen served as the experimental unit (5 pens/treatment with 50 birds/pen). Data was collected from 10 birds/pen. Sensory data was obtained from 7 panelists over 8 sessions and analyzed in two stages: first, determining the means of the panelists within each session, then evaluating the session means. Differences in yield, meat quality, and sensory attributes (texture, flavor, and basic taste) were determined using Duncan's test ($P \leq 0.05$) for the effects of system, breed, and their interaction. Meat quality was primarily affected by breed. Ross meat had greater yields ($P < 0.001$) and a higher incidence of myopathy (WB, $P < 0.001$; WS, $P = 0.021$; SM, $P = 0.003$) than SASSOs. SASSO exhibited a greater shear force, protein, fat, and collagen ($P < 0.01$), while Ross showed greater moisture in composition and more polyunsaturated fatty acids (%) ($P < 0.001$; $P = 0.021$). In the SP, reduction in saturated fatty acids (%) and improved oxidative stability were observed, possibly due to dietary diversity ($P = 0.007$; $P = 0.039$). No differences were detected in the O:L ratio and the percentages of Omega-3 and Omega-6. Sensory differences were minimal. Meat from CV was rated as juicier than the SP ($P = 0.023$). Additionally, the SASSO's meat was chewier than Ross's ($P = 0.006$). Both are within the normal range of juiciness and chewiness of chicken meat. The basic taste was not affected by any of the factors or their interactions. Overall, trade-offs exist between growth rate and meat quality. This highlights the importance of providing producers with a guide on selecting breeds/systems that strike a balance between performance and product quality to meet consumer expectations.

Keywords: Production Systems; Silvopastoral; Conventional; Broilers; Meat Quality

M70 Meat quality and shelf-life evaluation of turkey breasts under refrigerated storage

Camila Hammel Sobreira*^{1GS}, Jean C. Caceres², Jesse Grimes¹, Fernanda Santos¹, Yan Campbell¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; 2*North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United States

Turkey processing varies in its practices in each plant, and these differences impact the product quality and shelf-life. This study aimed to determine turkey breast meat quality and shelf life under refrigerated storage from 2 facilities (A and B). Two breasts per

treatment per day were evaluated on days 1, 6, 7, 8, 9, 10, 11, 12, and 13 for pH, color, microbial growth, and sensory attributes. Microbial growth was determined using total plate count (TPC) procedures, and sensory evaluation was performed through a quantitative descriptive analysis (QDA) (0–15 scale). A Random Complete Block Design was used for data analysis, with each trial (month) as blocks. Data were analyzed using GLM procedures in SAS (v.9.4), with means separated by Tukey's HSD at a 95% confidence level. TPC increased over time for both treatments, but facility A samples showed greater counts on each day, except day 1 ($P \leq 0.05$). Facility A had greater yeast counts on all days, except for days 1 and 13 ($P \leq 0.05$). pH did not differ between treatments ($P > 0.05$). Color differences were minimal, although A samples were brighter on days 7 and 13, while B samples were redder on days 9 and 13 and yellower on days 8 and 9. For sensory, facility A samples were juicier on days 1, 7, 11, 12, and 13, more tender on days 1, 6, 8, and 10, and mushier on days 1 and 8 ($P \leq 0.05$), whereas flavor attributes and overall acceptability declined similarly for both treatments. Facility B samples were pinker on day 6 ($P \leq 0.05$), while cooked color, raw and cooked aromas did not differ ($P > 0.05$). Overall, the facilities A and B samples produced distinct quality patterns over storage. Samples from A had greater microbial counts but more favorable sensory texture attributes, such as juiciness, tenderness, and mushiness. Based on color changes, sensory scores, total plate and yeast counts, the shelf-life for both facilities' turkey breasts under refrigeration is estimated up to 9 days. For best protein and water interaction and functionalities for yield and product quality purposes, breasts should be used for further processed products by day 7. These results indicate that trade-offs and variability in management practices across facilities may influence shelf-life and consumer-perceived quality of turkey breasts.

Keywords: Turkey breast; shelf-life; meat quality; cold storage; poultry processing

M71 Impact of Peracetic Acid Storage Conditions on *Campylobacter* Reduction on Chicken Breast

Pheron A. Collie^{*1GS}, Aaron R. Bodie¹, Marco Reina¹, Blesseth McDonald² ¹University of Georgia, Poultry Science, Athens,

Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States

Peracetic acid (PAA) is a vital antimicrobial disinfectant utilized broadly within the poultry industry. As such, producers buy PAA in large volumes to suffice their production over an allotted period of time. The goal of this study was to determine the effect of different storage conditions on the efficacy of PAA on *Campylobacter jejuni* subsp. *jejuni* (ATCC strain 81176) over time. To do this, a 240,000 ppm (24%) PAA solution was placed under three different storage conditions, sun, room, and 4 °C refrigeration over a 28-day period. This solution was split into triplicates for each condition. Chicken breast cutlets were weighed out at 50 grams per cutlet with three cutlets per treatment. The breast cutlets were then inoculated with an average 5.85 Log CFU/mL of *C. jejuni* each timepoint from prepared inoculums made with Bolton's Broth and allowed to sit for one hour to allow for the sufficient attachment of the *C. jejuni*. Simultaneously, PAA treatments had their initial concentration measured, recorded, and were then adjusted to a standard 300 ppm in bulk solutions in order to treat the breast cutlets after inoculation. Samples were dip treated with 300 ppm PAA treatments for 30 seconds and rinsed with buffered peptone water. Treatment rinsates were then spread plated on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated under microaerophilic conditions at 42 °C for 48 hours. Sampling occurred on Days 0, 7, 14, 21, and 28. Through days 0 to 28, the initial concentration of PAA across all conditions measured at 24% and by day 28 the 'sun' treatment condition remained consistent from the other conditions, consistently measuring at 24%. Though there was a numerical difference, results of a Tukey HSD analysis revealed no statistical difference between initial concentrations of the different treatment groups ($p > 0.05$). When analyzing PAA over time and storage, the mean log reduction decreased from 3.401 log CFU/mL at day 0 to 0.324 log CFU/mL by day 28 in the Refrigerator group. A two-way ANOVA analysis revealed a significant difference between *C. jejuni* reduction using PAA as a dip treatment based on day of usage ($p < 0.001$). These results suggest that storage duration of PAA reduces efficacy against *Campylobacter* on poultry meat.

Keywords: PAA; *Campylobacter*; Storage; Reduction

Food Safety I

M72 Predictive modeling of *Salmonella* Typhimurium behaviors on chicken wings during low temperature storage: Effects of heat, cold, and acid stress

Brenda J. Gonzalez^{*1GS}, Zhen Jia², Stephen N. White³ ¹Auburn, Poultry Sciences, Auburn, Alabama, United States; ²Auburn University, Poultry Science, Auburn, Alabama, United States; ³USDA, Agricultural Research Service, Athens, Georgia, United States

Salmonella is frequently exposed to various environmental stresses during poultry processing, which can alter the pathogen's behavior. In this study, we investigated the effects of stresses on the behavior of *Salmonella* Typhimurium (ST) and developed models for pathogen risk prediction. Heat, cold, and acid-stressed ST were induced by simulating poultry processing conditions to which the pathogen may be exposed. Both stressed and unstressed ST were inoculated on chicken wings and stored at abuse temperatures of 4, 10, and 20°C. ST populations were enumerated over time. The Huang model was developed to describe the growth of ST in response to stresses during low-temperature storage. Results showed that at 4 and 10°C for 30 days, both stressed and unstressed ST survived with around 1-2 log reduction in populations, while indigenous microflora grew well, reaching

above 5 log CFU/mL rinsate at day 3. At 20°C, notable differences were observed in the growth behavior of acid-stressed ST (ASST) compared to unstressed ST. ASST exhibited a significantly shorter lag phase (1.81 h) compared to unstressed ST (USST, 13.14 h), representing an approximately 7-fold reduction. The specific growth rate of ASST (0.22 h⁻¹) was also slower than that of USST (0.35 h⁻¹). Additionally, the maximum population achieved by ASST (6.08 log CFU/mL rinsate) was about 2.7 log units lower than that of USST (8.73 log CFU/mL rinsate). These findings demonstrate that acid stress can significantly alter ST growth kinetics. We also found that the developed Huang model was suitable for predicting the behavior of unstressed and stressed ST as well as indigenous microflora on chicken wings during storage. The model developed in this study could be used for *Salmonella* risk assessment and chicken wings' microbial shelf-life prediction. These findings provide critical insights into how environmental stresses influence pathogen behaviors and demonstrate the potential of predictive modeling for risk and shelf-life assessment.

Keywords: Predictive modeling; Stress response; *Salmonella*; Chicken wings

M73 *In vitro* evaluation of gallic acid against *Salmonella* Typhimurium

Indira Neupane*^{1GS}, Hanseo Ko¹, Ishwari Gyawali¹, Deependra Paneru¹, Woo Kyun Kim¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

Salmonella remains a major concern in the poultry industry, affecting public health and food safety. As the poultry production system continues to transition toward a No Antibiotic Ever (NAE) approach, there is a growing demand for identifying effective natural alternatives. Gallic acid (GA), a natural polyphenol compound found in fruits, plants, and nuts that exhibits antimicrobial and antioxidant properties, may serve as a promising natural antimicrobial compound against *Salmonella* in poultry. Therefore, a study was conducted to investigate the ability of GA as an antibacterial agent against *Salmonella* Typhimurium under *in vitro* conditions. The antibacterial efficacy of GA against *Salmonella* Typhimurium nalidixic acid-resistant (ST^{NR}) was evaluated using standard microbiological procedures. The minimum inhibitory concentration (MIC) was performed through the microdilution broth method across a range of GA concentrations (750–16,000 µg/mL). The minimum bactericidal concentration (MBC) was identified by plating concentration at or above MIC on Brilliant Green Sulfa (BGS) agar plates. Time-kill assay was performed to evaluate the bactericidal effect of GA over a 24h incubation time. The sub-lethal concentrations of GA (500–6,000 µg/mL) were used to evaluate its effect on bacterial swimming motility using the swim agar assay. All the assays include a control group (0 µg/mL GA). Data were analyzed with one-way ANOVA followed by Dunnett's post-hoc test with significance level set at $P < 0.05$. The results revealed that GA inhibited *Salmonella* Typhimurium in a dose-dependent manner, where the MIC was found to be 8,000 µg/mL, with a significant reduction in OD600 value ($P < 0.001$). The MBC was identified as 10,000 µg/mL. Time-kill kinetics showed that the concentration of GA above 8,000 µg/mL reduced bacterial counts after 2h, with complete elimination of *Salmonella* observed at 10,000 µg/mL within 8h of incubation (5.85 log₁₀ CFU/mL reduction). Additionally, the sub-lethal concentrations of GA significantly reduced the swimming motility of ST^{NR} ($P < 0.001$). In conclusion, GA demonstrated good antibacterial effect against *Salmonella* Typhimurium *in vitro*, supporting its potential as a natural antimicrobial candidate for an antibiotic-free poultry production system.

Keywords: *Salmonella*; *in vitro*; gallic acid; natural antimicrobial compound

M74 Role of Transcriptional Factors in the Intra-amoebic Survival of *Salmonella enterica* Serovar Typhimurium (SL1344)

Samantha G. Philip*^{1GS}, Greeshma Bharathan², Caroline Dawson¹, Hunter Sheffield¹, Stephen N. White³, Shabarinath Srikumar¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Auburn University, Poultry Science, Auburn, Alabama, United States*; ³*USDA-ARS U.S. National Poultry Research Center, Poultry Microbiological Safety & Processing, Athens, Georgia, United States*

Salmonella enterica serovar Typhimurium remains a major concern in poultry production due to its capacity to persist in diverse environmental reservoirs. Free-living protozoa, such as *Acanthamoeba* spp., are commonly found in poultry environments and can harbor *Salmonella* intracellularly. This protected niche may promote bacterial persistence and transmission. Although DNA-binding transcription factors (TFs) regulate *Salmonella* survival within macrophages, their roles in adapting to intra-protozoan conditions are not well understood.

This study examined two TFs Orf242, encoded within *Salmonella* Pathogenicity Island 2 (SPI-2), and Fur, a global iron regulator to determine their importance for intra-amoebic survival. *S. Typhimurium* SL1344 (wild type) and its deletion mutants Δ orf242 and Δ fur were co-cultured with *Acanthamoeba castellanii* (MOI 10:1). Intra-amoebic survival was assessed using a gentamicin-protection assay, and intracellular colony-forming units (CFU/mL) were quantified over a 16 h infection period. Statistical analysis was performed in RStudio using two-tailed Student's *t*-tests ($\alpha = 0.05$). Both Δ orf242 and Δ fur mutants exhibited significantly reduced intra-amoebic survival compared to the wild type. The Δ fur strain showed marked inhibition at 16 h ($p = 0.005982$), consistent with its essential role in scavenging iron within iron-restricted vacuoles. Similarly, Δ orf242 was significantly impaired at 16 h ($p = 0.0032$), indicating that Orf242 also contributes to intra-amoebic adaptation. Growth-curve analyses in LB, egg yolk, and chicken homogenate revealed no differences between SL1344 and Δ orf242, suggesting that Orf242's function is specific to intracellular environments. These findings highlight the importance of both Fur and Orf242 in enabling *Salmonella* to survive within *A. castellanii*. Targeting such transcriptional regulators may help develop pre-harvest control strategies to reduce *Salmonella* persistence and contamination in poultry systems.

Keywords: *Salmonella*; *Acanthamoeba castellanii*; transcription factors; intracellular survival; food safety

M75 Physiological characterization of different *Salmonella* serotypes in low-water food matrices

Elva Hernandez*^{1GS}, Sidra Nazeer¹, Greeshma Bharathan¹, Erin Olander², Ana Troncoso¹, Stephen N. White³, Shabarinath Srikumar¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Auburn University, Biological Science, Auburn, Alabama, United States*; ³*USDA-ARS U.S. National Poultry Research Center, Poultry Microbiological Safety & Processing, Athens, Georgia, United States*

Foods with low water activity (desiccated food/low aW foods) are considered safe for consumption. The low content of available water supposedly inhibits bacterial growth. However, pathogens such as *Salmonella* can survive in these environments and are linked to multiple outbreaks in products such as milk powder, infant formula, and egg yolk powder. These outbreaks highlight the need for serotype-specific investigations especially of uncommon serotypes to better understand survival in low-water food environments. This study aimed to evaluate the survival of eight *Salmonella* serotypes *S. Aberdeen*, *S. Agona*, *S. Anatum*, *S. Diarizonae*, *S. Oranienburg*, *S. Kentucky*, *S. Montevideo*, and *S. Typhimurium* under desiccation in TSB, milk powder, and egg yolk powder over 48 h. The known desiccation-tolerant strain *S. Typhimurium* ST4/74 was included as a reference. Each serotype was inoculated independently in triplicate into each matrix, and viable counts were measured at 0, 1, 2, 3, 4, 5, 8, 24, and 48 h. Differences between 0 h and 48 h were analyzed using Student's *t*-test ($p < 0.05$). Although all serotypes survived desiccation for 48 h, a significant decline in viability occurred across matrices. On average, only ~0.008% and ~0.009% of cells remained viable at 48 h, while egg yolk powder supported higher survival (~0.02%). *S. Diarizonae* and *S. Anatum* showed the lowest survival in egg yolk powder (~0.009%), while other serotypes reached ~0.02%. *S. Aberdeen* and *S. Typhimurium* had the highest viability (~0.04%) at 48 h in egg yolk powder. These findings confirm that desiccation tolerance in *Salmonella enterica* is both serotype- and matrix-dependent. The observed variation reflects physiological differences that may influence persistence and risk in dry-food production environments. Understanding these

survival patterns can improve risk assessment and inform control strategies to reduce *Salmonella* contamination in powder-based food systems, enhancing safety in powder-based products.

Keywords: *Salmonella* Enterica; Low-water foods; Survival; Serotype-specific responses; Viability

M76 Transcriptomic insights into the adaptive mechanisms of *Salmonella* Typhimurium during survival on chicken meat
Greeshma Bharathan^{*1GS}, Karoll Chinchilla², Lillian Dye¹, Hunter Sheffield¹, Michelle Hayden¹, Samantha G. Philip³, Aisha Madi¹, Priyanka Banerjee⁴, Shabarinath Srikumar¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Zamorano Institute, Honduras, Honduras*; ³*Auburn University, Poultry Science, Auburn, Alabama, United States*; ⁴*Auburn University, Department of Animal Science, Auburn, Alabama, United States*

Foodborne salmonellosis remains a major global public health concern, with poultry products serving as a leading vehicle for *Salmonella* transmission. The success of *Salmonella enterica* as a foodborne pathogen is closely tied to its ability to rapidly adapt to diverse and nutrient-limited environments encountered during food production and storage. To better understand the regulatory networks that facilitate this adaptation, we performed transcriptomic profiling of *S. Typhimurium* ST4/74 during survival on chicken meat. Bacteria were inoculated onto chicken meat, RNA was isolated at mid-lag, mid-log, and mid-stationary phases, and RNA-seq was conducted using the Illumina HiSeq platform, followed by comprehensive bioinformatic analysis. Transcriptomic data revealed a distinct induction of oxidative stress response pathways, including significant upregulation of catalase-encoding *kat* genes. Consistent with oxidative stress exposure, iron acquisition systems under the control of the Ferric Uptake Regulator (*Fur*) were also strongly induced. Functional studies supported these findings: both the Δfur and $\Delta feoB$ mutants were significantly impaired for survival on chicken meat ($P = 0.0005$ and $P = 0.0004$, respectively), highlighting the importance of iron homeostasis. All statistical analyses were performed using RStudio (version 4.4.2). In contrast, deletion of the siderophore biosynthesis gene *entC* did not impact survival, likely due to redundancy among *Salmonella* siderophore systems. Notably, manganese uptake via *sitA* was essential ($P = 0.005$), suggesting that the pathogen may substitute manganese for iron to mitigate oxidative damage. A highly induced small RNA, STnc3080, was identified and found to be antisense to a 38-bp region in the *nrdH* leader sequence. We propose that STnc3080 may post-transcriptionally modulate *nrdH*, encoding class Ib ribonucleotide reductases essential for DNA synthesis under oxidative conditions. Collectively, these findings reveal key regulatory mechanisms supporting *Salmonella* survival on chicken meat and highlight oxidative stress resistance as a critical driver of persistence in poultry-associated environments.

Keywords: *Salmonella*; adaptability; oxidative stress; *Fur*; small RNAs

M77 Impact of peanut skin supplementation and *Salmonella* challenge on gut microbiota of broiler chickens

Yabaiz Tahir^{*1GS}, Lin Walker¹, Justin Lowery¹, Lauren Anderson¹, Natalie Roberts¹, Thien Vu², Ondulla Toomer², Monika Weglarz³ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²*Food Science & Market Quality and Handling Research Unit, ARS, USDA, Raleigh, North Carolina, United States*; ³*Animal Biosciences & Biotechnology Laboratory, ARS, USDA, Beltsville, Maryland, United States*

Salmonella, a major foodborne pathogen in poultry, poses a human health risk. This study evaluated the outcomes of the presence of peanut skin (5%) in the diet on cecal and ileal microbiota in *Salmonella* Typhimurium-challenged birds. A 2 x 2 design was used to randomly assign 180-day-old 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), cecal and ileal contents were aseptically collected from 12 birds/treatment for microbiota analysis. Sequencing of the V3-V4 hypervariable region of the 16S ribosomal RNA (rRNA) was performed using Illumina's MiSeq platform and was analyzed using QIIME2 software. Statistical analysis was performed using nonparametric tests, with a significance level of $p < 0.05$. In the cecum, evenness, the number of amplicon sequence variants (ASVs), and Shannon Index did not differ significantly among dietary treatments. Regarding richness, a difference was observed between CON and PS groups ($p = 0.05$); however, richness was higher in non-challenged birds than in challenged birds ($p = 0.0004$). Unweighted UniFrac PERMANOVA indicated strong compositional shifts ($p = 0.001$), where challenged birds clustered away from the non-challenged groups. In the ileum, evenness was unaffected ($p < 0.05$); however, richness, ASVs, and Shannon Index in non-challenged birds were significantly different ($p < 0.05$) compared to challenged birds. PS also showed a significant difference ($q < 0.05$) compared to CON in all metrics except for Shannon Index ($q > 0.05$). Unweighted UniFrac showed distinct microbiota clustering in non-challenged groups compared to the challenged groups ($p = 0.001$), though less distinctly than in the cecum. Overall, CON and PS diets differed in initial metrics, suggesting differences in healthy microbiota structure; however, diversity was greatly reduced after *Salmonella* challenge. PS supplementation produced significant effects, especially in the ileum. These results suggest that infection is a major factor in the shift of intestinal microbiota, but dietary PS may promote microbial stability. Further research is in progress to clarify host microbiota interactions.

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

Food Safety II

M78 Effect of a chestnut quebracho tannins blend on *Salmonella* colonization in broiler chickens

Rabin Raut^{*1GS}, Sabin Poudel¹, Matthew Bailey¹, Shijinaraj Manjankattil², Jinquan Wang³, Zahidul Tushar¹, Rishav Kumar¹, Yagya Adhikari⁴, Samuel J. Rochell⁵, Karyl Hedden⁶, Dianna Bourassa¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Auburn University, Poultry Science, Auburn, Alabama, United States*; ³*Auburn University, Poultry Science, Auburn, Alabama, United States*; ⁴*Auburn University, Department*

of Poultry Science, Auburn, Alabama, United States; ⁵*Auburn University, Poultry Science, Auburn, Alabama, United States*; ⁶*Silvateam USA, New York, New York, United States*

Bioactive compounds in plant extracts can support gut health and help control *Salmonella* in poultry production. This study evaluated the effects of a chestnut quebracho tannin blend (TE; Silvafeed Nutri P, Silvateam S.P.A., Italy) on *Salmonella* recovery in broiler chickens. A total of 1,200 day old male chicks (Ross 708)

were assigned to 4 dietary treatments in a RCBD with 12 replicate pens of 25 birds each: (1) negative control (NC; unchallenged), (2) positive control (PC; challenged with nalidixic acid resistant *Salmonella Typhimurium* and *S. Infantis* at 10^5 CFU/bird on day 0), (3) PC + bacitracin methylene disalicylate (BMD; 100 mg/kg feed), and (4) PC + TE (1000 g/ton feed). Diets were formulated according to Ross 708 nutrient requirements for starter (d0-14), grower (d15-28), and finisher (d29-35) phases. Ceca, boot swabs, liver, spleen, and carcass rinsates were collected at d7, 14, 21, 28, and 35. Log CFU counts were analyzed by ANOVA with Tukey's HSD, while systemic dissemination and carcass prevalence were assessed by Chi-square, pairwise proportion tests, and logistic regression. TE reduced ceca counts compared to BMD at d7 ($P=0.018$) and d21 ($P=0.008$), but not at d14 ($P=0.96$) or d28 ($P=0.62$). At d21, TE had lower cecal *Salmonella* counts than PC ($P<0.001$), while BMD did not differ ($P=0.77$). At d28, neither TE nor BMD differed from PC ($P=0.20$ and $P=0.87$). At d35, most counts were below detection. Boot swabs were modestly lower in TE than BMD at d7 ($P=0.030$), but from d14 onward, TE, BMD, and PC did not differ ($P>0.05$). Systemic prevalence was high early, with 70-75% liver positives in challenged groups at d7 versus 0% in NC ($P<0.001$). At d14, TE reduced liver infection odds compared to BMD (OR 0.20, CI: 0.05-0.70, $P=0.016$), with 20.8% positives in TE, 56.5% in BMD, and 60.9% in PC. Spleen prevalence showed a tendency towards reductions in TE at d7 ($P=0.083$), but differences were not significant thereafter. Carcass rinsates remained high at d35 in challenged groups (BMD 79%, TE 83%, PC 100%) compared to NC (67%), with no reduction relative to PC ($P>0.05$). The chestnut quebracho tannin blend provided sporadic reductions in cecal *Salmonella* and transient systemic protection but did not consistently suppress colonization or reduce carcass contamination.

Keywords: chestnut and quebracho tannin extracts; broiler chickens; *Salmonella* challenge; systemic prevalence; carcass reinstates

M79 Effect of booster route on the efficacy of a live-modified *Salmonella* vaccine in broilers: impact on cecal colonization, organ prevalence, and carcass contamination

Arjmand Fatima*^{1GS}, Muhammad Naeem¹, Sabin Poudel¹, Jinquan Wang¹, Matthew Bailey¹, Maria Baca¹, Dianna Bourassa¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States

Vaccination is a key pre-harvest strategy to reduce *Salmonella* contamination in broilers. This study evaluated the effect of a live-modified *Salmonella* vaccine under wild-type (WT) exposure, comparing two booster routes (spray vs. drinking water) on cecal colonization, organ prevalence (liver and spleen), and carcass contamination. Six hundred broilers were randomly assigned to three treatments: T1 (vaccine spray + water booster), T2 (vaccine spray + spray booster), and T3 (sham spray + sham booster [spray + water]). Ceca, liver, and spleen samples were aseptically collected on days 4, 28, and 35, with carcass rinses on day 41. Birds in all groups were orally challenged on day 10 with 10^6 CFU/bird of *S. Enteritidis*, but the challenge strain was not subsequently recovered. Thus, cecal/carcass counts and organ prevalence post-challenge reflect WT *Salmonella*. Cecal/carcass counts were log transformed and analyzed using one-way ANOVA and liver/spleen prevalence by chi-square in R ($P \leq 0.05$). On day 4, cecal log₁₀ *Salmonella* counts did not differ among treatments (T1: 6.01 ± 0.33 ; T2: 6.36 ± 0.30 ; T3: 6.27 ± 0.29). Counts decreased across all treatments on day 28 and continued through day 35, with no significant differences among treatments. Liver *Salmonella* prevalence did not differ among treatments on day 4 (T1: 75%, T2: 62.5%, T3: 75%). By day 28, prevalence declined in T1 and T3 but remained stable in T2. On day 35,

prevalence decreased in all treatments, with T1 significantly higher than T3 (T1: 37.5%, T2: 29.2%, T3: 4.2%; $P = 0.018$). No treatment-related differences were observed in spleen *Salmonella* prevalence on day 4 (T1: 75%, T2: 87.5%, T3: 62.5%). On day 28, prevalence was higher in T1 than T3 (T1: 83.3%, T2: 62.5%, T3: 41.7%; $P = 0.012$). By day 35, prevalence declined across all treatments, with no significant differences observed among treatments. On day 41, carcass log₁₀ *Salmonella* counts were lower in T2 than T3 (T1: 1.32 ± 0.17 , T2: 0.94 ± 0.16 , T3: 1.60 ± 0.21 ; $P = 0.050$), indicating improved control with the spray booster. Overall, cecal and organ colonization reflected WT *Salmonella* clearance with age, and the spray booster modestly influenced carcass contamination at market age, suggesting the vaccine could help support food safety in broilers.

Keywords: *Salmonella*; broiler; live modified vaccine; booster route; cross contamination

M80 Impact of dietary organic acids and their derivatives on ileal microbiome of *Salmonella*-challenged broilers

Ruby Paudel*^{1GS}, Farazi Abinash Rahman¹, Shijinaraj Manjankattil¹, Dianna Bourassa¹, Jinquan Wang¹, Jundi Liu², Sabin Poudel¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²Eastmen Chemical Company, Animal Nutrition BU, Kingsport, Tennessee, United States

Current study was to evaluate the effects of organic acids and their derivatives on ileal microbiome of *Salmonella* challenged broilers. A total of 800 Ross 708 male broilers were randomly divided into four treatments with 8 replicates and 25 chicks per replicate. Birds were inoculated with a nalidixic acid resistant *Salmonella Typhimurium* at 10^5 Cfu/bird on d7. Four dietary treatments include T1) Control; T2) formic acid-based blend at 3 kg/mt; T3) short-/medium-chain monoglycerides blend at 1.5 kg/mt; T4) combination of T2 and T3. The ileum content from middle portion of ileum was collected on d 14 and d 35. The full-length 16S rRNA sequencing was done using MinION. MinKNOW and EPI2ME were used for base calling, demultiplexing, and taxonomic classification. Data were analyzed in R using Phyloseq package. Alpha diversity metrics were analyzed using ANOVA or Kruskal-Wallis test depending upon normality of data. Alpha diversity metrics Chao1 index was significantly impacted by the treatment on d 14, in which T1 has highest bacterial diversity compared to the T2 and T4 ($p = 0.0124$). However, no significant difference was observed in other alpha diversity metrics (Shannon, Simpson and Pielous evenness) and beta diversity (Bray-Curtis) between treatments on both d14 and d35. Whereas, Jaccard beta diversity showed a significant treatment effect on d14 ($p=0.003$), with T1 significantly different from other treatments. Differential abundance analysis of d14 ileum microbiome showed beneficial bacteria (*Bacillus nakamurai*, *Bacillus vallismortis*, and *Bacillus velezensis*) had higher abundance in T4 compared to other treatments. Whereas necrotic enteritis associated potential pathogenic bacteria (*Clostridium manihotivorum*) had highest abundance in T1. Additionally, organic acids and their derivatives supplementation significantly reduced butanol (*Clostridium beijerinckii*) and butyrate (*Faecalicatena fissicatena*) producing bacterial abundance compared to control. Overall, these results indicate that both formic acid based and short-/medium-chain monoglycerides blends decrease the bacterial diversity at early age, promote proliferation of beneficial bacteria, and decrease abundance of potential pathogenic bacteria in ileum during *Salmonella* challenged condition.

Keywords: ileum microbiome; full-length 16S rRNA; Formic acid; short-/medium-chain monoglycerides; broilers

M81 Cold plasma: The next-gen non-thermal, non-chemical intervention to improve raw poultry safety and shelf-life

Katherine Sierra*^{1GS}, Cameron Smith¹, Vianca Tashiguan¹, Micah T. Black¹, Amit Morey¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States

Conventional sanitizers and antimicrobials are effective against Salmonella and microbial spoilage but raise concerns over chemical residues and bacterial adaptations, highlighting the need for alternative solutions such as cold plasma (CP). CP is a non-thermal technology that generates reactive species with antimicrobial potential. This study evaluated CP using different machines against Salmonella and chicken spoilage in two independent experiments. In the first experiment, chicken skin was inoculated either with *S. Enteritidis*, Nalidixic Acid-Resistant *S. Heidelberg*, or *S. Typhimurium* (~4 log CFU/cm²), allowed to attach (30 min at 4 °C), and treated with CP (5.75 kV) for 0, 10, or 20 min under helium, hydrogen peroxide (H₂O₂), or combined conditions (n= 216). Samples were diluted with Buffer Peptone Water (BPW), plated in Xylose Lysine Deoxycholate (XLD), and incubated for 24h at 35 °C. In the second experiment, excised tissue sections (2×2×0.5 cm) from three chicken parts (breast, drumstick, and skin) with six treatments (control, H₂O₂ alone, 5- and 10-min direct plasma exposure (230 V), and 5- and 10-min plasma-activated H₂O₂) with three repetitions (n = 270). Samples were treated, placed in tubes, then vortexed with BPW, plated in Plate Count Agar (PCA), and incubated at 37 °C for 48h. Additionally, microbiome, biochemical, and phylogenetic tree analysis were performed to determine the impact of CP treatment on the microbial community. Data was analyzed by ANOVA to detect significant differences among treatments (p ≤ 0.05), and when significant, Tukey's HSD test was used for pairwise comparisons. Data indicated, 1) CP at plasma-activated H₂O₂ achieved up to >2 log, while helium or H₂O₂ alone had minimal effects. Notably, CP without H₂O₂ at 5.75 kV still reduced *S. Enteritidis* by 2.46 log after 20 min. 2) On chicken parts, 10 min plasma-activated H₂O₂ (230 V) achieved a microbial reduction of ~2.5 log, which was statistically greater than the control (p ≤ 0.05). The highest abundance of microbial spoilage found on chicken samples was *Pseudomonas*, *Staphylococcus*, *Pantoea*, and *Bacillus*, differing per treatment. Together, these results demonstrate the strong antimicrobial potential of CP against both pathogens and microbial spoilage on poultry products.

Keywords: Salmonella; Poultry Products; Microbial load; Cold Plasma; Microbiome Analysis

M82 Efficacy of a commercial high pressure inside out wash system on microbial loads during poultry processing

Shamar T. Simon*^{21GS}, Aaron R. Bodie², James A. Widmer², Twan Koenen² ¹University of Guyana, Faculty of Agriculture and Forestry, Georgetown, Guyana; ²University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Campylobacter contamination during poultry processing remains a challenge as unit operations such as scalding, defeathering and evisceration promote cross-contamination on carcasses. The increased microbial loads can impair the efficacy of chilling, often the final critical control point (CCP) for poultry harvest. The goal of this research was to determine whether applying a commercial Inside-Out carcass rinse immediately after feather removal reduces microbial load on the carcass after chilling. Three 30 broiler trials were processed at the University of Georgia's pilot poultry processing facility. Treatments were (1) A control of immersion chilling with no peracetic acid (PAA), (2) the Undine washer post-pick, (3) Undine washer post-pick and post-processing, (4) Undine washer post-pick + 200 ppm PAA and (5) immersion chilling with 200 ppm. *Campylobacter spp.* and *Enterobacteriaceae* (EB) were

the target microbial groups and they were isolated from poultry rinsates. EB were quantified utilizing EB Petrifilm and *Campylobacter spp.* was enumerated using mCCDA (Modified Charcoal Cefepazone Deoxycholate Agar) then incubated for 48 hours at 42°C. All samples were collected and processed in accordance with the USDA Microbiology Laboratory Guideline (MLG) and confirmed colonies were Log transformed for statistical analysis. Both the Undine washer and 200 ppm PAA had a significant effect on microbial load (one-way ANOVA P < 0.05). The greatest reduction was seen when the two interventions were combined achieving 1.5 Log CFU/ml lower than the control. For *Campylobacter spp.* a similar reduction occurred. A significant difference was seen when the Undine Washer and 200 ppm PAA was used (P < 0.05). Cramer's V showed a weak but statistically detectable association between reduction and treatment (Cramer's V = 0.10 ; 95% CI: 0.003-0.39). The Undine washer + 200 ppm PAA yielded the best results. Findings highlighted that upstream load reduction significantly influences antimicrobial efficiency in downstream processing, effectively reducing microbial load. Consequently, the introduction of a post picker wash can enhance the performance of antimicrobials. This supports sustainability in poultry processing by offering a low-chemical intervention focused on improving food safety.

Keywords: Food Safety; *Campylobacter*; Poultry processing; Carcass Washer; Peracetic acid

M83 Polyphenols are synergistic with peracetic acid in mitigating Salmonella in simulated poultry chilling conditions

Hunter Sheffield*^{1GS}, Greeshma Bharathan¹, Robert Colvin¹, Michelle Hayden¹, Samantha G. Philip¹, Shabarinath Srikumar¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States

Peracetic acid (PAA) is the most widely used antimicrobial in poultry processing plant chiller systems. Although PAA is a highly effective antimicrobial, increasing bacterial tolerance, high doses, and potential occupational health safety concerns underscore the need to identify novel compounds that can be synergistic with PAA, thereby allowing for the use of lower PAA levels. In this study, we utilized a small-scale immersion chiller water system to simulate industry practices and evaluate the synergistic effects of bioactive compounds such as 4-hydroxycinnamic acid (HA) and itaconic acid (ItA) with PAA on the mitigation of multi- drug-resistant *Salmonella enterica* Typhimurium DT104 (DT104). UV-sterilized chicken skin coupons were inoculated with DT104 and allowed to attach for 1 hour. The spiked skin coupons were treated with a tested low-efficacy baseline concentration of PAA (5 ppm), bioactive compounds alone, and PAA-bioactive compound (HA or ItA) combinations. Coupons were then submerged in the treatment solutions for up to 120 minutes at 4°C, with samples collected every 30 minutes and plated on XLD using standard spread plating for bacterial enumeration. While PAA (5 ppm) alone produced a limited reduction in bacterial counts (due to its low concentration), the combination with either or both bioactive compounds showed a reduction in bacterial counts. However, t-test function using RStudio (version 4.4.2) did not reflect any significant differences between the PAA alone versus the PAA-bioactive compounds combinations. The non-significant reduction could be due to the lack of bacterial multiplication at 4°C, raising the possibility that active multiplication of *Salmonella* is essential for the bioactive compound-based reduction of bacterial loads. Our results, however insignificant, underscore the importance of investigating bacterial physiology in antimicrobial intervention studies.

Keywords: Antimicrobial resistance; Phenolic acids; *Salmonella*; Poultry meat; peracetic acid

M84 Use of chemical agents to extend the lifetime of peracetic acid

Kortney Martin^{*1UG}, Daniel Sabo¹ *Georgia Tech Research Institute, Atlanta, Georgia, United States*

Peracetic acid (PAA) is often used 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 may be effective at extending PAA lifetime: 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) and ethylenediaminetetraacetic acid (EDTA). This study explored various concentrations of each chemical, individually and synergistically, in simulated chiller water containing a 1000 ppm cation mixture (magnesium, sodium, and potassium). All solutions were buffered to pH 9 to match industry standards using 4M NaOH and PAA was incorporated targeting an initial concentration of 160 ppm. Timing was started once pH reached 9.0 and initial samples were taken to determine PAA concentration using a MP-9700E colorimetric meter system. PAA concentration values were first recorded at set intervals from 0-90 minutes before the experiments ran overnight (17–22 hours), allowing for measurable PAA decay and thus more accurate half-life values. Calculations were completed using the half-life exponential decay formula. PAA's mean half-life in simulated chiller water was 126 minutes. Incorporating 200 ppm EDTA increased half-life to 2447 minutes (SD =151). 500 ppm, 1000 ppm, and 2000 ppm EDTA reduced half-life to 1792 minutes (SD =185), 1001 minutes (SD =316), and 0 minutes respectively. HEDP had a linear relationship with half-life: 15 ppm HEDP (\bar{x} =1223 minutes, SD=239); 25 ppm HEDP (\bar{x} =1264, SD=584); 35 ppm HEDP (\bar{x} =1617, SD=293); 100 ppm HEDP (\bar{x} =1799, SD=705). PAA's half-life was most extended by the combined effect of 15 ppm HEDP with 200 ppm EDTA, resulting in a 2792 minute half-life, SD=214. HEDP at 15 ppm with 500 ppm EDTA had a 2141 minute half-life (SD=149), and 15 ppm HEDP with 1000 ppm EDTA had a 336 minute half-life (SD=178). An analysis of variance test was conducted, indicating a significant effect of chemical use on PAA half-life; $F(13,27) = 100.51, p < 0.001$. This study shows that combined chemical use can greatly improve PAA's half-life. Further combination experiments are underway with optimum combinations to be implemented for an antimicrobial study to determine PAA's efficacy with longer half-lives.

Keywords: Peracetic acid; Decay Kinetics; Lifetime; Half-life; Immersion Chiller

M85 Non-invasive detection of Salmonella contamination in SPF White Leghorn eggs via VOC profiling at cold storage temperatures

Kandi A. Henry^{*1UG}, Christopher Heist¹, Caitlin Harris², Olga Kemenova¹, Daniel Sabo¹, Stephanie Richter¹ *Georgia Tech Research Institute, Atlanta, Georgia, United States*; ²*USDA-ARS, Poultry Microbiological and Processing Unit, Athens, Georgia, United States*

This study aims to characterize volatile organic compound (VOC) emissions from eggs stored at low temperatures as early biomarkers of contamination. We aim to identify microbial VOCs (mVOCs) produced by *Salmonella enteritidis* (SE) in eggs stored under standard hatchery conditions (17 °C) vs. commercial egg storage conditions (4 °C). By targeting VOCs specific to these conditions, we seek to establish biomarkers for detection prior to incubation and commercial distribution. SPF White Leghorn eggs were assigned to eight treatment groups (control vs. SE; yolk vs. albumen; 17°C vs. 4°C). Eggs were inoculated with PBS or PBS containing SE (10³ CFU/mL) and stored for three days. VOCs were collected from the same eggs on Days 0–3, with a subset

breakout from each group on Days 0–2. Breakout of the VOC collection eggs was conducted on Day 3. VOCs were captured using a sorption extraction device in sterile glass jars (90 min), analyzed by 2D gas chromatography–mass spectrometry (GC×GC–MS), and identified via the NIST 2017 spectral database. Egg contents were diluted with 1% BPW, plated on BGS, and incubated for 48 hr. Recovered SE counts were used as ground truth data for collected VOC profiles. Statistical analysis was performed using Principal Component Analysis (PCA) for VOCs and one-way ANOVA for SE counts. Results indicate that temperature is the main driver of VOC profile differentiation. High-temperature samples had increased VOC values regardless of SE status. At lower temperatures, both SE and control samples displayed mixed intensities with no consistent differences. PCA results revealed distinct separation between SE and control yolk samples at high temperatures. These findings suggest that at 17°C, SE's metabolic activity renders the egg a viable substrate for growth, producing distinct VOC profiles that can act as biomarkers for contamination. At 4°C, SE remains dormant with minimal metabolic activity, resulting in less pronounced VOC profiles. Despite the need for additional replicates, these preliminary results highlight the potential of mVOC profiling as an effective and non-invasive tool for early detection of contamination, therefore reducing the risk of pathogen transmission as well as enhancing hatchery biosecurity and the safety of the final product.

Keywords: Egg; Salmonella; VOC; Non-invasive; Temperature

M86 Antimicrobial resistance in Salmonella isolates recovered from poultry products of select retail grocery stores

Thomas Stokes^{*1GS}, Yagya Adhikari¹, Dianna Bourassa¹ *Auburn University, Poultry Science, Auburn, Alabama, United States*

Salmonella causes 1.35 million foodborne infections annually in the US. Growing levels of resistance make these *Salmonella* more difficult to treat. This study was designed to assess antimicrobial resistance (AMR) patterns of 114 *Salmonella* isolates recovered from poultry meat at retail. Isolates were categorized based on their recovery from poultry products of different production types (conventional, no-antibiotic-ever, organic, Halal, Kosher, and Asian/Latin). PCR confirmed *Salmonella* isolates were phenotypically tested for antibiotic susceptibility against 14 different antibiotics using the NARMS broth microdilution method, and the minimum inhibitory breakpoint values for each antibiotic (CMV5AGNF) were based on CLSI guidelines. Logistic regression analysis was used to establish the association between production category and resistance pattern. Models were fitted using Firth's bias-reduced logistic regression to account for complete separation of data. Then pairwise comparison between variables was conducted using emmeans package in R. The level of significance was set to $p \leq 0.05$ and 95% confidence interval of odds ratio was calculated. 98% of isolates were resistant to at least one antimicrobial, while 88% of isolates were multi-drug resistant. *Salmonella* isolated from conventional products were 97% AMR; all other categories were 100% AMR. Conventional was 87% MDR; organic was 95%, Asian/Latin was 73%, and all other categories were 100% MDR. No production category was significant for either AMR or MDR. There was no significant difference ($p > 0.05$) among the six production types based on the resistance pattern of *Salmonella* isolates for each antibiotic tested, except for AUG2 (Amoxicillin/clavulanic acid 2:1 ratio). The odds of detecting a *Salmonella* isolate resistant to AUG2 (MIC $\geq 32/16$ µg/mL) in Asian/Latin products (7/18) were significantly higher ($p = 0.0448$) as compared to the *Salmonella* isolates acquired from conventional products (0/62). These results show that consumer product labels do not correlate to a significant increase in overall resistance but can have risk factors with specific antimicrobials.

Investigation into the genetic AMR traits of *Salmonella* isolated from these products could further elucidate consumer risks by production type.

Keywords: Salmonella; Antimicrobial Resistance; Production Types; Retail Poultry Meat

M87 *Salmonella* prevalence on poultry meat products sourced from retail grocery stores

Maria Baca*^{1GS}, Jinquan Wang¹, Thomas Stokes¹, Yagya Adhikari¹, William Echeverria¹, Arjmand Fatima¹, Marissa Askew¹, Dianna Bourassa¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Understanding *Salmonella* prevalence and risk factors in retail poultry products is essential for improving current food safety protocols and principles. The main objective of this study was to estimate prevalence of *Salmonella* from a diverse group of chicken, turkey, and duck meat products from retail stores using intensive isolation procedures. 189 samples were collected and classified by species: chicken (165), turkey (21), and duck (3); part type: breast (39), thighs (30), wings (19), ground chicken (18), tenders (29), ground turkey (22), drums (22), and others (n< 5 each) including gizzard, head, heart, leg quarter, liver, neck and paws; and marketing type: conventional (78), non-antibiotic ever (NAE, 56), organic (33), and other specialty labels including air chilled, Kosher, Asian market, Latin market and Halal combined as others (n< 5 each). On collection day, 1 lb (~454 g) of each sample package was rinsed in 150 mL buffered peptone water for 1 min and incubated together for 24 h at 37 °C. *Salmonella* prevalence was determined using conventional culture-based methods including enrichment and plating on multiple selective media. A cross-sectional sampling design was used, grouping retail poultry samples by species, part type, and production type to evaluate *Salmonella* prevalence. Chi-square test was applied to production type and species, while Fisher's exact test was used for part type due to small sample numbers of positives (n<5) in R (R Core Team). Significance level was set at 0.05. Overall prevalence was 75% (142/189). *Salmonella* prevalence in chicken and turkey products was 75 and 76% (P = 0.5957). Among product types, prevalence ranged from 55% to 86%, with drums showing the highest prevalence and tenders the lowest but were not statistically different (P=0.0951). Breast, Wings, and Tenders had a prevalence of 77, 79, and 80%, respectively. *Salmonella* prevalence was also similar among marketing categories (P=0.1937) and ranged from 85% (organic) to 69% (conventional). Ultimately, *Salmonella* remains commonly present in retail poultry products, including breast, tenders, thighs, and wings. This highlights the continued need for effective interventions to reduce contamination in cut-up parts and to promote safe handling practices among consumers.

Keywords: food safety; poultry products; prevalence; safety; retail

M88 Detection and molecular characterization of *mcr-1* in multidrug-resistant *Escherichia coli* isolated from retail poultry meat

Jouman W. Hassan*¹, Anahita Ghorbani², Bledar Bisha², Issmat Kassem¹ ¹*University of Georgia, Center for Food Safety, Griffin, Georgia, United States*; ²*University of Wyoming, Department of Animal Science, Laramie, Wyoming, United States*

Poultry meat is a nutrient-rich and widely consumed commodity, yet its safety remains a concern. In addition to contamination with foodborne pathogens, poultry meat can carry antibiotic-resistant (AR) organisms and genes (ARGs). Antibiotics are widely used in poultry production to treat/prevent disease; however, in settings

with limited antimicrobial stewardship, they are also used for growth promotion. The overuse of these drugs has contributed to the spread of resistance, particularly to colistin, a last-resort antibiotic. Studies have reported the colonization of broiler chickens with the plasmid-borne mobile colistin resistance gene (*mcr*), but data on its detection on retail chicken remain limited. In this study, we aimed to assess the detection of *mcr-1* in *E. coli* recovered from retail chicken meat in Lebanon and to characterize the isolates using phenotypic resistance profiling and whole genome sequencing (WGS). Skinless chicken breasts (n = 151) were collected from retail markets and butcheries. Colistin-resistant *E. coli* was isolated using RAPID[®] *E. coli* 2 agar supplemented with colistin. Resistance was evaluated using the Kirby-Bauer disk diffusion assay, colistin resistance was confirmed by broth microdilution, and ARGs were detected using PCR. A subset of isolates was subjected to WGS for analysis. Biofilms were assessed using the crystal violet method, and conjugation assays were conducted to evaluate plasmid transfer to rifampicin-resistant *E. coli* DH5a and *Salmonella* Enteritidis. *mcr-1* was detected in 16 multidrug-resistant *E. coli*. Conjugation and plasmid analyses showed that *mcr-1* was carried on the globally disseminated IncX4 and IncHI2 plasmids. WGS data revealed that the isolates were genetically diverse (belonged to multiple sequence types) and carried various ARGs and disinfectant resistance genes. Moreover, extended beta-lactamases (*bla*_{TEM} and *bla*_{CTX-M}) were detected in 12 isolates. All isolates were predicted to be potential human pathogens and showed medium to strong biofilm formation. The data highlight the need for effective surveillance and targeted interventions across the broiler production chain and suggest that colonization with *mcr* at preharvest may persist to the retail stage, posing a public health and food safety concern.

Keywords: Poultry; *mcr-1*; multidrug resistant; plasmid; *E. coli*

M89 Prevalence of *Campylobacter* in chicken meat products of retail grocery stores: A cross-sectional study

Yagya Adhikari*¹, Maria Baca¹, Jinquan Wang¹, Matthew Bailey¹, Dianna Bourassa¹ ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States*

Campylobacter is a common cause of foodborne gastrointestinal infections in humans. More than 70% of these infections were attributed to contaminated poultry and meat products. To determine the *Campylobacter* prevalence in retail grocery stores, a cross-sectional study was conducted in the Southeast region of the U.S. Altogether, 103 chicken product samples from different batches were collected from 7 retail grocery stores. Samples were pre-screened with the 3M Molecular Detection System (MDS) following overnight pre-enrichment with 3M Campy Enrichment broth. The isolation of bacteria followed standard microbiological protocol using Bolton's broth and Campy Cefex agar as selective media. Data were analyzed in R using generalized linear model using logit link to determine the effects of sample types (breast, thighs, tenders, drums, wings) and production types (organic, antibiotic-free and conventional) on the odds ratio of *Campylobacter* prevalence. Pairwise comparisons were conducted to further separate odds ratio within a sample type or production type using 'emmeans' package with an adjustment of Tukey's method. The level of significance was set to p<0.05 and 95% confidence interval (CI) of odds ratio was also calculated. Chicken products acquired from grocery stores has an overall *Campylobacter* prevalence of 21% (22/103), with prevalence ranging from 8% in breast meat to 54% in wings. Moreover, the odds of *Campylobacter* detection in wings were 13 times (95% CI; 2.38 – 109.38) higher as compared to breast samples (p=0.0485). In case of different production types,

conventional, antibiotic-free and organic chicken products had a *Campylobacter* prevalence of 22%, 15% and 35% respectively. There were no significant differences in the odds of *Campylobacter* detection in one production type over another ($p > 0.05$). The prevalence results showed that the chicken meat products in the retail grocery stores could be contaminated with *Campylobacter* despite antimicrobials incorporated at processing, thereby causing a serious public health threat.

However, the actual bacterial load present in those chicken products was unknown. Further studies should be directed to determine the bacterial concentration and the antimicrobial resistance patterns of those bacterial isolates.

Keywords: *Campylobacter*; retail grocery stores; chicken meat products; foodborne infections

Metabolism & Nutrition I: Vitamins & Minerals

M90 Performance and physiological responses of laying hens to 32 weeks of phosphorus modulation

Raquel M. Horn*^{1GS}, Sergio L. Vieira²¹, Douglas D. Maria¹, Yuri J. Olabarriga¹, Walter E. Altevogt¹, Luana A. Lopes da Cunha³, Rodolfo Leiskosky¹ ¹*UFRGS, Porto Alegre, Rio Grande do Sul, Brazil*; ²*Phytobiotics, Porto Alegre, RS, Brazil*; ³*UFRGS, Animal Science, Porto Alegre, Rio Grande do Sul, Brazil*

Phosphorus (P) is essential for skeletal integrity, egg formation, and energy metabolism in laying hens, and dietary available P (AvP) can interact with phytase to modulate mineral utilization. This study aimed to determine how increasing AvP, with or without phytase, affects performance, nutrient digestibility, bone quality and mineral metabolism. A total of 324 Bovans White hens (18 wk) were assigned to a completely randomized 6×2 factorial arrangement (0.09, 0.16, 0.23, 0.30, 0.37, 0.44% AvP; 0 and 4,000 FYT/kg phytase), totaling 12 treatments with 27 replicates. Hens received a common diet from 18–25 wk and experimental diets from 26–57 wk. Performance was recorded in eight 28-d periods. At 57 wk of age, tibias ($n=15$ /treatment) and ileal digesta were collected to assess bone traits and nutrient digestibility. Blood samples were obtained to measure circulating Ca, P, alkaline phosphatase (ALP), parathyroid hormone (PTH), and calcitonin (CT). Additionally, egg yolks ($n=10$ /treatment) were analyzed for phosvitin, and total excreta and eggs were collected to determine Ca and P retention. Performance was analyzed using three-way ANOVA; all other responses with two-way ANOVA (SAS; $p \leq 0.05$). Phytase, AvP, and age influenced all the performance parameters ($p < 0.05$). Tibia mineral content and breaking strength increased as dietary AvP increased, especially when phytase was included ($p < 0.05$). Phytase improved P digestibility by 24.9%, whereas Ca digestibility responded only to dietary AvP, with an 18.1% increase between the lowest and highest levels ($p < 0.05$). Phytase elevated serum ALP by 47.5% and increased circulating Ca and P concentrations by ~26%, while AvP affected only serum P ($p < 0.05$). PTH and CT were influenced by phytase, and only PTH was responsive to AvP ($p < 0.05$). Egg yolk phosvitin increased with both higher AvP and phytase, from 2,888 to 9,829 $\mu\text{g/mL}$ and from 4,960 to 9,733 $\mu\text{g/mL}$, respectively. Phytase also improved P retention by 8.7% and increased apparent metabolizable energy by 234 kcal/kg ($p < 0.05$). Phytase enhances P utilization and metabolic efficiency in laying hens, but its benefits depend on dietary available phosphorus levels, as Ca:P balance critically modulates bone integrity, mineral metabolism, and overall physiological responses.

Keywords: phytase; physiology; available phosphorous; laying hens; mineral

M91 Manganese requirements of broilers chickens under phytase supplementation

Luana A. Lopes da Cunha*^{2UG}, Sergio L. Vieira¹², Walter E. Altevogt², Douglas D. Maria², Raquel M. Horn², Yuri J. Olabarriga², Rodolfo Leiskosky² ¹*Phytobiotics, Porto Alegre, RS, Brazil*; ²*UFRGS, Porto Alegre, Rio Grande do Sul, Brazil*

Manganese (Mn) supplementation in broiler diets is common practice, yet requirements under widespread phytase usage remain unclear. This study aimed to determine the Mn requirements of modern broilers fed diets supplemented with high levels of phytase. A total of 640 one-day-old male Cobb \times Cobb 500 chicks were allocated in 80 wire cages in a completely randomized 2×5 factorial design, evaluating diets with or without phytase (4,000 FYT/kg) and five graded levels of supplemental Mn (0 to 120 mg/kg, resulting in analyzed levels from 19.64 to 129.97 mg/kg). Each treatment had eight replicates with eight birds each. The study was conducted until 28 days of age. The following parameters were evaluated: zootechnical performance, total excreta and ileal content collection, leg parameters (valgus, varus, tibia rotation, gait score), tibia evaluations (morphometry, breaking strength, Mn content) and Mn concentration in liver and heart. Data were analyzed using two-way ANOVA via the GLM procedure of SAS. Significance was accepted at $P \leq 0.005$, with mean separation by Tukey's test. Responses to Mn were estimated using a linear and quadratic polynomial regression. No phytase \times Mn interaction was found for any variable ($p > 0.05$). Phytase supplementation did not influence ($p > 0.05$) the apparent ileal digestibility of dry matter (DM), crude protein, or metabolizable energy (AME). However, total tract DM retention and AME were higher ($p < 0.05$) in birds receiving phytase. Phytase also significantly improved body weight gain and feed conversion ratio ($p < 0.05$). In contrast, increasing dietary Mn levels had no effect ($p > 0.05$) on growth performance, leg deviations, or bone strength. However, Mn supplementation led to linear ($p < 0.05$) increases in tibia Mn concentration ($Y = 3.407 + 0.01676x$, $R^2 = 0.128$) and fecal Mn excretion ($Y = 0.822 + 0.0313x$, $R^2 = 0.666$). The results demonstrate that Mn supplementation beyond the basal level (~20 mg/kg) in corn-soybean diets with high-dose phytase does not enhance broiler growth or skeletal health. Phytase is crucial for maximizing performance, allowing for a substantial reduction in Mn inclusion to align production efficiency with reduced environmental impact.

Keywords: Manganese; phytase; requirement; digestibility; performance

Metabolism & Nutrition II: Amino Acids

M92 Effects of branched-chain amino acid and tryptophan ratio variations in diets containing corn, soybean meal, and concentrated corn protein on turkey poult performance, litter moisture, and blood plasma serotonin in a 42d grow out

Emily B. Estanich*^{1GS}, Elizabeth Lynch¹, Lucas E. Knarr¹, Alexis Renner¹, Joseph Moritz¹ ¹*West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States*

Research has shown that excess leucine (Leu) in poultry diets decreases bird performance due to antagonisms among branched-chain amino acids (BCAA). However, providing supplemental crystalline valine (Val) and isoleucine (Ile) has been shown to restore performance in diets with high Leu levels. Excess Leu may compete with tryptophan (Trp), leading to a reduction in serotonin synthesis and a subsequent decrease in feed intake. This study aimed to assess diets with varying BCAA ratios and Trp on hen poult performance, litter moisture, and blood plasma serotonin in a 42d grow out. A nutritionally adequate diet served as the positive control (PC). High corn gluten meal (HCG) inclusions were used to increase Leu:Lys ratios over 1.5 in four additional diets: an uncorrected HCG diet and diets corrected with Val+ Ile, Trp, or Val+ Ile+ Trp (All). A randomized complete block design was utilized with 13 replicate pens of 17 poults. Data were analyzed using JMP Pro 18.0 considering pen location within the room as the blocking criterion. Plasma serotonin concentration was determined via ELISA using a 4-parameter logistic regression. Significance was set at $P < 0.05$. Significant ANOVA results were further analyzed using Fisher's LSD test. During d0-21, the PC and HCG + All treatment yielded the highest FI and LWG ($P < 0.05$). Within d0-28, the HCG + All diet generated the lowest FCR ($P < 0.05$). However, statistical differences were lost between the PC and HCG treatment for LWG and FCR. The HCG + All diet produced similar or improved LWG and FI on d42 relative to the PC. Litter moisture was significantly different between treatments on d42 with the PC providing the highest moisture percentage ($P < 0.05$). Blood plasma results trended toward significance ($P = 0.0980$) with the PC showing the highest numerical level. The addition of Trp to the HCG diet did not improve blood plasma serotonin levels or feed intake. These data suggest that turkey hen poults appear capable of compensating for BCAA imbalances as they mature to 42d. An inclusion of Val, Ile, and Trp to diets with excess Leu, could be used to restore or improve bird performance in a 28d grow out. The increased cost of crystalline amino acids to balance use of concentrated corn protein should be considered.

Keywords: Branched-chain amino acids; Tryptophan; Plasma serotonin; Corn gluten meal

M93 Determination of digestible lysine requirements in modern male turkey poults during the brooder phase

Darby R. Boontarue*^{1GS}, John W. Boney¹ ¹*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States*

Digestible lysine (dLys) requirements (req) for male turkeys have not been updated in over two decades, despite lysine's critical role as the reference amino acid (AA). Two consecutive studies aimed to identify the dLys req of Nicholas Select male poults from 0-28 d based on weekly feed intake (FI), body weights (BW), live weight gain (LWG), mortality-corrected feed conversion ratio (FCRm) and daily dLys consumption. The preliminary trial (Trial 1), using poults from first-lay hens, evaluated six dLys levels (1.62%, 1.79%, 1.96%, 2.06%, 2.15%, and 2.22%) in a randomized block design (48 pens; 32 poults/pen). The subsequent trial (Trial 2) refined the range and evaluated six dLys levels

(1.60%, 1.68%, 1.76%, 1.84%, 1.92%, and 2.00%) plus two controls formulated to maintain AA ratios: Industry CON-1.68% and Predicted CON-1.84% (96 pens; 32 poults/pen). Apparent ileal amino acid digestibility (AIAAD) was determined for treatments 1.60%, 1.76%, 2.00%, and Industry CON. Trial 1 was analyzed using one-way ANOVA in JMP Pro 18 with Fisher's LSD ($P < 0.05$) for mean separation, and LS means were fitted using quadratic polynomial regression to estimate the dLys req at 95% of the vertex. Trial 2 used a mixed model with block nested within room to account for room variation. In Trial 1, poults fed 1.62% dLys had reduced FI, BW, and LWG ($P < 0.05$), while optimal responses occurred at 1.84% dLys. By d 28 in Trial 2, birds fed the Industry CON diet consistently had lower LWG and BW than all other treatments ($P < 0.05$), whereas graded dLys levels had similar FI, LWG, and BW. Although the 1.92% and 2.00% dLys had the highest dLys consumption ($P < 0.01$), BW and FCRm did not differ significantly from the other graded levels. Despite increasing dLys intake with each incremental dLys level, no plateau or quadratic response was observed, preventing req estimation within the tested range. AIAAD improved with higher dLys inclusion, while the Industry CON had the lowest digestibility. Overall, results suggest that modern male turkeys may have lower dLys req during the brooder phase under non-challenged conditions. Lower dLys inclusions should be evaluated while maintaining balanced AA ratios to prevent secondary limitations.

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

M94 Responses of male Cobb 500 broilers to digestible phenylalanine + tyrosine to lysine ratios in diets with or without intact protein ingredients

Kenneth B. Nelson*^{1GS}, Jason Lee², Benjamin Parsons¹, Mike Kidd¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*; ²*C.J. Bio America, Downers Grove, Illinois, United States*

Recent market introduction of feed-grade L-His HCl requires updated dietary aromatic amino acid needs for broilers. Three experiments were conducted to evaluate broiler responsiveness to digestible Phe + Tyr to Lys ratios (Phe+Tyr:Lys). Experiments (Exp) I and II each utilized 56 floor pens (8 treatments; 7 replicate pens) containing 11 birds/pen from 15 to 35 d, and Exp III utilized 40 battery cages (8 treatments; 5 replicate cages) containing 5 birds/cage from 10 to 17 d. In Exp I and II, basal diets (1.10% digestible Lys) were formulated using corn and intact protein ingredients to contain deficient Phe+Tyr:Lys. L-Phe replaced an inert filler to form Phe+Tyr:Lys ranging from 82 to 130 in increments of 8 in Exp I and 88 to 130 in increments of 7 in Exp II. Positive control (PC) diets were formulated to Phe+Tyr:Lys of 114 (Exp I) and 116 (Exp II) met with only intact protein. In Exp III, a purified cornstarch-amino acid basal diet (1.10% digestible Lys) was formulated to a Phe+Tyr:Lys of 46 met with only L-Phe. L-Phe replaced an inert filler to form Phe+Tyr:Lys ranging from 46 to 109 in increments of 9. Pen/cage bird and feed weights were measured at the beginning and conclusion of experimental periods to calculate BW gain, feed intake, and feed efficiency (FE). In Exp I and II, 4 birds per pen were randomly selected at 36 d for processing to determine carcass characteristics. All data were subjected to linear and quadratic regression analyses. Preplanned orthogonal contrasts were performed between the PC and surfeit Phe+Tyr:Lys and resulted in the PC birds having reduced peritoneal fat yield in Exp I and II ($P \leq 0.05$), but inconsistent live performance improvements. Increasing the Phe+Tyr:Lys linearly increased total breast meat yield ($P = 0.03$), but did not influence

any live performance parameters. Increasing the Phe+Tyr:Lys did not influence any live performance or carcass parameters in Exp II. In Exp III, quadratic responses were observed for BW gain, feed intake, and FE ($P \leq 0.01$). An optimal Phe+Tyr:Lys of 81 was estimated for FE using a linear broken-line model. These results indicate that the Phe+Tyr:Lys requirement for live performance in male broilers is substantially lower than the requirement for optimal carcass composition.

Keywords: broiler; nutrition; amino acid; phenylalanine; requirement

M95 Growth of broilers fed high soybean meal inclusion during moderate and warm ambient temperatures

Reuben Adejumo*^{1GS}, Elizabeth A. Lynch¹, Lucas E. Knarr¹, Emily B. Estanich¹, Joseph Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States

The surge in soybean meal (SBM) availability poised by the increase in production of biodiesel is projected to continue to lower the price of SBM and feed cost, prompting farmers to consider greater utilization of this valuable protein source in broiler diets. Consequently, optimizing dietary electrolyte balance (DEB) and amino acid (AA) ratio (non-essential/essential) is critical for sustaining growth performance and metabolic efficiency in broilers particularly under variable thermal environments. Two experiments evaluated the effects of DEB and AA ratio (Gly+Ser: Dig.lys=1.75) on the performance of broiler chickens under two different thermal environments: moderate and warm ambient temperatures from 0 to 38 d. A corn-SBM based diets containing; 1. normal DEB (230+ mEq/kg) and normal AA ratio with glycine, 2. normal DEB and low AA ratio without glycine, 3. high DEB and normal AA ratio with glycine, and 4. low DEB with normal AA ratio with glycine were fed to a total of 2576 male Ross 308 1-day-old chicks randomly allotted to 14 replicate pens (23 chicks per pen) for each of the four treatments in 2 separate studies to determine the influence of ambient temperatures on rearing environment via live bird performance. Diets were formulated to crude protein and digestible amino acid requirements of Ross 308 broilers, glycine supplementation was used to achieve normal AA ratio, high SBM inclusion was used to achieve high DEB. On day 38, live performance metrics were measured. All data were analyzed using JMP 19. Moderate temperature, 38 d live weight gain (LWG) and mortality corrected for FCR were best for normal DEB or low DEB with normal AA ratio (diets 1 & 4). Diet 2 relative to 1 and 4 increased feed intake FI, decreased LWG and increased FCR ($P < 0.05$). Diet 3 relative to diet 1 and 4 increased FI and FCR ($P < 0.05$). For warm ambient condition 38 d data, LWG and mortality corrected for FCR were best for diets 1 and 3 relative to diet 2 ($P < 0.05$). Normal DEB with low AA ratio decreased FI relative to diets 3 and 4 ($P = 0.00$). High DEB and low AA ratio can be detrimental to broilers when reared during moderate temperature, but high DEB was an advantage during the warm ambient conditions. Increasing SBM inclusion may optimize metabolic demands during warmer conditions.

Keywords: Ambient conditions; Soybean meal; Glycine; Serine; Potassium

M96 Effects of arginine and threonine levels on growth performance of broiler chickens with mild enteric stress

Addison Elstner*^{1GS}, Rosana A. Hirai¹, Audrey McElroy¹, Abigail Flores¹, Rahul Reddy¹, Kasey Young¹, Jason Lee², Roshan Adhikari², Chance Williams³ ¹Texas A&M University, Poultry Science, College station, Texas, United States; ²CJ Bio America, Downers Grove, Illinois, United States; ³Wayne-Sanderson Farms, Hoschton, Georgia, United States

The objective of this study was to evaluate the effects of varying dietary Arginine (Arg) and Threonine (Thr) levels supplied through free amino acids inclusion on broiler performance under mild enteric stress. A total of 2,268 day-old Ross YPM \times 708 male broilers were placed in 12 replicate floor pens (21 birds/pen; 0.0796 m²/bird) and fed a corn-soy diet with inclusion of corn dried distillers grains and meat bone meal for 49 days. All birds received a 2X-dose of Coccivac-B52 via spray vaccination before assignment to 1 of 9 treatments (Trts). This trial was a 3 \times 3 factorial design with main effects being digestible Arg (dArg) to digestible Lysine (dLys) (104, 112, or 120) and digestible Thr (dThr) to dLys (66, 69, or 72). Average body weight (BW), BW gain (BWG), feed intake/bird (FI), and feed conversion ratio (FCR) were measured on days 0, 21, 35, 42, and 49. Data was analyzed using PROC GLM in SAS 9.4, with a 3-way ANOVA and Tukey's test for means separation ($P \leq 0.05$). During the starter phase, elevated dArg:dLys ratios of 112 and 120 had lower FCR compared to the 104 dArg:dLys ratio. For d0-35, a Thr \times Arg interaction was observed as increasing dArg ratio from 104 to 112 and 120 decreased FCR when dThr ratio was 66 or 69, however, no improvement in FCR was observed with increasing dArg ratio in diets containing the highest level of Thr (72 dThr:dLys). Similar results for d0-42 FCR showed increasing dArg:dLys ratio decreased FCR except for the highest dThr:dLys. Across all phases, FI was highest for birds fed the 112% dArg diet and lowest for birds fed the 120% dArg diet. This difference in FI impacted BWG, however at the conclusion of the trial, no differences were observed in BW. Little impact was observed on live performance of birds due to dThr:dLys ratio. In conclusion, Thr \times Arg interactions showed that low to mid-range dThr supported performance without the need for higher dThr inclusion when paired with sufficient dArg. Furthermore, elevated dArg (112% or 120%) improved FCR during peak stress, whereas moderate dArg levels were sufficient in later cumulative phases. Additional studies should explore this model under severe enteric stress to fully understand the mechanistic effects of these free AA's on performance and gastrointestinal health.

Keywords: amino acids; arginine; broilers; growth performance; threonine

M97 Influence of dietary protein reduction with feed-grade amino acids on energy utilization and body composition of broilers

Jose R. Hernandez*^{1GS}, Jason Lee², Roshan Adhikari², Samuel J. Rochell¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²CJ Bio America, Downers Grove, Illinois, United States

This study evaluated the effects of dietary CP reduction through feed-grade amino acid incorporation on the energy utilization and body composition of broilers from 14 to 35 d of age. A total of 192 Ross 708 male broilers were randomly distributed in 32 battery cages (6 birds/cage) and fed a common starter from 1 to 14 d of age. On d 14, cages were standardized to 4 birds/cage with uniform body weights and assigned to 4 treatments (8 cages/treatment). Treatments consisted of diets with 4 CP concentrations (22, 21, 20, and 19%). The high (22%) CP diet contained feed-grade Met and Thr, with feed-grade Lys, Thr, Val, Ile, Arg, Trp, Gly, His, and Phe used to maintain minimum specifications for these amino acids as CP decreased. Three 48-h energy balance assays were performed from 19 to 21, 26 to 28, and 33 to 35 d to determine AME. Net energy (NE) was calculated using the comparative slaughter technique to determine body composition (BC) and retained energy 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 2

birds/cage at d 36. Ground carcasses were analyzed for energy, moisture, protein, fat, and ash content. For NE calculation, daily fasting heat production was calculated as 108 kcal/kg*BW^{0.70}. Data were analyzed by one-way ANOVA and linear and quadratic contrasts of dietary CP were performed. Statistical significance was set at $P \leq 0.05$. The AME of the diets increased linearly ($P < 0.05$) as CP concentration decreased during each period, with an average increase of 31 kcal/kg per percentage point reduction of dietary CP. On a DM basis, carcass fat tended to increase ($P < 0.10$) and carcass energy increased ($P < 0.05$) linearly as dietary CP was reduced. Energy retained as fat increased linearly ($P < 0.05$) as dietary CP decreased. Although energy retained as protein tended to decrease ($P < 0.10$), neither carcass protein content (% DM) nor total retained protein (g) were influenced by CP reduction. No differences were observed between treatments in NE ($P > 0.05$). These results indicate that reducing dietary CP at a given level of amino acid density increases the AME content of the diet and energy retained as fat without affecting total protein deposition.

Keywords: crude protein; amino acids; broilers; body composition; energy metabolism

M98 Interactive effects of different levels of crude protein and metabolizable energy on breast muscle myopathies and foot pad lesion score in Ross-708 broilers

Muhammad Ali*^{1GS}, Catherine Fudge¹, Nicolas Mejia-Abauza¹, Caitlin Harris², Roshan Adhikari³, Jason Lee³, Chongxia Chen¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States;* ²*USDA-ARS, Poultry Microbiological and Processing Unit, Athens, Georgia, United States;* ³*CJ Bio America, Downers Grove, Illinois, United States*

Protein and metabolizable energy are the two most expensive dietary components that can affect broiler growth, potentially by impacting muscle development and food pad health. This experiment aims to understand the effects of crude protein (CP) reduction in two different metabolizable energy (ME) level diets on broilers' breast muscle myopathies and foot pad health. Corn-soy-based diets were formulated for respective starter (0-14 d), grower (15-28 d), and finisher (29-42 d) phases, as standard ME and low ME (100 kcal/kg less than standard ME), and three different CP levels with optimal amino acid ratios (high, medium, and low), resulting in 6 treatments (8 replicates x 20 straight run Ross-708 broilers). At 41 d, ten birds/pen were selected for foot pad lesion scoring (FP). At 43 d, 8 birds/pen were processed for evaluation of wooden breast (WB), white striping (WS), and spaghetti muscle (SM). An ordinal logistic model was used to evaluate the main and interactive effects of CP, ME, and sex (CP*ME*sex) on myopathies and FP scores (CP*ME only). Spearman's rank correlation was used to analyze the correlation between the desired parameters. The P -value was set at <0.05 . No three-way interaction effect of CP, ME, and sex was observed on muscle myopathies ($P > 0.05$). However, CP and ME interaction effects were observed ($P < 0.05$) on WB and WS. In standard ME diets, CP reduction resulted in a higher incidence of WB scores 2 and 3, while in low ME diets, the trend was reversed for the same score. For WS, CP reduction resulted in a low incidence of WS score 3, irrespective of ME level. These can be correlated as positive correlation of WB ($r_s=0.45$, $P < 0.0001$) and WS ($r_s=0.46$, $P < 0.0001$) with pectoralis major weight. Additionally,

SM remained unaffected by the interaction or main effects of CP, ME, or sex ($P > 0.05$). For FP, in low-energy diets, CP reduction improved footpad health with more birds scoring 0; however, an opposite trend was observed in standard ME diets, which may correlate with litter moisture ($r_s=0.30$, $P < 0.0001$). In summary, dietary changes in ME and CP affected WB, WS, and FP; however, the effect was not similar in all dietary treatments, and these responses appear to be modulated through changes in pectoralis major weight and litter moisture.

Keywords: Broilers; Crude Protein; Energy; Foot pad lesions; Breast muscle myopathies

M99 Role of branched-chain amino acids on performance, egg, and bone quality in late-lay Hy-Line W-36 hens

Charis A. Waters*^{1GS}, Roshan Adhikari², Jason Lee², Ruben Kriseldi³, Michael Elliot⁴, Li Zhang¹, Kelley Wamsley¹, Pratima Adhikari¹ ¹*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States;* ²*CJ Bio America, Downers Grove, Illinois, United States;* ³*Aviagen, Huntsville, Alabama, United States;* ⁴*A&E Nutrition Services, Lititz, Pennsylvania, United States*

Branched-chain amino acids (BCAAs), including Ile, Leu, and Val, play a crucial role in the health and productivity of laying hens. The objective of this study was to investigate the effects of BCAA on the performance of Hy-Line W-36-laying hens during the late-lay period (67 to 83 wks of age). A total of 600 hens were housed in 200 conventional A-frame cages according to a 2³ full factorial circumscribed central composite design (CCD) with 20 treatments (8 factorial, 6 star, and 6 center points). Each treatment consisted of varying dVal:dLys (67 to 101), dIle:dLys (58 to 92), and dLeu:dLys (120 to 220) ratios; dLys was formulated at 0.70% per the Hy-Line W-36 management guide. Eggs were collected daily to determine hen-day egg production. Feed and eggs were weighed weekly to assess feed intake (FI), FCR, and hen-day egg mass. Egg quality was evaluated every 5 weeks; at 83 wks, one bird/pen was euthanized for tibia collection to determine tibia ash and tibial breaking strength (TBS). Data were analyzed as a CCD using the surface response option of JMP v. 19, and means were considered significant at $P < 0.10$. Feed intake increased (115.2g/bird/day; $P = 0.0981$; $R^2 = 0.68$) at moderate levels of dVal:dLys (86) and dIle:dLys (81) at the low dLeu:dLys (120) ratios. For egg quality, both Haugh unit (HU; 89.69; $P = 0.0159$; $R^2 = 0.80$) and relative albumen weight (65.56%; $P = 0.0142$; $R^2 = 0.80$) were maximized at the highest dVal:dLys ratio (101). However, relative albumen weight was maximized at the highest dIle:dLys (92) and dLeu:dLys (220) ratios, whereas HU was maximized at the lowest dIle:dLys (58) and dLeu:dLys (120) ratios, showing an opposing response pattern for Ile and Leu. Tibial breaking strength increased (217.97 N; $P = 0.0853$; $R^2 = 0.69$) at moderate levels of Val:Lys (93), lowest levels of Ile:Lys (58) and Leu:Lys (120) ratios. These findings demonstrate that adjusting the dVal:dLys and dIle:dLys ratios appropriately can help optimize FI, egg albumen, and TBS in late-lay hens. As dietary dLeu:Lys levels increase, corresponding adjustments in dVal:dLys and dIle:dLys are required to maintain optimal performance and bone quality.

Keywords: branched-chain amino acids; central composite design; egg weight; laying hens; performance

Metabolism & Nutrition III: Feed Additives

M100 Effects of dietary essential oil blend supplementation on the growth performance of broiler chickens

Hansol Kim*^{1GS}, Sammad F. Olayiwola¹, Taiwo Alabi¹, Michael J. Ford¹, Tuoying Ao², Jose Soto², Sunday Adedokun¹ ¹*University*

of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States; ²Alltech Inc., Nicholasville, Kentucky, United States

The objective of this study was to evaluate the growth performance of broiler chickens supplemented with an essential oil blend. A total of 360 one-day-old Cobb 500 byproduct male broiler chicks (initial BW = 42.4 ± 1.2 g) were randomly assigned to 5 dietary treatments in a randomized complete block design, with 12 replicate cages per treatment and 6 birds per cage. The dietary treatments consisted of a corn-soybean meal-based basal diet alone or supplemented with 100, 200, 400, or 600 mg/kg of a novel essential oil blend (EOB, Alltech Inc., Nicholasville, KY). Birds were fed a starter diet (d 0 to 9) and a grower diet (d 9 to 28), provided *ad libitum*, and housed in an environmentally controlled room. Growth performance was measured by phase and across the entire 28-d trial, with adjustments for mortality. Data were analyzed using the MIXED procedure of SAS. The IML procedure was used to generate coefficients for orthogonal polynomial contrasts, which were applied to evaluate linear and quadratic responses to dietary levels of EOB supplementation. A quadratic broken-line model (NLIN procedure of SAS) was used to estimate the optimal dietary supplementation level of EOB based on the gain-to-feed ratio (G:F). Statistical significance was declared at $P < 0.05$. From d 0 to 28, average daily gain and feed intake rose linearly ($P < 0.05$), and G:F was quadratically ($P = 0.002$) related to increasing levels of EOB. A quadratic broken-line analysis of G:F indicated that the optimal dietary supplementation level of EOB was 284 mg/kg ($P < 0.001$). In conclusion, supplementation of broiler diets with EOB resulted in growth performance benefits in a dose-dependent manner, with the quadratic broken-line model suggesting 284 mg/kg as the optimal level to maximize feed efficiency during the 28-d growing period. These findings indicate that EOB supplementation can serve as an effective nutritional strategy to enhance broiler productivity.

Keywords: broiler; essential oil blend; feed efficiency; growth performance

M101 Enhanced nutrient and energy utilization in broiler chickens supplemented with *Yucca schidigera*

Sammad F. Olayiwola*^{1GS}, Hansol Kim¹, Taiwo Alabi¹, Micheal J. Ford¹, Tuoying Ao², Jose Soto², Sunday Adedokun¹ ¹University of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States; ²Alltech Inc., Nicholasville, Kentucky, United States

Optimizing nutrient utilization is vital for the profitability and sustainability of poultry production, especially as the industry moves away from the use of antibiotic growth promoters. Given the phyto-genetic benefits of *Yucca schidigera*, this study evaluated the growth performance and nutrient utilization of broiler chickens fed whole *Yucca schidigera*. A total of 288 one-day-old male Cobb by-product breeder chicks (initial BW = 44.0 ± 0.9 g) were assigned to 4 dietary treatments in a randomized complete block design with 12 replicates per treatment and 6 birds per cage. The birds were fed corn-soybean meal-based diets supplemented with 0, 125, 250, or 500 mg/kg of whole *Yucca schidigera* (WYS, Alltech Inc., Nicholasville, KY) for the pre-starter (d 0-9) and starter (d 9-27) phases. The birds were raised in an environmentally controlled room and had free access to feed and water throughout the 27-d experimental period. Body weight gain, feed intake, and gain-to-feed ratio were determined. An external marker (TiO₂) was used in the diets to determine nutrient utilization variables that included dry matter, nitrogen, energy, apparent metabolizable energy (AME), and nitrogen-corrected apparent metabolizable energy (AMEn). Statistical analysis was performed using the SAS MIXED procedure (v.9.4, SAS Inst. Inc., Cary, NC), and polynomial contrasts were used to assess the linear and quadratic effects. Statistical significance was set at $P < 0.05$.

No differences were observed in the growth performance of broiler chickens during the 27-d growing period among WYS levels. The utilization of nitrogen and AME rose linearly ($P < 0.05$) with increasing levels of WYS in the diets, while the utilization of dry matter, energy, and AMEn had a quadratic relationship to increasing levels of WYS in the diets ($P < 0.05$). The results from this trial indicated that supplementing broiler diets with WYS enhanced nutrient and energy utilization of broiler chickens.

Keywords: AMEn; Broiler chickens; Nutrient utilization; Performance; *Yucca schidigera*

M102 Dietary supplementation of a synergistic blend of plant extracts, vitamins and highly bioavailable minerals mitigates embryonic mortality and newly-hatched chick dehydration

Mário Henrique S. Lopes*^{1GS}, Clarisse Techer², Vitor Pais¹, Sara Benavides¹, Melany Lovon¹, Christian Santos¹, Caio Barbalho¹, Byron Munoz¹, Lucio Araujo¹, Cristiane Araujo¹ ¹University of São Paulo, Department of Animal Science, Pirassununga, São Paulo, Brazil; ²miXscience, Bruz, France

Eggshell quality is a critical factor in breeder operations, ensuring protection against damage and contamination. Attention has been given to nutritional strategies that aid bone and eggshell mineralization, nutrient bioavailability, and promote gut and immune function. Eggshell integrity directly influences chick quality and farm profitability. This study aimed to assess the effects of Tecnoshell, a blend of active ingredients in breeder hen's diets. A total of 144 AP95 breeder hens were randomly distributed in three treatments: NC: Corn/soybean-meal diet; PC: NC+40ppm virginiamycin; TC: NC + 2000ppm Tecnoshell by Enviup (miXscience, France); Hens were distributed in 12 pens per group, four hens/pen, and fed from 23 to 65 wks of age. Feed and lightning were done as the lineage manual. At 43 and 53 wks of age, semen was collected from roosters of the same age, and hens were inseminated with pooled semen. Eggs were collected for a week post-insemination and set for incubation. Hatching rates were noted. Non-hatched eggs were analyzed for embryo mortality, in addition to infertile and pipped eggs. Chicks were weighted and measured at hatch and inspected for hydration status, hock and navel quality. Chick data were analyzed with ANOVA, while hatch and mortality rates were analyzed using Chi2 test with significance at 0.05. No differences in embryo mortality were observed during hatches, but more pipped eggs were observed from hens fed NC than in hens fed PC or TC at 53 wks of age. No differences in chick weight or length were observed from 43-wk-old hens, while chicks from PC and TC hens showed better chick hydration status than NC chicks (0% TC vs 22% NC). At the second hatch, despite chicks from TC hens showing lower weight than PC (48.3g TC vs 50.4g PC), they also showed the longest lengths between groups (19.2cm TC vs 18.8cm PC). No differences in fertility were observed in either hatches, but eggs from TC showed hatchability (74.6% TC vs 78.0% PC, 42% NC) and hatch of fertile (88.8% TC vs 90.7% PC, 45.9% NC) rates equivalent to PC at the first hatch, being both higher than hens fed NC. Our results suggest that Tecnoshell can increase chick length and reduce dehydration in newly-hatched chicks, contributing to better operational performance and profitability.

Keywords: Calcium pidolate; Breeder hen; embryonic mortality; Incubation

M103 Impact of pine bark inclusion on growth, intestinal health, and Evans Blue-measured tissue damage in *Histomonas meleagridis* infected turkey poults

Hamid Reza Rafieian-Naeini*^{1GS}, Venkata Prathap Reddy Keshavareddy¹, Seshidhar Reddy Gudidoddi¹, Hemanth Reddy Katha¹, Ishwari Gyawali¹, Indira Neupane¹, Woo Kyun

Kim¹ *University of Georgia, Poultry Science, Athens, Georgia, United States*

Histomonas meleagridis (HM) invades the ceca and liver of turkeys. No effective treatments have been available since 2017 due to FDA restrictions. Pine bark (PB), rich in phenolic compounds and tannins, may offer a natural alternative for controlling HM infections. This study aimed to evaluate the effects of dietary PB supplementation on growth, intestinal health, and tissue damage in turkey poult challenged with HM for 28 d. A total of 360 day-old male turkey poults were randomly assigned to six treatments with five replicates. T1, non-challenged + 0% PB; T2, non-chall + 0.2% PB; T3, non-chall + 0.4% PB; T4, challenged + 0% PB; T5, chall + 0.2% PB; and T6, chall + 0.4% PB. Challenge were performed through the cloaca with 10⁵ HM trophozoites on day 18. Indicators of treatment efficacy included infection rate, mortality, and lesion scores employed throughout the trial. Lesion severity was also quantified using a newly developed Evans Blue Dye (EBD) method to compare with traditional lesion scoring techniques. The data were analyzed using two-way ANOVA followed by Tukey's post hoc test ($P < 0.05$). The results showed that mortality was significantly affected and modulated by both HM infection and PB supplementation. Infection with HM increased mortality, whereas the addition of 0.2% PB significantly reduced mortality (13%) compared to the no-PB group (28%). Ceca lesion scores reduced it in the 0.2% PB group compared to the no-PB group ($P < 0.05$). Interestingly, EBD concentrations in the ceca and liver of challenged birds were markedly increased ($P < 0.0001$). Supplementation with 0.2% PB significantly decreased EBD concentration in challenged birds compared to those without PB supplementation (32% reduction). Growth parameters were significantly deteriorated by the HM main effect, whereas PB supplementation showed no significant influence, and the interaction effect was also not significant. In conclusion, dietary inclusion of 0.2% pine bark may serve as a natural, phytogetic, and safe feed additive to help combat *Histomonas meleagridis* infection in turkeys. Additionally, the newly developed Evans Blue Dye technique provides an objective and quantifiable method for assessing lesion severity compared to traditional semi-subjective lesion scoring approaches.

Keywords: *Histomonas meleagridis*; Pine Bark; Evans blue; Lesion score; Turkey

M104 Effects of a botanical feed additive and experimental housing conditions on longitudinal changes in broilers exposed to an acute feed withdrawal period prior to coccidiosis inoculation

Laney E. Froebel^{*1GS}, Mike J. Rincker², Ryan N. Dilger¹ *University of Illinois, Department of Animal Sciences, Urbana, Illinois, United States; ²DPI Global, Porterville, California, United States*

The antioxidant defense system plays a vital role in supporting broiler health during exposure to environmental and immunological stressors. In this study, a commercial feed additive [Micro-Shield™ (MS); DPI Global, Porterville, CA] and different experimental housing systems were evaluated for their longitudinal effects on endogenous antioxidants and overall health in broilers. The 30-d study (starter d 0-14, grower d 14-30) was conducted in a 2×4 factorial arrangement with factors including 1) housing context [2 levels; battery cages (BC) with raised-wire flooring or floor pens (FP) with fresh pine shavings] and 2) dietary treatment [4 levels; 1) control diet fed to birds not exposed to stressors (NC), 2) control diet fed to birds exposed to stressors (PC), 3) PC + 1,000 mg of MS/kg diet in the starter phase + 500 mg of MS/kg diet in the grower phase (MSL), and 4) 1,000 mg of MS/kg diet in the starter and grower phases (MSH)]. A total of 720

male Ross 708 chicks at 2 d post-hatch were assigned to 1 of 8 treatment groups: 1) BC-NC, 2) BC-PC, 3) BC-MSL, 4) BC-MSH, 5) FP-NC, 6) FP-PC, 7) FP-MSL, 8) FP-MSH. Treatment groups raised in BC or FP were allotted to 10 or 8 replicates, respectively, each housing 10 birds. A multiple mild stressors challenge was imposed by birds undergoing a 12-h feed withdrawal prior to a coccidiosis vaccine challenge on study d 15, which was designated as 0 d post-inoculation (DPI). All data were analyzed by a 2-way ANOVA using SAS and effects were considered significant when $P < 0.05$. Additionally, area under the curve values were calculated using SAS to serve as a summative, longitudinal measure of serum markers. On 9 DPI, there was a main effect of dietary treatment where the MSH treatment had higher ($P < 0.05$) serum catalase levels compared with its counterparts. There was a main effect of dietary treatment where NC birds had the highest ($P < 0.05$) serum superoxide dismutase levels on 12 DPI. Additionally, there was a main effect of housing context on 12 DPI where BC-housed birds had higher ($P < 0.05$) serum superoxide dismutase levels compared with FP-housed birds. These results suggest that MS supplementation and experimental housing context influence the broiler antioxidant defense system during periods of stress.

Keywords: antioxidant; broiler; health; polyphenol; saponin

M105 Evaluation of *Bacillus*-based direct feed microbials on the performance and egg quality of laying hens from hatch to 40 weeks of age

Bethany Alexander^{*1GS}, Samantha Huffer¹, Brian Dirks², Hector Leyva-Jimenez², Michael Persia¹ *Virginia Tech, Radford, Virginia, United States; ²United Animal Health, Sheridan, Indiana, United States*

An experiment was conducted to determine the effects of direct-fed microbials (DFM) on Hy-Line W-36 laying hens from hatch to 40 wk of age. Birds were fed one of four diets: a control diet with no DFM (NC), NC + 3.68×10⁵ cfu/g Novela® ECL (ECL), NC + 7.34×10⁴ cfu/g Novela® (Nov), and NC + 7.4×10⁴ cfu/g Amnil® (Amn). In total, 864 chicks were allocated to 12 replicate cages of 18 chicks. During the pullet phase, body weights (BW) and mortality corrected feed conversion ratio (FCR_m) were determined every 3 wk. Data were analyzed using one-way ANOVA in SAS, if $P \leq 0.05$, means were separated using Fisher's LSD test. All DFMs increased BW (164.7, 173.6, 170.8, and 172.7 g/pullet) and improved FCR_m (2.320, 2.200, 2.215, and 2.211 g feed/g egg mass) in comparison to NC ($P \leq 0.05$) for NC, ECL, Nov, and Amn, respectively. Mortality was increased over the first 3 wk, with ECL resulting in a mortality rate nearly half that of the NC and other treatments (NC = 4.63% ECL = 2.31%, Nov = 4.63%, Amn = 4.17%). From 0 to 18 wk, no differences were observed in pullet BW or FCR_m, but ECL reduced mortality in comparison to NC and other treatments numerically. At 18 wk of age, a subpopulation of randomly selected birds was maintained on the same experimental treatments and moved from pullet to laying hen cages, resulting in 192 hens housed 3 per cage across 16 replicate cages for each treatment. Egg production and FCR were determined every 2 wk, body weights every 4 wk, and egg quality was measured every 4 wk starting at 24 wk. At the end of the experiment, all remaining hens were euthanized for abdominal fat pad (AFP) weight. Performance and egg quality data were analyzed using repeated measures with ANOVA in SAS ($P \leq 0.05$). Hens fed ECL improved FCR (1.690, 1.663, 1.679, 1.689 for NC, ECL, Nov, and Amn, respectively; $P = 0.04$) in comparison to NC. Hens fed ECL increased Haugh units ($P = 0.02$), but reduced egg specific gravity ($P = 0.01$) in comparison to NC-fed hens. Relative AFP was similar between NC and ECL, but was reduced with Nov and Amn (2.7, 2.4, 2.1, 2.2%, respectively;

P = 0.04). Overall, DFM supplementation was able to improve chick performance over 0 to 3 wk, and ECL reduced pullet mortality and improved laying hen FCR when fed from hatch to 40 wk of age.

Keywords: DFM; pullet; laying hen; performance; egg quality

M106 Evaluation of a *Bacillus*-based direct-fed microbial (DFM) on performance and housefly proliferation in broiler chickens

Isabella R. Villarreal^{*1GS}, Amber MacInnis², Hector Leyva-Jimenez³, Brian Dirks³, J.K. Tomberlin², Gregory Archer¹ ¹Texas A&M University, Poultry Science Department, College Station, Texas, United States; ²Texas A&M University, Department of Entomology, College Station, Texas, United States; ³United Animal Health, Sheridan, Indiana, United States

Poultry farms employ strategies to control house flies partly to reduce the risk of disease spread. This study evaluated a *Bacillus*-based DFM Amni[®] (United Animal Health, IN) on broiler performance, as well as its impact on the survival of house fly larvae. A complete randomized design was used to test two treatments, each consisting of 48 replicate cages of 6 male chicks each. The treatments were: 1) Basal Diet (CON) and 2) Basal + DFM (7.4x10⁴ cfu/g feed; AMN). Body weight (BW), feed consumption (FC), and feed conversion (FCR) were measured weekly (0-21d). Poultry manure was collected on d14, d18, and d22. To assess the survival of immature house flies, the study involved feeding 100 one-day-old house-fly larvae either CON manure or manure from birds fed the DFM. House fly larvae were provided with 18 g of manure every other day until mature third instar larvae were observed. Daily observations were made for pupation. After recording the pupae, the emerging flies were removed and counted daily. Observations continued until 3 consecutive days of no adult emergence. Additionally, an oviposition choice assay of adult flies was evaluated using 3 cage replicates per time point. Proximate analysis of the manure was conducted on d18 and d22. ANOVA was used to determine treatment differences (P<0.05). Cumulatively, the BW of the AMN group was numerically higher (P=0.352) by 1.6%. Weight-adjusted FCR was improved (P=0.046) by 2.5% in the AMN group. Manure analysis at d 18 showed lower (P<0.05) pH, humidity, Zn, ADF, and NDF in the AMN group. On day 22, pH and Zn were lower (P<0.05) in the DFM group, while P trended (P=0.052) to be lower in AMN manure. A difference (P<0.05) was determined for survivorship to the pupal stage. Survival to the pupal stage decreased an average of 32.2% in the AMN group compared to the CON; however, survival from the pupal to the adult stage was not affected. No differences were observed between treatments in the oviposition assay (P>0.05). However, a numerical reduction of 40.6% was observed for the AMN group. In conclusion, the DFM improved the cumulative performance of broilers, and the manure from the DFM-fed birds decreased the

house fly population by decreasing the survival rate of larvae to the pupal stage.

Keywords: Direct-fed microbial; House Fly; Broiler; Performance; Manure

M107 Evaluating the effects of Dacitic tuff breccia (DTB) and phytase on egg production parameters in late production brown laying hens

Tracy Omasanuwa^{*1GS}, Ishab Poudel¹, Rebecca Wysocky¹, Ramon Malheiros¹, Jon Ferrel², Kenneth E. Anderson¹ ¹North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United States; ²Azomite Mineral Products, Inc, Frankfort, Indiana, United States

Age-related decline in egg production and quality remains a major challenge in the poultry industry as hens are maintained in longer production cycles, often leading to economic losses due to increased shell breakage and poorer egg grades. The use of natural feed additives with mineral-enhancing and antioxidant properties has been shown as potential strategies to mitigate this issue. This study evaluated the effects of Dacitic Tuff Breccia (DTB), a natural mineral-rich complex silica ore, containing over 70 trace minerals and phytase supplementation on egg production and eggshell quality in late-production laying hens. A total of 630 Hy-Line Brown hens (50 weeks of age) were assigned to a completely randomized 2 × 3 factorial design, with two levels of phytase (0 and 10,000FYT/kg) and three levels of DTB (0.00%, 0.25%, and 0.50%), with 30 replicates of 21 hens per treatment. The duration of the study was from 50 to 100 weeks of age, with each 4-week interval considered a period. The parameters measured include hen-day egg production (HDEP), hen-housed egg production (HHEP), feed intake (FI), feed conversion ratio (FCR), mortality, and physical egg quality: weight (EW), Haugh unit (HU), albumen height (AH), shell breaking strength (EBS) and elasticity (ESE), vitelline membrane strength (VMS) and elasticity (VME). Data were analyzed using the PROC GLIMMIX in SAS 9.4, with significance determined at P ≤ 0.05 and means separated using least-square means test. There was no significant effect (P > 0.05) on egg production, egg weight, feed intake or feed conversion ratio on either DTB or Phytase supplementation alone. However, a significant interaction between DTB and Phytase was observed for HDEP (P = 0.0349) and mortality (P = 0.0073). Without phytase, hens fed 0.25% and 0.50% DTB showed higher production than the control group. In general, the results indicate that moderate DTB supplementation (0.25%) can enhance egg production in diets without phytase. However, those benefits were reduced when phytase was included in diet with DTB. Notably, DTB and phytase had no adverse effect on the internal or shell egg quality, indicating that DTB can be safely used as a natural mineral additive to enhance production in late-laying hens.

Keywords: Egg Production; Brown Laying Hens; Feed Additive; Dacitic tuff breccia; Phytase

Metabolism & Nutrition IV: Feed Additives

M108 Modeling and optimization of productive capacity and pellet quality based on known diet formulation and pelleting parameters that can affect feed manufacturing

Lucas E. Knarr^{*1GS}, Elizabeth Lynch¹, Emily B. Estanich¹, Reuben Adejumo¹, Holly L. Mahala¹, Gail L. McAndrew¹, Jon Ferrel², Joseph Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²Azomite Mineral Products, Nephi, Utah, United States

Considerable data are available on the effects of ingredients, grinding techniques, conditioning parameters, and pellet die

specifications on pelleting efficacy and efficiency. Some factors, such as rock phosphate inclusion rate, have seldom been evaluated for their effect on the pelleting process. Little effort has been made to create large-scale models to help predict productive capacity (PC) and pellet quality (PQ). These models could assist nutritionists and feed manufacturers in matching appropriate pelleting parameters to diet formulations to achieve target PC while also maximizing PQ. Therefore, the objective of this experiment was to model the influence of Azomite[®] (AZM; 0.00 to 0.50%), mixer-added fat (MAF; 0.25 to 1.75%), dicalcium phosphate (DCP; 0.00 to 1.90%), and conditioning temperature

(CT; 68.3 to 85.0°C) on pelleting PC and PQ. Experimentation was conducted in a blocked, four-factor central composite design with 3 replications. Data analyses were completed using response surface methodologies, up to three-way interaction terms were evaluated, and ambient temperature (AT) was used as a covariate. Relative factor influence using dependent resampled inputs was examined for the overall, PC, and PQ models, and the highest-order interactions were illustrated via surface plots. Diet formulation, CT, and AT accounted for 45.95%, 45.29%, and 8.76% of the total influence across all responses, respectively. Increasing AZM or MAF negated the negative effects of DCP and increased overall PC (AZM*DCP*CT: $P=0.015$; MAF*DCP*CT: $P=0.008$). Increasing AZM mitigated the negative impacts of increasing MAF on pellet durability at low to mid-level CTs but further decreased durability at high CTs (AZM*MAF*CT: $P=0.031$). Friction and low PC associated with high DCP and/or low CT increased the pellets to fines ratio (P:F), and low DCP and high CT decreased P:F (DCP*CT: $P=0.039$). In summary, including AZM may be a more cost-effective method to increase manufacturing efficiency and efficacy when little room is available in the diet, given the PQ decrease associated with MAF and the relatively small increase in PC at a similar inclusion rate. Increasing CT may be an effective way to increase PC and PQ; however, high hot-pellet moisture may cause an overall decrease in PQ.

Keywords: Pellet Production Rate; Pellet Quality; Feed Manufacturing Optimization

M109 Further evaluation of effects of microencapsulated copper sulfate on the performance of 0 to 35 d old broiler chickens raised on used litter

Jennifer A. Blair^{*1GS}, Joel Samuels², Winston Samuels², Michael Persia¹ ¹*Virginia Tech, Blacksburg, Virginia, United States*; ²*Maxx Performance, Roanoke, Virginia, United States*

An experiment was conducted to determine the effects of microencapsulation (M) of copper sulfate (CS) on broiler performance, intestinal health and copper excretion. Male Ross 708 chicks were housed in floor pens and randomly assigned to treatments with 18 replicate pens of 23 chicks. Treatments included birds raised on used litter seeded with coccidial oocysts and fed experimental diets including negative control (NC) diet (without supplemental CS); NC + 200 g/MT of 100% CS (200CS; 200g CS/MT); NC + 260 g/MT of 70% MCS (182MCS; 182g CS/MT); and NC + 200 g/MT of 70% MCS (140MCS; 140g CS/MT). The used litter was generated by mixing CocciVac® B52 into feed and allowing those birds to shed oocysts into the litter for 21d before the start of the experiment. Body weight gain (BWG) and mortality corrected feed conversion ratio (FCRm) were calculated for 0 to 10, 0 to 28, and 0 to 35d. On D14 and D28, 1 bird per pen was assayed for serum FITC-dextran. On D10, D28, and D35, 1 bird per pen was euthanized for gizzard scoring and litter samples were collected from each pen for dry matter determination. Data were analyzed using ANOVA in JMP Pro 18.0, and means were separated using Student's T-test. All CS treatments increased BWG compared to the NC for 0 to 28d ($P \leq 0.05$) and 0 to 35d ($P = 0.08$). Body weight gain was increased in comparison to the NC by 67, 92 and 50g over 0 to 28d and 42, 67, and 12g over 0 to 35d for 200CS, 182MCS, and 140 MCS, respectively. All CS treatments decreased FCRm compared to the NC over 0 to 10d ($P = 0.09$), 0 to 28d ($P < 0.05$), and 0 to 35d ($P = 0.08$). Mortality corrected feed conversion ratio was reduced in comparison to the NC by 0.05, 0.03, and 0.01 over 0 to 10d, 0.03, 0.04, and 0.03 over 0 to 28d, and 0.02, 0.03, and 0.02 over 0 to 35 d for 200CS, 182MCS, and 140 MCS, respectively. Although CS was able to increase BWG and decrease FCRm, gastro-intestinal

health was unaffected as gizzard scores, FITC-dextran, and litter dry matter were not altered by CS supplementation ($P > 0.05$). All CS sources were able to increase broiler BWG and improve FCRm over the 35-day period and did so without altering gastro-intestinal health.

Keywords: broiler; microencapsulation; coccidiosis; copper sulfate; performance

M110 Evaluation of the effects of ZinMet® (zinc methionine complex) as a partial replacement of inorganic zinc sources on broiler performance in Ross 708 broilers

Myla Partin^{*1GS}, Emily Lawrence¹, Don McIntyre², Dima White², T.J. Biggs², Gabriella Beck¹, Elisha Young¹, Antonio Fuentes¹, Victoria Ayers¹ ¹*Tennessee Technological University, Agriculture, Cookeville, Tennessee, United States*; ²*Global Animal Products Inc., Amarillo, Texas, United States*

Organic trace minerals, such as zinc methionine complex (ZinMet), offer higher bioavailability, allowing poultry to absorb and utilize nutrients efficiently. This study evaluated the effects of ZinMet as a partial substitute for inorganic zinc sources on broiler growth performance and carcass traits. A total of 1,104 male Ross 708 broilers were allocated to one of 48 floor pens and assigned to one of three dietary treatments for 42 days: (1) a control diet containing 80 ppm total zinc (40 ppm zinc sulfate and 40 ppm zinc hydroxychloride), (2) a diet replacing zinc sulfate with 40 ppm ZinMet and 40 ppm zinc hydroxychloride, and (3) a diet replacing zinc hydroxychloride with 40 ppm ZinMet and 40 ppm zinc sulfate. Each treatment had 16 replications. All data were analyzed in a randomized complete block design with the pen location determining the block. The experimental unit was 1 pen containing 23 Ross 708 male broiler chicks. The PROC GLM procedure of SAS was used to analyze data by 1-way ANOVA. Means were then further separated using Fisher's least significant difference post hoc comparison when the ANOVA was significant ($P \leq 0.05$). Birds fed ZinMet + zinc sulfate exhibited increased feed intake (FI) and decreased feed conversion ratios (FCR) from days 0–14, compared to controls ($P < 0.05$). Birds fed ZinMet + zinc hydroxychloride demonstrated increased FI and live weight gain (LWG) over 42 days, along with increased uniformity at days 14, 35, and 42 ($P < 0.05$). Additionally, ZinMet with zinc hydroxychloride resulted in greater yields in whole carcass (WOG), pectorals minor weight, and total breast yield compared to other treatments ($P < 0.05$). Both ZinMet-containing diets increased FI, LWG, and decreased FCR. These findings suggest that ZinMet, particularly in combination with zinc hydroxychloride, enhances broiler growth performance and carcass characteristics, offering a viable alternative to conventional inorganic zinc sources.

Keywords: Zinc; Zinc-Methionine; Ross 708; Broilers; ZinMet

M111 Multi-omics assessment of cecal contents from broilers fed different feed additives across development

Ana Fonseca^{*1GS}, Sophia Kenney¹, Samantha Seibel¹, John W. Boney¹, Erika Ganda¹ ¹*Pennsylvania State University, Animal Science, State College, Pennsylvania, United States*

As poultry production shifts away from antimicrobial growth promoters (AGPs), integrating microbial, resistome, and metabolomic responses is essential to evaluate antimicrobial-free feeding strategies. This study characterized the effects of a probiotic, an essential oils blend, a glycan mixture, and a prebiotic on the cecal microbiome, resistome, and metabolite profiles of broilers. 3,072 Cobb 500 one-day-old chicks were randomly allocated to 96 pens, with eight replicates of 32 broilers per pen, and raised to day 43. Treatments consisted of six groups: basal diet,

a basal diet with Bacitracin Methylene Disalicylate (BMD), a basal diet with an essential oils blend, a basal diet with a probiotic (*Bacillus subtilis*), a basal diet with a prebiotic (Yucca and Quillaja saponins), and a basal diet with a complex glycan mixture. Twenty-four birds (four per treatment) were sampled at d1, d10, d25 and d43 for 16S rRNA microbiome, shotgun AMR and NMR-based metabolomics (d1, d10 and d43) on cecal content. Statistical comparisons for alpha (Kruskal–Wallis), beta (PERMANOVA) diversities and Random Forest test were performed. The dominant genera included *Bacteroides*, *Faecalibacterium*, and *Mediterraneibacter*. Microbial alpha diversity did not differ by treatment (Shannon: $P = 0.30$), or age (Shannon: $P = 0.84$), and beta diversity was not affected by treatment or age ($P > 0.05$). Tetracycline, and aminoglycoside were the most abundant antibiotic resistance gene class found, with profiles remaining largely stable across feed additives relative to the control diet. ARG alpha diversity was unaffected by treatment but varied with age ($P = 0.001$), while ARG beta diversity differed by treatment ($P = 0.034$) and by age ($P = 0.001$). NMR-based quantification identified 40 metabolites with different profiles, showing significant effects of treatment ($P = 0.001$), and feeding phase ($P = 0.001$). Treatments (basal diet, antibiotic, and essential oils), and feeding phase (starter, grower and finisher) induced alterations in succinate, fumarate, glycerol, nicotinate and glutamate. Overall, age was a major factor of the resistome and metabolites but not the cecal microbiome. These findings support the feasibility of using natural feed additives to promote poultry health without relying on AGPs.

Keywords: Feed Additives; Microbiome; Resistome; Metabolites

M112 Impact of phytase and stibiotic inclusion on BCO/lameness and underlying mechanisms

Nusrat Jahan*^{1GS}, Elizabeth S. Greene¹, Lulu Liu¹, Carrie Walk², Sami Dridi¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States;* ²*AbVista, Marlborough, United Kingdom*

Bacterial chondronecrosis with osteomyelitis (BCO) is a major cause of lameness and welfare loss in modern broilers, and nutrition and bone interactions are increasingly viewed as a key point for reducing this problem. In this study, we evaluated whether a high dose of phytase and a stibiotic (Signis) could lessen BCO related bone damage while maintaining growth and processing performance and explore associated bone gene expressions. A total of 1,920 broilers were reared to 56 days in a four factor design with two flooring systems (litter or ramp), two basal diets [positive control (PC) vs. negative control (NC; reduced calcium and phosphorus with 500FTU phytase)], phytase (0 vs. 3,000 FTU/kg), and stibiotic (0 vs. 100 grams/tonne), generating eight dietary treatments. Performance parameters (body weight, feed intake, feed conversion ratio), lameness, mortality, and femoral head lesion scores were recorded throughout the trial. On day 50, femoral heads from a subset of birds were collected for RNA extraction, qPCR, and Western blot analysis, and on day 56 birds were processed to measure blood gases and electrolytes, carcass yield, and breast myopathy scores. Four-way and three-way ANOVA for performance parameters, carcass, blood, and lesion traits were analyzed in JMP Pro version 18, and one-way and two-way ANOVA for gene expression data were run in GraphPad Prism version 8, with significance declared at $P < 0.05$. Across treatments, performance, lameness, and mortality were not significantly affected, indicating that neither phytase nor the stibiotic compromised performance. In contrast, femoral BCO lesions were improved with phytase, as birds receiving the phytase showed milder femoral head damage than unsupplemented-birds. Blood gas and electrolyte profiles indicated that normal birds differed from lame birds in pH, electrolytes, and ionized calcium.

Femoral bone gene expression showed that osteoclast stimulating factor 1 was higher in normal than in lame femoral heads. In conclusion, our results indicate that high dose phytase improves femoral bone health and reduces BCO severity in fast growing broilers, and ongoing work on additional bone markers will help clarify the underlying mechanisms and guide future nutrition and leg health interventions.

Keywords: broiler chickens; lameness; bacterial chondronecrosis with osteomyelitis; phytase; stibiotic

M113 Effect of phytase supplementation to corn-soybean meal-based diets containing increasing levels of non-phytate phosphorus in broiler chickens

Ashton M. Croudep*^{1UG}, Sammad F. Olayiwola¹, Taiwo Alabi¹, Jacqueline Jacob¹, Michael J. Ford¹, Sunday Adedokun¹ ¹*University of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States*

The objective of this study was to evaluate the effects of supplementing phytase to corn-soybean meal (C-SBM)-based diets containing increasing levels of non-phytate phosphorus (nPP) from dicalcium phosphate on growth performance, nutrient digestibility, and digesta pH in 23-day-old broiler chickens. A total of 196-day-old broiler chicks were randomly assigned to 4 dietary treatments in a randomized complete block design, with 7 replicate cages per treatment and 7 birds per cage. Birds received a nutritionally adequate C-SBM-based starter diet from d 0 to 9. Beginning on d 9 through to d 23, birds were fed experimental diets containing 1.73, 2.51, 3.30, and 4.35 g/kg nPP, with each diet supplemented with 1,500 FTU/kg phytase. Growth performance was measured on d 0, 9, 14, and 23. On d 23, ileal digesta was collected from all birds in each cage from the distal two-third of the ileum, while the digesta pH from different sections of the gastrointestinal tract (GIT) was measured from one bird per cage. Statistical analysis was performed using the MIXED procedure of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). Polynomial contrasts were used to determine the linear and quadratic effects of dietary treatments, with statistical significance set at $P < 0.05$. Increasing dietary nPP resulted in significant improvements in growth performance. Between d 9-14, ADG, ADFI, and G:F increased linearly ($P < 0.05$), and from d 14-23, both ADG and ADFI showed linear ($P < 0.05$) response. Across the entire study (d 9-23), ADG and ADFI increased linearly ($P < 0.05$) while a tendency for quadratic increase ($P = 0.083$) was observed for G:F. Increasing dietary nPP reduced ($P < 0.05$) mineral digestibility with a linear decrease ($P = 0.009$) in P digestibility. Energy digestibility and digesta pH in the sections of the GIT examined were unaffected by the dietary treatments. In conclusion, increasing dietary nPP in the presence of phytase enhanced growth performance but reduced Ca and P digestibility, indicating that high levels of nPP in the diet improved growth performance but also compromised mineral digestibility in broiler chickens.

Keywords: broiler chickens; growth performance; nutrient digestibility; phosphorus; phytase

M114 Effects of probiotic blends on growth performance in broilers challenged with *C. perfringens* raised in used litter

Matthias Intriago*^{1GS}, Charles L. Hofacre²¹, Matthew K. Jones³, Chongxiao Chen¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States;* ²*Southern Poultry Research Center, Watkinsville, Georgia, United States;* ³*Southern Poultry Research Group, Watkinsville, Georgia, United States*

The current study evaluates a probiotic blend (SFF Probiotic EQ Plus) composed of *Bacillus subtilis* and *Bacillus licheniformis* on growth performance and gut health in broilers raised on used litter

and exposed to a *Clostridium perfringens* challenge. A total of 1,344 one-day-old male Ross 708 birds were placed in 48 pens, with 28 birds/pen, and raised until 43 days. They are assigned to 4 dietary treatments (12 replicates/treatment): a non-medicated challenge control (T1), BMD at 55 g/ton (T2), SFF Probiotic EQ Plus at 0.01% (T3), and 0.1% (T4). A mild necrotic enteritis (NE) model was created using a combination of coccidia vaccination and *Clostridium perfringens* challenge via feed at d15, 22, and 29 (1×10^{10} CFU/pen). Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded on d0, 15, 29, and 43. Necrotic enteritis lesion scoring was conducted on d16, 23, and 30. Data were analyzed with general linear procedures using ANOVA with a comparison of means using least significant difference (LSD) at a $P < 0.05$. From d 0-15, high-probiotic treatment had higher BWG than the challenge control and BMD treatments, with low-level probiotic treatment intermediate. Meanwhile, low-probiotic treatment has a lower FCR than BMD. From d0-29, similar to previous phase, high-probiotic treatment maintained higher BWG, and lower FCR than the challenge control. For the overall period (d0-43). BMD yielded the highest BWG and the lowest FCR, with two probiotic treatments intermediate. Meanwhile, low-probiotic treatment had the lowest mortality, followed by the high-probiotic treatment. No difference in lesion scoring was found on days 16 and 23. Still, on d30, the high-probiotic group had significantly lower lesion scores than the control, with the low-probiotic and BMD groups intermediate. Cumulative lesion scores showed the same trend. In summary, probiotics showed improvement in growth performance and gut lesions during *Clostridium perfringens* challenges, with higher levels demonstrating greater efficacy. The lower level showed better improvements in overall mortality. Future studies may optimize dosing by applying higher inclusion in early diets and tapering to the lower level during late grow-out for improved cost efficiency.

Keywords: Broiler Performance; Direct-Fed Microbials; Gut Health; Used Litter; Necrotic Enteritis

M115 Effect of genetically engineered *Bacillus subtilis* expressing diadenylate cyclase (dac A) in *Salmonella* challenged broiler birds

Praveen Reddy Kasu^{*1GS}, Waqas Ishaq², Chengyuan Fang^{3,4}, Revathi Shanmugasundaram⁵, Jiahe Li³, Ramesh Selvaraj¹
¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States; ³University of Michigan, Biomedical Engineering, Ann Arbor, Michigan, United States; ⁴Harbin Medical University, Esophageal and Mediastinum, Harbin, Heilongjiang, China; ⁵U.S. National Poultry Research Center, Agricultural Research Service, Toxicology and Mycotoxin Research Unit, Athens, Georgia, United States

Genetically engineered spore forming probiotic, *Bacillus subtilis* (*Bs*) carrying an enzyme diadenylate cyclase (*dac A*) act as immune enhancer against pathogens like *Salmonella*. *DacA* catalyzes ATP to form c-di-AMP, that acts as a pathogen associated molecular pattern which stimulates the innate immune system through stimulator of interferon genes. This study evaluates the impact of genetically engineered *B. subtilis* supplementation on production performance, intestinal permeability, and *Salmonella* loads in ceca, liver, and spleen. A total of 192 one-day-old Cobb 500 chicks were randomly allocated into four treatment groups: Control, *Salmonella* challenge *Bs*^{GFP} + *Salmonella* challenge and *Bs*^{dacA} + *Salmonella* challenge with eight replicates per group. *Bs*^{GFP} acts as a probiotic control. On days 0 to 4 post-hatch, birds were orally gavaged with *Bs*^{dacA} and *Bs*^{GFP} spores at 1×10^9 spores/bird. On day 14,

birds were challenged with *Salmonella* Enteritidis at 10^8 CFU/bird. Data was analyzed using one-way ANOVA. On day 21 and 28, no significant differences in body weight gain and feed conversion ratio (FCR) were observed between treatment groups ($p > 0.05$). In liver and spleen, *Bs*^{GFP} and *Bs*^{dacA} groups showed approximately 65% and 55% reduction in *Salmonella* loads respectively compared to the *Salmonella* challenged group ($p < 0.05$). Similarly, cecal *Salmonella* load was 10% less in *Bs*^{GFP} treatment group compared to *Salmonella* challenged group ($p < 0.05$). There were no significant differences in gut permeability across the treatment groups ($p > 0.05$) but in *Bs*^{GFP} and *Bs*^{dacA} treatment groups there was a numerical decrease in gut permeability compared to *Salmonella* challenged groups. This observed decrease in gut permeability in *Bs*^{GFP} and *Bs*^{dacA} groups suggests that *B. subtilis* inhibits the translocation of pathogens from intestinal lumen to systemic circulation. In conclusion, *Bs*^{dacA} effectively reduced *Salmonella* load in liver and spleen and had no negative impact on production performance suggesting an effective probiotic intervention.

Keywords: *Bacillus subtilis*; *Salmonella*; Broilers; Immune enhancers; Genetic engineering

M116 Engineered *Bacillus subtilis* expressing anti-FliC nanobodies reduces the *Salmonella* load in challenged broilers

Waqas Ishaq^{*1GS}, Praveen Reddy Kasu², Chengyuan Fang^{3,4}, Jiahe Li³, Revathi Shanmugasundaram⁵, Ramesh Selvaraj¹
¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States; ³University of Michigan, Biomedical Engineering, Ann Arbor, Michigan, United States; ⁴Harbin Medical University Cancer Hospital, Department of Esophageal and Mediastinum, Harbin, Heilongjiang, China; ⁵United States Department of Agriculture, Toxicology and Mycotoxin Research Unit, Athens, Georgia, United States

Probiotic bacteria *Bacillus subtilis* can be engineered to produce nanobodies targeting the Flagellin-C (FliC) virulence factor of *Salmonella* Enteritidis (SE) that aids in bacterial motility and host intestinal cell invasion. This study evaluated the effectiveness of anti-FliC nanobody-producing *Bacillus subtilis* on production performance, post-infection caeca, liver and spleen *Salmonella* load, and gut permeability of SE-infected broilers. A total of 192-day-old Cobb-500 were randomly allocated to four treatments: Uninfected + Basal, SE + Basal, SE + *Bacillus subtilis*^{GFP} (*Bs*^{GFP}) and SE + *Bacillus subtilis*^{FliC} (*Bs*^{FliC}). *Bs*^{GFP} served as an empty vector, whereas *Bs*^{FliC} produced anti-FliC nanobodies. Birds in the control group received oral gavage of PBS, whereas (*Bs*^{GFP}) and (*Bs*^{FliC}) were gavaged with *Bacillus* spores at 1×10^9 spores/ml from D0 to D5 post hatch. SE infection was induced by inoculating 1×10^8 CFU/bird of *Salmonella* Enteritidis on D14 through oral gavage. Data were analyzed using one-way ANOVA. The means were separated using Tukey's HSD test. At 28 days of age, no statistically significant differences ($p > 0.05$) in feed intake, body weight gain, and feed conversion ratio were found. On 6 dpi, there were significant differences ($p < 0.05$) in caeca, liver, and spleen *Salmonella* load among different treatment groups. Birds in the *Bs*^{FliC} group had a 1.9 MPN log/g lower cecal *Salmonella* load in comparison with *Salmonella*-challenged birds, whereas a reduction of 1.5 MPN log/g was detected in comparison with *Bs*^{GFP}. Similarly, a lower *Salmonella* load of 1.7 MPN log/g was detected in the liver and spleen of *Bs*^{FliC}-supplemented groups in comparison with *Salmonella*-challenged birds. No significant differences ($p > 0.05$) in gut permeability were found among the treatments post-infection. In conclusion, *Bs*^{FliC} demonstrated its nanobody-mediated intervention strategy to reduce the *Salmonella* load in ceca and its systemic spread without affecting the key

production performance indices and can be used with other feed additives to reduce the *Salmonella* loads in poultry.

Keywords: Nanobodies; *Salmonella*; Probiotics; Flagellin-C; *Bacillus subtilis*

M117 Effects of a *Saccharomyces cerevisiae*-based postbiotic on *Salmonella* Typhimurium growth in vitro

Paniz Zarghami Dastjerdi*^{1GS}, Gabriella C. Ribeiro¹, Aden Smith¹, Rami A. Dalloul¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

Postbiotics are defined as inanimate bacterial and/or microbial fermentation components that are beneficial to the host. They can reduce bacterial colonization, including *Salmonella* Typhimurium. This study evaluated the inhibitory effects of a *Saccharomyces cerevisiae*-based postbiotic against *Salmonella enterica* serotype Typhimurium. Frozen bacterial stocks stored (10 µL) were added to 10 mL of buffered peptone water at an initial concentration of 3.9-4.2 log₁₀ CFU/mL at hour zero. The treatments were: CON (control with no postbiotic), P-T0 (1.6 µL/mL postbiotic added at hour zero), and P-T4 (1.6 µL/mL postbiotic added at the beginning of exponential growth [4 h]). Cultures were incubated at 37°C for 12 h in nutrient broth with hourly sampling. At each time point, aliquots were serially diluted and plated on XLD agar to determine viable counts (log₁₀ CFU/mL). All growth-curve data, including log₁₀ CFU/mL, generation time, specific growth rate, and area under the curve (AUC), were normalized by subtracting each replicate's hour-0 bacterial concentration before analysis. Therefore, all reported values represent true bacterial growth over 12 h and are not influenced by initial inoculum differences and were analysed using one-way ANOVA with Tukey's post-hoc test ($P \leq 0.05$). P-T0 produced the strongest inhibition. The mean 12-h increase in normalized colony count (Dlog₁₀ CFU/ml) was P-T0 (3.18 ± 0.72) < P-T4 (3.90 ± 0.07) < CON (5.14 ± 0.56), with P-T0 being significantly lower than the control ($P = 0.0115$). Normalized AUC reduced in P-T0 (23.7 ± 8.40), which is significantly lower than the control group (38.2 ± 1.60, $P = 0.0362$). Growth rate was also reduced, from 0.545 ± 0.158 h⁻¹ (CON) to 0.490 ± 0.105 h⁻¹ in P-T0 ($P = 0.0477$). In contrast, adding the postbiotic at 4 h (P-T4) did not show any significant difference in the growth kinetics. These results demonstrate that the yeast-derived postbiotic has a measurable inhibitory effect on *S. Typhimurium*, particularly when applied at hour zero by strongly reducing biomass accumulation and slowing growth kinetics. Timing-dependent efficacy highlights the need for strategic application when considering postbiotic-based interventions for *Salmonella* control.

Keywords: postbiotic; *Saccharomyces cerevisiae*; *Salmonella* Typhimurium; growth kinetics; bacterial inhibition

M118 Dose-dependent effects of protected organic acids on growth performance, *Salmonella* colonization, and gut health in broilers challenged with *Salmonella* Typhimurium

Hanseon Ko*¹, Indira Neupane¹, Venkata Prathap Reddy Keshavareddy¹, Ishwari Gyawali¹, Kwang Yong Lee², Woo Kyun Kim¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²MorningBio, Cheonan, Chungcheongnam-do, Korea (the Republic of)

Salmonella is one of the major foodborne pathogens in broiler production, posing a substantial threat to public health. This study aimed to evaluate the effect of dietary protected organic acids (POA) on growth, *Salmonella* colonization, gut integrity, and immune response in broilers challenged with *S. Typhimurium* (ST). A total of 450 one-day-old broilers were allocated to 30 cages for 21 d. Treatments comprised a non-challenged control and

four ST-challenged groups with different dietary POA supplementation levels (0, 0.05, 0.1, and 0.2%). All birds in ST-challenged groups were challenged with ST on d 1. Growth performance, cecal ST load, ST prevalence in different organs, ileal morphology, and gene expression of tight junction proteins and cytokines were evaluated. One-way ANOVA with Tukey's post hoc test was performed to compare all treatment groups, and orthogonal polynomial analysis was conducted to assess the dose-dependent effect of POA. Fisher's exact test was used for ST prevalence, with adjustments using the Bonferroni correction. The results revealed that dietary POA supplementation at 0.1% and 0.2% improved body weight gain by approximately 9.8% compared to the ST-challenged control from d 14 to 21 ($P < 0.05$). Increasing POA levels also resulted in linear improvements in feed conversion ratio from d 14 to 21 and d 0 to 21 (Linear, $P < 0.05$). Moreover, dietary POA levels linearly reduced cecal ST load on d 4, 14, and 21 among the ST-challenged groups (Linear, $P < 0.05$). In addition, ST prevalence in the spleen and liver was lower in birds receiving 0.1% POA compared to the ST-challenged control on d 21 ($P < 0.05$). On d 21, dietary POA levels linearly increased villus height, crypt depth ratio, and relative expression of claudin-4 in the ileum (Linear, $P < 0.05$). In summary, most key measured parameters exhibited a dose-dependent response, and the most pronounced benefits were observed at 0.1% POA supplementation. Therefore, the current results demonstrate that dietary POA could be a potential strategy for controlling *Salmonella* in broiler production by improving gut health parameters and overall performance.

Keywords: Organic acids; Feed supplement; *Salmonella*; Gut health; Broiler

M119 Effects of Ginger, Roselle and their combination on carcass and meat lipid oxidation in broiler chickens

Adejoke A. Mako*¹, Adekoyejo S. Oyegunwa¹ ¹Tai Solarin University of Education Nigeria, Animal Science, Ijebu Ode, Ogun State, Nigeria

The paradigm shift towards making livestock production as natural as possible has necessitated increased research into the use of plants with medicinal and other bioactive components as feed additive in livestock production. Therefore, this study was designed to evaluate the effect of Ginger, Roselle and their blends on carcass and meat lipid oxidation quality of broiler chickens. A total of 90, one-day-old Arbor acres broiler chicks were randomly divided into 6 groups of 5 birds each in a Completely Randomized Design. Six identical diets (each representing a treatment) were formulated such that Treatment 1(Control) had no phyto-supplementation while diets 2, 3, 4, 5 and 6 were each supplemented with 1% of either ginger, roselle or their combination as highlighted. Treatment 1(Control diet) was without ginger or roselle, treatment 2 had 1% dried ginger, treatment 3 had 1% dried roselle, treatment 4 had 1% dried of ginger-roselle [25:75], treatment 5 had 1% dried ginger-roselle [50:50], and treatment 6 had 1% dried ginger-roselle [75:25]. At the end of the eight weeks feeding trial, 2 birds per replicate were purposively selected for meat quality evaluation. The data obtained were subjected to analysis of variance (ANOVA). The analysis was conducted using the general linear modelling procedure (SAS, 1999) while Duncan Multiple Range Test was used to separate means where variation occurred (Duncan, 1955). High level variation were recorded across all the treatments at weekly evaluation intervals, with treatment 6 having the highest level of lipid oxidation at Weeks 1 (1.88) and 4 (3.13). Treatment 1 (Control) had the lowest lipid oxidation at Week 1 (0.82), while treatment 4 had the lowest level of lipid oxidation at Week 4 (2.25). It can be concluded from the findings that treatment 4

reduced abdominal fat deposition in the carcass and lipid oxidation up till four weeks of storage thereby promoting healthier carcass composition and preservation of meat quality.

Keywords: ginger; roselle; lipid oxidation; carcass; broiler chicken

Machine Learning/AI

M120 Predicting processing age for density-compliant turkey production using machine learning

Brendan O. Heinichen*^{1GS}, Peter Ferket¹ *North Carolina State University, Prestage Department of Poultry Science, Wake Forest, North Carolina, United States*

Commercial turkey producers supplying certified premium markets must maintain stocking density below third-party certifier mandated thresholds (10 lbs/ft² or ~48.8 kg/m²) throughout production. Current scheduling approaches rely on historical averages that may not account for flock-specific variation, risking non-compliance or premature processing. This study developed a machine learning framework to predict optimal processing age for meeting density certification requirements using early-stage flock data from a commercial integration. Historical production records (n = 1,991 product flocks over 15 years) were analyzed using five modeling approaches: gradient boosting machines, random forests, linear regression, deep learning, and automated machine learning. Models predicted density as (head placed × expected livability × actual weight) / area using only variables available at 12 weeks to enable actionable early predictions. Expected livability was 0.87 based on certifier guidance using historical flock performance. Linear regression with L1 regularization achieved best performance (validation R² = 0.861, test R² = 0.808, temporal validation R² = 0.942), outperforming complex ensemble methods. An age optimization algorithm was developed to determine processing timing for achieving target density of 10 lb/ft². Age predictions showed mean absolute error of 3.27-3.50 days across independent validation sets, with 75% of predictions within ±5 days of actual processing age and minimal systematic bias (-0.067 days). Feature importance analysis confirmed that bird numbers per area (Head Placed and Area) dominated predictions (95% combined importance), while Age contributed 3%. This approach demonstrates practical implementation of machine learning for production scheduling in certified turkey systems. The methodology uses routinely collected data and open-source tools (R with h2o package), providing a replicable approach for commercial integrators. Processing age predictions with ±3.5 day accuracy consistent with operational scheduling flexibility while reducing certification compliance risk. The research demonstrates the complete analytical pipeline from data preparation through model deployment, emphasizing practical considerations for industry implementation.

Keywords: turkey; stocking density; machine learning; certification; processing optimization

M121 Path optimization and commercial feasibility of a quadruped robot for floor egg collection in cage-free facilities

Aravind Mandiga*^{1GS}, Guoming Li¹, Tianming Liu¹, Ramviyas Nattanmai Parasuraman¹, Ramana M Pidaparti¹, Venkat Umesh Chandra Bodempudi¹, Samuel E. Aggrey¹ *University of Georgia, Athens, Georgia, United States*

Floor eggs represent 5-6% of daily production in cage-free systems, requiring labor-intensive manual collection. This study evaluated commercial feasibility of autonomous quadruped robots through mathematical path optimization and economic analysis in a 50,000-hen facility producing 2,000 daily floor eggs. A Unitree Go1 robot with 10 kg payload capacity was analyzed for two scenarios: 50-egg and 77-egg payloads. Power consumption

calculations using cost-of-transport relationships yielded 56.4 W and 60.4 W for respective payloads at optimal speed (0.34 m/s), providing 16.45 and 15.4 hours daily operation with seven charge cycles. Boustrophedon cellular decomposition optimized routing in a 380 m × 18.2 m facility with aviary-constrained access to conveyor belts. Robot coverage width (0.646 m) was determined from arm geometry: 0.4 m robotic arm at 0.356 m height providing 0.183 m reach per side plus 0.28 m robot width. For 50-egg capacity, optimized routing with 20 segments (19 m each, 78 m coverage per segment) totaled 10,745.8 m daily travel. For 77-egg capacity, 13 segments (29.2 m each, 118.74 m coverage per segment) totaled 7,805.44 m travel. Both remained within maximum travel capacities of 13,664 m (50-egg payload) and 12,357 m (77-egg payload), demonstrating operational feasibility for complete daily floor eggs collection and transfer in commercial facilities. Economic analysis showed initial investment of \$3,504-9,304 with \$800 annual operating costs (\$48.91 electricity, \$750 maintenance). Assuming manual collection costs of \$0.03 per egg (\$21,900 annually), the system generates \$21,100 net annual benefits with 61-162 day return on investment. Robot accessibility analysis confirmed robot dimensions (0.114 m crouched, 0.241 m standing) permit full facility access under aviary structures meeting EU requirements of at least 0.45 m clearance between litter floor and aviary levels. The 77-egg payload configuration provides superior operational efficiency through reduced travel distance while maintaining battery constraints, demonstrating commercial viability for cage-free production systems.

Keywords: mathematical modeling; cage-free systems; egg collection; economic feasibility; path optimization

M122 Integrated virtual screening, ADMET analysis, and molecular dynamics reveal African natural compounds as promising FAdV inhibitors

Amina Kardoudi*^{1GS}, Abdelouheb Kardoudi², Salahedine Redoune², Faouzi Kichou¹, Siham Fallahi¹ *Institute of Agriculture and Veterinary Medicine Hassan II, Avian Pathology and Public Health, Casablanca, Casablanca, Morocco; ²Institute Pasteur of Morocco, Casablanca, Morocco*

The *Adenoviridae* family includes mammalian *Mastadenoviruses* and avian *Aviadenoviruses*, which are responsible for a wide range of diseases such as inclusion body hepatitis (IBH) and hydropericardium syndrome (HHS) in poultry. A wide variety of bird species, including chickens, pigeons, and psittacines, have been reported to harbor avian adenoviruses. These viruses are highly contagious and spread rapidly among flocks through mechanical, horizontal, and environmental transmission routes, with no effective antiviral treatment currently available. Targeting the fiber proteins involved in viral attachment and host cell entry, therefore, represents a promising strategy to control infection. In this study, a structure-based virtual screening of 14,330 African natural compounds was performed using two regional databases: the African Natural Products Database (ANPDB) and the South African Natural Compound Database (SANCDDB). The screening was conducted against four viral targets Fiber-1 (PDB ID: 7W83) and Fiber-2 (PDB ID: 2VTW) of *FAdV-4*, and the Short-Fiber (PDB ID: 2IUM) and Long-Fiber (PDB ID: 7X5T) of *FAdV-1*. From this process, the top 7,000 compounds per target were selected based on docking scores and interaction energy, and 10 shortlisted candidates were

subsequently evaluated through ADMET profiling to assess pharmacokinetic behavior, toxicity, and chemical stability. Three lead compounds were finally identified: mol_SANCDDB_245 (targeting *FAdV-4 Fiber-1*, -10.0 kcal/mol), mol_ANPDB_2908 (targeting *FAdV-1 Long-Fiber*, -10.5 kcal/mol; and *FAdV-4 Fiber-2*, -10.4 kcal/mol), and mol_ANPDB_6449 (targeting *FAdV-1 Short-Fiber*, -9.8 kcal/mol). These ligands exhibited stronger binding affinities than the reference compound Dehydroepiandrosterone (DHEA, -7.7 kcal/mol) and displayed favorable physicochemical and pharmacokinetic properties. Molecular dynamics simulations (500 ns) confirmed the thermodynamic stability and conformational persistence of the ligand-protein complexes, validating their potential as stable inhibitors. Altogether, this integrative study highlights the promising antiviral potential of African natural compounds against *FAdV-1* and *FAdV-4*, providing a solid computational foundation for future *in vitro* and *in vivo* validation studies in avian virology.

Keywords: FAdV; Fiber protein; ADMET; ANPDB; SANCDDB

M123 Computer vision-based forecasting of body weight and carcass in tom turkeys

Mireia Molins^{*1GS}, Kai-Teng Hou¹, Luis Giron¹, John W. Boney¹, Enrico Casella¹ ¹*Pennsylvania State University, Animal Science, State College, Pennsylvania, United States*

Accurately forecasting turkey body weight (BW) can improve productivity, enable more precise feeding strategies, and support sustainable poultry production. However, achieving individual accurate BW predictions is a challenging task due to its labor-intensive and time-consuming nature. While computer vision (CV) and artificial intelligence (AI) have shown great potential in estimating poultry BW, to the best of our knowledge, no studies have attempted to forecast future BW using these technologies. In this study, we conducted a longitudinal observational trial to evaluate the potential of CV to forecast BW in tom turkeys. Color and depth images were captured at two frames per second using an Intel RealSense D435 camera, with 30 toms housed in a single pen. A top-down depth camera, mounted on the ceiling, ensured consistent measurements while minimizing obstructions from animal movement and reducing sensitivity to the bird's orientation relative to the camera. The animals were monitored from day 37 to day 133, with manual BW measurements recorded five times per week. We trained an instance segmentation model (mAP50-95(M) = 0.8) to extract RGB and depth information of each bird, while removing the background, which may add noise. A morphological opening operation with a 5x5 kernel was applied to the depth image to smooth out the noisy depth data. Then, ResNet models of different sizes (18, 34, 50) were trained using all available input images (RGB, depth, and mask). Several parameter configurations were evaluated to find the optimal parameters, and data was split by animal ID on each day with a ratio of 80:10:10 (training, validation, and testing, respectively). Our preliminary results showed promising performance: R² of 0.97 and MAPE of 8.18% estimating BW on the same day. When forecasting BW one week ahead and two weeks ahead, we achieved an R² of 0.95 and 0.92, and a MAPE of 10.74% and 11.07%, respectively. These preliminary results suggest that AI and CV can be used to forecast BW in tom turkeys, offering significant benefits to the poultry industry by reducing stress on the animals and optimizing efficiency in the barns.

Keywords: Computer Vision; Bodyweight estimation; Bodyweight forecasting; Machine Learning; Turkeys

M124 Spectral imaging systems combined with machine learning can improve poultry food safety and predict wing quality

Micah T. Black^{*1GS}, Katherine Sierra¹, Vianca Tashiguano¹, Rudro Deb Sardar¹, Chinelo S. Onah-Kachiside¹, Thiago Belem¹, Laura Garner¹, Fartash Vasefi², Moon Kim³, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*SafetySpect, Inc., Sherman Oaks, California, United States*; ³*USDA-ARS, Beltsville, Maryland, United States*

Impending changes in the *Salmonella* poultry safety requirements and pressures to improve throughout is paving the way for processors to explore novel technologies such as spectral imaging. Existing imaging systems in the poultry processing sector are limited in their abilities to observe phenomenon visible to the human eyes. We demonstrate that fluorescence imaging system with machine learning can detect invisible fecal contamination on raw poultry and eggs as well as can be used to predict wing quality. Broiler carcasses and shell eggs were inoculated with fecal matter and imaged with a handheld spectral camera in a controlled, dark setting before inoculation, after contamination, and after washing. *Salmonella* Typhimurium was inoculated in chicken carcass fecal slurry remained present (97.14%) on post-wash contaminated areas using GeneUp as further analysis, emphasizing the camera's ability to reveal contamination missed during human inspection. Classification of chicken carcasses successfully detected residual contamination following rework, achieving 100% precision, 83.3% recall, and 99.4% accuracy. With CNN classification for contaminated eggs, we achieved 100% precision, 66.7% recall, and 100% average precision. Chicken wings were evaluated to capture the degree of bruising found on whole and partial wings. Spectral imaging paired with CNN algorithms determined classification into bruised/non-bruised wings with 100% accuracy. A color segmentation model was successfully built to automatically detect and encapsulate the bruised area using Red, Green, Blue (RGB) scale, followed by calculating the percent wing area and bruise percentage. Integrating fluorescence-based spectral imaging technologies powered with machine learning in poultry and egg processing operations could strengthen product quality, support FSIS compliance, and ultimately reduce *Salmonella* risk throughout the supply chain.

Keywords: Fecal contamination; Wing quality; Spectral imaging; Food safety; Salmonella

M125 Freshness classification and spoilage prediction of raw poultry during supply chain using bacterial counts and volatile organic compounds

Vianca Tashiguano^{*1GS}, Katherine Sierra¹, Micah T. Black¹, Haitao Li², Kate Trout³, Mahamoud Almasri⁴, Hoa Hoang⁵, Tatijana Fisher⁶, Laura Garner¹, Sungeun Cho¹, Zhen Jia¹, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*University of Missouri, College of Business Administration, St. Louis, Missouri, United States*; ³*University of Missouri, Department of Nutrition and Exercise Physiology, Columbia, Missouri, United States*; ⁴*University of Missouri, Department of Electrical Engineering and Computer Science, Columbia, Missouri, United States*; ⁵*University of Missouri, Food and Agricultural Policy Research Institute, Columbia, Missouri, United States*; ⁶*Lincoln University of Missouri, State Extension Specialist-Poultry, Jefferson City, Missouri, United States*

Cold chain disruptions can shorten the shelf life of fresh chicken by speeding up microbial growth. Traditional microbiological tests are accurate but slow, destructive, and impractical for real-time monitoring. Electronic-nose (E-nose) systems offer a rapid, non-destructive alternative by detecting volatile organic compounds (VOCs) associated with bacterial activity and spoilage. This study

combines microbial data, VOC profiles, and machine-learning models to predict spoilage progression and classify freshness levels. Tray-packed raw chicken breast fillets obtained from a commercial poultry supplier were subjected to temperature abuse (TA) conditions of 4°C for 30 min, followed by 1 hour at either 30°C or 37°C, in five repeated cycles. Microbial growth data were used to train a feedforward neural network (FNN) for shelf-life prediction. Data processing, regularization, and bootstrapping techniques are applied to improve model robustness and predictive accuracy. To classify chicken samples as Fresh, Moderate, or Spoiled based on VOC profiles, three models were applied. A Random Forest Categorization (RFC) used binary VOC presence and revealed key spoilage markers. A Logistic Regression (LR) model incorporated quantitative features, including the number of VOCs per sample, their relevance index, and metadata. Meanwhile, a Multinomial Naïve Bayes (MNB) model leveraged VOCs' categorical identities to capture qualitative spoilage signatures. The FNN accurately predicted aerobic growth under temperature abuse, with the fastest increase under the 37°C treatment. Predictions aligned with observed counts (MSE = 0.951, $R^2 = 0.647$). Shelf-life simulations showed earlier spoilage under higher temperatures, reaching 7 log CFU/g roughly three days earlier than controls. Classification models also performed well; LR achieved $R^2 = 0.76$ and MNB $R^2 = 0.68$. Although RFC had lower overall accuracy (56.5%), it achieved 100% recall for spoiled samples when tuned, supporting its usefulness for early spoilage identification. Integrating machine learning with VOC and microbiological data provides a stronger tool for the stakeholders in the poultry cold chain to detect spoilage and predict shelf-life of raw chicken meat in the supply chain.

Keywords: Short-Temperature Abuse; Feedforward neural network; Random Forest Categorization; Logistic Regression; Multinomial Naïve Bayes

M126 Estimate weekly feed intake and body weight gain in caged broilers using 3D Fecal Pointcloud

Yu Wang*^{1GS}, Ziyuan Zhao¹, Iyabo Oluseyifunmi¹, Gregory Archer¹, Giri Athrey¹, Samson Oladokun¹, Ziteng Xu¹ ¹Texas A&M University, Poultry Science, Bryan, Texas, United States

Feed conversion rate (FCR) is one of the most important economic traits for poultry industry because feed accounts for approximately 70% of the total production costs. FCR reflects the relationship between feed intake (FI) and body weight gain (BWG) and is traditionally measured by manually weighing the residual feed and birds. These methods are time-consuming and labor-intensive. Since fecal output is positively correlated with FI, it is possible to estimate feed intake through fecal weight. However, weighing feces is time-consuming manual process making it less practical under commercial conditions. Therefore, this study aims to measure fecal volume with a depth camera as a practical, non-contact alternative for indirectly estimating FI and BWG. The experiment involved 480 broiler chickens (Cobb 500) housed in 40 battery cages (12 birds/cage) for 28 days. A metal tray was placed underneath each cage to collect feces sample in a 24-hour interval. Spilled feed was removed from the tray by tiling the tray over. Feed intake and body weight were measured weekly. An Intel RealSense L515 depth camera was mounted 50 cm above the tray, providing a vertical top-down view of the feces. A processing pipeline was developed to estimate the fecal volume through the point cloud data. Daily measured fecal weight and volume were summed up every 7 days. Multiple linear regression analysis was applied to examine the feasibility of predicting weekly FI and BWG using feces volume and week of age as input. The results

show strong relationships between fecal weight, fecal volume, FI and BWG. As expected, fecal volume and experimental week is strongly correlated with fecal weight ($R^2 = 0.95$, MAPE = 17.21%). Weekly accumulated fecal volume is positively correlated with weekly FI ($R^2 = 0.96$, MAPE = 10.16%), suggesting that fecal traits can provide valuable insight for estimating feed consumption. Feces volume and week of age show a strong correlation with weekly BWG ($R^2 = 0.95$, MAPE = 12.97%). In conclusion, the presented study showed that feces volume can provide insights to broiler's productive performance such as feed intake and body weight gain. The method can be applied to caged poultry farming operations to estimate feed intake and body weight gain for each cage.

Keywords: Fecal Volume; Computer Vision; Feed Intake; Depth Camera; Precision Poultry Farming

M127 A computer vision approach for tracking the platform using behavior of chickens in commercial broiler houses

Anjan Dhungana*^{1GS}, Bidur Paneru¹, Samin Dahal¹, Emily Nemerovsky², Katy Tarrant³, Lilong Chai¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²California State Polytechnic University, Pomona, Department of Animal and Veterinary Sciences, Pomona, California, United States; ³Fresno State University, Animal Science and Agricultural Education, Fresno, California, United States

Environmental enrichment, such as elevated platforms, is used in broiler houses to address welfare concerns related to leg health, inactivity, and natural behaviors. Although previous studies have examined how platforms affect broiler welfare and activity, quantifying their use in commercial settings remains difficult due to large flock sizes. Manual assessment is possible but impractical, time-consuming, and prone to human errors. Automated monitoring is needed to better understand enrichment use and optimize platform design and placement. This study presents a new method for monitoring platform usage by chickens in a commercial broiler house using deep learning. The house was measured 103.6 m L × 14.6 m W and housed 19,400 broilers. Square wooden platforms measuring 0.28 m² were placed. Videos were collected from day 6 to 16 using Amcrest SmartHome cameras. From the videos, over 6,000 images were extracted and split into training, validation, and testing sets (70:20:10), and five You Only Look Once 11 (YOLO11) object detection models (i.e., 11n, 11s, 11m, 11l, 11x) were trained. Among these, YOLO11n performed best with a precision of 100%, recall of 85%, average precision of 83.6% and F1-score of 85%. Despite having high precision, presence of dust impaired the model's ability to accurately detect every bird on the platform. The trained YOLO11n model was integrated with the ByteTrack algorithm to track individual bird's activity. The system was integrated into a web-based application built using Streamlit to analyze uploaded videos and generate time-based reports automatically. This study demonstrated that computer vision and deep learning can provide a reliable and automated approach for monitoring chickens' platform use behaviors such as frequency and time in commercial broiler houses. Additionally, this method would minimize the labor need for manual observation of bird-enrichment interactions and facilitate data-driven decisions to optimize enrichment design in poultry houses.

Keywords: broiler welfare; computer vision; deep learning; enrichment design

Metabolism & Nutrition V: Enzymes

M128 Admixture of xylanase and glucanase impacts the expression of hepatic protein metabolism genes after a mixed *Eimeria* challenge

Olanrewaju Ayinde^{*1GS}, June Hyeok Yoon¹, Rama D. Kolli¹, Oluyinka A. Olukosi¹, Adebayo Sokale² ¹*University of Georgia, Poultry Science, Athens, Georgia, United States* ²*Minor Outlying Islands; BASF, Ludwigshafen, Germany*

We reported previously that supplementing carbohydrases in a mixed *Eimeria* challenge when birds are fed a corn-soybean meal diet resulted in higher overall weight gain, with no effect on villi histomorphology, short-chain fatty acid profile, and plasma FITC-d. The current study investigates gene expression responses of inflammatory cytokines, nutrient transporters, and liver protein turnover genes, when birds are fed corn-soybean meal diet supplemented with 50 or 100 g/ton blends of xylanase and glucanase during an *Eimeria* challenge. A total of 960-day-old Cobb broiler chicks were divided into four treatments: 1) Control, 2) mixed *Eimeria* challenge (CH), 3) CH + 50g/ton xylanase and glucanase, and 4) CH + 100g/ton xylanase and glucanase with ten replicates each. On d 15, the challenged group (treatments 2 – 4) were orally gavaged with 1ml of a mixed *Eimeria* solution. Spleen, cecal tonsils, and jejunal mucosa tissues were collected from one bird per pen on d 21, and liver on d 42. Pen was the experimental unit, and data were analyzed using one-way ANOVA. On d 21, carbohydrase supplementation and *Eimeria* challenge had no effect on the expression of pro-inflammatory (IL-1 β , IFN- γ , and iNOS) and anti-inflammatory (IL-10) cytokines in the spleen and in the cecal tonsils, except for the pro-inflammatory gene iNOS in the cecal tonsil, which tended to be upwardly expressed in the challenged treatment without carbohydrases ($P = 0.066$). Also, there was no treatment effect on oocysts shedding among the challenged treatments on d 21. On d 42, protein synthesis genes (4EBP1, mTOR, and IGF1) and protein degradation gene (FBXO9) showed a significantly lower ($P < 0.05$) expression in the liver in the challenged treatment supplemented with 100g/ton carbohydrase compared with the unchallenged treatment ($P < 0.05$). Collectively, although carbohydrase supplementation did not alter acute phase inflammatory genes expression, the downward expression of FBXO9 may suggest reduced hepatic protein degradation, which can indicate increased protein accretion in the carbohydrase supplemented challenged treatment as indicated by the increased weight gain in the compensatory phase.

Keywords: *Eimeria*; xylanase; glucanase; gene expression; gut health

M129 Optimal phytase supplementation levels for broilers differ by thermal condition for phosphorus utilization but not for growth or bone traits

Julianne K. Stiles^{*1UG}, Hansol Kim¹, Michael J. Ford¹, Sunday Adedokun¹ ¹*University of Kentucky, College of Animal and Food Science, Louisville, Kentucky, United States*

The objective was to determine the optimal level of phytase supplementation in broiler diets under thermoneutral (TN) and heat stress (HS) conditions. A total of 336 d-old male broiler chicks were randomly assigned to a 2 \times 4 factorial arrangement consisting of two environmental conditions (TN vs. HS) and four dietary phytase levels (0, 500, 1,000, or 1,500 FTU/kg). Birds were reared at 28.0-30.0 °C from d 0 to 7; from d 7 to 21, HS birds were subjected to cyclic heat stress (34.0-37.5 °C for 8 h/d and 25.5-29.0 °C for the remaining 16 h/d), whereas TN birds were maintained at 25.5-29.0 °C throughout. All diets were corn-soybean meal-based and met or exceeded nutrient requirements, except for being deficient in calcium and phosphorus (P). Average

daily gain (ADG), tibia breaking strength, and apparent total tract utilization (ATTU) of P were measured. Data were analyzed to assess the main effects of environmental condition, the quadratic effect of phytase supplementation, and their interaction. A one-slope broken-line regression was used to estimate the optimal phytase level, and differences between TN and HS were tested by two-sample Z-test. Statistical significance was declared at $P < 0.05$. No interaction between environmental condition and phytase supplementation was observed. From d 0 to 21, ADG increased ($P < 0.001$) quadratically with phytase supplementation. The estimated optimal phytase levels for ADG were 614 \pm 44 FTU/kg under TN and 554 \pm 43 FTU/kg under HS, with no difference ($P = 0.715$). Tibia breaking strength increased quadratically ($P < 0.001$) with phytase, with optimal levels of 697 \pm 224 FTU/kg (TN) and 598 \pm 213 FTU/kg (HS), with no difference ($P = 0.564$). Phosphorus utilization exhibited quadratic response ($P < 0.001$) to increasing phytase supplementation. The estimated optimal phytase level for ATTU was 536 \pm 73 FTU/kg (TN) and 987 \pm 76 FTU/kg (HS), with the latter being higher ($P = 0.029$). In conclusion, phytase supplementation improved growth performance, bone mineralization, and P utilization in a quadratic manner regardless of temperature conditions. However, the optimal phytase level differed among response criteria, suggesting that nutrient utilization and growth responses are not equally sensitive to phytase under varying physiological demands.

Keywords: Bone; broiler; heat stress; phytase; utilization

M130 Inorganic phosphate can be omitted from broiler starter diets barring an appropriate calcium inclusion

Elizabeth A. Lynch^{*1GS}, Lucas E. Knarr¹, Emily B. Estanich¹, Reuben Adejumo², Joseph Moritz¹ ¹*West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States*; ²*West University, Animal, Food and Nutritional Sciences, Morgantown, West Virginia, United States*

The inclusion of inorganic phosphorus (P) in the form of finite rock phosphate sources increases feed cost and environmental impact, but addition of phytase may in part mitigate these issues. The objective of this study was to evaluate a commercially available phytase on bird performance in d0-21 starter diets that omit dicalcium phosphate (DCP) with increasing calcium (Ca) inclusion in either corn/soybean meal (CSB) or CSB + wheat middling diets. A positive control (PC) diet (CSB + DCP) was formulated with 0.5% calculated non-phytate phosphorus (nPP) and 0.95% total Ca. Five levels of Ca (0.45, 0.58, 0.70, 0.83, and 0.95), two substrates (CSB or CSB + wheat middlings at 0.2% or 0.26% calculated phytate-P, respectively), and two phytase inclusions (0 or 1,500 FTU/kg) with 0% DCP (0.12% nPP) were used to create the NC treatment scheme. All diets contained a commercially available xylanase. Birds were weighed and separated into 176 groups to create uniform initial weights of 8 chicks per cage. Treatments were randomly assigned, and data were analyzed using JMP Pro 18.0 considering raised wire cage location within the room as the blocking criterion. Significance was set at $P \leq 0.05$, and significant ANOVA results were further analyzed using Tukey's HSD. As calculated ratios of Ca:nPP widen in the diets that omit DCP, (3.46 to 7.31), LWG and FI decreased, and FCR and mortality percentage increased, regardless of substrate, when compared to a PC ($P < 0.05$). However, the addition of phytase restored bird performance to that of the PC when Ca was provided at 0.58% or higher in CSB + wheat middlings diets and 0.70 or 0.83% in CSB diets. Therefore, it can be postulated that diets can omit DCP with a 0.12% nPP if corn/SBM diets are formulated to provide an appropriate Ca:nPP and a quality phytase is utilized. These results demonstrate the

complexity and relevance of the phytate-P/phytase/nPP/Ca relationship for nutritionists, allied industry, and integrators.

Keywords: Phytase; Inorganic Phosphate; Dicalcium Phosphate; Broiler Performance; Calcium

M131 Redefining calcium requirements in laying hens through phytase superdosing and low-phosphorus diets

Deependra Paneru*^{1GS}, Hanseo Ko¹, Ishwari Gyawali¹, Daniel Lee¹, Anil Gautam¹, Jose Otavio Sorbara¹, Woo Kyun Kim¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

Excessive dietary calcium (Ca) impairs phosphorus (P) utilization through Ca-phytate complex formation, requiring higher inclusions of both minerals in layer diets. This increases feed cost and environmental P excretion. Phytase superdosing offers a promising approach to eliminate Ca-phytate antagonism by rapidly degrading phytate and liberating bound Ca, potentially allowing dietary Ca to be lowered while maintaining lower inorganic P supplementation. However, the safe lower limit of Ca under such conditions has remained undefined. Therefore, a study was conducted to identify the minimum Ca requirement under phytase superdosing. A total of 80 Hy-Line Brown hens at 52 weeks of age were randomly assigned to 5 dietary treatments (8 replicates × 2 hens), consisting of graded total Ca levels (4.18, 3.14, 2.36, 1.77, and 1.33%; 4.18% = breeder recommendation) with constant low available P (0.21%) and an enhanced *E. coli* 6-phytase superdose (1,500 FTU/kg) for 8 weeks. Hen-day egg production (HDEP), egg weight, eggshell thickness, and feed intake were recorded. At week 8, whole-body bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry, whereas femur and keel BMD were quantified using micro-computed tomography. Data were subjected to one-way ANOVA, and minimum Ca requirements were estimated using broken-line regression analysis ($P < 0.05$). HDEP and average egg weight were unaffected until the breakpoints of 1.86 and 1.80% Ca, respectively, below which both parameters declined significantly ($P < 0.01$). However, eggshell thickness remained stable only up to 2.84% Ca and then decreased linearly ($P < 0.001$). Whole-body BMD was preserved above 2.26% Ca, with significant demineralization at lower levels ($P < 0.001$). Femur BMD remained stable above 1.90% Ca, whereas keel BMD was maintained until 1.77% Ca ($P < 0.05$). Below 1.77% Ca, hens showed behavioral signs of Ca deficiency (aggression and shell-eating). In conclusion, superdosing of an enhanced *E. coli* 6-phytase at 1,500 FTU/kg in low-phosphorus diets enables total dietary Ca to be safely reduced to 2.84% in mid-late lay Hy-Line Brown hens, without compromising egg production, egg weight, eggshell thickness, and skeletal integrity, while also decreasing feed cost and phosphorus excretion.

Keywords: laying hens; calcium; phytase superdosing; eggshell; bone mineralization

M132 Effects of enzyme supplementation on excreta nutrient profiles and egg quality of post-peak production white laying hens

Elizabeth Meza Robinson*^{1GS}, Hansol Kim¹, Jacqueline Jacob¹, Michael J. Ford¹, Sunday Adedokun¹ ¹*University of Kentucky, Animal & Food Sciences, Lexington, Kentucky, United States*

There is a desire for organic fertilizer sources (such as animal manures), due to excess nutrients from inorganic NPK fertilizers causing environmental concerns. The objective of this study was to investigate the effects of dietary enzyme supplementation on the nutrient profiles of poultry excreta from post-production white laying hens to be used as organic fertilizer in greenhouse crops, while also evaluating egg quality to ensure no depletion. A total of 180 white laying hens were randomly assigned to 5 dietary treatments (Diet A:PC diet meeting all nutrient requirements; Diet B:NC diet with reduction of Ca and P; Diet C:NC with 600FTU/kg phytase; Diet D:NC with 50g/MT xylanase and 50g/MT mannanase; Diet E:NC with 600FTU/kg phytase, 50g/MT xylanase, and 50g/MT mannanase) with 6 replicate cages per treatment and 6 birds per cage. The trial ran for 16 weeks with eggs collected for quality analysis at weeks 0, 6, 12, and 16. At the end of the study, excreta was collected from each pen for nutrient analysis. The egg quality data were analyzed using the IML procedure of SAS. Least squares means of each treatment were calculated, and pairwise comparisons were performed using the least significant difference method with Tukey's adjustment. Nutrient profiles of excreta were evaluated using percent difference, to assess the amount of N, P, K, and Ca excreted from each diet. At week 6, egg yolk color from Diet A was considered significantly higher ($p < 0.001$) than the other diets; egg height showed a significant difference between Diets C and D ($p < 0.05$) with no difference in Diets A, B, and E; and egg weight was considered significantly higher in Diet A compared to B with no differences between Diet A and Diets C, D, and E. No significant differences in egg quality were shown at weeks 0, 12, and 16. There was a 32.4% reduction in excreted P and 27.0% reduction in Ca from Diet B when compared to Diet A. It was also shown that the inclusion of enzymes in the diets reduced the amount of P in the excreta by an additional 3.9% (Diet C), 10.5% (Diet D), and 8.3% (Diet E). To conclude, this study shows that exogenous enzymes significantly reduce P excretion and can reduce environmental impact of mineral run-off from excreta fertilizer without risking egg quality.

Keywords: poultry; layer; mineral; exogenous enzyme; excreta

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M133 Assessing calibration transferability and impact of particle size on near infrared reflectance spectroscopy predictions of nitrogen corrected true metabolizable energy and digestible amino acid content in solvent extracted and mechanically expelled soybean meal

Thomas Jones*^{1GS}, Cassandre Juzaitis-Boelter², Chongxiao Chen¹, Adam Davis¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*; ²*Bruker Optics, Madison, Wisconsin, United States*

Previously, we reported that near infrared reflectance spectroscopy (NIRS) can predict digestible amino acid (dAA) and nitrogen corrected true metabolizable energy (TME_N) content of ground solvent extracted (SE) and mechanically expelled (ME) soybean meal (SBM) samples with predictive accuracy comparable to the

margin of error associated with rooster bioassays. These calibrations were developed on a Bruker Multipurpose Analyzer (MPA), a dual-channel FT-NIR instrument; however, in commercial settings such as feed mills, the single-channel TANGO-R is more commonly used, as it employs a more streamlined graphical user interface in comparison to the MPA. The current research assesses the practical implementation of our original calibrations by examining the influence of particle size and inter-instrument variation on predictive consistency. Spectra for 33 SE and 10 ME SBM samples were analyzed. Each sample was divided into two aliquots. One aliquot was ground using a 1093 Cyclotec Sample Mill (0.5-mm screen) and analyzed on a TANGO-R at a feed mill. The other aliquot was ground with a Retsch ZM200 centrifugal grinder (1.0-mm screen) and analyzed using our MPA, and the same TANGO-R was transported to our

laboratory. Both instruments were equipped with calibration models established in our initial research. Statistical differences were evaluated using paired *t* tests. Significant differences ($P < 0.05$) were observed for TME_N and nearly all dAAs across grind size and between instruments, but mean differences were marginal (<5% of analyte average) and directionally consistent. Predictions from the 0.5-mm grind and TANGO-R were almost universally lower than their paired comparisons. These results indicate that grind size and inter-instrument variation exert minor, predictable effects on TME_N and dAA predictions. Since these differences follow a consistent direction rather than a random distribution, analyte-specific bias correction for instrument and/or particle size can be applied to the original calibrations. Consequently, NIRS could provide a reliable and transferable method for determining TME_N and dAA content of SE and ME SBM in poultry across Bruker FT-NIR platforms and sample preparation conditions.

Keywords: Poultry; Soybean Meal; Ingredient Analysis; Feed Formulation

M134 Metabolizable energy concentrations of resistant starches fed to 21-day-old broiler chickens using the regression and difference methods

June Hyeok Yoon*^{1GS}, Rama D. Kolli¹, Olanrewaju Ayinde¹, Sujitha Veeraganti¹, Oluyinka A. Olukosi¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

The objective of the study was to determine the metabolizable energy (ME) concentrations of raw potato starch (RPS), high-amylose cornstarch (HCS), and banana starch (BS) using the regression and difference methods in 21-day-old broilers. A total of 180 male broilers were allocated to 6 dietary treatments in a randomized complete block design with six replicates (five birds/cage). Birds received a corn-soybean meal (SBM) starter diet. On d14, birds were fed six different experimental diets consisting of: 1) corn-SBM-based basal diet, 2) basal+150 g/kg RPS, 3) basal+300 g/kg RPS, 4) basal+200 g/kg HCS, 5) basal+400 g/kg HCS, and 6) basal+300 g/kg BS. The resistant starches (RS) replaced portions of the basal diet, but the proportion of energy-yielding feedstuffs (corn, SBM, and soybean oil) in all diets remained constant. Titanium dioxide was included as an indigestible marker, and excreta were collected the last two days of the study to determine the apparent ME values (AME) of experimental diets. Test ingredient-associated ME intake [kcal dry matter (DM)] was regressed against test ingredient intake (kg) to determine the ME and nitrogen-corrected ME (ME_N ; kcal/kg DM) of RPS and HCS using the regression method. The difference method was used for determining AME value of BS. Orthogonal polynomial contrasts were used to assess the linear and quadratic effects of RPS and HCS levels on AME values in experimental diets. The AME and AME_N in experimental diets decreased linearly ($P < 0.05$) as the dietary RPS and HCS levels increased. The determined ME and ME_N values of RPS and HCS using the regression method were 1,731 and 1,709 kcal/kg DM, and 1,753 and 1,780 kcal/kg DM, respectively, in 21-d-old broilers. The AME and AME_N values of BS using the difference method were 1,681 and 1,645 kcal/kg DM. The calculated AME value in experimental diets, using the determined ME values of RS was close to the analyzed values. In conclusion, the ME values of RS are relatively low, and these values are predictable in the corn-SBM-based diets. The RS is highly resistant to enzymatic hydrolysis in the intestine and may serve as a functional fiber to enhance hindgut saccharolytic fermentation in broiler chickens.

Keywords: raw potato starch; high-amylose cornstarch; banana starch; metabolizable energy; regression method

M135 Variation in metabolizable energy and amino acid content could limit the utilization of insect meals in commercial poultry diets

Kiley Stout*^{1GS}, Coleman J. Hatmaker¹, Adam Davis¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Given their high energy and/or protein content as well as the ability to commercially produce them throughout much of the world, insect meals have gained considerable attention for their potential use in livestock diets to decrease the utilization of cereal grains and soybean meal (SBM) in these diets. The goal of the current research was to determine the nitrogen corrected true metabolizable energy (TME_N) and digestible amino acid content of commercially produced black soldier fly (*Hermetia illucens*) larva (BSFL), yellow mealworm (*Tenebrio molitor*), and cricket (*Acheta domesticus*) meals to determine their potential suitability for incorporation into commercial poultry diets. Ten BSFL, 5 mealworm and 3 cricket samples were analyzed for dry matter, gross energy, nitrogen corrected true metabolizable energy (TME_N), crude protein and both total and digestible amino acid content. The TME_N and digestible amino acid content of the insect meal samples was determined by the intact and cecectomized rooster bioassays, respectively. Nutrient values of different insect meals were analyzed by ANOVA and differences were considered significant when P was < 0.05 . The TME_N content did not significantly differ between the 3 insect meal types. On an as is basis, the TME_N values of the BSFL samples ranged from 3,598 to 5,142 kcal/kg, while the crude protein values ranged from 31 to 46%. For mealworms, the TME_N values varied from 3,762 to 4,816 kcal/kg, while crude protein values ranged from 44 to 58%. The TME_N values of the dried cricket samples ranged from 2,667 to 4,002 kcal/kg, while crude protein values ranged from 61-65%. The amino acid digestibility coefficients of BSFL and mealworms were comparable to SBM, while the values for crickets were lower than SBM, but, due to the higher concentration of total amino acids in crickets relative to SBM, the total digestible content of each amino acid in the cricket samples was equal or greater than soybean meal. While the tested insect meals are energy dense and high in available amino acids, the tremendous variation in the TME_N and amino acid content of the different commercial samples of the same type of insect meals would potentially inhibit their use in commercial poultry diets.

Keywords: true metabolizable energy; black soldier fly larva; yellow mealworm; cricket

M136 Effects of feeding varying levels of mycotoxin-containing diets on diet choice of broiler chickens

Eleanor G. Tipton*^{1UG}, Hansol Kim¹, Jacqueline Jacob¹, Michael J. Ford¹, Sunday Adedokun¹ ¹University of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States

This study evaluated the effects of feeding diets with varying levels of mycotoxins on feed preference of broiler chickens. Three experimental diets were formulated as corn-soybean meal-based diets in which regular corn was replaced with mycotoxin-contaminated corn fines (mycotoxin contents were 28,323, 3,059, and 682 ppb for fumonisin, vomitoxin, and zearalenone, respectively) at 0% (D0), 50% (D50), or 100% (D100) substitution levels. Four dietary treatments (T1, D0 vs. D0; T2, D0 vs. D50; T3, D0 vs. D100; and T4, D50 vs. D100) were evaluated. A total of 224 one-day-old Cobb 500 broiler chicks were randomly assigned to 4 treatments with 8 replicate cages/treatment and 7 birds/cage. Two feeders corresponding to the assigned diets were placed on opposite sides within each cage, and all feed and water were provided *ad libitum*. To avoid location bias, feeder positions were switched on d 2, 4, 7, 9, 11, 14, 16, and 18. Feed intake was

measured on d 7, 14, and 21. Feed intake per period (% consumed) was calculated for each diet within the same treatment, and a paired t-test was performed to evaluate differences in feed intake between the 2 diets. A cage was considered the statistical unit. Significance was set at $P < 0.05$. In T1 (D0 vs. D0), no differences in feed intake were observed between the 2 diets during any feeding phase (49.7 vs. 50.3%). In T2 (D0 vs. D50), no difference was observed during d 0–7, however, from d 7–14 and d 14–21, birds fed D0 consumed more feed ($P < 0.05$) than those fed D50 (57.5 vs. 42.5%). In both T3 (D0 vs. D100) and T4 (D50 vs. D100), feed intake was lower ($P < 0.01$) in birds fed D100 compared with D0 and D50, respectively, across all feeding phases (72.2 vs. 27.8% for T3 and 60.3 vs. 39.7% for T4). In conclusion, during d 0–7, broiler chicks showed no feed preference when offered diets containing a low concentration of mycotoxin, likely because younger chicks are less capable of distinguishing between feeds with or without mild contamination. However, as the mycotoxin concentration increased or after d 7, feed intake decreased regardless of concentration, suggesting that as broilers aged, they became more able to recognize and avoid mycotoxin-contaminated feed, with stronger aversion observed at higher contamination levels.

Keywords: Broiler; Feed intake; Mycotoxin; Palatability

M137 Evaluating the effects of cricket meal inclusion on Ross-308 male broiler performance and apparent ileal amino acid digestibility

Christian K. Crabtree^{*1GS}, Darby R. Boontarue², John W. Boney² ¹*Pennsylvania State University, Animal Science, Patton Township, Pennsylvania, United States;* ²*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States*

Cricket (*Acheta domesticus*) meal (CM) may serve as a sustainable alternative protein source in poultry diets due to its favorable amino acid (AA) profile. However, its AA digestibility and optimal inclusion rate remain unclear. This study determined the effects of graded CM inclusion on broiler performance and apparent ileal amino acid digestibility (AIAAD) from d1-21. A total of 450-d-old Ross 308 male broilers were randomly allotted to one of 45 cages (10 birds/cage). The five dietary treatments (0%, 2.5%, 5%, 10%, 15% CM) contained 0.2% titanium dioxide (TiO₂) and were arranged in a randomized complete block design with nine replicate cages per treatment. Body weight (BW), live weight gain (LWG), feed intake (FI), and mortality-corrected feed conversion ratio (FCR) were determined from d1-21. After recording d21 BW, broilers were provided feed for 6 hours after which 3 birds/cage were euthanized for distal ileal digesta collection. Pooled digesta samples per cage were frozen and freeze-dried. Feed and digesta were analyzed for TiO₂ and AA content to calculate AIAAD. Particle size analysis of treatments was conducted in duplicate. Data were analyzed using one-way ANOVA in SAS, and significant treatment effects were further separated using Fisher's LSD. Control-fed birds had the lowest BW and LWG. Birds provided either 5% or 10% CM weighed and gained the most. Birds consuming either 2.5% or 15% CM weighed and gained more than control-fed birds but less than birds provided 5% or 10% CM ($P < 0.001$ and $P < 0.001$, respectively).

Although descriptive, particle size decreased from 981 μm to 845 μm as CM inclusion increased from 0% to 15%. Met digestibility improved when CM was included ($P < 0.001$). Trp digestibility was highest ($P < 0.001$) when CM was included at 15% while Val digestibility was lowest ($P = 0.025$) when CM was included at 15%. Thr digestibility was highest when 5% or 10% CM was included ($P = 0.008$). Digestibility of Ala, Ser, Tyr, and Tau was reduced at 15% CM inclusion ($P < 0.001$). In conclusion, a clear reduction in broiler performance was supported by the reduction in AIAAD coefficients of several AA when CM was included at 15%. These data suggest CM can be used as an alternative protein source that optimizes LWG and BW when included at either 5% or 10%.

Keywords: Insect; alternative Protein; Sustainability; Digesta

M138 Comparison of the growth potential, carcass yield, and body composition of two modern broiler genotypes with a heritage meat-type chicken

Erasmio A. Flores Granados^{*1GS}, Bryan I. Fancher², Samuel J. Rochell¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States;* ²*Aviagen, Huntsville, Alabama, United States*

This study compared the growth performance, carcass yield, and body composition dynamics of two modern broiler genotypes (Ross YP \times Ross 708 and Ross \times Ross 308) with a heritage genotype that has been unselected since the 1950's (New Hampshire \times Columbian [NHC]). Male chicks (1,760 total) were placed in pens designated for performance ($n = 12$ pens/genotype) or sampling ($n = 8$ pens/genotype) measurements. Ross genotypes were reared to 56 d, whereas the NHC birds were reared to 63 d to achieve at least 2 kg final BW. Feed intake (FI), BW gain (BWG), and feed conversion ratio (FCR) were determined weekly. Carcass parts were measured weekly (1 bird/pen), and body composition was determined biweekly (1 bird/pen). Treatments were arranged as a randomized complete block design with pen as the experimental unit and pen location as a random blocking effect. A one-way ANOVA was performed using the MIXED procedure (SAS Institute Inc., Cary, NC) with significance declared at $P < 0.05$. Growth traits were modeled using nonlinear Gompertz equations for live weight, physical parts, and chemical components, and allometric coefficients were estimated to describe tissue and nutrient deposition. Modern genotypes exhibited greater ($P < 0.05$) BW, BWG, FI, and lower ($P < 0.05$) FCR across all ages compared with NHC, with mature weights (Wm) exceeding those of NHC by more than twofold. Breast muscle growth showed strong positive allometry in all genotypes, with Ross YP \times Ross 708 achieving the highest relative weight ($P < 0.05$) and the highest asymptotic breast weight as predicted with the Gompertz model. Heritage birds had higher ($P < 0.05$) relative weight of wings, head, neck, paws, and feathers, and higher ($P < 0.05$) allometric coefficient for ash and lipid content, indicating slower maturation and greater late-stage lipid deposition. Modern genotypes maintained higher ($P < 0.05$) protein concentration throughout growth. These findings demonstrate advances in growth potential and nutrient allocation between modern and heritage broiler strains and provide updated comparisons for carcass and body composition modeling.

Keywords: broiler; heritage; growth modeling; Gompertz; allometry

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M139 Regional differences in the digestible amino acid content of corn grown in Georgia

Allen B. Mann^{*1GS}, Thomas Jones², Adam Davis¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United*

States; ²*University of Georgia, Poultry Science, Athens, Georgia, United States*

Corn is the primary constituent of most poultry diets in the United States, and thus differences in its nutrient profile can influence bird

growth, egg production and nutrient excretion. Although Georgia does not rank among the top 20 corn-producing states, locally produced corn is used by the state's poultry industry, as it is one of the most common row crops within the state. This study evaluates regional differences in the metabolizable energy and digestible amino acid content of corn produced in Georgia. Corn grown north of Atlanta was classified as North Georgia corn, and corn grown south of Atlanta as South Georgia. Corn samples from different farms across North Georgia (n=26) and South Georgia (n=20), all from the 2024 production year, were analyzed for dry matter, gross energy, nitrogen corrected true metabolizable energy (TME_N), crude protein, and total and digestible amino acid content. The TME_N and digestible amino acid content of the corn samples was determined by the intact and cecectomized rooster bioassays, respectively. Statistically significant differences were determined using a one-way ANOVA with a significance level of $P < 0.05$. On an as is basis TME_N did not differ between the North and South Georgia corn samples, but the crude protein content was significantly greater in corn samples from North Georgia than South Georgia (8.9 versus 7.6%). Although the amino acid digestibility coefficient for all amino acids did not differ between the North and South produced corn samples, total content of alanine, aspartic acid, cysteine, glutamic acid histidine, isoleucine leucine, phenylalanine, proline, seine, threonine tyrosine and valine were significantly greater on as is basis in North Georgia grown corn than in South Georgia grown corn. Subsequently, the digestible content of all these amino acids was also significantly greater in corn produced in North Georgia, except for cysteine. Moisture content was significantly greater in the North Georgia corn samples than the South Georgia samples (13.5 versus 12.4%). The results indicate that the digestible amino acid content for poultry was superior for corn produced in North Georgia in 2024 relative that produced in South Georgia.

Keywords: Poultry; True metabolizable energy; Crude Protein

M140 Effects of varying inclusions of duckweed on Ross 308 male broiler performance and apparent ileal amino acid digestibility

Evangeline I. McGargle*^{1UG}, Jordan D. Brown¹, Darby R. Boontarue², Rachel A. Brennan¹, John W. Boney² ¹*Pennsylvania State University, Alexandria, Pennsylvania, United States;* ²*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States*

Duckweed (DW) is a floating, aquatic plant that can be easily grown on water and removed via skimming. Duckweed's ability to grow rapidly and double its mass every 16-48 hours as well as concentrate crude protein makes it a potential sustainable protein source in poultry diets. This study evaluated broiler performance and apparent ileal amino acid digestibility (AIAAD) in response to increasing DW meal inclusion (0%, 5%, 10%, and 15%) from d0-21. A control (0% DW) and 15% DW diet were formulated and included 0.2% titanium dioxide (TiO₂) as an indigestible marker. Portions of these diets were blended to achieve intermediate DW inclusions of 5% and 10%. Dietary treatments were provided to 12 replicate cages with each cage containing 10 broilers. Treatments were arranged in a randomized complete block design. On d21, feed intake (FI), live weight gain (LWG), body weight (BW), and mortality-corrected feed conversion ratio (mcFCR) were determined. Following broiler performance determination, birds were provided their dietary treatments for six hours. Then, three birds per cage were euthanized for distal ileal digesta collection. Digesta samples were pooled per pen, frozen until further analysis, and then freeze-dried prior to amino acid and TiO₂ analysis. Data were analyzed using one-way ANOVA in the GLM procedure of SAS. Means were separated using Fisher's LSD when $P < 0.05$.

Broiler LWG and BW were highest when birds were provided either 5% or 10% DW and lowest when birds were fed the control diet. Both LWG and BW were intermediate when birds were provided 15% DW ($P=0.028$ and $P=0.31$, respectively). The AIAAD coefficients for His, Phe, and Leu worsened as DW inclusion increased while the Thr digestibility coefficient improved above control fed birds, regardless of the DW inclusion ($P<0.05$). The AIAAD of all analyzed non-essential amino acids worsened as DW inclusion increased to 15%. In summary, d1-21 broiler LWG and BW suggest that DW can be included up to 15%. However, the intermediate performance response and AIAAD detriment at higher inclusions supports DW inclusion up to 10%. These data warrant additional experimentation in a full grow out to market weight to further assess performance and processing yield impacts of DW inclusion.

Keywords: alternative ingredient; sustainability; nutrient profile

M141 Evaluating black soldier fly larvae meal as an alternative sustainable protein source for broiler growth and gut health

Ishwari Gyawali*^{1GS}, Deependra Paneru², Seshidhar Reddy Gudidoddi¹, Hamid Reza Rafieian Naemini¹, Indira Neupane¹, Woo Kyun Kim¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States;* ²*University of Georgia, Poultry Science, Athens, Georgia, United States*

Black soldier fly larvae (BSFL) meal is a promising sustainable alternative to soybean meal (SBM) in poultry diets, offering valuable nutrients and natural antimicrobial benefits that support broiler growth and gut health. A study was conducted to investigate the effects of incorporating BSFL meal into broiler diets on growth performance and gut health. A total of 432 one-day-old male Cobb500 chicks were randomly assigned to six dietary treatments with 6 replicates (12 birds/rep). The treatments included a control diet containing 0% BSFL (standard corn-SBM diet) and experimental diets with increasing levels of BSFL meal at 2.5, 5, 7.5, 10, and 12.5%. Growth performance and gut health parameters were analyzed over 28 days feeding period. Data were analyzed using one-way ANOVA and orthogonal polynomial contrasts were used to assess dose-response trends ($P < 0.05$). Our results showed that BSFL inclusion up to 12.5% did not compromise growth performance compared to the standard corn-SBM diet. Interestingly, BSFL inclusion at 5% had the highest body weight (BW) and body weight gain (BWG) compared to 12.5% inclusion ($P = 0.0357$). Feed intake was numerically higher in 5% BSFL group compared to other BSFL inclusion levels and standard corn-SBM diet ($P = 0.1471$), while feed conversion ratio (FCR) remained consistent across treatments ($P > 0.05$). In the duodenum, villus height was comparable among all treatment groups ($P > 0.05$). However, crypt depth increased significantly with 12.5% BSFL inclusion compared to 0, 2.5, and 5% inclusion levels ($P = 0.0038$). The villus height-to-crypt depth (V/C) ratio was highest at 5% BSFL inclusion compared to 12.5% ($P = 0.0281$). In the jejunum, a quadratic trend was observed for villus height, which increased with BSFL inclusion up to 5% before decreasing at higher inclusion levels (quadratic, $P = 0.0336$). The jejunal V/C ratio also showed quadratic responses which increased with BSFL inclusion up to 7.5% before decreasing at higher inclusion levels (quadratic, $P = 0.0094$). In conclusion, BSFL meal can be effectively incorporated into broiler diets as a sustainable protein source. While 12.5% inclusion did not adversely affect growth performance, a 5% inclusion level appears to optimize growth and gut health parameters.

Keywords: Black soldier fly larvae; broiler performance; gut health; sustainable protein

M142 Energy value of hemp heart fines for broiler chickens determined using the regression method

Samuel O. Oluwayemi*^{1GS}, Ayodeji Aderibigbe¹ *Ohio State University, Department of Animal Sciences, Wooster, Ohio, United States*

The fluctuations in the cost and availability of conventional feed ingredients have led to renewed interest in alternative feedstuffs that can enhance the sustainability of poultry production. Industrial hemp and its byproducts have emerged as potential candidates due to their favorable nutrient profiles, and some aspects of their nutritive value have been reported in poultry. Hemp heart fines (HHF) are characterized by high energy (6,217 kcal/kg DM), high fat (40.6%), moderate crude protein (28%), and fiber levels (NDF 19.6%, ADF 12.4%) making it a nutrient-dense co-product with potential as an alternative energy source. However, limited information is available on their digestible and metabolizable energy contribution for poultry. Hence, this study was conducted to determine the ileal digestible energy (IDE), ME, and MEn of HHF for broiler chickens using the regression method. Three diets were formulated: a corn-SBM reference diet and two assay diets in which HHF replaced energy-yielding ingredients at 80 or 160 g/kg of diet. Each diet was fed to 8 replicate cages of 10 broiler chickens per cage from day 17 to 21 post-hatching. Titanium dioxide was included as an indigestible marker for indirect determination of energy utilization by the index method. Excreta collection was conducted during the last 3 d of the experiment followed with euthanasia by CO₂ asphyxiation to retrieve ileal digesta. Data was analyzed by the ANOVA using the GLM procedure of SAS. The apparent ileal digestibility of gross energy (GE) and IDE (kcal/kg DM) in test diets linearly increased ($P < 0.05$) with increasing substitution with HHF. Similarly, the ME and MEn (kcal/kg DM) of diets linearly increased ($P < 0.05$) with increasing substitution with HHF. The regression-derived estimates for HHF are 5,659, 4,958, and 4,748 kcal/kg DM for IDE, ME, and MEn, respectively. This corresponds to 76–91% of the total GE in HHF. These results provide the first estimates of the energy value of HHF in broiler diets, indicating high digestible and metabolizable energy potential comparable to other conventional high-fat co-products.

Keywords: hemp; broiler; metabolizable energy; feed ingredient

M143 Application of palm oil in broilers nutrition

Allison Jun T. Kawaoku*^{1GS}, Nicolas Mejia-Abaunza¹, Catherine Fudge¹, Muhammad Ali¹, Federico Etcheverry¹, Adam Davis¹, Ronald Pegg², Chongxiao Chen¹ *University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²University of Georgia, Department of Food Science, Athens, Georgia, United States*

Compared to other plant oils, palm oil (PO) is produced in highest volume with the least land use. This study compares the use of dietary PO to soybean oil (SO) on growth and meat characteristics in broilers. In a floor pen trial, 900 one-day-old male Cobb500 chicks were randomly assigned to 5 treatments, with 9 replicates per treatment. Corn-soy-based diets were formulated for starter (d0-14), grower (d15-28), and finisher (d29-42) phases to meet Cobb 500 broiler nutrition specifications with SO being replaced on a caloric basis with various PO products: 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 the fatty acid (FA) profile of the oil samples were determined. At 14, 28, and 42d, body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined. At d43, 8 birds per pen were processed for carcass yields with breast muscle collected to assess color, pH, and FA profile. Data was

analyzed using ANOVA with Tukey's HSD used for mean separation ($P < 0.05$). All PO products had higher TMEn compared to SO, except PALMOL. The UFA:SFA ratio for SO was 5.47, HOPO exhibited a balanced ratio of 1.94, with a higher content of MUFA among the PO products. The lowest ratio was for CPO (1.10). HOPO had comparable growth performance to SO. During d0-14 PALMOL had lower BW ($P = 0.0154$) and BWG ($P = 0.0139$) compared to SO and higher FCR compared to HOPO and SO ($P = 0.0001$). Growth parameters did not differ in later dietary stages. Meat pH, color, and carcass weight did not differ between SO and PO. Abdominal fat pad weight was greater in PALMOL than SO ($P = 0.0091$). RBDPO wing yield was less than SO and PALMOL ($P = 0.0128$). RBDPO and CPO had higher *Pectoralis minor* % yield than PALMOL ($P = 0.0199$). Broilers fed SO had higher PUFA%, and lower MUFA% and SFA% in breast muscle compared to the PO treatments ($P < .0001$). Among the PO, SFA% content in CPO was higher than in HOPO and PALMOL ($P < .0001$). This study supports PO's use as an alternative oil source in broiler diets, and this is especially true for HOPO, which has a high energy value, balanced FA profile, and similar growth and carcass characteristics to SO.

Keywords: palm oil; soybean; energy; fatty acid; broiler

M144 Impact of hammermill tip speed and screen size on corn particle size distribution and uniformity

Urias Castillo*^{1GS}, Wilmer Pacheco¹, Eva Guzman¹ *Auburn University, Poultry Science, Auburn, Alabama, United States*

Grinding is the primary process regulating particle size in feed manufacturing and represents a significant proportion of total energy consumption in feed mills. Particle size influences pellet quality, nutrient utilization, and poultry performance. This study evaluated the impact of hammer tip speed (m/s) and screen size (mm) on the geometric diameter average (GMD) and geometric standard deviation (GSD) of ground corn. The experiment used a 3 x 3 factorial arrangement combining three hammermill tip speeds (91, 73, and 55 m/s) and three screen hole diameters (4.76, 6.35, and 7.94 mm) for a total of 9 treatments. For each treatment, four homogenized subsamples were collected from each 136 kg batch of ground corn to assess particle size characteristics. The GMD and GSD were determined according to ASABE Standard S319.4. Data were analyzed as a completely randomized 3 x 3 factorial design using the GLIMMIX procedure of SAS, with screen size, tip speed, and their interaction specified as fixed effects. Means were separated using Tukey's test at a significance level of $P < 0.05$. As hammer tip speed decreased, GMD (873, 946, 1205 μm ; $P < 0.05$) and GSD (1.80, 1.95, 2.09, $P < 0.05$) increased, indicating a coarser and more heterogeneous grind at lower hammer tip speeds. Corn ground with the 7.94 mm screen produced a coarser ($P < 0.05$) GMD compared to 6.35 mm and 4.75 mm screens (1,062 vs. 980, 983 μm , respectively). Screen size also affected GSD, with the largest screen generating the most variable particle distribution (2.07 vs. 1.95 and 1.82). A significant tip speed x screen size was detected for GMD ($P < 0.05$). At higher tip speeds (91 and 73 m/s), GMD did not differ among screen sizes; however, at the lowest tip speed (55 m/s), increasing screen size from 4.75 or 6.35 to 7.94 mm increased GMD (1,172 and 1,138 vs. 1,306 μm). These results demonstrate that tip speed is the primary driver of particle size and uniformity, while the effect of screen size becomes more pronounced at lower hammer tip speed.

Keywords: particle size; hammermill; tip speed; screen size

M145 Comparing blended feeding to phase feeding strategy: effects on growth performance, carcass yield, and meat characteristics in broilers

Nicolas Mejia-Abaunza^{*1GS}, Catherine Fudge¹, Muhammad Ali¹, Federico Etcheverry¹, Allison Jun T. Kawaoku¹, Alleya Collins¹, JT Pope², Chongxiao Chen¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*; ²*House of Raeford, Rose Hill, North Carolina, United States*

Broiler has specific nutrient requirements that change daily. However, modern systems use phase feeding, which could lead to periodic under- or over-supply of nutrients. This study evaluated the effect of a feeding program on growth performance, carcass yield, and meat quality in broilers. This feeding program is achieved by blending high- and low-nutrient-concentration diets from d 8 to 29 to meet their daily nutritional requirements. A total of 480 one-day-old Ross 708 male chicks were raised in floor pens. Birds were randomly assigned to one of two feeding programs, with 20 birds per pen and 12 pens per treatment. The control group followed a two-phase program with starter (d0-18) and grower diets (d19-36). The blended feeding group received a starter diet from d 0-7, and a mixture of starter and grower diets with a decreasing ratio from d 8 to 29, and a grower diet from d 30-36. Body weight (BW) and body weight gain (BWG) were measured on d 7, 18, 29, and 36. Daily feed intake (FI) was recorded during the blended period and measured at d 7 and 36. On d 36, two birds per pen were selected for organ index. On d 37, ten birds per pen were processed for carcass yields and part weights. Data were analyzed using a t-test at the $P < 0.05$ significance level. From d 8 to 18, FI was higher in the blended feeding group ($P = 0.001$), but no differences in BW, BWG, or FCR ($P > 0.05$). However, during d 8 to 29 and d19 to 29, the blended feeding group showed significant improvements in BWG and FCR with no differences in FI ($P < 0.05$). Consequently, the overall period (d 0-36), the blended feeding group improved BWG ($P = 0.047$) by 39g/bird, and FCR by 7 points ($P < 0.001$), accompanied by a reduction in FI ($P = 0.011$). For meat characteristics, no differences were found except that fat pad weight ($P = 0.031$) was lower in the blended feeding group. No difference in organ index was observed. In conclusion, blended feeding improved growth performance by better aligning with daily nutrient requirements and reduced fat pad accumulation. Further research is necessary to explore the mechanism and further optimize the blending ratios and duration.

Keywords: Blended feeding; growth performance; meat characteristics; organ index; broiler

M146 Impact of sorghum particle size on growth performance, gizzard weight and pH in broiler chickens

Victoria Wilson^{*1GS}, R. Scott Beyer², Haley Otott¹, Chad Paulk¹ ¹*Kansas State University, Grain and Food Science, Manhattan, Kansas, United States*; ²*Kansas State University, Animal Sciences and Industry, Manhattan, Kansas, United States*

Sorghum is a cereal grain that serves as an energy source in broiler diets when it is competitively priced for feed formulation. The objective of this study was to evaluate sorghum particle size (mean geometric diameter, dgw; PS) effects on growth performance, gizzard weight, and pH in broilers from 4-49 d of age. A total of 864 COBB by-product breeders were allotted to 72 pens ($n = 10/\text{pen}$) at 4 days of age and treatments were randomly assigned to pens within location block. Treatments consisted of corn (C), or sorghum (S) based diets at variable target PS of, 400, 600, 800, 1000 or 1200 microns. Actual PS were measured as C 850 μm and S 414, 606, 821, 1046, and 1124 μm , respectively. Body weight gain (BWG), and feed intake (FI) data were collected for day 4-12, 13-28, 29-39, and 40-49 with feed conversion ratio (FCR) being calculated for each phase and adjusted for mortality. Diets were fed in crumble form d 4-12 and pellet form d 13-49. On d 49, two average birds per pen were selected for gizzard pH and weight collection. Data were analyzed using PROC GLIMMIX

procedure of SAS as a RCBD and tested for linear and quadratic contrasts in sorghum-based diets. Dunnett's test was performed for the comparison of each S diet to the C treatment. Overall (d 4-49) birds fed sorghum-based diets had increased ($P < 0.05$) BWG and FI ($P < 0.01$) compared to those fed the corn-based diets, resulting in no evidence of difference in FCR ($P = 0.08$). Increasing sorghum particle size tended to increase (linear, $P = 0.059$) FI and BWG (quadratic, $P = 0.056$) of broilers, with the majority of the improvements in BWG occurring when PS was increased from S1000 to S1200. Broilers fed the S400 diets had decreased ($P < 0.05$) relative gizzard weight (RGW) compared to those fed C800. Broilers fed diets with increasing sorghum PS up to S1000 had increased (quadratic, $P < 0.01$) RGW. Gizzard pH decreased as S-PS increased (linear, $P = 0.05$), with only S400 having a greater pH than C800 ($P < 0.05$). In conclusion, broilers fed the sorghum based diets had increase BWG and FI compared to those fed the corn based diets. Broilers fed the S1200 diets resulted in the greatest FI and BWG. Increasing the sorghum PS up to S1000 increased RGW and decreased gizzard pH.

Keywords: sorghum; particle size; gizzard; growth performance; broilers

M147 Ontogeny of small intestinal morphology in Cobb 500 broiler chickens

Emmanuel Alagbe^{*1GS}, Jung Y. Sung¹, Jonathan Pasternak¹², Olayiwola Adeola¹ ¹*Purdue University, Animal Sciences, West Lafayette, Indiana, United States*; ²*University of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States*

Intestinal development in broiler chickens plays a key role in efficient nutrient absorption. Morphological features such as villus height, crypt depth, villus perimeter, and villus area indicate the absorptive capacity of the intestine, and these indices change dynamically as birds grow. Hence, this study investigated postnatal small intestinal maturation in Cobb 500 broiler chickens from d 0 to 28. On d 0, birds were randomly assigned to 16 replicate cages of 8 birds per cage with body weight (BW) as a blocking factor. Jejunal and ileal tissues were collected from one bird per cage on d 0, 7, 14, 21, and 28 for intestinal morphology measurements. A 5×2 factorial arrangement was used, with age (0, 7, 14, 21, and 28 d) and intestinal section (jejunum and ileum) as the two factors. Data were analyzed using the MIXED procedure of SAS. There was a positive linear effect of age ($P < 0.01$) on villus height, villus perimeter, and villus area. There was a quadratic effect of age ($P < 0.01$) on villus base width, villus mid-width, crypt depth, and villus height-to-crypt depth (VH/CD) ratio. The villus height, villus area, and VH/CD ratio were greater ($P < 0.05$) in the jejunum relative to the ileum. There was a linear effect of age ($P < 0.01$) on luminal area, total cross-sectional perimeter, and total cross-sectional area of broiler chickens. Villus height, perimeter, and area were strongly positively correlated with other intestinal morphology indices in the jejunum and ileum ($P < 0.01$). In terms of allometric relationships, all intestinal morphological indices exhibited a slower growth rate relative to the BW of birds from d 7 to 28. In conclusion, this study reveals coordinated structural changes in the intestinal mucosa that are likely important for supporting the rapid postnatal growth in fast-growing broiler chickens. It also provides a baseline for age-related intestinal maturation and links morphological development to nutritional strategies in broiler chickens.

Keywords: total cross-sectional area; broiler chickens; allometry; morphology; intestinal development

M148 Effect of insoluble grit supplementation on growth and gastrointestinal development of Hy-Line Brown pullets

Maria Alvarenga*^{1GS}, Mireille Arguelles-Ramos¹, Ahmed Ali¹ ¹*Clemson University, Animal and Veterinary Sciences, Clemson, South Carolina, United States*

Efficient gastrointestinal development is essential for optimal nutrient utilization in pullets, and insoluble grit may enhance gizzard function and digestive capacity during the rearing period. The objective of this study was to evaluate the effects of insoluble grit supplementation on growth performance and gastrointestinal development of Hy-Line® Brown pullets and its relevance to improving digestive efficiency during rearing. A total of 450 pullets were randomly allocated to three treatments: no grit (CON), 3% grit mixed in feed (MIX), or ad libitum grit (LIB), each with five replicate pens of 30 birds, from hatch to 20 weeks of age. Feed intake and body weight were collected at multiple ages, while gastrointestinal organ weights, digesta retention, and intestinal segment lengths were measured at 20 weeks. Data were analyzed using one-way ANOVA with Tukey's HSD test ($p \leq 0.05$). Feed intake did not differ among treatments at 6–8 weeks ($p = 0.696$; $p = 0.096$) but increased in grit-supplemented pullets from 11 weeks onward ($p = 0.034$). By 20 weeks, MIX and LIB birds consumed 104.7 ± 5.09 g and 108.4 ± 8.95 g, respectively, compared with 91.5 ± 6.55 g in CON ($p = 0.025$). Body weight was similar in early rearing ($p > 0.05$), but grit supplementation increased BW at 14 weeks ($p = 0.039$), 18 weeks ($p = 0.027$), and 20 weeks ($p = 0.042$), with MIX pullets consistently heaviest. Grit also increased gizzard weight ($p = 0.028$), proventriculus digesta retention ($p = 0.031$), and gizzard retention ($p = 0.041$). Total intestinal length was greater in MIX and LIB birds (169.4 ± 4.3 cm; 169.0 ± 4.6 cm) than in CON (158.0 ± 3.9 cm; $p = 0.044$). In summary, insoluble grit supplementation stimulated gizzard mass, increased digesta retention, and enhanced intestinal development, contributing to improved growth performance. Incorporating grit directly into the feed produced the most consistent benefits, indicating that sustained uniform ingestion is more effective than voluntary access. Overall, grit inclusion represents a simple and economical strategy to support digestive function and prepare pullets for the nutritional demands of the laying period.

Keywords: Insoluble grit; gizzard development; pullet; gastrointestinal tract; intestinal morphology

M149 Age-dependent responses of laying hen strains to heat stress: Impacts on blood gas profile and performance during post-peak phase

Taiwo J. Alabi*^{1GS}, Sammad F. Olayiwola¹, Hansol Kim¹, Michael J. Ford¹, Jacqueline Jacob¹, Sunday Adedokun¹ ¹*University of Kentucky, Animal and Food Sciences, Lexington, Kentucky, United States*

This study investigated age-dependent responses in blood gas parameters and performance traits of laying hen strains exposed to cyclic heat stress (CHS). A total of 240 sixteen wk-old pullets (120 Brown and 120 White) were assigned in a RCBD in a 2 (strain: White vs. Brown) x 2 (temperature: thermoneutral, TN vs. CHS) factorial arrangement with 6 hens per replicate, and 10 replicates per strain in each room. The same hens were sampled at 41, 49, and 57 wk of age for repeated-measure analysis. All birds were fed the same corn-soybean meal-based diet that met or exceeded their nutrient requirements. All hens were on the same temperature (TN 23.9 °C) throughout duration of the study, except for hens on CHS maintained at 33.9 °C for 8 hr/day on weekdays. Blood samples were collected from the first six replicates per strain in each room after 4 hr of heat exposure and analyzed using an I-STAT blood gas analyzer for pH, pCO₂, pO₂, HCO₃⁻, Na⁺, iCa, and hematocrit. Response variables measured include average daily feed intake (ADFI), hen-day egg production (HDP), feed conversion ratio (FCR), and egg mass. Data were analyzed using the MIXED

procedure of SAS for the 2x2x3 factorial, and orthogonal polynomial contrasts were applied to assess linear and quadratic age effects. Rectal temperature was higher ($P < 0.05$) in hens under HS and decreased linearly ($P < 0.01$) with age. Blood pH increased ($P < 0.05$) under HS, with higher values in white hens. Measured pO₂ and saturated O₂ showed a quadratic response ($P < 0.01$) with age, increasing then decreasing across the sampling period. The Na⁺ concentration showed a three-way interaction and a linear response ($P < 0.01$) with age. The iCa showed a quadratic response ($P < 0.05$) with age, increasing then decreasing across the sampling period. The ADFI increased quadratically ($P < 0.05$) with age, while FCR showed a similar nonlinear trend ($P < 0.05$) under HS; HDP decreased linearly ($P < 0.05$) with age. In conclusion, age modulates thermoregulatory and electrolyte responses in hens under HS, with white hens exhibited stable blood gas balance and higher thermotolerance. Early post-peak hens showed better FCR under HS, but aging reduced productivity, highlighting age and strain-specific needs.

Keywords: bird strain; blood gas; cyclic heat stress; laying hen; performance

M150 Evaluating the dose-response effects of a bacillus-based probiotic in tom turkeys under commercial-like conditions

Luis Giron*^{1GS}, Mireia Molins¹, Darby R. Boontarue¹, Brian Dirks², Hector Leyva-Jimenez², John W. Boney¹ ¹*Pennsylvania State University, Animal Science, State College, Pennsylvania, United States*; ²*United Animal Health, Sheridan, Indiana, United States*

Direct-fed microbials (DFM) are recognized as a potential alternative to antibiotic growth promoters (AGP) in poultry production. Although many studies have reported benefits of DFM for growth performance in poultry, limited information is available regarding their efficacy in turkeys under commercial conditions. This study evaluated the effects of Novela® ECL, a *Bacillus*-based DFM, on turkey performance and processing yield under commercial-like conditions. A total of 720 Nicholas Select tom turkeys were randomly assigned to one of three dietary treatments: control, control + 0.0125% Novela® ECL (1.84×10^5 cfu/kg), or control + 0.025% Novela® ECL (3.68×10^5 cfu/kg). Birds were raised on fresh litter (d 0–36) and then transferred to grow out pens containing used litter (d 37–126). Treatments were arranged in a randomized complete block design with eight replicate pens of 30 birds per treatment. Growth performance, including body weight (BW), live weight gain (LWG), feed intake (FI), feed conversion ratio (FCR), and mortality were recorded across phases. At d 126, one bird per pen was processed to measure carcass and parts yield. Data were analyzed by ANOVA (SAS), with means separated using Fisher's LSD test when $P \leq 0.05$. During early production, poulters were exposed to commercial stressors such as litter transition, bird density, and relocation to a different facility, mimicking the two-stage production system commonly used in commercial turkey production. By the end of Starter 2 (d 0–42), poulters fed 0.025% Novela® ECL were 74 g heavier and gained 72 g more than those fed 0.0125%, and 45 g heavier with 42 g higher gain than control birds. As birds adapted and the intensity of commercial stressors decreased over time, control fed birds exhibited compensatory growth, resulting in similar BW to other dietary treatments by d 126. No effects were observed on flock uniformity, mortality, or processing yields. In summary, Novela® ECL at 0.025% improved early growth under typical commercial stressors, suggesting that DFM efficacy may be greatest when birds are exposed to environmental or management stressors typical of commercial production.

Keywords: Turkey production; direct-fed microbials; stressors; growth; performance

Environment & Management II

T151 Assessment of climatic changes on broiler production in Oyo state, Nigeria

Johnson A. Olupona*¹ ¹*Federal College of Animal Health and Production Technology, Ibadan, Animal Production, Ibadan, Oyo, Nigeria*

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 while majority (80.0) of the respondents had tertiary education; however only 56.0% were broiler farmers. 90.0% practice deep litter system. Results further shows that all the respondents are aware of climate change and its effect on the birds. The farmers noticed high temperature and sunshine intensity has increased in the last five years. Also, majority (92.0%) strongly agreed that excessive heat makes the birds spread their wings, leading to lower feed intake and increase mortality. The weather is no more predictable and this 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 thereby leading 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 ($x^2=68.048$), feed resources used ($x^2=346.721, p=0.011$), types of housing ($x^2=30.113, p=0.036$) have significant relationship on the challenges of Climate change among the respondents. Farmers need to be prepared for uncertainty and develop other adaptation strategies, and governments to be involved in climate change issues. Also integrating climate change into the curriculum of agriculture in our institutions

Keywords: Broiler production; Climate change; Smallholder farmers; Adaptation strategies; Curriculum

T152 Investigating the baseline litter microbiome of turkeys over time from three producers in Iowa to enhance flock health

Cassie L. Ettinger*¹, Elizabeth M. Beilke², Casey E. McGinley¹, Michael B. Rhys¹, Jake K. Byrnes¹ ¹*Barnwell Bio, New York, New York, United States;* ²*West Liberty Foods, West Liberty, Iowa, United States*

The turkey microbiome remains understudied despite the critical role of the gut microbiome in health and performance. Establishing the characteristics of a healthy baseline microbiome is essential for identifying turkeys experiencing dysbiosis (disease states). To tackle this, Barnwell Bio collaborated with three turkey producers in Iowa to characterize the litter microbiome of their operations. Boot sock samples, an easy, non-invasive method for collecting both feces and litter, provided aggregate barn microbiome samples. One barn per operation was monitored weekly for ~10 weeks throughout the growout. Deep metagenomic sequencing was performed on weekly samples using Element AVITI short-read technology, generating over 50 million reads per sample. The

data was then analyzed using Barnwell Bio's proprietary bioinformatic pipelines. We compared the microbiomes across all three operations, benchmarking alpha diversity, beta diversity, and relative abundance patterns week over week. We found that healthy turkeys had microbiomes that showed similar alpha diversity (microbial richness) across operations (Kruskal-Wallis, $p > 0.05$). While beta diversity (microbial community composition) differed significantly by operation (PERMANOVA, $p < 0.05$), with each operation exhibiting a unique and generally stable microbiome signature during healthy periods. At one operation, we observed two adverse health events characterized by high mortality and antibiotic treatment. These events led to a sharp drop in alpha diversity, a concomitant increase in the relative abundance of commensal bacteria and subsequent recovery to an altered community composition. We also benchmarked the diversity of antibiotic resistance (AMR) genes and virulence factor genes at each operation, revealing different background potentials across operations, likely related to barn history and differing management practices. In conclusion, this work establishes healthy turkey litter microbiome baselines across Iowa operations, revealing shared health features while acknowledging operation-specific signatures. Observed shifts during adverse events demonstrate microbiome monitoring's potential for early health detection and guiding management decisions to optimize microbiome recovery and bird performance.

Keywords: Microbiome; Biosurveillance; Turkeys; Monitoring; Metagenomics

T153 Feasibility of rearing pullets in mobile pasture units over midwestern summer months

Sara Cloft*¹, Jared Trask¹, Javier S. Garcia², Marisa A. Erasmus¹, Darrin M. Karcher¹ ¹*Purdue University, Department of Animal Sciences, West Lafayette, Indiana, United States;* ²*USDA Agricultural Research Service, U.S. National Poultry Research Center, Athens, Georgia, United States*

Continuous access to outdoor range is a requirement of pasture-based systems that raises multiple logistical challenges. Mobile housing units that can be moved throughout a pasture offer advantages to providing a fixed outdoor space, but have limited inside space to protect from predators and provide environmental controls. In most pasture systems, pullets are reared indoors and moved outside when transitioning to lay. Transitioning pullets to pasture at the end of rearing may impact how many birds choose to utilize pasture. However, recommendations for mobile unit management and research into pullet rearing in such units are nonexistent. Our study reared 1,200 ISA brown pullets from hatch in common grower rooms until 6, 10 or 16 weeks of age (woa), when they were moved to pasture units (Mobile Chicken House Model 300). At each age, 300 pullets were placed in one of three mobile units with 109 ft² fenced pasture space available per bird. Placed pullets were locked inside the mobile units for 72 hours (h), trained to return to the units at night for 96 h, and monitored for an additional 72 h to assess night training. Pullets were evaluated for welfare and body condition in conjunction with placement ages and each group was weighed weekly throughout. Statistical analysis of growth consisted of z-test to compare to ISA guide and ANOVA between groups. Predation from hawks was the primary cause of mortality, at 12 woa mortality in the 6 woa group was 16% compared to only 3% in the 10 woa group. Heat stress was another concern in this study as heat index exceeded 27 C on 29 days. Due to this heat, the 10 woa group were released onto pasture after only 48 h. The 16 woa group had the poorest night trainability with birds remaining outside after the training period, whereas the other units had no birds outside. Body weights (BW) across all

groups were significantly lower than ISA guide BW at 6 and 10 woa; however, at 16 woa, despite challenges, pullets placed on pasture were heavier than ISA guide BW and pullets reared inside ($P < 0.0001$). Our project is continuing through the lay period to assess the effect of pullet age of pasture introduction on performance and range utilization, but pullets reared on pasture have similar COV for BW as those inside (7.8% vs. 8.4%).

Keywords: Pasture; Pullets; Predation; Outdoor Access; Growth Performance

T154 Evaluation of lytic phages for biocontrol of *Enterococcus faecalis* in poultry

Alba Frias-De-Diego*¹, Manuel Jara³, Mitsu Suyemoto², Rocio Crespo² ¹Tennessee Technological University, Biology, Cookeville, Tennessee, United States; ²North Carolina State University, Population Health and Pathobiology, Raleigh, North Carolina, United States; ³Tennessee Technological University, School of Environmental Sciences, Cookeville, Tennessee, United States

Enterococcus faecalis, by itself or as a mixed infection with other bacteria such as *Escherichia coli*, is responsible for up to 50% of the embryo and early chick losses in commercial poultry production. The prevalence of multidrug resistance in *Enterococci* makes conventional antibiotic treatment challenging. Bacteriophage therapy offers a promising alternative, utilizing viruses as biocontrol agents to specifically target and destroy pathogenic bacteria. Enterococcal phages have been known for over 70 years and are employed to control multidrug-resistant infections in several animal species, including humans. For this study, strain-specific bacteriophages were isolated from 24 litter samples from multiple broiler houses. The isolated phages demonstrated high lytic activity, and yielded an average of 6.6×10^{11} PFU/mL on double-agar overlays (0.6% Todd-Hewitt Broth agar), with plaques ~1 mm in diameter. Fisher's Exact test indicated no cross-reactivity between the isolated *E. faecalis* phages and other bacteria such as *Staphylococcus aureus*, *E. coli*, or *Pseudomonas* ($p < 0.01$) indicating a narrow host range. Each isolated phage was also screened against different strains of *E. faecalis* to ensure specificity (Fisher's exact test; $p < 0.01$). The whole genome of the phages was sequenced using Illumina and compared to other sequences at the National Center of Biotechnology Information (NCBI) for classification and identification of variant regions with previously described phages. In conclusion, these data indicate high-titer, lytic activity of phages isolated from poultry litter against *E. faecalis*. These findings provide a strong basis for further investigation into the therapeutic potential of these phages as a suitable method for controlling this pathogenic bacterium, and potentially leading to reduce of bird mortality in commercial poultry operations.

Keywords: *Enterococcus faecalis*; bacteriophage; biocontrol; poultry; hatchery

T155 Optimizing a BSL-2 *in vitro* litter model to evaluate IndigoLT™ amendment effects on *Salmonella Typhimurium* dynamics

Elena G. Olson*¹², Brett Hale³, Steve Ricke¹ ¹University of Wisconsin, Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States; ²Supreme EGO LLC, Middleton, Wisconsin, United States; ³AgriGro, Doniphan, Missouri, United States

Poultry litter can be a major environmental reservoir for *Salmonella*. IndigoLT™ (ILT) is a liquid litter amendment developed to mitigate microbial contamination through biological and physicochemical modification. A biosafety-level-2 (BSL-2) *in*

vitro model was established to assess ILT's direct effects on *S. Typhimurium* growth kinetics under controlled conditions. Phase 1 validated sterile calcium carbonate ("chalk")-used to attach *S. Typhimurium* for inoculation as a neutral carrier. Four chalk doses (0, 130, 1,300, and 13,000 mg per 120 g litter) were incubated for 10 days at room temperature. Aerobic, lactic acid bacteria, and Gram-negative counts (TSA, MRS, MAC) and pH were analyzed via one-way ANOVA. Phase 2 used the validated 1:100 w/v chalk ratio to inoculate litter with nalidixic acid-resistant *S. Typhimurium* chalk (64 ng/μL). Litter received ILT at 12.5 or 0 % and was incubated 7 days at ~27°C. *Salmonella* was enumerated on XLD plates containing 64 ng/μL nalidixic acid at days 1, 3, and 7. Phase 3 quantified direct ILT-*Salmonella* interactions using a 96-well kinetic assay across four ILT doses (0, 6.25, 12.5, 25 %). Optical density (OD600) was recorded every 10 min for 24 h at 37°C. Growth curves were fit to Gompertz/Baranyi models; μ_{max} , λ , and AUC that captures the rate and extent of growth were analyzed by linear mixed-effects models with Tukey HSD post-hoc tests. Chalk dose did not affect microbial counts or pH ($P > 0.05$), validating its neutrality as an inoculum carrier. Using this ratio, ILT-treated litter reduced *Salmonella* from day 1 to 3 ($P = 0.023$) and trended lower by day 7 ($P = 0.053$). In the 96-well assay, ILT caused dose-dependent inhibition: medium ($R^2 > 0.8$) and low ($R^2 > 0.9$) doses decreased μ_{max} ($P < 0.05$) and AUC ($P < 0.0001$), and λ was moderately prolonged ($P = 0.03$) in the low dose group, while the high dose completely suppressed visible growth. The BSL-2 *in vitro* pipeline provides a controlled framework for quantifying amendment effects on pathogen kinetics. ILT demonstrated distinct, dose-dependent reduction of *S. Typhimurium* growth, supporting its potential as a litter-based pathogen control strategy. Future work will extend this model to evaluate indirect, metabolite-mediated effects of ILT.

Keywords: Poultry litter; *Salmonella Typhimurium*; BSL2 *in vitro*; pathogens; litter amendments

T156 Broiler breeder feathering during the rearing and laying phases is affected by target body weight and the length of the starter feed period

Edgar O. Oviedo-Rondón*¹, Gabriele Jardim Kinchin¹, Artur De Freitas Ribeiro¹, Emily Crawford², Shivi Rao², Jesus Arango² ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²Cobb Genetics, Siloam Springs, Arkansas, United States

Poor feather development during rearing and hen feather loss during the laying phase can cause decreased fertility, as increased scratches may reduce voluntary mating. Target body weight (BW) dictates feed 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 commercial pullets were split into 16 pens. Corn-soy-wheat bran diets were fed in a dark-house during the rearing phase, with feed allocations to achieve target BWs and adjusted weekly to maintain them. Pullets were moved to a laying house. Feathering was scored at 6, 9, 14, 20, 25, 30, 35, 40, and 45 wk of age by experienced personnel. During rearing, scores (S) ranged from 1 = fully feathered to 3 = areas without feathers. In the laying phase, scores ranged from 0 to 5 (S0 = fully feathered and S5 = bald thighs). Data was analyzed using ordinal logistic regression and contingency analysis, with BW as a covariate. At 6 wk of age, the ST affected ($P < 0.05$) feathering; 34.9% of pullets had S1 and 13.4% had S3 when fed ST for 6 wk, while 42.5% of pullets had S1 and 7.8% S3 when ST was fed for 4 wk. At 9 and 14 wk,

interaction effects ($P < 0.001$) were detected. At 9 wk, 18.3% of pullets with L BW had S1 when fed ST for 6 wk, while 34.6% had S1 when fed ST for 4 wk. No differences were observed in H pullets. At 13 wk, 80.2% of H pullets had S1 when they were fed ST for 6 wk, while only 58.8% of pullets fed ST for 4 wk had S1. No differences due to ST length were observed in L pullets and 85% of pullets had S1. No treatment effects ($P > 0.05$) were observed at 20, 25, and 30 wk when almost all pullets had perfect feathering. At 35 and 40 wk, rough, broken feathers and bald areas in the backs were observed, but no treatment effects ($P > 0.05$) were detected. At 45 wk, the ST affected ($P < 0.05$) feathering independently of target BW, and 70.4% of the hens fed starter for 6 wk had S0, S1, and S2, while 64.7% of the hens fed starter for 4 wk had similar scores. In conclusion, target BW and ST affected feathering development, and 6 wk of starter feed may improve feathering at 45 wk.

Keywords: Feathering; broiler breeders; target body weight; starter phase; pullets

T157 Influence of housing system on the eggshell microbiome

Fernanda Santos^{*1}, Monika Weglarz², Jennifer Kerr³ ¹*North Carolina State University, Food, Bioprocessing and Nutrition Sciences Department, Raleigh, North Carolina, United States;* ²*USDA-ARS, Animal Biosciences and Biotechnology Laboratory, Agriculture Research Service, Beltsville, Maryland, United States;* ³*Notre Dame of Maryland University, Biology Department, Baltimore, Maryland, United States*

This study investigated the influence of two different egg production housing systems, conventional cage house (CH) and aviary (cage-free house, CFH), on the eggshell microbiome over a full production cycle. In the CH system, eggs roll out of cages onto belts, while in the CFH system, hens are free-range with access to nests, and eggs roll out of the nest onto a belt. A total of 120 eggs from CH and 240 eggs from CFH were collected and transported to the lab. The eggs were washed individually, and pooled wash samples (three egg washes/genomic sample) were stored at -80°C until processing. Whole Genome Sequencing (WGS) was performed to analyze the eggshell wash microbiome. After quality control and trimming, taxonomic classification was performed using Kraken2 and Bracken 2.5. Further analysis using HUMAnN3 provided estimates of gene family and metabolic pathway abundance. Finally, QIIME2 was used for alpha and beta diversity analyses and differential abundance analysis (ANCOM). The WGS analysis revealed significant differences in microbial diversity and composition between the two housing systems. The evenness was significantly higher ($p < 0.05$) in CH than in CFH. In CFH samples, evenness was higher in eggs collected directly on the belt, suggesting that exposure to the aviary floor leads to a higher variation in the eggshell microbiome. Pairwise PERMANOVA confirmed a significant difference in microbial diversity based on the house type ($p < 0.05$). A clear distinction between the microbial communities of CH and CFH samples was also visualized using Principal Coordinate Analysis (Bray-Curtis-Emperor-Bracken method). The ANCOM analysis identified 38 species that showed a significant difference in abundance between

the house systems, including *Staphylococcus*, *Corynebacterium*, *Fusarium*, *Acinetobacter*, and *Pasteurella*. In conclusion, the WGS analysis of eggshell wash samples showed a significant difference in microbial diversity between house systems. No specific pathogenic species were found to differ significantly between house systems

Keywords: Eggshell; microbiome; housing systems

T158 Energy and nutrients concentrate diet as strategy to mitigate the deleterious effect of cyclic environment heat stress in broilers production

Kaique M. Gomes¹, Lucimauro Fonseca¹, Fernanda G. Silva¹, Matheus G. Silva¹, Wender V. Fernandes¹, Horácio S. Rostagno¹, Arele A. Calderano¹, Melissa I. Hannas^{*1} ¹*Universidade Federal de Viçosa, Animal Science, Viçosa, Minas Gerais, Brazil*

Broiler chicks were used to evaluate the potential use of a higher energy and nutrient concentrated diet to mitigate deleterious effect of cyclic environment heat stress on growth and corporal composition. A total of 288 male Cobb 500 broilers chicks with 18 d-old ($834\text{g} \pm 5,42$ of initial body weight) were randomly divided into 4 treatments with 12 replicates and 6 birds per cage in a four control climate chambers. TN group was maintained at a thermoneutral condition ($23 \pm 1^{\circ}\text{C}$) and feed a corn and soybean meal basal diets (BD) formulate to achieve nutritional requirements proposed by Hannas et al. (2024) for male broilers from d18-27 and d27-38, HS group was subjected to 9-h of thermal stress (32°C) and feed a BD, TN-PF group was maintained at TN condition and received the same amount of basal diets than HS and HS-CD group was subjected to HS condition and received concentrated diets (CD) in energy and nutrients, formulated with 3,350 and 3,410 kcal/kg ME and 1.38 and 1.28% dLys, respectively, from d18-27 and d27-38. Broilers were evaluated for feed intake (FI), weight gain (WG), final body weight (BW) and feed conversion ratio (FCR). Body composition was measured using dual-energy X-ray absorptiometry on d38, after 8-h fasting before slaughtering birds. Data were submitted to ANOVA at 5% significance level, differences were determined using Tukey test ($P < 0.05$). From 18-38 d broilers FI, WG, final BW and FCR were affected by treatments ($p < 0.001$). TN group had higher FI ($P < 0.001$) than the other groups, also HS and TN-PF have intermediate FI and HS-CD the lowest value. Broilers in TN and HS-CD exhibit similar final BW and WG, and higher ($P < 0.001$) than HS and TN-PF. HS-CD broilers have lower FCR ($P < 0.001$) followed by TN and HS with intermediate values, and TN-PF broilers showed higher FCR compared with all treatments. On carcass composition broilers in TN and HS-CD showed similar carcass weight, lean mass, water, protein, fat and ash amount, those were higher than broilers in groups HS and TN-PF ($P < 0.05$). Thermal cyclic heat stress from d 18-38 impaired broiler performance and carcass composition, but the use of higher energy and nutrients concentrated diets was an effective strategy to mitigate the deleterious effect of cyclic environment heat stress.

Keywords: amino acids; challenge; hot; poultry; protein deposition

Metabolism & Nutrition VIII: Enzymes

T159 Effects of a phytase and NSP enzyme blend on growth performance and feed efficiency of Cobb 500 broilers fed diets with reduced nutrient density

Shameer Rasheed^{*1}, Mark LaVorgna¹, Mahmoud Masadeh¹, Gary Reznik¹, Kristy Dorton¹ *Devenish Nutrition, Fairmont, Minnesota, United States*

Three floor-pen studies were conducted to evaluate a proprietary enzyme blend containing phytase and NSP enzymes (ExtractaZyme, EZ; Devenish Nutrition, Fairmont, MN) on growth performance and feed conversion of Cobb 500 broilers fed diets formulated with reduced energy, calcium, and phosphorus. Day-old straight-run chicks (n = 1,500 in Study 1; 1,500 in Study 2; and 2,500 in Study 3) were randomly allocated to pens (50 birds/pen; 10 reps/treatment; 0.88 sq.ft/bird). Birds were fed corn-soy pelleted diets containing Zoamix. Treatments consisted of a Positive Control diet (PC), a Negative Control diet (NC) formulated with reduced nutrients (-50 kcal/lb energy; -0.16% Ca; -0.18% P), and the NC diet supplemented with EZ at 0.50 lb/ton. Study 3 included two NC diets with lower energy densities (-37 and -50 kcal/lb). Performance variables included body weight (BW), weight gain (WG), feed conversion ratio (FCR), and adjusted FCR. Data were analyzed as a one-way ANOVA using SAS with pen as the experimental unit. Means were separated using Fisher's protected LSD with significance declared at $P \leq 0.05$. Across all studies, NC diets decreased ($P \leq 0.05$) BW and worsened ($P \leq 0.05$) FCR compared with PC diets, confirming the effect of nutrient reduction. Supplementation of EZ consistently restored or surpassed performance compared to PC. In Study 1, EZ improved BW (6.40 vs. 5.87 lb; $P < 0.0001$), WG (0.160 vs. 0.147; $P < 0.0001$), FCR (1.49 vs. 1.51; $P = 0.0007$), and adjusted FCR (1.483 vs. 1.5; $P < 0.0001$) compared to NC. Study 2 showed similar outcomes with improvements in BW (6.95 vs. 6.56 lb; $P = 0.003$) and adjusted FCR (1.44 vs. 1.49; $P = 0.002$) compared to NC. In Study 3, EZ improved ($P \leq 0.02$) BW (4.94 vs. 5.66 - 5.75; $P = 0.004$) and adjusted FCR (1.69 vs. 1.587 - 1.57; $P = 0.04$) in both -37 and -50 kcal/lb NC diets, bringing performance equal or superior to PC. Numerical reductions in mortality were also observed in enzyme-supplemented treatments across studies. Overall, adding EZ helped broilers improve growth and feed efficiency, even under reduced nutrient density. These results demonstrate that EZ can support nutrient utilization, maintain bird performance, and offer cost-effective nutritional strategies in commercial broiler production systems.

Keywords: enzyme; phytase; NSPase; feed conversion; nutrient uplift

T160 Effects of an organic-approved phytase on growth performance and feed efficiency of Cobb 500 broilers fed diets with reduced calcium and phosphorus

Cody Flores^{*1}, Shameer Rasheed¹, Mark LaVorgna¹, Mahmoud Masadeh¹, Kristy Dorton¹ *Devenish Nutrition, Fairmont, Minnesota, United States*

Two studies were conducted to evaluate the efficacy of an organic-approved phytase (D-PHY Organic; D-PHY; Devenish Nutrition, Fairmont, MN) on growth performance and feed efficiency of Cobb 500 broilers fed diets formulated with reduced calcium and phosphorus. D-PHY is a 3-phytase derived from *Aspergillus niger* designed to enhance phytate phosphorus digestibility and nutrient utilization. In Study 1 (40 d), 2,000 straight-run broilers were randomly assigned to 40 floor pens (50 birds/pen; 10 reps/treatment; 0.88 sq.ft/bird). Birds were fed corn-soy pelleted diets. Treatments included: Positive Control (PC), Negative Control (NC; 0.16% less Ca and 0.18% less P), and NC + D-PHY

at 1,000 or 1,500 FTU/kg feed. In Study 2 (21 d), 512 male broilers were housed in 64 cages (8 birds/cage; 8 reps/treatment) and fed corn-soy mash diets. Treatments included PC, NC (0.13% less Ca and P), and NC + D-PHY at 500, 1,000, 1,500, 2,000, 2,500, or 3,000 FTU/kg. Performance variables included body weight (BW), weight gain, feed intake, feed conversion (FCR), and adjusted FCR. Data were analyzed as a one-way ANOVA using SAS with pen or cage as the experimental unit. Means were separated by Fisher's protected LSD ($P \leq 0.05$). In Study 1, the NC diet reduced BW ($P = 0.0006$; 5.45 vs. 5.96 lb) and worsened adjusted FCR ($P = 0.001$; 1.623 vs. 1.542) compared with PC. Supplementation with D-PHY increased BW to 6.08 lb and improved FCR to 1.498 and adjusted FCR to 1.488. This represented improvements of 7.9 points in FCR and 13.4 points in adjusted FCR versus NC, and 5.0 points in FCR and 5.4 points in adjusted FCR versus PC. In Study 2, increasing phytase levels from 500 to 3,000 FTU/kg increased ($P = 0.08$) BW from 1.83 to 1.93 lb compared with PC birds (1.78 lb). Feed efficiency improved in a dose-dependent manner with FCR improving ($P = 0.08$) from 1.219 (NC) to 1.160 at 2,500 FTU/kg and adjusted FCR improving ($P = 0.04$) from 1.225 (NC) to 1.152 — an improvement of 7.3 points vs. NC and 5.0 points vs. PC. Overall, D-PHY improved growth and FCR in broilers fed Ca and P-deficient diets. The consistent improvement in FCR demonstrates that D-PHY is an effective phytase solution for both conventional and organic production systems, supporting sustainable and cost-efficient nutrition programs.

Keywords: organic phytase; broiler; feed conversion; nutrient uplift; phosphorus

T161 Supplementation of β -mannanase in combination with phytase and carbohydrase improved growth performance of broiler chickens

Adebayo Sokale^{*1}, Bradley Turner², Brett Lumpkins³, Kendre Stringfellow¹, Anna Fickler⁴ *1BASF, Florham Park, New Jersey, United States; 2Poultry Research Partners, LLC, Athens, Georgia, United States; 3Southern Poultry Feed & Research, Inc., Athens, Georgia, United States; 4BASF SE, Ludwigshafen, Germany*

Exogenous enzymes have been demonstrated to improve nutrient utilization. This study aimed to investigate the effects of dietary supplementation of β -mannanase (mannanase) on top of a phytase and carbohydrase on the growth performance of broilers fed a diet reduced in Metabolizable Energy (ME) and amino acids (AA). A total of 1536 one-day-old Cobb 500 male broilers were distributed into 48 floor pens (32 birds \times 12 pens/treatment) and randomly allotted to 4 dietary treatments in a complete randomized design. The treatments (T) were: T1=Positive control (PC) formulated to be nutrient adequate following Cobb500 (2022) nutrient levels recommendations, T2=Negative control (NC) formulated by reducing the PC diet by 45 kcal/kg ME and 5% AA, T3= NC supplemented with mannanase A (NC+A), and T4= NC supplemented with mannanase B (NC+B). All diets contained phytase (1000 FTU/kg) and a xylanase + β -glucanase admixture, with matrix applied. Diets were corn-soybean meal based and fed in 4 phases: starter (d0-14), grower (d15-28), finisher (d29-42), withdrawal (d43-56). Body weight (BW), feed intake and mortality were measured d14, 28, 42, 56. Body weight gain (BWG) and feed conversion ratio adjusted to a common BW (FCR) were calculated. Data were analyzed using Mixed model of JMP PRO 17 and means were separated using Tukey's HSD test ($P < 0.05$). There was a significant effect ($P = 0.001$) of mannanase on FCR at 0-14 d, with the NC showing the highest FCR in comparison to other treatments. At 0-28 d, there was a significant treatment effect on BWG ($P = 0.008$) and FCR ($P < 0.001$). The NC treatment showed reduced BW compared to other treatments, and

FCR was significantly lower in PC and NC+A compared to NC and NC+B. There was a significant treatment effect on FCR at 0-42 d ($P=0.006$) and 0-56 d ($P=0.017$). The FCR differed significantly between NC, NC+A and PC, with NC+B as intermediate. In conclusion, this study demonstrated that energy and AA reduction negatively impacted growth performance, and the addition of mannanase compensated this reduction. Additionally, when used in the same diet with reduced energy and amino acids, there tended to be a difference in the response of birds to the two mannanase.

Keywords: β -mannanase; broilers; NSP-enzymes; performance; phytase

T162 Dietary protease mitigates the negative effects of trypsin inhibitors on digestive function and gut health in broilers beyond super-dose phytase. 2. Jejunum and pancreas transcriptomics

Luis F. Romero*¹, Laura M. Payling¹, Rasha Qudsieh², Hugo Romero², Frances Yan² ¹BIOFRACTAL, Loule, Portugal; ²Novus International Inc., St. Charles, Missouri, United States

Soybean meal (SBM) trypsin inhibitor (TI) levels can compromise protein utilization in poultry diets, and the interaction between dietary protease and super-dose phytase remains under evaluation. This study investigated the effects of CIBENZA® DP100 (protease) supplementation in diets varying in TI content in presence of 1,500FTU/kg phytase. Birds were fed corn-SBM-based diets arranged as a 2×2 factorial (two TI levels in feed based on average of all feed phases: normal (1.47mg/g) and high (2.57mg/g); two protease levels: 0 and 250g/MT). Tissue samples from pancreas and jejunum were collected from Ross 308 male broilers at 24d of age (10 birds per treatment). mRNA was sequenced (Illumina, 150 PE, poly-A, 20M reads/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) using an *adj* $P<0.05$ threshold. A transcriptomics-based index of chicken intestinal health (Gut SAVVY™ Index, GSI; BIOFRACTAL, Portugal) was used to quantify the effects of protease from -100% (poor health) to +100% (robust health effect). Transcriptomic data confirmed that TI strongly impaired digestive function and induced stress in both tissues. In jejunum, TI suppressed the expression of enzymes responsible for protein (QPA score = -1.8), fat (QPA score = -1.7), and carbohydrate digestion (QPA score = -1.7), along with immune sensing activation including TLR4 (QPA score = +1.4). In the pancreas, TI triggered oxidative stress induced senescence, apoptosis, and suppression of insulin signaling. Protease supplementation at 250 g/MT restored digestive enzyme expression, improved nutrient transporter activity, reduced immune activation, and reduced oxidative stress induced senescence in the intestine, with a positive GSI (+37%) versus the TI treatment. In the pancreas, protease reduced cellular stress responses and restored insulin production and signaling. These findings demonstrate that protease inclusion (250 g/MT) effectively mitigated the negative effects of dietary TI on digestive and endocrine function even though phytase was added at the super-dose level in all diets, optimizing intestinal health and systemic metabolism in broilers.

Keywords: protease; trypsin inhibitors; soybean meal; broiler; transcriptomics

T163 Effect of multi-carbohydrase or protease supplementation in reduced energy diets on growth performance and nutrient digestibility of broilers

Andres F. Bolivar Sierra*¹, Gretta Larson¹, Alejandra Forero Salamanca¹, Ashir F. Atool¹, Rob Patterson², Jorge Y. Perez Palencia², Crystal L. Levesque¹ ¹South Dakota State University, Animal Science, Brookings, South Dakota, United States; ²CBS Bio Platforms Inc, Calgary, Alberta, Canada

Supplementation with multi-carbohydrase and protease has improved fiber, metabolizable energy, and amino acid digestibility in monogastric. However, evidence on their potential energy release in reduced-energy broiler diets is limited. This study evaluated the effect of carbohydrase and/or protease supplementation in reduced-energy diets on growth performance and nutrient digestibility of broilers. A total of 810 one-day-old male chicks (40 ± 2.2 g) were distributed to 45 floor pens (18 birds/pen) in a completely randomized design, using pen location as a blocking factor. Pens were assigned to one of five dietary treatments: positive control (PC), reduced energy diet (RED1), RED1 + multi-carbohydrase (RED2), RED1 + multi-protease (RED3), and RED1 + multi-carbohydrase + multi-protease (RED4). The PC was formulated to meet breed recommendations in a brood to finish program of 3 phases (2 weeks each) and RED diets were formulated at 3.5% less AME than PC. Feed and birds were weighed at the end of each phase to assess performance. Between d14–21 diets contained 0.3% titanium oxide. Fresh feces were collected during d20–21 and analyzed along with diet samples for dry matter (DM), gross energy (GE), nitrogen (N), and titanium to determine apparent total tract digestibility (ATTD). Data were analyzed using PROC GLIMMIX in SAS. Over the entire trial there was no difference among treatments for BW ($2,509 \pm 100$ g), daily gain (61 ± 3 g), daily feed intake (91 ± 4 g), or ATTD of GE (82 ± 2 %) and DM (76 ± 1 %). The PC had lower ($P < 0.05$) FCR than RED1, RED3, and RED4 during phase 2 (1.25, 1.33, 1.32, 1.36, 1.41 ± 0.03 for PC and RED1–4, respectively) and the entire trial (1.45, 1.51, 1.47, 1.51, 1.52 ± 0.02) but was similar to RED2. The ATTD of N was higher ($P = 0.1$) in PC and RED4 compared with RED3, although similar with RED1 and RED2 (72, 70, 67, 64, 74 ± 3 %). While enzyme supplementation did not enhance broiler performance, the multi-carbohydrase had lower FCR with respect to the other reduced energy diets. The lower N digestibility observed in RED3 may suggest limited efficacy of the multi-protease under reduced dietary energy conditions.

Keywords: dietary energy; enzymes; feed additives; growth efficiency; poultry

T164 Effect of feed Supplementation with a Muramidase on productivity of commercial hens during the first phase of the laying cycle

Antoine Meuter*¹, Irene Eising¹, Marta I. Gracia², Guillermo Cano Lopez², Tamara Loeffler¹ ¹Nononesis, Vertou, France; ²IMASDE, Madrid, Spain

In the early phase of the production cycle for commercial layers, there is a critical window that lays the groundwork for the flock long-term's productivity and health. During this initial stage, producers face unique challenges – from ensuring proper nutrient intake and uniform growth to managing early stressors that can impact future laying rates. Addressing these early-phase hurdles is key to optimizing overall flock performance and egg quality down the line. Muramidase (EC 3.2.1.17) which is an enzyme that hydrolyzes bacterial cell wall peptidoglycans, has gained increasing attention for its role in improving gut functionality and nutrient utilization. Previous studies have shown that muramidase supplementation can improve feed efficiency and productivity in broilers or turkeys. Muramidase has now been evaluated in layers. The aim of the study was to evaluate the effect of dietary muramidase supplementation on the performance of Isa Brown

laying hens during early production. A 140-day randomized complete block design was conducted with 210 Isa Brown hens (22 weeks old), allocated to 42 cages (5 hens/cage, 21 replicates/treatment). Two treatments were compared: control (CON; basal diet) and muramidase (MUR) at 30,000 LSU (F)/kg feed. Data were analyzed using a General Linear Model (GLM) with Tukey's post-hoc test (SPSS v27.0). The cage was the experimental unit. Statistical significance was set at $P \leq 0.05$. MUR supplementation significantly increased laying index (95.1% vs. 90.9%, $P=0.0051$) and egg mass (57.3g/hen/d vs. 55.0g/hen/d, $P=0.0132$) compared to control. Feed conversion per kg egg (2.17 vs. 2.27, $P=0.0189$) and per dozen eggs (1.57 vs. 1.65, $P=0.0062$) were also improved in layers fed with MUR. No significant differences were observed in egg weight, feed intake, or mortality. By supporting feed efficiency, muramidase shows great potential to sustainably optimize performance of hens from point of lay up to peak production and beyond.

Keywords: Muramidase; Enzyme; Layers; Productivity

T165 Efficacy of a purified fumonisin esterase (FUMzyme®) in reducing fumonisin exposure in broiler chickens

Verena Starkl¹, ¹*dsm-firmenich, Getzersdorf, Lower Austria, Austria*

Fumonisin, secondary metabolites produced by various *Fusarium* species on corn, are among the most prevalent mycotoxins worldwide. In poultry nutrition, corn is the primary contributor to elevated fumonisin concentrations in feed. Data from the dsm-firmenich Mycotoxin Survey (2025) indicate that the 2025 U.S. corn harvest exhibited a high fumonisin prevalence of 80%, with an average contamination level of 4,711 ppb and a maximum concentration of 44,162 ppb. Mitigating fumonisin exposure during digestion represents the most cost-effective strategy to reduce its detrimental effects on poultry. Current approaches include adsorption and enzymatic biotransformation; however, to date, only one purified fumonisin esterase (FDA CFR §573.485) is authorized for use in poultry feed in the United States. FUMzyme® catalyzes the hydrolysis of fumonisins (FB1, FB2, FB3) into non-toxic or less toxic partially and fully hydrolyzed derivatives. Regulatory approval requires, among other criteria, demonstration of efficacy in reducing fumonisin levels within the animal. To address this, biomarker studies were conducted using 288 Ross 308 broilers (mixed sex; initial body weight ≈ 45 g), randomly allocated to eight pens per treatment group (12 birds per pen). Treatments included: a non-contaminated control diet, a fumonisin-contaminated (15 ppm fumonisins) diet (FUM) and a fumonisin-contaminated diet supplemented with FUMzyme® (15 ppm fumonisins + 15 U FUMzyme®/kg feed). After 35 days, FB₁ concentrations in crop content were reduced by 61% in the FUMzyme® group compared to the FUM group. Furthermore, non-toxic HFB₁ levels were approximately tenfold higher in the FUMzyme® group than in the untreated FUM group. Statistics

were carried out using T version 3.6.3.. The level of significance was $p \leq 0.05$. These findings demonstrate that purified fumonisin esterase (FUMzyme®) effectively reduces fumonisin exposure in poultry, providing a viable solution for mitigating fumonisin-related risks in feed.

Keywords: fumonisin; mycotoxin; biotransformation; fumonisin esterase; broiler

T166 Dietary protease mitigates the negative effects of trypsin inhibitors on digestive function and gut health in broilers beyond super-dose phytase. 1. Performance

Rasha I. Qudsieh^{*1}, Hugo Romero-Sanchez¹, Frances Yan¹ ¹*Novus International, Inc, Raleigh, North Carolina, United States*

Soybean meal (SBM) trypsin inhibitor (TI) levels can compromise protein utilization in poultry diets, and the interaction between dietary protease and super-dose phytase remains under evaluation. This study investigated the effects of CIBENZA® DP100 (protease) on top supplementation in diets varying in TI content in the presence of 1,500 FTU/kg phytase. A total of 1,224 Ross 308 male broilers were allocated to four dietary treatments arranged as a 2 × 2 factorial (two TI levels in feed based on average of all feed phases: low (1.47 mg/g) and high (2.57 mg/g feed); two protease levels: 0 and 250 g/MT). Phytase was added to all diets at 1,500 FTU/kg. Each treatment had 17 replicate pens with 18 birds per pen. Diets were corn-SBM-based, pelleted at 80 °C, and fed over 56 days (starter 0–14 d, grower 14–24 d, finisher 24–42 d, withdrawal 42–56 d). Performance and carcass data were analyzed by two-way ANOVA. For the overall period from 0–56d, increasing TI significantly reduced body weight gain (BWG; $P = 0.01$) by 227 g and increased feed conversion ratio (FCR; $P = 0.08$) by 9 points, while increasing pancreas weight by 20% ($P = 0.02$). Although diets were supplemented with super-dose of phytase, supplementation with 250 g/MT protease mitigated the negative effects of high TI on BWG and FCR, restoring 140 g and 4 points of BWG and FCR, respectively. Under low TI conditions, additional protease did not improve performance ($P > 0.05$). For the main effect of protease, supplementation with 250 g/MT protease increased tenderloin weight ($P = 0.03$) by 10 g, and improved carcass yield ($P = 0.05$) by 1% compared to non-supplemented group. There was no effect of TI on carcass weight or yield. These results indicate that supplementation of protease in diets containing low TI content and 1,500 FTU/kg phytase did not generate additional benefits on performance, however, when TI levels were increased to 2.57 mg/g, protease supplementation at 250 g/MT enhanced growth efficiency even in the presence of 1,500 FTU/kg phytase. Therefore, phytase super dose of 1500 FTU/kg cannot replace protease supplementation in diets with high TI content.

Keywords: Broiler; trypsin inhibitor; protease; phytase super-dose; performance

SCAD III

T167 Using the WaterFowl Alert Network to identify waterfowl habitat and waterfowl abundance in close proximity to commercial poultry farms across the U.S.

Matt Hardy^{*2}, Tristan Bond², Joseph Gendreau¹², Jaclyn Smolinsky², Maurice Pitesky¹², Jeff Buler²³ ¹*University of California, Davis, Poultry Health and Reproduction, Davis, California, United States;* ²*AgriNerds, Inc, Davis, California, United States;* ³*University of Delaware, Newark, Delaware, United States*

The current HPAI outbreak has led to the depopulation of over 175 million domestic poultry with a total economic impact of over \$3 billion dollars and counting. Since waterfowl are the primary reservoir of avian influenza viruses, understanding how waterfowl abundance/presence changes over space and time is an essential component of poultry husbandry, biosecurity, and risk assessments. The WaterFowl Alert Network (WFAN) represents the world's first tool to predict daily waterfowl distributions at fine (250m) spatial resolution. The tool currently covers the entirety of the Atlantic and Mississippi flyways within the USA and strategic parts of the Central and Pacific Flyways. Specifically, the WFAN

uses a combination of radar and telemetry-based machine learning models to predict waterfowl abundance and occupancy, respectively, via satellite imagery of land cover, weather, and other environmental variables. Here we present redacted data quantifying off-farm risk factors of waterfowl abundance and habitat around 40 farms in 3 of the 4 waterfowl migration flyways. Off-farm risk factors include daily predicted waterfowl abundance and occupancy (i.e. dynamic biotic factors) and static abiotic and biotic factors of proximity to water bodies (e.g. lakes, reservoirs, ponds, rivers, creeks, sewage treatment ponds, wastewater lagoons, quarries), agricultural fields, and other poultry and dairy operations. Results of mean waterfowl density within 4km of each farm show the greatest abundance of waterfowl in the months of November and March. From a spatial perspective we see the highest waterfowl abundance in California. To understand the qualitative risk for the surrounding natural and built environment we did an assessment using satellite imagery overlapping our WFAN model within a 4km buffer of each farm included in the study. Of the 40 farms included in this study high waterfowl abundance was found in agricultural areas with rice, corn, soybeans and winter wheat. These data demonstrate the dynamic nature of waterfowl roosting abundance around a subset of commercial poultry operations in the U.S. and the value of collecting these data to better understand the spatio-temporal risk of HPAI spillover.

Keywords: HPAI; Waterfowl; Offsite risk factors; WaterFowl Alert Network

T168 Uh oh! Dead hens: An exercise in common sense in diagnostic poultry medicine

Jason Sousa*¹, Dallas Clontz² ¹Amick Farms, Monetta, South Carolina, United States; ²Veterinary Diagnostic Pathology, Polkton, North Carolina, United States

A two-house farm with a flock of 26-week-old broiler breeder hens presented for a sudden increase in mortality. Hen mortality on this farm had been mildly elevated for the three days prior to the sudden and exponential increase in mortality. The mild increase in mortality had occurred concurrently with administration of oxytetracycline and neomycin in the water for treatment of bacterial infections secondary to histomoniasis. The grower reported that sick birds could easily be identified and seemed to convulse just prior to death. Clinical examination revealed a low percentage of morbid birds with generalized weakness/lethargy, swollen heads, and dyspnea. Otherwise, the majority of the flock appeared healthy. Gross lesions included hepatomegaly/splenomegaly, serosanguineous subcutaneous exudate, swollen heads, edematous cloacas, urolithiasis, and pale hearts and kidneys. Differential diagnosis included: avian influenza (AI), Newcastle disease virus, avian metapneumovirus (aMPV), infectious bronchitis virus, fowl cholera, gangrenous dermatitis, Selenium/Vitamin E deficiency, calcium tetany, Calcium/phosphorus imbalance, infectious coryza, and *Mycoplasma gallisepticum* (MG). Diagnostic samples collected included fresh/frozen tracheas, livers, and spleens; formalin fixed tissues for histopathology; and feed samples. PCR testing was negative for AI, MG/MS, and aMPV from tracheal samples. Calcium, phosphorus, Vitamin E, and Selenium were all within the formulated values on feed analysis. Histopathology findings were consistent with an acute systemic bacterial infection with intralosomal gram positive rods. Culture of livers and spleens confirmed presence of *Clostridium septicum* in pure culture. Treatment with oxytetracycline and neomycin was discontinued and penicillin treatment was initiated, which successfully reduced the mortality. Clinical reasoning in prioritizing differential diagnoses and diagnostic testing will be discussed in addition to

how to utilize the differential diagnosis to guide treatment decisions in poultry medicine.

Keywords: differential diagnosis; diagnostics; mortality; gangrenous dermatitis

T169 Mapping litter beetle (*Alphitobius diaperinus* Panzer, 1797) movements in a laboratory model and on a poultry research farm

Joseph A. Adeyinka*^{1GS}, J Beckmann², A Schultz², Kenneth S. Macklin⁴, Ruediger Hauck¹³ ¹Auburn University, Pathobiology, Auburn, Alabama, United States; ²Auburn University, Entomology and Plant Pathology, Auburn, Alabama, United States; ³Auburn University, Poultry Science, Auburn, Alabama, United States; ⁴Mississippi State University, Poultry Science, Mississippi, Alabama, United States

The litter beetle (*Alphitobius diaperinus* Panzer, 1797) and its larvae, the lesser mealworm, are common darkling beetles in poultry environments. They serve as a mechanical vector for Salmonella, Marek's disease virus, avian influenza virus, and other pathogens. In addition, beetles damage insulation, contaminate feed, and reduce feed efficiency when eaten by the birds. Currently, there is little knowledge of how far and fast litter beetles travel, which would be valuable information for control programs. This study mapped *A. diaperinus* distribution and movement in a laboratory model and in broiler houses and on a poultry research farm. Locomotion trials showed that single beetles fled from their point of release, traveling on average 62.5 cm within 10 minutes. Group movement was density-dependent, with beetles released in groups of 30 beetles traveling farther than beetles released in groups of 10 beetles (ANOVA, $P < 0.05$). For field data, sampling was conducted across four broiler houses over a 12-month period at Auburn University's Charles Miller Farm. A total of 26 cylindrical PVC pipe traps were positioned equidistantly within and around the litter zones in Houses 1A, 2, 4A, and 4B. Traps were inspected weekly to record beetle capture numbers. Trap data showed high beetle concentrations indoors in all four sampled houses. The highest beetle densities were consistently observed in House 1A and House 2, particularly within active litter zones, while House 2 exhibited moderate fluctuations corresponding to bird placement and removal cycles. No beetles were detected in outdoor or perimeter traps, indicating confinement to litter microhabitats. Seasonal variations revealed that captures peaked during summer and early fall, declining in winter as ambient temperatures decreased, reflecting a strong environmental influence on beetle activity and movement. Fewer than 30% of ~2,500 marked beetles were recaptured by Day 3, indicating rapid dispersal or concealment in litter. Most recaptures occurred in littered pens, with limited movement across open areas. Overall, *A. diaperinus* remains confined to litter microhabitats yet spreads quickly within houses, underscoring the need for continued ecological mapping to guide sustainable control strategies in broiler farms.

Keywords: Alphitobius diaperinus; broiler biosecurity; mark recapture; beetle movement

T170 Where are the bugs hiding? Mapping *Enterococcus faecalis* hotspots in a broiler hatchery

Nathaniel Miller*^{1GS}, Autumn Gregg¹, Erin Nemivant², Suzanne McComb⁴, Algis Martinez³, Kate Hayes³, Patti Bryant¹, Martha Pulido-Landinez¹ ¹Mississippi State University, North Augusta, South Carolina, United States; ²Oregon State University, Corvallis, Oregon, United States; ³Cargill, Wayzata, Minnesota, United States; ⁴Pecking Around Consulting, Athens, Alabama, United States

Enterococcus faecalis (EF) is increasingly recognized as an opportunistic pathogen in commercial broiler production, contributing to embryo and early chick mortality and poor performance in birds less than one week old. Its persistence and antimicrobial resistance underscore the need to identify hatchery contamination sources to improve sanitation strategies. This study evaluated the prevalence and distribution of EF in a commercial broiler hatchery in Mississippi to identify reservoirs requiring enhanced cleaning and disinfection. A cross-sectional design was used to assess eighteen sample types representing potential contamination or transmission sites: feathers and visibly clean and dirty eggs from the egg room; feathers and eggs in incubators; hatcher residues; air samples from incubator, hatcher, and chick processing halls; chick processing belts; cleaned chick boxes; tray wash dip tank; transport trays (with and without chicks); and yolk sac and cloacal swabs from pips and cull chicks. Samples were inoculated in BHI broth, directly plated on CNA agar, and incubated overnight at 37°C under microaerophilic conditions. Bacterial identification was performed by MALDI-TOF-MS-Vitek technique. Bacterial growth was evaluated using a semi-quantitative scoring system. Nearly all sampled areas tested positive for EF. In total, 95% of eggs were positive (storage room and incubator eggs). All feathers and hatch residues were positive, with variations in the type of growth. Air samples from incubator halls showed light growth, whereas hatcher and chick processing areas exhibited heavy growth. Among chick samples, 26.7% of yolk sacs and 100% of cloacal swabs were positive, with the highest incidence of yolk sac infection occurring in chicks from the oldest parent flock. Cleaned chick boxes were also contaminated. The tray wash dip tank and cleaned hatch trays were the only EF-free sites. In conclusion, EF was widespread throughout the hatchery environment, with major sources identified as eggshell surfaces, feathers, and airborne particulate matter. Improved egg pack hygiene and hatchery biosecurity and targeted cleaning protocols in egg-handling and chick-processing areas are essential to reduce the environmental persistence and early chick colonization of the bacteria.

Keywords: *Enterococcus faecalis*; hatchery; disinfection; biosecurity; broilers

T171 Transmission of variant infectious bronchitis virus field virus, DMV/1639, in Mass vaccinated and unvaccinated broilers

Cory W. Yarbrough^{*1GS}, David M. Wills¹, Brian J. Jordan³, Holly S. Sellers², Maurice Raccousier¹ ¹*University of Georgia, Athens, Georgia, United States;* ²*Ceva Animal Health, Lenexa, Kansas, United States;* ³*Zoetis, Parsippany, New Jersey, United States*

Infectious bronchitis (IB) remains an economically important and significant respiratory disease caused by infectious bronchitis virus (IBV), affecting all three segments of the poultry industry. The continual occurrence of point mutations and recombination events results in the emergence of new IBV strains, creating complexity in vaccination strategies targeting circulating strains. In this study, transmission of variant IBV field virus, DMV/1639, was evaluated in vaccinated broilers as the virus transmitted within individual passages and subsequent passage groups. Ten consecutively hatched passage groups consisting of two vaccinated and two non-vaccinated sub-groups per passage group were used. Day-old chicks were vaccinated with a commercial IBV Mass vaccine. At 21 days of age, the initial passage group was challenged with DMV/1631 field virus. Variant IBV field virus was investigated by clinical signs and virus detection post-challenge. At 4 days following exposure to variant IBV field virus, higher average clinical sign scores and increased quantities of challenge virus were detected in non-vaccinated sub-groups

among the 10 transmission passage groups. At 7 and 11 days following exposure to DMV/1639 field virus, non-vaccinated sub-groups continued to exhibit increased severity of clinical signs and higher levels of detected challenge virus within the transmission passage groups. At 14 days following challenge, similar levels of field challenge virus were detected in vaccinated and non-vaccinated sub-groups in all passage groups, indicating that viral replication had substantially decreased. Reducing the frequency of IBV infections and viral replication subsequently decreases transmission of IB, contributing to a decline in the emergence of variant strains. Emergence of novel IBV variants further complicates vaccination-based IBV control efforts.

Keywords: Infectious Bronchitis Virus; DMV/1639

T172 Development of a SINV self-amplifying replicon vaccine expressing H5 for protection against highly pathogenic avian influenza

Suman Bhattarai^{*1GS}, Brittany Henry¹, Anish Sapkota¹, Lok Raj Joshi¹² ¹*University of Georgia, Department of Population Health, Athens, Georgia, United States;* ²*University of Georgia, Precision One Health Initiative, Athens, Georgia, United States*

High pathogenic avian influenza (HPAI) continues to cause severe losses in poultry, underscoring the need for a safe, scalable and broadly protective vaccination platform. Current vaccines have significant limitations: killed vaccines lack broad protection, live vaccines carry reversion risks and mRNA vaccines are economically impractical for poultry industry. To address these limitations, we developed a next-generation Sindbis virus (SINV) self amplifying replicon vaccine designed to deliver influenza haemagglutinin (HA) antigens with the goal of enabling protection, strong immunogenicity and low-cost production. First, using a SINV reverse genetics system, we engineered replicon constructs expressing the hemagglutinin (HA) gene of a highly pathogenic avian influenza virus (H5N1) in two formats: a replication competent construct expressing the HA under sub genomic promoter and a replication deficient construct in which structural genes of SINV were replaced by the HA gene. In avian DF1 cells, we confirmed successful expression of HA protein from both replicon constructs by an immunofluorescence assay. In addition, we were able to rescue virus within 48hrs of post transfection. Recombinant SINV was successfully rescued and passaged, and subsequent infection assays in DF1 cells further verified the replicon functionality and HA expression. We further assessed vaccine delivery feasibility by generating virus-like particles (VLPs) through co-transfection of the replicon and packaging plasmids. These VLPs successfully delivered replicon RNA into DF1 cells, resulting in detectable HA expression. These findings establish a SINV self-amplifying RNA platform for expressing influenza HA antigen and support its potential application in the poultry industry. Development of a stable producer cell line for scalable VLP manufacturing is currently ongoing and in-vivo immunogenicity and protection studies are planned.

Keywords: Self amplifying replicon vaccine; Virus-like particles; Stable cell line expression; Haemagglutinin; Highly pathogenic avian influenza

T173 Evaluation of the effect of novel combinations of postbiotic and phytogenic compounds on *Histomonas meleagridis* transmission and performance in turkey poults

Diego Cortes^{*1GS}, Aaron Forga¹, Kasey A. Matusik¹, James Higueta¹, Taylor Schubauer¹, Karen Echevarria¹, Melissa Martinez¹, Maci Oelschlager², Manuel Da Costa², Benjamin Parsons¹, Danielle Graham¹ ¹*University of Arkansas, Fayetteville, Arkansas, United States;* ²*Cargill, Savoy, Illinois, United States*

The purpose of the study was to investigate the effect of novel combinations of postbiotic and phytogetic compounds on *H. meleagridis* horizontal transmission and performance in turkey poult hens. At d0, 400 poulters were randomized, tagged, weighed, and allocated across four groups: T1 Challenged Control; T2 Challenged with postbiotic A+phytogetic single compound; T3 Challenged with postbiotic A+phytogetic multi-compound; and T4 Challenged with postbiotic B+phytogetic nucleus combination. A standard starter diet was provided from d0-7 followed by an amino acid-deficient (56% restriction) corn-soybean meal diet from d7-34 to promote transmission of *H. meleagridis* in a battery cage model. Products were included in respective diets at 1.6lbs/ton from d0-34. Each group had 10 cages (10 poulters/cage). On d14, density was reduced to 8 poulters/cage, and 2 poulters/cage were intracloacally challenged with 10^5 *H. meleagridis* cells (PHL strain)/0.5mL (seeders). Seeders were commingled with 6 naïve poulters (contacts) until d27. Poulters and feed were weighed at d0, d7, d14, and d27 (seeder termination) or d34 (contact termination) to calculate BW, BWG, and FCR. Cecal and liver lesion scores (LS) were recorded for mortalities or at termination (d27 or d34). ANOVA was used to determine significant differences for performance parameters with means being further separated using Tukey HSD test. Chi-square was used to identify differences for horizontal transmission or mortality. Statistical significance was set at $P < 0.05$. No significant differences in performance were observed between treatments post-challenge. Although not significant, T4 seeders had the lowest average liver and cecal LS compared to T1 seeders. T2 and T4 contacts had markedly lower liver LS compared to the T1 contacts. The average cecal LS was significantly reduced in contacts from T2, T3, and T4 compared to T1. Horizontal transmission was significantly reduced for contacts in groups T2 (62%), T3 (57%), and T4 (63%) compared to the challenge control T1 (80%). Histomonosis-related mortality was statistically higher for contacts in T1 compared to T2, T3, and T4. These results indicate that postbiotic and phytogetic compounds may reduce *H. meleagridis* horizontal transmission, LS severity, and mortality in turkey poulters.

Keywords: Histomonas; histomonosis; postbiotics; phytogetics; turkeys

T174 Effect of dextran sodium sulfate administration on *Histomonas meleagridis* horizontal transmission

Jesus A. Maguey¹, Andressa Stein¹, Raul Marcon¹, Roberto Senas¹, Billy M. Hargis¹, Danielle Graham¹, Juan D. Latorre*¹ ¹University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States

Histomonosis is a protozoal disease caused by *Histomonas meleagridis* and characterized by the presentation of severe typhlohepatitis. Unfortunately, no commercial treatments or vaccines are currently available. On the other hand, dextran sodium sulfate (DSS) is a sulfated polysaccharide used to study ulcerative colitis in animals. Based on previous research suggesting a delayed pro-inflammatory immune response in turkey poulters against *H. meleagridis*, the objective of this study was to evaluate the impact of DSS administration on the presentation of enterohepatic lesions and transmission of *H. meleagridis*. Two independent experiments were conducted using a horizontal transmission model. Each experiment used 6 replicates of 6-10 female turkey poulters per group. In experiment 1, treatment groups included 1) Corn, 2) Wheat middlings (WM), 3) Capsaicin 0.002%, and 4) WM+DSS 0.75%. In experiment 2, treatments were 1) Corn, 2) Corn+DSS 0.75%, 3) WM, 4) WM+DSS 0.75%, 5) WM+DSS 0.4%, and 6) WM+DSS 0.45g/mL gavage. DSS was administered in the drinking water 24h before challenge. On day 8

of age, 50% of the poulters were intracloacally challenged with 100,000 histomonads/0.5 mL, and wax paper was placed in the battery cages to promote transmission. At the end of the study, liver and cecal lesion scores, as well as the percentage of transmission, were obtained after examination of all the animals. Horizontal transmission, mortality, and frequency of lesions data were analyzed using the chi-square test of independence. BW and BWG were analyzed using ANOVA in a complete randomized design with significance at $P < 0.05$. Means were further separated using Tukey's test. No detrimental effects on performance parameters were observed when DSS was administered ($P > 0.05$). Interestingly, only animals drinking water with 0.75% DSS showed a reduction in horizontal transmission (86% vs 15%) and lower cecal lesion scores (1.94 vs 0.24) in comparison to positive controls in both experiments ($P < 0.05$). In conclusion, administration of 0.75% DSS appears to mitigate horizontal transmission and lesion scores in the current set of experiments. Further research is required to understand the interaction of intestinal epithelial inflammation and its role in histomonosis prevention or severity.

Keywords: Histomonas meleagridis; dextran sodium sulfate; transmission; lesions; turkey

T175 Immune outcomes following early vaccination in chicks

Rodrigo A. Gallardo*¹, Robert Hernandez¹ ¹University of California, Davis, Population Health and Reproduction, Davis, California, United States

The chicken immune system is immature at hatch. Its main deficiencies are in heterophil and natural killer function, reducing phagocytic and oxidative capabilities. In addition, the number of T, B cells and MHC class II positive cells are reduced, gradually increasing until 8 to 9 weeks. These developmental characteristics pose challenges to day-of-age vaccination which is a practice often chosen because allows mass immunization of birds shortly after hatch. This project evaluated the immune responses induced by two vaccines administered at DOA (IBV Mass and NDV enteric) and characterized the immune cell populations following vaccination and subsequent challenge with a heterologous IBV-DMV1639 strain. After vaccination, macrophages were activated, non-conventional T cells expanded, and both tear and serum IgA and IgY levels increased compared with unvaccinated controls. These results indicate early mucosal immune conditioning; however, the cellular composition of this response remains incompletely defined. Following the heterologous challenge, vaccination modulated mucosal immunity, and the IBV Mass vaccine reduced DMV1639-induced pathology. When IBV and NDV vaccines were administered together, the largest increase in tracheal CD3+ T cells was observed. The presence of resident macrophages and T cells in dual-vaccinated chickens may support enhanced recall responses, reducing the need for additional macrophage recruitment during infection. These findings are important for understanding and optimizing early immune responses in chicks.

Keywords: Early vaccination; NDV enteric; Immunology; Vaccination; IBV

T176 VG/GA Newcastle Vaccine administered with coccidiosis vaccination improves gut health during a necrotic enteritis challenge

Christa K. Goodell*¹, John McCarty¹, Matthew K. Jones², Ramesh Selvaraj³, Tim J. Johnson⁴, Charles L. Hofacre³, Richard Phillips¹ ¹Boehringer Ingelheim, Duluth, Georgia, United States; ²Southern Poultry Research Group, Watkinsville, Georgia, United States; ³Southern Poultry Research Center, Watkinsville,

Georgia, United States; ⁴University of Minnesota, Department of Veterinary and Biomedical Sciences, Saint Paul, Minnesota, United States; ⁵University of Georgia, Athens, Georgia, United States

The Villegas–Glisson/University of Georgia (VG/GA) vaccine strain of Newcastle disease virus (NDV) is considered primarily enterotropic, though it also replicates in the respiratory tract of chickens. Enterotropic VG/GA vaccine strain induces strong Interferon γ (IFN γ), Th1, and Th2 immune responses in the gut, which can be expected to co-protect against other intestinal infections like coccidiosis. The objective of this study was to evaluate the efficacy of the VG/GA vaccine in improving gut immune response during a necrotic enteritis challenge. A total of 1400 birds were randomly allotted to 1. Negative control; 2. Precocious coccidia vaccine [CV]; 3. *Clostridium perfringens* challenge [CP]; 4. CV + CP [NE]; 5. VG/GA vaccine [VG/GA]; 6. VG/GA + CP; 7. VG/GA + NE in 8 replications. VG/GA vaccine (AVINEW®, Boehringer Ingelheim, Duluth, GA) and CV (VAXXILIVE® COCCI 3; Boehringer Ingelheim) were administered according to label dose on D0. On D 12, 13, and 14, 1×10^8 CFU/bird CP was mixed in chicken feed. Data were analyzed using ANOVA and means were separated using Tukey's HSD ($p < 0.05$). On D42, birds in VG/GA, VG/GA + CP, and VG/GA + NE had comparable BWG to the negative control group. On D15, birds in the VG/GA and VG/GA + CP groups did not exhibit a significant increase in gut permeability compared to the negative control group. On D15, birds in the VG/GA, VG/GA + CP, and VG/GA + NE groups showed no difference in jejunal Claudin mRNA levels compared to the negative control group. On D15, birds in the VG/GA groups had a significant ($p < 0.05$) increase in the cecal tonsil IL-1, IFN γ , and IL-21 mRNA and spleen IL-1 and IFN γ mRNA levels compared to the negative control groups. By D7, both VG/GA and CV vaccinated birds showed more abundant beneficial bacteria in cecal contents at the phylum level, and by D15, VG/GA, VG/GA + CP, and VG/GA + NE birds showed more abundant beneficial bacterial genera, less *E. coli* and *Clostridium* genera, and genera known to indicate gut maturation, compared to non-vaccinated control groups. It can be concluded that the VG/GA vaccine stimulated both local and systemic IL-1 and IFN γ mRNA levels, beneficially impacted gut microbial population, and reversed loss in production performances during a NE challenge.

Keywords: coccidiosis; vaccine; necrotic enteritis; VG/GA strain newcastle; cytokine

T177 Differentiation between vaccine and wildtype Avian Metapneumovirus (aMPV) subtypes A and B using the full G gene sequence amplified from clinical samples by a nested PCR assay

Maria Chaves¹, Eman Gadu¹, Silvia Carnaccini¹, Yuko Sato¹, Mohamed El-Gazzar*^{1GS} ¹Iowa State University, Veterinary Diagnostic and Production Animal Medicine, Ames, Iowa, United States

The use of live attenuated vaccines against avian metapneumovirus (aMPV) subtypes A and B has recently been authorized in commercial poultry. To effectively control aMPV, the poultry industry must be able to accurately track wildtype infections and differentiate them from live vaccines. Therefore, a reliable diagnostic tool that can distinguish aMPV wildtype infection versus a vaccine is necessary to make decisions regarding the prevention and control of the disease. The G gene in aMPV is known for its high sequence variability, and the G gene-based sequence typing remains the foundation of aMPV subtyping and phylogenetic classification. Previously developed assays can partially amplify the G gene of subtypes A and B, in two separate

assays. Partial G gene sequences can differentiate between subtypes A and B, but cannot differentiate between vaccine and wildtype. Whole genome sequencing (WGS) can achieve this differentiation but remains costly and time-consuming. We developed a nested PCR assay capable of directly amplifying the full length of the G gene of subtype A and/or B subtypes in one assay, enabling differentiation of vaccine from wildtype. Comparing all sequences from both GenBank and from clinical samples were conducted and two sets of primers were designed: (1) an outer primer set, targeting conserved interspacer regions outside the G gene used for the first round of 40 cycles of PCR amplification and (2) an inner primer set, inside the flanking regions of the G gene using the PCR product from the first round as a template for the second round of 40 cycles of PCR amplification. This approach has successfully amplified the full length of the G gene of subtypes A and B, independently or mixed in clinical samples with both low and high viral loads. Sanger sequencing followed by similarity and phylogenetic analysis clearly distinguished all authorized live attenuated vaccines in the US from wildtype infections of both subtypes A and B. Compared to other approaches, such as WGS, nested PCR amplification is practical, rapid and cost-effective, which renders the differentiation accessible to the poultry industry. Addressing this diagnostic gap allows timely outbreak response, informed vaccination strategies, and effective disease containment.

Keywords: Avian Metapneumovirus; Vaccine vs wildtype differentiation; Nested PCR sequence typing; Full length G gene; Direct amplification from clinical samples

T178 Isolation and characterization of Egg Drop Syndrome '76 from U.S. field samples

Han-Yang Wang¹, Lucas Goble¹, Samara Wartella¹, Eman Gadu¹, Laura E. Hinojosa¹, Daniel Wilson³, Luke Trimble³, Mark Mouw³, Yuko Sato¹, Mohamed El-Gazzar¹, C J. Caceres², Silvia Carnaccini*¹ ¹Iowa State University, VDPAM, Ames, Iowa, United States; ²Iowa State University, VMPM, Ames, Iowa, United States; ³Wilson Veterinary Co., Needham, Indiana, United States

Egg Drop Syndrome 1976 (EDS-76) is an emerging infectious viral disease that affects laying chickens and leads to a significant decline in quality and number of eggs. EDS-76 was recently reported in the United States in Pennsylvania in 2018 and since then has rapidly spread to commercial and backyard chicken flocks in Indiana (2021), Michigan (2022) and Missouri (2024). Currently, virus isolation attempts in various diagnostic laboratories in the US have been unsuccessful leaving the producers to import vaccines based on foreign EDS-76 isolates. Importing vaccines is less than ideal because antigenic differences between isolates can compromise protection. Additionally, sourcing vaccines globally is a complex process that introduces significant logistical and regulatory challenges for egg producers. Viral isolates are necessary for validating diagnostic assays, studying disease pathogenesis and developing inactivated and modified live vaccines. Therefore, the purpose of this research is to develop and standardize a successful method of virus isolation and propagation of EDS from field samples. Suspension made from clinically affected and PCR-positive (Ct < 20) egg shells rinses, egg shell membranes and cloacal swabs were filtered, and inoculated at various dilutions onto various substrates (cell cultures and embryonated eggs through various routes) and incubated at 37°C for up to 10 days. Embryonated eggs and cells were monitored daily for mortality and for cytopathic effect (CPE), respectively. The cell culture supernatant and allantoic fluid were collected every 24 hours until the end of each experiment and tested for hemagglutination activity and presence of viral DNA by qPCR. Next Generation sequencing was performed to amplify and

characterize the whole genome of the isolates and test for purity of the stock. Virus isolation was successful and confirmed by declining qPCR Ct values over time in tissue culture and embryonated eggs, presence of increased hemagglutination activity, and negative staining electron microscopy visualization of Adenovirus-like virus particles. In conclusion, this attempt represents the successful isolation of EDS-76 in the US with proof of virus propagation and sets up the foundational work for the development of future vaccines.

Keywords: Egg Drop Syndrome; Virus isolation; methods

T179 Evaluating the role of vaccination and oocyst management in modulating immune responses of *Eimeria*-challenged turkey poult

Demilade I. Ibiwoye*¹, Philip Yeboah¹, Thaciane L. Amaral¹, Yabaiz Tahir¹, Tracy Omasanuwa¹, Carissa Gaghan², Shelly Nolin¹, Raveendra Kulkarni², Lisa Bielke¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*North Carolina State University, Department of Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, North Carolina, United States*

Mitigating the economic burden of coccidiosis requires effective prevention measures such as vaccination and medications. This study aimed to assess the effects of *Eimeria* vaccination and intermittent amprolium treatment on growth performance, fecal oocyst shedding, and immune response in tom poult challenged with mixed *Eimeria* species. It was hypothesized that vaccination and amprolium treatments would reduce fecal oocyst shedding, improve growth performance, and modulate the immune response in poult challenged with *Eimeria*. 345 poult were randomly assigned to three groups: (a) non-vaccinated, non-inoculated (NVNI), V group vaccinated with 40% of poult receiving 100 oocysts of *E. adenoides*, *E. gallopavonis*, and *E. meleagridis* on d1, and (c) VA group vaccinated with 40% of poult receiving 100 oocysts of mixed *Eimeria* on d1 and medicated with amprolium from d8 to d12. On d21, V and VA were inoculated with 25,000 oocysts/poult of mixed *Eimeria* species. Performance was measured on d1, d21, and d28, while fecal oocyst shedding was monitored from d6 to d28 at 2-day intervals. Lesions in the ceca and duodenum were scored on d28. Immune responses were assessed by mRNA expression (IFN γ , IL-10, IL1 β , CD40, FOXP1, IL-6, and TGF β 2) analysis in the ceca and cecal tonsils, and immune cell profiling in the cecal tonsils. Data were analyzed using one-way ANOVA, with significance at $P < 0.05$. There were no significant differences ($P > 0.05$) in weight gain between the treatment groups. Fecal oocyst shedding was significantly reduced in the treatment groups, with the VA group showing the lowest oocyst count ($P < 0.05$) on d10, d14, d18, d26, and d28. Immune analysis revealed that the most significant immune modulation occurred in the VA group. Overall, vaccination combined with intermittent amprolium treatment improved performance, reduced oocyst shedding and mitigated intestinal damage while modulating the immune response, thereby offering an effective vaccination

strategy for inducing protective immunity with lowered risk of clinical coccidiosis.

Keywords: Eimeria; Poults; Vaccination; Amprolium; Coccidiosis

T180 Functional benefits of a high-flavonoid corn line (PennHFD1) on gut health of broilers subjected to a mild enteric challenge

Vinicius Buiatte*¹, Mahmoud A. Mahmoud¹, Ana Fonseca¹, Tyler Lesko², Bryan Panek², Dervan Bryan¹, Surinder Chopra², Alberto Gino Lorenzoni¹ ¹*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States;* ²*Pennsylvania State University, Plant Science, University Park, Pennsylvania, United States*

Flavonoids are plant-derived metabolites that have been described to improve the intestinal health and performance of chickens raised in antibiotic-free systems. Feed ingredients rich in flavonoids can be utilized as functional feedstuffs in poultry diets with potential health-promoting effects, including antioxidant and anti-inflammatory effects. The purpose of this study was to investigate the functional effects of a specialty high-flavonoid corn line (PennHFD1, Penn State University) on broiler chickens subjected to a mild intestinal challenge model. In a 2x2 factorial experimental design, 480 male broilers were assigned to one of the four treatments: 1. Standard diet (corn-soybean-based) formulated with a near-isogenic corn line (A-line); 2. Standard diet + PennHFD1; 3. Challenge diet (containing wheat, fishmeal, and lard) + A-line; 4. Challenge diet formulated with PennHFD1. Growth performance, gut permeability (FITC-d), oxidative stress biomarkers, and ileal gene expression of key proteins were evaluated at various time points over 21 days. Statistical analyses were performed with a mixed-effects ANOVA in R Studio, and significance was claimed when $P \leq 0.05$. Broilers fed the Challenge diet with PennHFD1 obtained a 15.3% higher body weight gain ($P < 0.01$) and a 13-point reduction in feed conversion ratio ($P < 0.01$) compared to those fed the A-line. No differences between corn types were observed in chickens fed the Standard diets. The Challenge diet significantly increased intestinal permeability ($P < 0.01$) and lipid peroxidation ($P < 0.01$), and birds fed the PennHFD1 obtained lower gut permeability ($P = 0.04$), reduced ileal gene expression of tight junctions (CLDN-1) ($P < 0.01$), and increased gene expression of mucin-2 ($P < 0.01$), which was associated with an improved intestinal barrier function. In conclusion, these findings demonstrated that dietary PennHFD1 improves intestinal health and increases growth performance in broilers under mild intestinal stress. The use of near-isogenic lines strengthens the attribution of these effects to the flavonoid content of PennHFD1, supporting the use of flavonoids as a nutritional strategy to improve poultry production in the antibiotic-free era.

Keywords: Enteric Health; Polyphenol; Antioxidant; Inflammation; Maize

Metabolism & Nutrition IX: Feed Additives

T181 The impact of *Macleaya cordata* extract on broiler chicken performance: A meta-analysis

Jose Otavio Sorbara¹, Cleverson Souza², Dimitri Freitas*², Rafael Hermes², Sergio L. Vieira³ ¹*University of Georgia, Poultry Science, Hoschton, Georgia, United States;* ²*Phytobiotics, Eltville, Germany;* ³*Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Macleaya cordata extract (MCE) is a source of alkaloids benzoquinolinic and has been proposed as a natural alternative to

antibiotic growth promoters. A meta-analysis was performed to quantify the impact of MCE (commercially known as Sangrovit) on broiler performance. The objective was to evaluate the overall effect of MCE supplementation versus a control diet across multiple independent trials conducted in different countries, years, and experimental conditions. For each response variable—feed conversion ratio (FCR), average daily gain (ADG, g/d), and cumulative in-farm mortality (%)—a linear mixed model (LMM) was fitted using the R software with the lme4 and lmerTest

packages. Each trial was treated as a random source of variation to account for between-study heterogeneity. A total of 36 reports were reviewed and data extracted; these yielded 72 paired comparisons (Control vs Control+MCE). Most data came from trials run in the USA (36), Brazil (30), Thailand (16), Germany (14), South Africa (8), and Mexico (6), with additional comparisons from Australia, Japan, the Netherlands, Poland, Russia, Saudi Arabia, and Turkey (four from each), and from France, Iran, and Israel (two from each). Half of the comparisons involved a defined challenge (coccidia, *Clostridium perfringens*, heat stress, high stocking density, or mycotoxin), and half reported no challenge. Trial duration ranged from 10 to 56 days of age, and 83% lasted ≥ 34 days. MCE did not significantly affect ADG (Control 61.86 vs MCE 63.10 g/d), but showed a significant improvement in FCR (Control 1.689 vs MCE 1.648; $\Delta = -0.041$; $p < 0.001$) and reduction in mortality (Control 6.1% vs MCE 4.6%; $\Delta = -1.5$ percentage points; $p < 0.001$). The FCR size response is comparable to those expected from antibiotic growth promoters and other feed additives. The magnitude of the mortality reduction is both statistically and operationally meaningful, warranting further investigation into which mortality categories are most affected and the production contexts that maximize the benefit. These findings demonstrate that MCE supplementation can enhance feed efficiency and survivability in broiler production systems.

Keywords: Gut; Cocci; Performance; Additive; Clostridium

T182 Effects of *Macleaya cordata* alkaloids (Sangrovit® Extra) on live performance of male broilers

Rafael Hermes^{*3}, Jose Otavio Sorbara², Sergio L. Vieira³, Dimitri Freitas³, Julia Schmitt¹, Verena Determann¹ ¹*Phytobiotics Futterzusatzstoffe GmbH, Eltville am Rhein, Germany*; ²*University of Georgia, Poultry Science, Hoschton, Georgia, United States*; ³*Phytobiotics, Porto Alegre, RS, Brazil*

Isoquinoline alkaloids (IQs), derived from *Macleaya cordata*, exhibit anti-inflammatory properties that can support performance, carcass quality and resilience of broilers. This study aimed to assess the effects of an IQs blend (Sangrovit® Extra (SE)), on the growth performance of male broilers, 1 to 42 d. The trial was conducted at Freie Universität Berlin, Germany, using 600 male Cobb 500 broilers. Birds were housed in floor pens (1.7 × 1.9 m; 0.22 m²/bird) with a final stocking density of 16 kg/m² in the end. The study had starter (1–14 d), grower (15–28 d) and finisher (29–42 d) feeds formulated with corn, soybean meal and wheat. Feeds were pelleted and treatments were graded inclusions of the additive: 0, 45, 60 and 120 mg SE/kg feed. Data were analysed by one-way ANOVA using SPSS software. Treatment means were compared using a Tukey test, probability of $p \leq 0.05$ was accepted as statistically significant, and p-values ranging from 0.05 to 0.10 were considered as trends. The results showed a dose-dependent response to the supplementation of SE with an optimal efficacy observed at approximately 120 mg/kg feed, where significant improvements in body weight gain (+2.8%; $p = 0.001$) and a trend towards reduced feed-to-gain ratio (-2.2%; $p = 0.056$) were detected compared to the control group. Lower inclusion levels (45 and 60 mg/kg feed) did not significantly affect growth performance under institute conditions with a low stocking density. Mortality was not significant and around 4%. Overall, the results demonstrate that supplementation with SE at 120 mg/kg feed effectively enhances growth performance in male broilers. This study demonstrated a clear dose-dependent improvement in broiler growth performance with SE supplementation. These effects were likely linked to the anti-inflammatory and health-promoting properties of the IQs present in SE, supporting overall broiler performance.

Keywords: Broiler; Isoquinoline alkaloids; phytogetic; Sangrovit; performance

T183 Effect of feeding a novel phytogetic feed additive on growth performance of broilers: a pooled ANOVA analysis

Mickie Metz^{*1}, Logan Erb¹, Prashant Mishra¹, Stacie Crowder¹, Morgan Thayer¹, Ellen Davis¹ ¹*Fortiva, Rochester, Minnesota, United States*

The dietary inclusion of phytogetic products has become more common, particularly as the use of antimicrobials for growth promotion is no longer permitted. Among the emerging alternatives, phytogetic additives such as Remify (Fortiva, Arden Hills, MN) have demonstrated consistent and repeatable improvements in broiler growth performance across both challenged and non-challenged environments. Remify is a proprietary blend of patented phytogetics selected through extensive *in vitro* screenings based on their ability to modulate inflammation and improve growth performance in broilers. The objective of this pooled anova analysis was to evaluate the impact of Remify on the growth performance of broiler chickens reared under non-challenged conditions, drawing on data from four independent floor pen studies. The variables included in the analysis were body weight (BW), body weight gain (BWG), mortality adjusted feed conversion (mFCR), and percent mortality. Diets for all trials were formulated to meet or exceed nutritional recommendations for broilers from d0-42 and were provided in a three-phase feeding program. In each trial, Treatment 1 (T1) was a control diet devoid of gut health additives and Treatment 2 (T2) contained 0.5 lbs/ton of Remify. The two treatments were arranged in a randomized complete block design, with the experimental unit being one pen of broilers. Analysis of variance was completed using the mixed procedure of SAS and least squares means were compared using Fisher's least significant difference ($P < 0.05$) (9.4; Cary, NC). There were no differences in percent mortality between T1 and T2 (6.2% vs. 6.4%) and there were no differences in mFCR d0-14. However, birds provided T2 had significantly improved mFCR d14-28 by 4.61-points when compared to those provided the control (1.3898 vs. 1.4359, $P < 0.05$). In addition, birds provided T2 had significantly improved mFCR d28-42 by 3.11-points when compared to the control (1.5415 vs. 1.5726, $P < 0.05$). Overall, birds provided T2 had a 3.33 point improvement in mFCR in the 42d trial period compared to the control (1.4051 vs. 1.4384, $P < 0.05$). These data indicate that Remify supplementation yields consistent and reproducible growth performance improvements in broilers, even under non-challenged conditions.

Keywords: Phytogetic; Broiler; Feed conversion

T184 Effect of a PhytoComplex additive (Fytera Perform) on performance of more than 900,000 commercial broilers

Ellen Hambrecht¹, Sandra van Kuijk¹, Marlien de Kock¹, Nienke de Groot^{*1} ¹*Trouw Nutrition, Amersfoort, Netherlands*

Fytera Perform (FP; Trouw Nutrition, The Netherlands) contains the full array of target actives from clove, cinnamon and oregano natural essential oils. Several studies with FP under controlled research conditions found that broilers supplemented with FP consistently had an improved feed conversion ratio and body weight gain (e.g. de Bruin et al., 2023; Sampath et al., 2025; Flores et al., 2025). Results from Sampath et al. (2025) and Park et al. (2025) suggest that FP may support intestinal health by modulating inflammation and maintaining gut integrity. Commercial conditions, including high stocking densities and rapid growth rates, are indeed known to trigger intestinal inflammation, which diverts nutrients away from growth towards immune response. The objective of this study was to assess the effect of FP in broilers

under commercial conditions in a large-scale field trial. A total of approximately 1.8 million broilers (either Ross 308 or Cobb 500) were enrolled in the trial, equally distributed across two production cycles, 16 houses (each with around 58,000 birds), and two dietary treatments: (1) CONTROL, a corn-soy-based commercial diet, or (2) FP, the same diet supplemented with 25 g/t FP throughout the production cycle. Average slaughter age was 36.6 days. Data for the overall trial period were analyzed in SAS using PROC MIXED considering house as the experimental unit with treatment as fixed effect. Slaughter age, round and genetics were included as covariate. Post-hoc multiple means comparisons were performed using the Tukey-Kramer adjustment. There were no interactions between genetics and dietary treatment. There were no statistically significant differences in feed intake or mortality (100.4 vs. 102.2 g/day and 3.1 vs. 3.5% for FP and CONTROL, respectively). FP resulted in higher final body weights (2.31 vs. 2.27 kg), average daily gains (63.1 vs. 61.9 g/day), and European Production Efficiency Factor (EPEF; 383 vs. 362) compared to the CONTROL ($p < 0.05$). Uncorrected feed conversion ratio was improved by almost 6 points for the FP treatment (1.592 vs 1.651, $p < 0.0001$). In conclusion, Fytera Perform improved broiler performance in this field trial, confirming previous results from studies under research conditions.

Keywords: Phytogenic; Broiler; Commercial conditions; Growth performance; inflammation

T185 Effects of polyherbal supplementation on growth performance and hepatic health in broilers

Cinta Sol¹, Felipe Horta¹, Javier Gonzalez*¹, Bernardo F. Iglesias^{2,3} *Nuproxa Switzerland Ltd., Etoy, Vaud, Switzerland;* ²INTA-EEA Pergamino, Sección Avicultura, Área de Producción Animal, Pergamino, Argentina; ³ECANA-UNNOBA, Área de Producción Animal, Pergamino, Argentina

Liver health plays a central role in nutrient metabolism, detoxification, and overall physiological resilience in poultry production. Under intensive rearing conditions, the liver is

frequently challenged by several stressors that impair its metabolic and regenerative capacity, ultimately affecting performance and productivity. This study evaluated the effects of dietary supplementation with a natural polyherbal mixture (PHM; LivoLiv™, Nuproxa Switzerland Ltd.) rich in quercetin and andrographolides, on performance and hepatic metabolic indicators in broiler chickens. A total of 540 one-day-old male Cobb500 chicks were randomly assigned to three treatments (n=10 reps; 18 birds each): 1) CON: Basal diet; 2) PHM250: CON + PHM at 250 g/t; and 3) PHM500: CON + PHM at 500 g/t. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were recorded weekly. At 34d, blood samples from two birds per replicate were collected for biochemical evaluation of hepatic and lipid profiles. Data were analyzed by ANOVA and means compared using Fisher's LSD test ($\alpha = 0.05$). All analyses were performed using InfoSTAT v2020. Supplementation with PHM improved growth performance relative to CON. At 34d, PHM500 birds showed higher BW than CON (2312^b, 2344^{ab} and 2378^ag, for CON, PHM250 and PHM500; $P = 0.04$) while PHM250 achieved the best FCR (1.482^a, 1.448^b and 1.478^a, for CON, PHM250 and PHM500; $P < 0.01$). Birds fed PHM500 displayed increases in serum AST, albumin, cholesterol ($P < 0.05$), and ALT ($P = 0.08$), suggesting enhanced hepatic activity and lipid metabolism rather than liver damage, as the values remained within physiological ranges. Such changes are consistent with improved hepatocellular metabolism, nutrient utilization, and lipid mobilization. The quercetin and andrographolides present in the PHM may contribute to these effects through antioxidant and hepatoprotective mechanisms. In conclusion, dietary supplementation with LivoLiv™ improved hepatic function and metabolic resilience in broilers, supporting better liver health and productive performance.

Keywords: broiler performance; hepatic metabolism; polyherbal supplementation; liver health; quercetin and andrographolides

Metabolism & Nutrition X: Amino Acids

T186 Influence of the timing and amount of amino acid intake from 25 to 46 d post-hatch on the performance and yield of Ross YP × 708 broilers

Samuel J. Rochell*¹, Chance Williams², Mark Lemons², Roshan Adhikari³, Jason Lee³ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Wayne-Sanderson Farms, Oakwood, Georgia, United States; ³CJ Bio America, Downers Grove, Illinois, United States

The influence of the timing and amount of digestible amino acid (AA) intake during the finisher 1 (F1) and 2 (F2) periods on the performance and meat yield of male Ross YP × 708 broilers was evaluated. Beginning at 25 d post-hatch, birds were provided 1 of 6 treatments based on a factorial arrangement of 2 digestible AA densities in the F1 (100 or 110%) period from 25 to 36 d × 3 AA densities (85, 100, of 115%) during the F2 period (36 to 46 d). Amino acid density was formulated relative to primary breeder recommendations, and ratios of digestible essential AA relative to Lys were similar across diets within phase. Treatments were replicated with 12 pens of 25 birds, and 8 birds per pen were processed at 47 d. Data were analyzed by 2-way ANOVA with F1 and F2 AA density as main effects. Amino acid density did not influence body weight gain (BWG) during the F1 period, though birds fed the 110% diet had lower ($P < 0.05$) feed intake (FI) and FCR than birds fed the 100% diet. During the F2 period, only main effects of F2 AA density were observed, with no carryover or interactive effects ($P > 0.05$) of F1 AA density. Specifically, BWG

was lowest for birds fed 85% AA density, highest for those fed 115% AA density, and intermediate for those fed 100% AA density. Feed intake of birds fed the 100 and 115% AA diets were similar, but were both lower ($P < 0.05$) than those fed the 85% diet. Feed conversion ratio was reduced ($P < 0.05$) in a stepwise manner by increasing AA density during the F2 period. For the overall experimental period, BWG and FI were only influenced by F2 AA density and followed the same trends observed in that phase, whereas FCR was independently reduced ($P < 0.05$) by increasing F1 or F2 AA density. Breast meat weight and yield were influenced ($P < 0.05$) by F2, but not F1, AA density with both measurements being lowest for birds fed the 85% AA diet (24.2%), highest for those fed the 115% diet (24.76%), and intermediate for those fed the 100% AA diet (24.54%). Relative fat pad weight was reduced ($P < 0.05$) in an independent and stepwise manner by increasing F1 or F2 AA density. In conclusion, these data demonstrate the potential to adjust dietary AA density in response to market conditions as birds approach target processing weights.

Keywords: amino acid; broiler; lysine; intake; yield

T187 Validation of a simplified indicator amino acid oxidation procedure for evaluating lysine utilization in relation to egg production in aged broiler breeders

Diego Martinez¹, Cole Umberson¹, Carol Ponce-de-Leon², Oscar H. Aguilar¹, Lindsey Conaway³, Erik Pollock³, Craig N. Coon*¹ ¹University of Arkansas, Department of Poultry Science,

Fayetteville, Arkansas, United States; ²Independent Researcher, Fayetteville, Arkansas, United States; ³University of Arkansas, Stable Isotope Laboratory, Fayetteville, Arkansas, United States

This study evaluated a simplified procedure for the Indicator Amino Acid Oxidation (IAAO) technique to assess digestible lysine utilization in aged broiler breeders with varying egg production. Twelve female breeders of a new genetic line (CDP5), over 80 weeks old and producing 0–0.7 eggs/day, were housed in 12 respiratory chambers equipped with periodic breath sampling and adapted for 2 days to a balanced control diet. Hens received six dietary treatments (0.23, 0.33, 0.42, 0.89, 0.99, and 1.09% dig. LYS), one per day, completing all treatments over 6 days. Half of the daily feed was divided into thirteen 5-g meals offered every 30-min; the remainder was fed after the IAAO procedure. Meals 1–4 and 6 contained no isotope; meal 5 provided a priming dose of 4.5 mg L-[1-¹³C]phenylalanine (¹³C-PHE) per kg BW; meals 7–13 supplied 1 mg/kg BW each. Air samples were collected at 30-min intervals (13 per hen/day), starting before meal 2, and analyzed for ¹³CO₂ enrichment by isotope-ratio mass spectrometry (IRMS). One ¹³C enrichment curve per hen/day was generated, and mean baseline and plateau ¹³C values were calculated using two alternative approaches: A (all 13 samples) and B (3 predetermined baseline and 3 plateau samples). Corresponding $\Delta\delta^{13}\text{C}$ values were computed as plateau minus baseline for each approach. Data (n = 72 per approach) were analyzed in JMP Pro 16 with a mixed model including calculation approach as a fixed effect and egg production rate as a random effect. A simple regression compared approach A (measured) with approach B (predicted). Validation criteria included the mixed-model approach effect, prediction error, and R². Approaches A and B yielded similar $\Delta\delta^{13}\text{C}$ values (P>0.05), with prediction error <10% and R²>0.80. Lysine utilization was affected by egg production rate (P<0.05). In conclusion, the simplified approach is suitable for assessing digestible amino acid utilization in old breeders, and egg production rate influences their digestible lysine requirements.

Keywords: Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; Lysine; Isotope

T188 Application and validation of a simplified indicator amino acid oxidation procedure to assess essential amino acid utilization during peak production in broiler breeders

Diego Martinez*¹, Oscar H. Aguilar¹, Carol Ponce-de-Leon², Lindsey Conaway³, Erik Pollock³, Craig N. Coon¹ ¹University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ²Independent Researcher, Fayetteville, Arkansas, United States; ³University of Arkansas, Stable Isotope Laboratory, Fayetteville, Arkansas, United States

This study evaluated a streamlined version of the Indicator Amino Acid Oxidation (IAAO) technique to characterize essential amino acid (AA) utilization in broiler breeders at peak production. Twelve 30-week-old hens of a new genetic line (CDP5) were placed in respiratory chambers and adapted for 2 days to a control diet. A mixed-model Latin-square-type arrangement (6 diets × 6 hens per tested AA) was applied to assess each digestible AA (LYS, TSAA, ARG, ILE, THR, VAL, TRP, and CP), evaluating two AA at a time. For each AA, hens received six diets containing three limiting and three excessive concentrations, one diet per day, completing the sequence within 6 days. Eighty percent of the daily feed was divided into thirteen 8-g meals fed every 30 min; the remaining 20% was fed after completing the IAAO assay. Meals 1–4 and 6 were isotope-free; meal 5 contained a priming dose of 4.5 mg L-[1-¹³C]phenylalanine (¹³C-PHE) per kg BW; meals 7–13 provided 1 mg/kg BW each. Air samples were collected at 30-min intervals (13/hen per day), starting before meal 2, and analyzed for ¹³CO₂ enrichment via isotope-ratio mass spectrometry. For

each hen/day, a ¹³C enrichment profile was generated, and mean baseline and plateau ¹³C values were estimated using two calculation strategies: A (all 13 samples) and B (3 predetermined baseline plus 3 plateau samples). $\Delta\delta^{13}\text{C}$ was calculated as the plateau–baseline difference for each method. Data (n = 288 per approach; 36 per AA) were analyzed in JMP Pro 16 using a mixed model with calculation approach, AA tested, and their interaction as fixed effects, and testing order as a random effect. A simple regression compared approach A (observed) with approach B (predicted). Validation criteria involved the mixed-model approach effect, prediction error, and R². Approaches A and B showed similar $\Delta\delta^{13}\text{C}$ values (P>0.05) with no AA × approach interaction (P>0.05), prediction error <10%, and R²>0.85. The SD of the minimum dietary concentration at maximum utilization was also calculated and was found comparable between approaches (P>0.05). In conclusion, the simplified IAAO calculation method is appropriate for evaluating digestible amino acid utilization in breeders at peak production and can provide confidence intervals that capture flock variability.

Keywords: Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; Isotope; Peak production

T189 KOH protein solubility is relevant in the manufacture of mechanically extracted soybean meal by dry extrusion

Nelson Ruiz*¹, Carl M. Parsons², Benjamin Parsons³, Jason Buseman⁴ ¹Nelson Ruiz Nutrition, LLC, Suwanee, Georgia, United States; ²University of Illinois, Animal Science, Urbana, Illinois, United States; ³University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ⁴Anderson International Corporation, Stow, Ohio, United States

The objective of this research was to define the quality parameters that correlate with poultry amino acid digestibility (AAD) in a set of samples derived from commercial lots of mechanically extracted soybean meal (MESBM). A total of nine samples corresponding to an equal number of lots were selected. KOH protein solubility (KOHPS) was conducted at Dairyland Labs (Arcadia, WI) utilizing the standardized procedure by Ruiz et al. (2025) with only one modification: grinding of the sample was done with a cryomill to generate a consistent particle size. Trypsin inhibitor activity (TIA) was measured at Eurofins Labs (Des Moines, IA) utilizing the AOCS (2020) method. In vivo AAD was conducted at the Animal Science Lab at the University of IL. Data were analyzed using the GLM procedure in SAS (2023) to assess correlations between KOHPS values & digestible amino acid (DAA) coefficients as well as correlations between TIA values & DAA coefficients. The significance value for all analyses was P≤0.05. A wide range of KOHPS values (83.73, 82.71, 77.21, 76.27, 74.56, 73.44, 66.01, 63.78, 38.60%) was highly correlated (R²=0.66; r=0.81; P=0.0076) with their corresponding digestible LYS (dLYS) coefficients (0.86, 0.88, 0.85, 0.80, 0.85, 0.80, 0.80, 0.81, 0.77). The KOHPS was also significantly correlated (P≤0.05) with dASP & dGLU coefficients. In contrast with solvent extracted SBM, for MESBM there was a non-significant correlation with dARG (P≥0.05). A wide range of TIA values (12.85, 11.50, 10.50, 7.59, 16.67, 15.38, 14.40, 5.03, 3.50 TUI/mg) was observed in the 9 MESBM samples, but TIA was inversely correlated only to digestible MET (R²=0.49; r=0.70; P=0.0358). Even though Ruiz et al. (2023) reported that in vivo AAD was inversely correlated to the trypsin and chymotrypsin inhibitor contents in solvent extracted SBM, those SBMs were high in KOHPS (~80%). In the present study the three highest TIA values (16.67, 15.38, 14.40 TUI/mg) are in samples with low KOHPS values (74.56, 73.44, 66.01%, respectively) explaining why no other inverse correlations between TIA and DAA coefficients are observed. In

conclusion, these data indicate that KOHPS is the predominant QC parameter in MESBM to assess its nutritional quality.

Keywords: KOH protein solubility; Trypsin inhibitor activity; Digestible lysine; Mechanically extracted soybean meal; Solvent extracted soybean meal

T190 The effect of breed, varying digestible lysine concentrations, and elevated amino acid ratios on the performance and processing metrics of broilers during a 42-day production period

Opeadura T. Osunbami*¹, Corey Johnson¹, Anthony Quant¹, Tanner Wise¹, Elizabeth Miller¹, Nickki Tillman² ¹*Kalmbach Nutritional Services, Upper Sandusky, Ohio, United States*; ²*Nutritional Statistics LLC, Buford, Georgia, United States*

This study evaluated the effects of digestible lysine (Dig Lys) concentration, amino acid (AA) ratios, and broiler breed on growth performance, carcass yield, and economic performance of broilers over a 41-day period. A total of 5,760 straight-run broilers (Ross 308 and Ross 308 AP) were allotted to 96 pens (60 birds/pen) in a 2 × 2 × 2 factorial arrangement with 2 breeds (Ross 308 vs. Ross 308 AP), 2 Dig Lys levels (standard vs. high), and 2 AA ratio levels (standard vs. high). Birds were fed four dietary phases (starter, grower, finisher, and withdrawal) formulated to meet or exceed Aviagen recommendations. Body weight and feed intake (FI) were measured on days 0, 10, 21, 32, and 41, and four birds per pen (two males and two females) were processed on day 42 for

carcass yield evaluation. Data was analyzed using a three-way ANOVA, with pen as the experimental unit and location as a blocking factor. Results showed no significant three-way interactions among breed, Dig Lys, and AA ratio, and for any measured parameters. However, breed significantly affected overall performance, as Ross 308 AP broilers exhibited higher ($P < 0.05$) body weight gain (BWG; 3.07 vs. 2.84 kg) and FI compared with Ross 308. The Dig Lys concentration influenced ($P < 0.05$) BWG and mortality-adjusted feed conversion ratio (FCRc) during the starter and grower phases, but the effect was not evident thereafter. Similarly, AA ratio impacted ($P < 0.05$) BWG during the starter phase and FCRc across both starter and grower phases. Processing yields were also affected by breed ($P < 0.05$) as Ross 308 AP had greater breast, thigh, drumstick, and wing yields than Ross 308, and this was consistent in both the male and female birds. Economic analysis revealed that Ross 308 AP achieved higher ($P < 0.05$) income over feed cost compared with Ross 308, whereas Dig Lys concentration and AA ratio had minimal influence. In conclusion, broiler breed exerted the strongest influence on production outcomes, whereas adjusting Dig Lys or AA ratios beyond standard levels provided limited benefits beyond the grower phase. Further studies evaluating these interactions under stress or varying environmental conditions could refine amino acid precision formulation strategies for modern broiler strains.

Keywords: amino acid; breed; broiler; feed efficiency; yield

Metabolism & Nutrition XI: Vitamins & Minerals

T191 Zn–Methionine Hydroxy-Analogue Chelate supplementation improves carcass quality in broilers under commercial conditions

Ana C. Ferreira*³, Kelen Zavarize², Liris Kindlein¹, Kelvin Prado³, Elisangela Gottardo³ ¹*UFRGS, Porto Alegre, Brazil*; ²*Novus, Indaiatuba, Brazil*; ³*Bello Alimentos, Itaquiraí, Brazil*

Broiler productivity is now judged not only by growth metrics but also by carcass and cut quality; condemnations and visual defects reduce chain efficiency and must be factored into nutritional strategies. Usage of lower dosages of Zn in the form of Methionine-Hydroxy-Analogue Chelate can be an alternative due to its higher bioavailability. The objective of this study was to evaluate the effect of replacing inorganic (Zn Sulfate) by reduced levels of organic minerals (Zn Methionine-Hydroxy-Analogue Chelate) on the skin quality, incidence of footpad, and carcass condemnations in broilers. The trial was conducted under commercial field conditions in the Central-West region of Brazil, which is predominantly tropical, using a randomized block design with control and treated flocks monitored over a four-month production period. In total, approximately 8 million broiler chickens received supplemented diets and were distributed into two groups: control - 120 ppm of zinc sulfate and test - 40 ppm of Zn-methionine hydroxy analog chelate. Analyses were conducted to evaluate incidence of arthritis, repugnant aspect, and skin dermatitis in carcasses. In addition, the occurrence of breast burn and broken skin (skin lesions) was recorded as indicators of carcass quality. Footpad dermatitis was also assessed scores (A/B and severe cases). The results indicated that supplementation with Zn–Methionine Hydroxy-Analogue Chelate significantly reduced the incidence of arthritis (1.14% vs 3.26%; $p < 0.01$), repugnant aspect (0.31% vs. 1.05%; $p < 0.0001$), and skin dermatitis in carcasses destined for disposal (0.43% vs. 2.56%; $p < 0.05$), when compared with the control group. A reduction was also observed in breast burn (18.2% vs. 32.6%) and broken skin (13.5% vs.

25.2%) in supplemented birds, representing an improvement of approximately 40% in the main causes of condemnation. For footpad dermatitis, a higher proportion of feet were classified as A/B in the Zn–Methionine Hydroxy-Analogue Chelate group, with a reduction in severe cases. In conclusion, supplementation with 40 ppm of zinc in the form of Zn–Methionine Hydroxy-Analogue Chelate contributed to improved Skin and footpad quality, reduced condemnations, and improved efficiency of meat production under commercial conditions in Brazil.

Keywords: footpad dermatitis; carcass condemnations; skin lesion; arthritis; organic mineral

T192 Effect of calcium pidolate supplementation and double-buffered sodium butyrate formulation in diets without phosphate and low Ca-P level on broiler performance, bone quality and intestinal histomorphology

Laure Duboc¹, Clemence Marecaille¹, Romain Bouvet³, Julián Melo*^{1,2} ¹*Dietaxion SAS, Le Loroux-Bottereau, France*; ²*UNLu, Tecnología, Luján, Argentina*; ³*Zootests, Ploufragan, France*

Calcium pidolate (PID) improves active transport of calcium (Ca) and can increase indirectly phosphorus (P) digestibility. On the other hand, the early release of butyric acid shown by double buffered sodium butyrate (DBSB) can improve feed digestibility, performance and intestinal health of broilers. The objective of this study was to evaluate the effect of dietary supplementation of PID and formulation of DBSB in diets without phosphate and low Ca-P level on the performance, bone quality and intestinal histomorphology of broilers. A total of 320 one-day-old male broiler chicks (Ross 308) were allocated in floor pens in a completely randomized block design (8 replicates/treatment). Starter (0-12d), grower (12-28d) and finisher (28-35d) diets were used. Control diets (CTRL) corresponded to the nutrient industry levels (500 FTU). Low Ca-P diets (LCP) were reduced in average by a 20% Ca_T and P_{dig} using for formulation a 2% Energy-Protein

Matrix for DBSB. PID (PIDOLin PCa, Dietaxion) and DBSB (BUTYLIn® 54, Dietaxion) were included at 300g/MT (1-12d) and 600g/MT (0-35d), respectively, including also 1500 FTU of phytase in the starter diet and 1000 FTU in the other diets. Feed intake and body weight (BW) were measured to calculate feed conversion ratio (FCR). At 35d, 8 broilers per treatment were slaughtered to measure villi length (VL) and crypt depth (CD) in the jejunum, tibia weight (TW) and strength (TS). Parametric data were analyzed using ANOVA and non-parametric data through Kruskal-Wallis test. BW, FCR, TW and TS between treatments were not statistically different ($P>0.05$), showing LCP birds only numerically higher BW, TW and TS ($P>0.05$). FCR was similar at the end of the experiment ($P>0.05$). LCP broilers had higher VL/CD ratio (+17%, $P<0.05$) than CTRL. In conclusion, the supplementation of 300 ppm of PID (0-12d) and the formulation of 600 ppm of DBSB in broiler diets combined with a high dose of phytase enabled to formulate diets with low level of Ca-P, keeping performance and bone traits at a similar level, but also improving gut health. The combination of PID, DBSB and high dose phytase then can be considered an effective strategy to feed cost savings and excretion reduction.

Keywords: broilers; butyrate; pidolate; phosphate; phytase

T193 Effect of different calcium sources and concentrations on the performance, blood minerals and tibia bones strength of layer pullets

Fausto Solis^{*1}, Ahmad Mehaad¹, Terry Meyer¹, Chris Olinger¹ ¹*The Wenger Group, WNSIC, Lancaster, Pennsylvania, United States*

Leg diseases in the United States have caused economic losses of more than 80 million US dollars in the broiler industry. Many nutritional strategies have been evaluated to improve bone strength that can mitigate the leg problems in poultry. Calcium carbonate is the most common source of calcium in livestock feeding. However, additional sources of calcium (calcium chloroide, calcium pantothenate, calcium sulfate, and calcium borogluconate), have been evaluated on the performance, blood mineral, and tibia bone strength. At nine weeks of age, calcium propionate increased body weight compared to calcium carbonate. The other mineral sources do not have difference compared to calcium carbonate. Calcium propionate also increased the blood calcium content to 12.93 mg/dL from 12.00 mg/dL of calcium carbonate. A follow up research study was conducted with the purpose of evaluating the effects of the source and percentage (%) of calcium in the pre-lay ration of caged layer pullets. The sources of calcium tested were calcium carbonate included at 100% and calcium carbonate at 70% and calcium propionate at 30% of inclusion. The % of calcium tested were 2.00%, 2.25%, 2.50% and 2.75% in a factorial arrangement of a randomized complete experimental design. The data were analyzed with the Statistical Analysis Software (SAS) and mean separation was carried with Tukey Multiple Range test with 5% of error. The thickness and the wideness of the bones were not significantly ($P>0.05$) different in any of the sources or content of calcium. However, the maximum flexure load of the bones increased ($P<0.05$) from 12.37 kgf in the 2.75% 100% calcium carbonate to 12.98 kgf, 13.15 kgf, 12.46 kgf and 13.06 kgf when the calcium carbonate was included at 70% y calcium propionate at 30% with the calcium contents of 2.00%, 2.25%, 2.50% and 2.75%, respectively. There was not significant difference of Bone Mineral Density (BMD) for any of the treatments formed by calcium sources and content. The bone surface increased from 61 mm² in the 100% calcium carbonate to 77 mm² in the 70% calcium carbonate and 30% of calcium propionate. In conclusion, the alternate calcium sources to calcium

carbonate may contribute to improving bone strength and potentially reducing leg health issues in poultry.

Keywords: Sources; Calcium; Bone; Tibia; Poultry

T194 The use of NanoSIMS and Synchrotron techniques to explain eggshell resistance in aged layers

Iris Henriquez Valido²³, Maria Angels Subirana², Stéphane Durusoy^{*1}, Yron Joseph Y. Manaig⁴, Stéphane Faucher², Pascale Senechal², Peter Moonen², Sandra Mounicou², Dirk Schaumlöffel² ¹*Animine, Annecy, France;* ²*Université de Pau et des Pays de l'Adour, Pau, France;* ³*Universitat Autònoma de Barcelona, Barcelona, Spain;* ⁴*Animine, R&D, Barcelona, Spain*

Old layer hens have eggs with a thinner shell, increasing the problems related to broken eggs and shell defects. This study evaluated the effect of trace metal (TM) supplementation on eggshell quality and developed a bioimaging method to understand TM incorporation into the eggshell and membrane of aged layers. A total of 936 White Leghorn hens (60 weeks) were allotted to three treatments (12 replicates of 26 birds): a negative control (NC) diet without TM supplementation (43 ppm Zn, 36 ppm Mn, close to NRC (1994) requirements), a Zn diet (NC + 90 ppm Zn from ZnO; HiZox, Animine), and an Mn diet (NC + 110 ppm Mn from MnO; ManGrin, Animine). After 20 weeks, eggs were analyzed for fracture force and shell thickness ($n=200$ eggs/treatment), and correlative imaging (X-ray tomography, LA-ICPMS, NanoSIMS; $n=1$). Pieces of membrane were separated from the eggshell and analyzed by ATR-FTIR. Fracture force and shell thickness were analyzed by the model $X_{ij} = \mu + \alpha_i + \epsilon_{ij}$ (X , measured trait; μ , average; α , effect of I treatment; ϵ , residual), and means separated by LSD test (SAS Software). Shell thickness was improved ($P<0.05$) by both Zn and Mn supplementation (0.35 mm) in comparison to NC (0.34 mm). However, fracture force was more increased ($P<0.05$) when hens received Mn diet (37 N) than Zn or NC diets (35 N in average). The development of correlative imaging technique (X-ray tomography and LA-ICPMS) showed that Mn and Zn are located in the outer membrane of the eggshell and in the cuticle, whereas Ca is mainly found in the palisade and mammillary layers. NanoSIMS images indicated greater Ca presence in Mn-supplemented eggs, possibly due to Mn influence on Ca-binding proteins, explaining the higher fracture force. Synchrotron FTIR showed increased polysaccharide signals in membranes and nucleation sites from Mn-fed hens. These findings, although based on a single imaging sample, suggest basal Zn and Mn levels are insufficient to maintain shell quality. Mn supplementation exerts a stronger effect than Zn, improving eggshell integrity likely via enhanced membrane composition and Ca-binding processes. Further research is needed to confirm Mn's role in shell biomineralization mechanisms.

Keywords: egg quality; manganese; zinc; NanoSIMS; layers

T195 Gene expression explained the benefits of HMTBa-Chelates of zinc and manganese under different copper programs on broiler performance

Hugo Romero-Sanchez^{*1}, Frances Yan¹, Bob Buresh¹, Melanie Roux¹, Laura M. Payling², Luis F. Romero² ¹*Novus International, Chesterfield, Missouri, United States;* ²*Anh-Innovation, Biofractal, Loule, Algarve, Portugal*

A 49-day broiler study evaluated the replacement of inorganic zinc (Zn) and manganese (Mn) with HMTBa-cheated mineral (MMHAC) under varying copper (Cu) sources and levels on growth performance, carcass, and gene expression. A factorial design combining three Cu sources—tri-basic copper chloride (TBCC, 125 ppm), MMHAC Cu (30 ppm), and Cu sulfate (10 ppm)—with two Zn/Mn sources (sulfates at 100/100 ppm or

MMHAC at 40/40 ppm), was analyzed by GLM procedure. Each treatment had 15 replicates of 20 Ross 708 male broilers per pen, challenged with a 5x coccidia vaccine at 16d. At 28d, liver and *pectoralis major* samples were collected from the high Cu sources for gene expression analysis. mRNA was sequenced (Illumina, 150 PE, poly-A enriched, 20M reads per sample) and gene abundance calculated using Kallisto. Differentially expressed genes were tested (Deseq2), followed by a topology-based Quantitative Pathway Activation method (Biofractal, Portugal) using an $\text{adj}P < 0.05$ threshold. High Cu inclusion, from either TBCC (125 ppm) or MMHAC Cu (30 ppm), significantly ($P < 0.001$) enhanced average daily gain (ADG) and feed conversion ratio (FCR), at 49d, compared to low Cu sulfate (10 ppm). MMHAC Zn/Mn supplementation significantly ($P < 0.05$) improved BW at 28d and FCR and ADG between 14–28 d, compared with sulfates Zn/Mn, regardless of the Cu level and source. No significant Cu \times Zn/Mn interactions were detected, indicating consistent benefits of chelated Zn/Mn across Cu programs. Birds receiving MMHAC Cu (30 ppm) maintained performance comparable to TBCC at 125 ppm while reducing proventricular lesions at 49d. Gene expression analysis demonstrated that chelated Zn/Mn upregulated liver genes related to mTOR signaling and the TCA cycle, while in muscle, it reduced amino acid catabolism, supporting lean tissue accretion. Chelated Cu further modulated hepatic Fe and Cu transport genes, reduced stress sensitivity, and favored nutrient-driven anabolic signaling. Overall, MMHAC Zn/Mn supplemented at 40 ppm effectively replaced 100 ppm inorganic sources, enhancing growth, FCR, and proventricular integrity. In TBCC-fed broilers, HMBa-chelated Zn/Mn activated genes related to protein accretion, limited amino acid degradation, and inhibited lipid metabolism.

Keywords: Chelate; Copper; Zinc; Manganese; Gene expression

T196 Efficacy of a natural Vitamin E and organic Selenium Complex (E Sel Power) compared with synthetic Vitamin E on productivity, egg quality, and antioxidant status in commercial laying hens

Paramartha Roy*¹ *Indian Herbs Specialties, Chandigarh, Chandigarh, India*

Metabolism & Nutrition XII: Feed Additives

T197 Effects of a commercial triple-strain *Bacillus*-based probiotic on egg quality, performance, welfare and livability of laying hens

Tamara Loeffler*¹, A. Meuter¹, M. Schwartz¹, Dan Moore² *¹Novonesis, Parkland, Florida, United States; ²Colorado Quality Research, Wellington, Colorado, United States*

Laying persistence in commercial hens requires constant attention to egg quality, feed efficiency, and bird welfare throughout the laying cycle. Nutritional interventions that establish a balanced gut microbiota from the first day of life can positively influence long-term productivity and health. This study evaluated the impact of dietary supplementation with a commercial triple-strain *Bacillus*-based probiotic on production performance, egg quality, livability, and welfare in laying hens. A total of 360 Leghorn day-old chicks were assigned to two treatments: 1) control group (CON) receiving a standard diet, and 2) Probiotic group (PRO, 1.6×10^6 CFU/g of feed) from D1 up to 42 weeks of age. Each treatment comprised 30 replicates, with two cages per replicate and three hens per cage. Data were analyzed using a general linear model (JMP 18 Software) and Dunnett test was used for means comparison. Hens fed with PRO showed better overall performance compared to CON ($p < 0.05$) with higher hen-day egg production (87.3 vs. 85.9 %), higher hen-housed production (85.9 vs. 84.1 %) as well as

Vitamin E is a critical lipid-phase antioxidant in poultry, essential for membrane stability & egg quality in commercial layers. A 20-week controlled study was conducted to evaluate the efficacy of a natural Vit. E supplement fortified with an organic selenium complex (E Sel Power M/S Indian Herbs, India) compared with synthetic Vit. E in BV300 layers. A total of 150 birds were assigned to three diets, T1: non-supplemented control (basal ration without Vitamin E); T2: basal ration with synthetic Vitamin E; T3: basal ration with E Sel Power. Five replicates of 10 birds/group were maintained from 16 to 35 weeks at an accredited CRO in Bengaluru, India. Performance indices (egg production, HHE) were recorded weekly. Egg quality (weight, yolk index, shell traits) & antioxidant properties were assessed at weeks 27 and 35. Antioxidant status was evaluated by FRAP assay of albumen, yolk & Vit. E concentration in yolk was quantified. All data were statistically analysed using Snedecor & Cochran procedures at $p < 0.05$. E Sel Power produced a significant improvement in layer productivity. Mean egg production between weeks 25–35 was highest in T3 (92.12%) compared with T2 (90.93%) and T1 (83.81%). Egg weight and yolk index showed similar enhancement in T3. (FRAP) assay demonstrated a clear increase of antioxidant capacity in both albumen and yolk from birds receiving E Sel Power. Albumen FRAP values were markedly higher in T3 (14.06 $\mu\text{g/g}$) versus T2 (8.72 $\mu\text{g/g}$) and T1 (4.26 $\mu\text{g/g}$). Yolk FRAP was greatest in T3 (599.39 $\mu\text{g/g}$) followed by T2 (461.32 $\mu\text{g/g}$) & T1 (296.90 $\mu\text{g/g}$). Yolk Vit. E concentration also increased substantially in E Sel Power group (0.37 $\mu\text{g/g}$) than control (0.26 $\mu\text{g/g}$), indicating superior deposition efficiency and antioxidant protection. Vitamin E is a major lipid-soluble antioxidant, & its enrichment in yolk is consistent with higher FRAP values observed in yolk from treated birds. This suggests improved protection of yolk lipids against oxidation and an added nutritional benefit for consumers. Supplementation with E Sel Power significantly enhanced productivity, egg quality & intrinsic antioxidant capacity of layers, outperforming synthetic Vit. E. The results establish E Sel Power as an effective, safe & sustainable alternative for modern layer nutrition.

Keywords: Egg quality; Antioxidant status; Natural Vitamin E; Organic selenium; FRAP

better feed efficiency with a lower feed consumption per dozen eggs (1.242 vs. 1.276 kg). Regarding egg quality parameters, egg weight was significantly increased ($p < 0.05$) with PRO (56.44 vs. 55.89 g). However, there was no significant difference between PRO and CON for Haught unit score (87.751 vs. 86.421, respectively) and eggshell thickness (0.420 vs. 0.413 mm, respectively). Furthermore, PRO group showed higher circulating levels of serotonin (+20 %) and lower mortality rate (-4 %) compared to CON ($p < 0.05$). In conclusion, continuous feed supplementation with a triple-strain *Bacillus*-based probiotic from D1 post-hatch enhanced productivity, egg quality, and welfare of hens without compromising egg quality reinforcing its potential role in sustainably improving layer production.

Keywords: Bacillus; layer; egg quality; probiotic; welfare

T198 Decrease feed costs and maintain the production performance of laying hens by using phytogetic feed solutions
Manu De Laet*¹, Vivek Kuttappan¹, Yann Fournis¹ *¹Cargill, Inc., Wayzata, Minnesota, United States*

For laying hens, feed costs are crucial and account for 60–70% of the total costs. Raw material prices have increased dramatically versus historical norms. Thus, it is necessary to optimize layer diet

formulations. Phytochemicals can improve nutrient digestion and utilization. Based on several digestibility studies managed by Cargill, nutritional matrix values were developed for phytochemicals. In this trial, the effect of a phytochemical blend (based on a combination of spices, bitter substances, essential oils, and saponins), used with nutritional matrix values, was evaluated by measuring the production performance of 200 ISA Brown laying hens, 20 replicates with 5 birds/replicate for each treatment from 19 to 34 wks of age. The objective was to achieve the same production parameters with a lower feed price. Two dietary treatments were tested: 1) control feed and 2) control + 150 g/MT phytochemical blend from 19 to 34 wks of age (nutritional matrix values from 25 until 34 wks). At the onset of lay, a rapid increase in nutrients is necessary as the birds must still cover nutrient requirements to reach adult body weight but also to achieve a rapid increase in egg weight and reach peak production of close to 1 egg per day. As birds can use all possible nutrients at the start of lay, the phytochemical blend was added on top of the feed from 19 to 25 wks and the nutritional matrix values were used from 25 wks onwards. From 19 to 25 wks of age, the laying rate (+2.42%) and FCR (+1.0 point) increased when the phytochemical blend was added on top of the diet. From 26 to 34 wks of age, when the nutritional matrix values were used, the laying rate (+0.62%) increased, and FCR did not change. All results were not significantly different. In this experiment, the nutritional matrix values from 25 wks onwards allowed a reduction in feed price by 2-3% without performance losses. The ROI (current value of investment/cost of investment) of the phytochemical blend in this trial was 3.3.

Keywords: Phytochemicals; Matrix values; Laying hens

T199 Postbiotics–phytochemical synergy supports improved layer production performance during APEC challenge

Vivek Kuttappan^{*1}, Yann Fournis¹, Manuel Da Costa¹, Dan Moore², Woo Kyun Kim³, Marc Decoux¹ ¹Cargill, Inc., Wayzata, Minnesota, United States; ²Colorado Quality Research, Inc., Wellington, Colorado, United States; ³University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Colibacillosis, caused by Avian Pathogenic *E. coli* (APEC), severely affects commercial poultry flocks by increasing mortality, reducing body weight and feed efficiency, lowering egg production, and posing food safety risks. This study evaluated the impact of combining a *Saccharomyces cerevisiae* fermentation-based postbiotic (SCFP) with phytochemical (Biostrong™ C-Protect, Cargill, Inc.) on mitigating APEC severity in laying hens. Two trials were conducted: Trial 1 involved 50-week-old layers (n=180 birds/treatment) over 42 days with an APEC challenge ($10^{7.5}$ CFU/bird) on day 21, while Trial 2 used 79-week-old layers (n=28 birds/treatment) over 56 days with a higher APEC dose (5×10^9 CFU/bird) on day 28. Birds in the control group (CON) received a basal diet, while the treatment group (TRT) received Biostrong™ C-Protect at 1.15 lb/ton of feed on-top. Both trials followed a randomized block design and were analyzed using SAS PROC GLIMMIX, with treatment as a fixed effect and pen nested within block as a random effect. In Trial 1, TRT birds showed higher (P<0.05) hen-day egg production (HDEP) before the APEC challenge. Although HDEP declined post-challenge in both groups, TRT maintained a numerical advantage (P=0.1). In Trial 2, no pre-challenge differences (P>0.05) were observed, but post-challenge TRT birds had better (P<0.05) HDEP and higher egg mass (P<0.05). Lung tissue analysis revealed a ~1 log₁₀ CFU/g reduction in *E. coli* load in TRT birds compared to CON (P>0.05). Overall, the SCFP and phytochemical combination supported layer performance under both challenged and unchallenged conditions, with more pronounced benefits following APEC exposure.

Keywords: Avian Pathogenic *E. coli* (APEC); Postbiotic; phytochemical; Hen-day egg production (HDEP); layers

T200 Influence of a symbiotic additive *Bacillus sp.*-based probiotic and yeast cell wall-derived prebiotic supplementation on intestinal health response in laying hens

Jose J. Yanquen Velasquez^{*1}, Jheinson A. Almanza Hernandez¹, Yuliana M. Medina Sandoval¹, Angee M. Ramirez Ruiz¹, Diana C. Mogollon², Mayra Diaz Vargas³ ¹Nutrexcol S.A.S., Research, Development and Innovation, Armenia, Quindio, Colombia; ²Universidad del Tolima, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia; ³Universidad Cooperativa de Colombia, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia

Intestinal health is essential in poultry production and depends on multiple factors. For a long time, growth promoter antibiotics (GPA) have been used to enhance poultry production and prevent gut diseases. However, several studies have shown an unfavorable impact of these molecules on intestinal integrity, gut-linked immune response and microbiota balance. Consequently, many feed additives and natural based products have been evaluated as alternatives. The objective of this study was to evaluate the influence of a symbiotic additive *Bacillus sp.*-based probiotic and yeast cell wall-derived prebiotic (Modubiot Pro®), compared with negative control (without GPA) and positive control (GPA), on intestinal health responses between 32 to 38 weeks in laying hens challenged at 35 weeks with a high dose medication. A total of 192 laying hens at 32 weeks were randomly allocated into 12 floor pens (16 hens/pen). Data were analyzed using one-way ANOVA in a completely randomized design. Mean separation was performed using Tukey's test and Kruskal-Wallis test was used for nonparametric statistics. At 35 weeks, significant differences were found (p = 0.0352) in the mucosal immune response between PC and the symbiotic additive (36.08 vs 16.58 mg/dl IgA). Three weeks later, no statistical differences were observed. In the histomorphometry evaluation at 38 weeks, villus height (VH), crypt depth (CD) and the ratio VH:CD showed no statistical differences; however, the mean in CD and VH:CD were similar between the PC and the additive. At 38 weeks, the mucosal absorption function was evaluated by measuring blood serum carotenoids, showing no statistical differences among treatments, and the values of the PC and the symbiotic additive were similar. Regarding the evaluation of microbiota response, alpha diversity analysis showed that the symbiotic additive was the most homogeneous, while the PC exhibited the highest richness. On the other hand, in NC and PC groups the most abundant genus was *Propionispora* (16.3% & 15.5%) compared with symbiotic additive, in which the most abundant genus found was *Lactobacillus* (24.2%). Overall, the inclusion of a symbiotic additive modulated mucosal immunity during dysbiosis, promoting intestinal integrity and a beneficial microbiota in laying hens.

Keywords: *Bacillus sp.*; yeast cell wall; growth promoter antibiotics; microbiota

T201 *Fusarium spp.* mycotoxins, a challenge for the Latin-American poultry industry. Use of an organoclay as antimycotoxin adsorbent to prevent their toxic effects

Aaron E. Lopez Aguilar^{*1}, José Antonio Fierro², Patricia Gómez², Juan Carlos Medina² ¹Sanfer Salud Animal, Feed Additives, Ciudad de México, Ciudad de México, Mexico; ²Nutek, Toxicology Laboratory, Tehuacán, Puebla, Mexico

Mycotoxin polycontamination in grains is common. Avian species are considered resistant to fusariotoxins intoxication. Experimental studies have agreed that the main effect directly

affects intestinal integrity and immune response. The objectives were to evaluate the efficacy of an organoclay (Zeotek®, ZK, Nutek) in two experiments, reducing the toxic effects of consuming feed contaminated with mycotoxins for 8 weeks: Fumonisin B1 (FB1), Deoxynivalenol (DON) and Zearalenone (ZEA) in laying hens. Both experiments were carried out in the Toxicology experimental unit of Nutek. In the first experiment, feed was contaminated with the three mycotoxins (MMC), FB1: 3500 ppb, DON: 1500 ppb and ZEA: 150 ppb. Forty-five 58-week-old laying hens were used, distributed into three treatments with five replicates of three hens each. T1, negative control without ZK and without MMC. T2, positive control with MMC, without ZK. T3 with ZK and MMC. The percentage of laying, egg weight, egg mass were measured daily, and shell strength, feed consumption, and effect on yolk pigmentation were measured weekly until the end of the experiment. For both experiments, the data obtained were analyzed by ANOVA using SYSTAT statistical software, and *Tukey test* was used to define the difference between means. Significance was determined at $p < 0.05$. Results: the inclusion of the ZK counteracted the negative effects in animals that consumed the mycotoxin mixture, mainly on egg production, number of eggs, egg mass and shell fragility. There was no feed rejection by the hens, nor any effect on yolk pigmentation, between treatments. For the second experiment a commercial diet was used with contamination such, FB1: 637 ppb, DON: 604 ppb and ZEA: 65 ppb. Thirty-36-week-old laying hens were used, distributed into two treatments (T1 and T3) with five replicates of three hens each, same weeks and parameters were evaluated. Results: the inclusion of ZK counteracted the negative effects in animals that consumed the mycotoxin mixture, number of eggs, egg mass and shell fragility. The other parameters were not affected. Discussion and conclusion: the concentrations of MMC used in these studies affected the parameters evaluated. The addition of the antimycotoxin additive reduced the toxic effects.

Keywords: Mycotoxins; polycontamination; fusariotoxins; poultry; organoclays

T202 Efficacy of a dietary supplement, B.I.O. Tox® Activ8, to support broiler performance metrics when exposed to Fumonisin and Deoxynivalenol

Matthew K. Jones*¹, Charles L. Hofacre¹, Cornelia Becker², Revathi Shanmugasundaram³, Anthony Pokoo-Aikins³ ¹*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ²*Biochem, Lohne, Germany*; ³*United States Department of Agriculture, Toxicology & Mycotoxin Research Unit, Athens, Georgia, United States*

Given the importance to both animal and human safety, poultry producers routinely test major feed ingredients to ensure no or low mycotoxin contamination. Hot spots of higher concentration as well as the rate of finished feed moving through an integrated broiler operation makes it difficult to attribute a specific batch of contaminated feed with clinical or subclinical mycotoxicosis symptoms. This highlights an opportunity for effective neutralization of mycotoxins in the final feed. The current scientific trial evaluated a dietary supplement (TB, B.I.O.Tox®Activ8, Biochem) in diets contaminated with mycotoxin under commercial field conditions. Four treatments included negative control (NC), second control group (NC+TB) with 0.2 % TB, positive control (PC) contaminated with ~3ppm fumonisin and ~4ppm deoxynivalenol, and trial group (PC+TB) with the same multi-contamination as PC plus 0.2 % TB. Each treatment was represented by 8 replicate floor pens of 15 male Ross broiler chickens. All groups received coccidia vaccine and were placed on reused litter. Birds and feed were weighed on day 0, 14, 28, and 37 to calculate broiler performance metrics. By day

14, groups with added mycotoxin had lower feed intake than the unchallenged groups. By 28 days, the enteric stress peaked from the toxins and enteric pathogens in the reused litter. The body weight gain was lower in PC (0.901kg^B) compared to NC (1.060 kg^A). PC+TB was statistically intermediate (0.949 kg^{AB}). PC had significantly lower feed intake than the unchallenged groups. By day 37, PC maintained significantly lower body weight gain (1.509 kg) compared to NC (1.757 kg). PC also had lower feed intake than the unchallenged groups. TB was able to bring body weight gain and feed intake values up to statistically intermediate levels in PC+TB. Total mortality in PC on reused litter was 17.5%^A, which was greater than NC, 5.83%^B. Similar to performance metrics, mortality in PC+TB (9.17%^{AB}) was statistically indistinguishable from the unchallenged control, NC. The consistent numerical outcomes in body weight gain and feed consumption combined with mortality values similar to the unchallenged control suggest the binder supported broilers with chronic exposure to low levels of mycotoxins in the feed.

Keywords: Mycotoxin; Binder; Enteric Health; FUM; DON

T203 Omics analysis of the effects of paraprobiotics *Lactiplantibacillus plantarum* ABG0016 on gastrointestinal function in broiler chickens

Hiroya Kondo*¹, Daisuke Matsui², Charles L. Hofacre³, Matthew K. Jones³, Hideaki Sueoka¹, Shiori Iino¹, Mari Haneta¹, Takako Fukuda¹, Iki Taketani¹, Tsuyoshi Tonoue¹, Izuru Shinzato¹, Junya Kouroki¹, Takahiro Nii⁴ ¹*Sumitomo Chemical Co., Ltd., Tokyo, Japan*; ²*Valent BioSciences Co., Chicago, Illinois, United States*; ³*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ⁴*Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan*

Paraprobiotics, inactivated microbial cells that benefit gut health, are promising alternatives to antibiotic growth promoters. This study aimed to evaluate, using omics analysis, the effects of paraprobiotics *Lactiplantibacillus plantarum* strain ABG0016 on nutrient absorption markers and gut microbiota in the gastrointestinal tract of broilers. A total of 16 3-day-old male broiler chicks (ROSS 308) were randomly assigned to 2 treatments (8 birds/treatment) and reared until 21 days of age. Treatments were Ctrl: Control fed the basal diet, ABG: Ctrl + 10 mg/kg ABG0016. The basal diet met the breeder's nutrient recommendations. ABG0016 was administered via feed. Serum, jejunal tissue and ileal digesta were collected from all birds at 21 days of age. Data were analyzed using t-test ($P < 0.05$). During the rearing period, BWG was numerically higher in the ABG group, although the difference was not significant. Proteomic analysis of jejunal tissues suggested that pathways related to lipid metabolism, intestinal epithelial barrier function, and immune function were activated in the ABG group. Lipidomics analysis of serum free fatty acids revealed a decrease in free fatty acid levels in the ABG group, suggesting enhanced lipid metabolism and/or suppressed lipolysis from adipose tissue. Quantitative PCR analysis of nutrient transporter expression in jejunal tissues showed that the expression level of iFABP, a fatty acid transporter, was significantly increased in the ABG group. Analysis of the gut microbiota using ileal digesta revealed that the relative abundance of beneficial bacteria, *Romboutsia* and *Lactococcus*, was significantly increased in the ABG group. Analysis of short-chain fatty acids (SCFAs) in the ileal digesta revealed that the concentrations of formic acid and acetic acid were significantly increased in the ABG group. A high correlation was observed among iFABP expression levels, the relative abundance of gut microbiota, and SCFAs concentrations. These findings suggest that administration of paraprobiotics ABG0016 contributes to the modulation of immune function, indirect improvement of gut

microbiota composition, and enhancement of nutrient absorption capacity in the intestinal epithelium through the actions of SCFAs produced by beneficial bacteria.

Keywords: Broiler; Paraprobiotics; Probiotics; Omics; Nutrition

T204 Effects of paraprobiotics *Lactiplantibacillus plantarum* strain ABG0016 on growth performance, immune response and intestinal parameters in broilers under a mild necrotic enteritis challenge

Daisuke Matsui^{*1}, Hiroya Kondo², Charles L. Hofacre³, Matthew K. Jones³, Takahiro Nii⁴, Shiori Iino², Mari Haneta², Takako Fukuda², Iki Taketani², Tsuyoshi Tonoue², Izuru Shinzato², Junya Kouroki² ¹*Valent BioSciences LLC, Libertyville, Illinois, United States*; ²*Sumitomo Chemical Co., Ltd., Tokyo, Japan*; ³*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ⁴*Hiroshima University, Graduate School of Integrated Sciences for Life, Hiroshima, Japan*

Paraprobiotics, inactivated microbial cells that benefit gut health, are promising alternatives to antibiotic growth promoters. This study aimed to determine the optimal dose of paraprobiotics *Lactiplantibacillus plantarum* strain ABG0016 and evaluate its effects on growth performance, immune response and intestinal parameters in broilers under a mild necrotic enteritis challenge. A total of 2,000 day-old male broiler chicks (ROSS 708) were randomly assigned to 80 pens for 5 treatments (16 pens/treatment) and reared until 43 days of age. Treatments were T1: Negative Control (NC) fed the basal diet, T2: NC + 55 mg/kg Bacitracin-Methylene Disalicylate, T3: NC + 2 mg/kg ABG0016, T4: NC + 5 mg/kg ABG0016, T5: NC + 10 mg/kg ABG0016. The basal diet met the breeder's nutrient recommendations. All birds received a live coccidiosis vaccine at the commercially recommended dose on the day of hatch and were placed on reused litter. Additionally, birds were challenged with a strain of *Clostridium perfringens* at 15, 22 and 29 days. Growth performance was measured at 15, 29 and 43 days. Serum, spleen, and ileal and cecal digesta were collected from one bird per pen at 16 and 43 days. Data were analyzed using one-way ANOVA followed by Fisher's LSD test ($p < 0.05$). At 43 days, T4 and T5 had significantly higher BWG than T1 and T3, while T2 showed the intermediate value. T2 showed significantly lower FCR than T1 and T3, while T4 and T5 showed the intermediate values. Mortality did not differ significantly among the treatments. Spleen cytokine gene expression analysis showed significant upregulation of IL-12 and IL-10 in T4 and T5, and T4 showed significantly higher expression of IFN- α and IFN- β , compared to other treatments. 16S rDNA analysis indicated a significant increase of *Lactobacillus* in the ileal digesta of T4 and T5 compared to T2. In the cecal digesta, the analysis revealed a significant increase in *Faecalibacterium* in T3 and *Lactobacillus* in T2, T4, and T5 compared to T1, positively correlated with isobutyric and formic acid concentrations, respectively. These findings indicate that ABG0016 modulates immune response, alters gut parameters indirectly, and improves growth performance in broilers at 5 or 10 mg/kg.

Keywords: broiler; paraprobiotics; probiotics; microbiome; immunity

T205 A blend of triterpenic saponins, polyphenols and coated calcium butyrate improves the performance and *Eimeria*-challenged broilers

David Harrington^{*1}, Michael D. Sims², Nemanja Todorovic¹ ¹*Nuance Biotechnology, Lausanne, Switzerland*; ²*Virginia Diversified Research, Harrisonburg, Virginia, United States*

Calcium butyrate and sweet chestnut polyphenols are known to enhance animal performance, while triterpenic saponins exhibit anti-*Eimeria* activity. Their combination may provide a novel strategy to support intestinal health in broilers. This study evaluated the effects of a protected calcium butyrate combined with *C. sativa* polyphenols and triterpenic saponins (PHY) on broiler performance. A total of 630 as-hatched Ross 308 broilers were allocated to three treatments (7 pens/treatment; 30 birds/pen): CON (control, basal diet), PHY (basal + PHY, 450 g/t), and SAL (basal + salinomycin, 60 g/t). Birds were reared from d0–42 on reused litter. Additives were included from day 0. On d14, all birds were orally challenged with mixed *Eimeria* spp. and euthanased 6 days later for lesion scoring (LS). Oocyst per gram feces (OPG $\times 10^3$) were determined on d21, d28 and d42. Body weight gain (BWG) was measured on d14, d21 and d42 and mortality adjusted-FCR (MFCR) for d0-14, d0-21 and d0-42. Mortality was recorded daily. Data were analyzed by ANOVA and transformed for normality where needed; significance was set at $P < 0.05$. On d14, BWG did not differ ($P = 0.375$) among CON, PHY, and SAL (500, 501, 510 g). At d28, SAL birds gained more than CON (1330 vs 1214 g; $P < 0.05$), while PHY (1259 g) was intermediate. By d42, BWG in SAL exceeded PHY and CON (2438, 2238, 2225 g; $P < 0.05$). For d0–14, FCR was lower ($P < 0.05$) in PHY than in CON and SAL by up to 4.5%. For d0-28, MFCR differed across groups (1.55, 1.49, 1.45; $P < 0.05$), and by d0–42, SAL and PHY improved MFCR versus CON by 6.2% and 2.1% ($P < 0.05$). Total LS values were 5.10, 3.38, and 1.05 for CON, PHY, and SAL, respectively ($P = 0.704$). OPG counts on d21–28 were up to 86% lower in SAL, with PHY intermediate and not significantly different. Similar numerical trends occurred on d42 ($P = 0.055$). In conclusion, combining protected calcium butyrate with polyphenols and saponins reduced the impact of *Eimeria* challenge and improved feed efficiency, though less than salinomycin. This natural blend may represent a promising alternative for maintaining broiler performance and gut health.

Keywords: Calcium butyrate; Polyphenols; Broilers; Saponins; *Eimeria*

T206 Effect of virginimycin or virginiamycin combined with Biostrong DualTM on broiler gut microbial composition and performance

Anne I. Goderis^{*1} ¹*Cargill, Velddriël, Belgium, Netherlands*

This study investigated the effect of virginiamycin (VIRG) or a combination of VIRG, a postbiotic and essential oils on growth performance and gut microbiota profile of broilers. A total of 180 male ROSS 308 birds were randomly allocated to 36 cages and fed starter and grower diets based on corn and soybean meal. Three dietary treatments were tested for 21 days: a control treatment (CTR), a standard diet supplemented with 20 ppm (VIRG) or VIRG combined with 400 ppm Biostrong DualTM (VIRG_DUAL). Body weight and feed intake were recorded per feeding phase and feed conversion ratio (FCR) was calculated. Performance data were analyzed using mixed model in R. Individual cloaca swabs were collected for microbiota analysis and analyzed using a customized fluorescence microarray GalleonTM with preselected biomarkers for performance, health and food safety. Relative intensity for each bacteria probe was submitted to ANOVA in a factorial arrangement with fixed effect of diet. Pairwise comparisons between standardized LS means were made for each bacteria and variable combination adjusting for FDR test with $p = 0.05$. The results showed that both VIRG and VIRG_DUAL treatment significantly reduced FCR vs CTR from 0-21 days. FCR was significantly lower for VIRG_DUAL compared to VIRG from 0-21 days. At 21d fat pad yield was significantly higher for birds fed VGM while this was counteracted by combining VGM with

DUAL. Microbiota results showed that VIRG significantly increased the relative abundance of *Bacteroides vulgatus*, *Bifidobacterium*, *Enterobacteriaceae*, *E. coli*, *Salmonella* and *Shigella* and decreased the abundance of *Lachnospiraceae* and *Lactobacillus crispatus*. The shifts in microbial profile may partly explain the positive effect of VIRG on performance. Combining VIRG with DUAL resulted in numerically higher abundance of *Lachnospiraceae* while lowering abundance of *Bacteroides vulgatus*, *Enterobacteriaceae*, *Salmonella*, *E. coli* and *Shigella* compared to VIRG. These results suggested that DUAL promotes beneficial microbes while mitigating the negative effect of VIRG on undesired bacteria such as *E. coli* and *Salmonella*, resulting in higher feed efficiency and lower fat pad yield.

Keywords: virginiamycin; postbiotic; essential oils; microbiota

T207 Comparative effects of BioCholine, Synthetic Choline Chloride and Betaine on growth performance, Serum Biochemical Parameters and Hepatic BHMT Gene Expression in broilers

Shivi Maini*¹, Kamlesh More² ¹*Indian Herbs Specialties, Chandigarh, Chandigarh, India;* ²*Dr. B.V. Rao Institute of Poultry Management & Technology, R&D, Pune, Maharashtra, India*

Choline is an essential nutrient in poultry, a methyl donor supporting phospholipid synthesis, lipoprotein transport, and hepatic lipid metabolism (Shahsavari et al., 2020). Conventional choline chloride (CC), poses challenges including hygroscopicity, oxidative degradation & potential nutrient antagonism in premixes (Hossain et al., 2021). Natural phosphatidylcholine sources such as BioCholine® (M/S Indian Herbs, India) are gaining attention due to superior bioavailability and stability (Jadhav et al., 2022). Betaine (BET) is another methyl donor but differs in physiological role and metabolic utilization. A trial evaluated efficacy of CC, BioCholine & BET on growth performance, serum homocysteine levels and liver betaine-homocysteine methyltransferase (BHMT) gene expression in broilers. 440 day-old VenCobb broiler chicks were randomly allocated to four dietary treatments: (T1) negative control without choline or BET; (T2) CC at 1000 g/ton; (T3) BioCholine at 200 g/ton; & (T4) BET at 500 g/ton, with 10 replicates per treatment. Birds were reared on deep litter for 42 days under standard management. Weekly body weight (BW), feed intake, FCR, mortality, incidence of FLS were recorded. On day 42, blood samples (3 birds/trt) were collected for serum homocysteine analysis using ELISA. Liver samples were collected for BHMT gene expression analysis via qPCR. All data were subjected to statistical analysis following Snedecor and Cochran procedures, with significance determined at $p < 0.05$. By day 42, BioCholine supplementation resulted in significantly ($p < 0.05$) higher mean BW (2650 g) compared with CC (2590 g) & control (2530 g). BioCholine-T3 showed the lowest FCR (1.56) compared with CC and BET (1.60 each). BHMT gene expression was highest in T3 (2.54-fold), followed by T2 (1.33-fold) and T1 (1.00-fold). Serum homocysteine levels were lowest in T3 (1564.69 ng/mL) than T1 (2234.20 ng/mL) & T4 (1681.89 ng/mL). BioCholine exhibited superior efficacy over synthetic choline chloride and betaine in improving growth performance, enhancing methylation efficiency (via BHMT expression), and reducing homocysteine levels. These findings highlight BioCholine as a promising natural alternative to synthetic choline sources for optimizing broiler metabolic health and performance.

Keywords: Lipid metabolism; methylation; BHMT; homocysteine; Choline

T208 Exploring the effect of a copper feed additive on the litter microbiome of broilers challenged with coccidiosis over time

Jake K. Byrnes*¹, Dan Moore³, Casey E. McGinley¹, Michael B. Rhys¹, Cassie L. Ettinger² ¹*Barnwell Bio, San Diego, California, United States;* ²*Barnwell Bio, New York, New York, United States;* ³*Colorado Quality Research, Wellington, Colorado, United States*

Copper has long been recognized for its role in poultry health. Beyond its nutritional benefits, it possesses antimicrobial properties and is included in poultry feed as a growth promoter and to mitigate certain pathogens during enteric stress. In this study, we used metagenomic sequencing to explore the impact of a copper feed additive on the litter microbiome of broilers challenged with coccidiosis throughout a full growout. A total of 115 Cobb 500 broiler chickens were randomly assigned to the negative control (0 ppm copper) or a copper chelate food additive treatment (60 ppm copper), consisting of five floor pens with 23 birds per pen on used litter. Broilers were challenged with a 5X dose of a vaccine containing *Eimeria acervulina*, *E. maxima*, and *E. tenella* on day 0. Three replicate boot sock samples per treatment were collected on days 1, 6, 13, 20, 27, 34, 41, 48, and 55. Deep metagenomic sequencing was performed using Element AVITI short-read technology and analyzed using Barnwell Bio's proprietary bioinformatic pipelines. Strong temporal trends in relative abundance were observed for both groups, with a shift after day 20 toward a stable microbiome, consistent with normal development. Beta diversity showed differences over time (PERMANOVA, $p < 0.05$) but not by treatment ($p > 0.05$). More nuanced treatment changes were identified by investigating specific taxa over time using ANCOM-BC2 and DESeq2. *Paraclostridium sordellii* was higher in abundance in controls consistently over time ($p < 0.05$). *Bifidobacterium* spp. showed decreased abundance during early development in the treatment ($p < 0.05$). More *Enterococcus cecorum* was observed at the end of growout in the control ($p < 0.05$). Finally, a shift in *Eimeria acervulina* cycling was noted with controls exhibiting an earlier and higher peak in abundance ($p < 0.05$). In summary, we observed targeted taxonomic shifts rather than broad diversity changes, including decreases in *Bifidobacterium* spp. and *Paraclostridium sordellii*, along with time-dependent effects on *Enterococcus* and *Eimeria*. These findings suggest that while copper feed additives may not dramatically alter overall microbial community structure, they can induce specific changes in certain taxa, including those with pathogenic potential.

Keywords: Broiler; Litter; Copper; Microbiome; Metagenomics

T209 Effects of nano phytogenic feed additive as an alternative to synthetic anticoccidials on oocyst count, intestinal integrity and histomorphometry in broiler chickens challenged with *Eimeria* spp.

Jheinson A. Almanza Hernandez^{1,2}, Angee M. Ramirez Ruiz*¹, Jose J. Yanquen Velasquez¹, Yuliana M. Medina Sandoval¹, Lina M. Peñuela Sierra², Luz C. Fandiño² ¹*Nutrexcol S.A.S., Research, Development and Innovation, Armenia, Quindio, Colombia;* ²*Universidad del Tolima, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia*

Coccidiosis can cause several intestinal damages in poultry production because some phases of parasite's life cycle develop intracellularly. Currently, producers use synthetic anticoccidials to reduce replication, but it is also necessary to improve intestinal health after treatment. Therefore, it is important to evaluate alternative additives for coccidiosis control and maintain the proper function of intestinal tract. This study aims to investigate the effects of three dosages (T3:300, T4:400 and T5:500 ppm) of a nano additive (Coxiout Pro®), compared to a negative control (NC: basal diet without anticoccidials) and positive control (PC: starter - 3.75 ppm maduramicin + 40 ppm nicarbazin, and grower

- 60 ppm salinomycin), on oocyst count, intestinal integrity and histomorphometry in broiler chickens challenged with 7×10^4 of *Eimeria* spp. A total of 600 male-chicks were randomly allocated in 60 floor pens (10 chicks/pen). The data were analyzed using one-way ANOVA in a completely randomized design. Mean separation was performed using Tukey's test. Results obtained for 35 days showed that nano additive at all dosages reduced the oocyst count to similar levels to positive control ($< 9,000$ oocyst per gram (OPG)), and exhibit statistical differences compared with negative control which was over 70,000 OPG ($p < 0.05$). Intestinal permeability was measured with FITC-D marker; the best treatment was PC compared with the NC and T3 (1.34 vs 1.88 vs 1.79 $\mu\text{g/ml}$) ($p < 0.05$) and there was no statistical difference compared with T4 and T5. On the other hand, carotenoids in blood serum were evaluated as an indicator of gut function and showed statistical difference between PC and NC (44.92 vs 33.15 $\mu\text{g/ml}$) ($p < 0.05$). Histomorphometry showed statistical differences in villus height (VH) with similar results for PC, T4 and T5; crypt depth (CD) with similar results for NC, T3, T4 and T5, and the best measure in ratio VH:CD was obtained with T4 (6.75) ($p < 0.05$). In conclusion, the use of a nano phytogetic feed additive could be used as an alternative to synthetic anticoccidials at dosages between 400 to 500 ppm throughout all live production in broiler chickens, reducing the oocyst count and providing positive effects on intestinal health parameters.

Keywords: nano phytogetic feed additive; coccidiosis; oocyst count; FITC-D

T210 Effect of XTRACT 6930 and feeding regimen on male, female, and straight-run broilers

Andrea Hanson*¹, Mohamad Mortada¹, Zhikai Zeng¹, Joshua Flees¹, Jose Charal¹, Kelsey Machens¹, Jason Hickman¹ ¹ADM, Montgomery City, Missouri, United States

This trial evaluated the effects of a combination of capsaicin, cinnamaldehyde, and carvacrol (XTRACT 6930, XT) on broilers of different sexes and feed programs. A total of 1,152 d-old, sexed chicks (Ross 708) were assigned to 1 of 4 scenarios: 1) FF: females fed diets formulated for females (FD), 2) FS: females fed diets for mixed sex (straight run, SR), 3) MS: males fed SR, and 4) SS: mixed sex fed SR. Half of all pens received diets with XT at 100 ppm or without XT (CON). Each treatment was replicated by 9 pens (16 birds/pen). Body weight (BW) and feed intake (FI) were recorded at phase changes on d 9, 27, 34 and 42 to calculate BW gain (BWG) and FCR. Phase 1 diets were similar among sexes, but FD contained ~50 kcal/kg less ME and ~5% less digestible amino acids than SR during phases 2-4. Four birds/pen were harvested on d 43 to obtain hot carcass, breast, tenderloin, and fat pad weights (wt). Data were analyzed to assess fixed effects of sex by diet scenario, XT, and their interaction, with random effect of block and covariate of initial BW. Significance was set at $P \leq 0.05$, and $0.05 < P < 0.10$ was a tendency. Overall, XT numerically reduced mortality (7.7 vs 10.3%, $P=0.13$) and increased tenderloin (3%, $P=0.03$), and fat pad (6%, $P=0.08$) wt compared with CON. The FD impaired FCR for FF compared with FS (2%, $P=0.03$). However, XT improved FCR (1.9%, $P=0.03$) and tenderloin wt (6%, $P=0.04$) compared with CON for FF, but not FS. In MS, XT increased BWG (4.1%) and FI (3.7%) compared with CON ($P < 0.03$). Feeding XT numerically increased d 42 BW (2.5%, $P=0.11$), breast wt (4%, $P=0.17$), and tenderloin (5%, $P=0.08$) wt for MS. Feeding XT to SS numerically increased FI (2.8%, $P=0.10$), FCR (1.4%, $P=0.11$), and tenderloin wt (4.8%, $P=0.13$), and increased breast (7.3%, $P=0.04$) and fat pad (18%, $P=0.01$) wt compared with CON. Overall, XT supplementation numerically improved mortality and increased tenderloin wt regardless of gender. However, performance

responses varied by scenario: XT increased BWG in MS, but overall FCR only improved in FF. Our results indicate XT improved nutrient bioavailability in females fed low-density diets, but a nutrient oversupply masked effects of XT in females fed SR. Future research should elucidate impacts of XT on nutrient utilization.

Keywords: broiler; carcass data; capsaicin; cinnamaldehyde; carvacrol

T211 Effect of coccidiosis vaccination status and essential oil administration on performance, oocyst secretion, and lesion scores of broilers subjected to a coccidiosis challenge

Timothy Broderick*¹, Carson Scharlau¹ ¹Ralco, Marshall, Minnesota, United States

Essential oils (EO) are used in poultry production for their gut health benefits, and are reported to improve animal health and growth performance. Phenolic compounds such as carvacrol possess antimicrobial properties, including oocysticidal activity against *Eimeria* species. Coccidiosis vaccines introduce viable *Eimeria* oocysts that facilitate an immune response through a low level infection and subsequent oocyst cycling. In this study, we investigated the effect of EO administration and coccidiosis vaccination on growth performance, oocyst shedding, and lesion scores in broiler chickens over a 28 day production period. 440 day old broilers were allocated into 20 pens according to a randomized complete block design in a 2x2 factorial arrangement based upon cocci vaccination status (unvaccinated or 1x spray vaccination at hatch) and water supplementation of a commercial EO product (0ml L⁻¹ or 0.3ml L⁻¹). All birds were orally challenged at 14d with 1ml of 10x cocci vaccine. Growth performance, oocyst counts, and lesion scores were analyzed by SAS using 2-way ANOVA and GLM, with Fisher's LSD for mean separation. Interactions were observed between vaccination status and EO administration for BW at 14d ($P=0.03$) and 28d ($P=0.02$). At both timepoints, average BW was greater in broilers receiving both EO and vaccine compared to untreated birds or those only receiving vaccine. Feed intake from 14-28d was significantly greater in broilers receiving EO ($P=0.01$). FCR at 14d was lower in broilers administered EO ($P=0.04$), whereas an interaction was observed for 14-28d FCR ($P=0.05$) with broilers receiving vaccines and EO being statistically similar to, but numerically higher than, broilers only administered vaccine or EO. Vaccinated broilers tended to have greater oocyst shedding at 7d ($P=0.08$) and 14d ($P=0.06$) compared to unvaccinated birds. Broilers administered EO tended to have lower duodenal lesion scores ($P=0.10$) at 22d. Our data suggest EO can be co-administered with cocci vaccines without impairing vaccine cycling at 7d and 14d. Additionally, co-administration could mitigate early performance loss associated with vaccine administration and improve health outcomes during a coccidiosis challenge.

Keywords: Coccidiosis; Essential Oil; *Eimeria*; Phytonutrient; Vaccine Interaction

T212 Impact of grape and green tea extracts on the antioxidant status and quality of broiler meat

Mayra Diaz-Vargas*¹, Juan A. Javierre^{2,3}, Carlos F. Duque-Ramirez¹ ¹Universidad Cooperativa de Colombia, Medicina Veterinaria y Zootecnia, Ibagué, TOLIMA, Colombia; ²Layn ingredients, Bogotá, D.C., Bogotá DC, Colombia; ³Tezcol SAS, Bogotá, D.C., Bogotá DC, Colombia

This study aimed to determine whether dietary green tea extract (TEA) or grape seed extract (GRAPE) enhance antioxidant enzyme activity and meat quality in broiler chickens, given the relevance of natural polyphenols as alternatives to synthetic

antioxidants in poultry production. A total of 408 broilers were assigned to three diets (PCG, TEA, GRAPE) in a completely randomized design (model: $Y_{ij} = \mu + T_i + e_{ij}$). Antioxidant enzymes (SOD, CAT, GSH-Px) were analyzed by ANOVA after verifying normality (Shapiro-Wilk) and homoscedasticity (Bartlett) at 5% significance; Tukey tests were used for multiple comparisons ($p < 0.05$). Meat quality traits (pH, color, WHC, cooking loss, texture, TBARS) were evaluated by factorial ANOVA ($3 \times 4 \times 2$ or $3 \times 2 \times 2$, depending on variable). Liver SOD, CAT and GSH-Px activities showed no treatment effect at 21 or 42 days (ANOVA, $p > 0.05$). In muscle, TEA reduced CAT at 42 days (8.47 ± 1.05 U/mg), significantly lower than PCG (13.03 ± 0.39 U/mg) and GRAPE (12.15 ± 1.00 U/mg; Tukey, $p < 0.05$), while SOD and GSH-Px remained unchanged ($p > 0.05$). Meat quality responses differed during storage. GRAPE stabilized breast pH across 15 days (6.50–6.58) compared with PCG (7.07; treatment \times time interaction, $p < 0.05$). Control birds showed the highest lightness (L^*), particularly in thigh meat (69.42 vs. 48.13

and 49.79 for GRAPE and TEA in trays), indicating paler meat; both extracts significantly reduced L^* values ($p < 0.05$). Lipid oxidation was lower in supplemented groups: in tray-stored breast meat at day 15, TBARS reached 0.10 mg MDA/kg in PCG vs. 0.06 mg/kg (GRAPE, TEA; $p < 0.05$). Under vacuum, TEA yielded the lowest TBARS (0.05 mg/kg). Shear force at day 15 decreased from 4.35 kgf (PCG) to 3.70 (GRAPE) and 3.18 (TEA; $p < 0.05$), reflecting improved tenderness. In conclusion, although dietary polyphenols did not enhance liver antioxidant enzyme activity, TEA selectively reduced muscle CAT, and both extracts significantly improved meat oxidative stability, color uniformity, and breast tenderness during storage. These findings support the potential of TEA and GRAPE as natural antioxidant additives that enhance meat quality without compromising enzymatic homeostasis.

Keywords: antioxidant enzymes; meat quality; CAT; GSH-Px; TBARS

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T213 Dexamethasone (DEX) challenge to induce intestinal dysbiosis and BCO

Katherine A. McCormick^{*1}, Yemi Burden¹, Adrienne Woodward¹, Hector Leyva-Jimenez¹, Karely Cantu¹, Brian Dirks¹ ¹United Animal Health, Sheridan, Indiana, United States

Bacterial chondronecrosis with osteomyelitis (BCO), induced by bacteria including *S. aureus* and *Enterococcus* spp. causes necrotic degradation within the proximal heads of the femora, tibiae, and other bones, along with growth depression in poultry. Glucocorticoids, including DEX, can trigger intestinal dysbiosis leading to BCO. The objective of this study was to evaluate the effect of DEX via feed or injection on broiler productivity, spleen microbial loads, and the incidence of BCO lesions. Male broilers were randomly allotted to one of six dietary treatments arranged in a 2×3 factorial of DEX route (IM vs FEED) and time (NO vs EARLY vs LATE). DEX IM was administered at 1.5 mg DEX/kg of BW on d 6 and 8 (EARLY) or d 20 and 22 (LATE). DEX FEED was administered at 1.0 mg DEX/kg of diet from d 6 to 14 (EARLY) and d 20 to 28 (LATE). Each treatment included 9 replicates with 9 birds/pen in an environmentally controlled room. The feeding program consisted of 2 dietary phases (starter d 0-14; grower d 14-35). All pens and feed were weighed weekly from d 0 to 35. One bird/pen was evaluated for lesion scoring of the femoral and tibial head on d 14, 28, and 35, and the spleen was sampled to evaluate *S. aureus*, *E. coli*, total coliforms, and *Enterococcus* microbial counts. Performance data and lesions were analyzed with Mixed Procedures of SAS, with main effects of route and time, while microbial loads were calculated as \log_{10} cfu/spleen and analyzed using One-way ANOVA. Compared to IM, FEED reduced end BW by 15.05% (2,488.3 vs 2,113.7 g, respectively, $P < 0.01$) and increased FCR by 8.70% (1.38 vs 1.50 g/g, respectively, $P < 0.01$) regardless of time. Compared to NO DEX, LATE reduced body weight by 16.45% (2,548.3 vs 2,129.0 g, respectively, $P < 0.01$) and increased FCR by 14.71% (1.36 vs 1.56 g/g, respectively, $P < 0.001$) regardless of route; administration of DEX EARLY was intermediate. No treatment differences were observed with spleen microbial loads. Some numerical increases were noted in BCO lesions with DEX regardless of route or time. Addition of DEX was an effective model to suppress growth performance; DEX FEED increased BCO lesions, causing a more severe condition.

Keywords: Dexamethasone; challenge model; BCO; broiler; performance

T214 Nutritional changes determined by the presence of fumonisins in corn: a new approach

Denize Tyska^{*1}, Cristina T. Simoes¹², Adriano Mallmann¹, Isadora Fabris Laber¹², Carlos A. Mallmann² ¹Pegasus Science, Santa Maria, Brazil; ²Federal University of Santa Maria (UFSM), Santa Maria, Brazil

Mycotoxin contamination in corn, particularly fumonisins (FUM) produced by *Fusarium* spp., poses not only toxicological risks but also potential impacts on the nutritional composition of the grain. Despite advances in food safety monitoring, the nutritional consequences of contamination exclusively by FUM remain little explored. A total of 408 corn samples were analyzed and divided into two groups: 204 samples that presented low contamination (LOW group, mean FUM = 549.18 μ g/kg) and 204 samples contaminated by FUM (FUM group, mean FUM = 4,958.21 μ g/kg). Additional mycotoxins – total aflatoxins, zearalenone and deoxynivalenol - were present at low levels in both groups. Mycotoxins analyses were performed using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Nutritional parameters, such as crude protein (CP), ether extract (EE), starch, and total amino acids (TAA) were evaluated using near-infrared spectroscopy (NIRS). All statistical analyses were performed using the software JASP. All evaluated parameters differed significantly ($p < 0.001$) between groups. The variables most affected by FUM contamination were damaged grains ($r = 0.588$), CP ($r = -0.442$), and gross energy ($r = -0.574$), which exhibited the greatest magnitude of change between groups. Smaller but significant reductions were recorded for EE ($r = -0.260$) and starch ($r = -0.204$). Some TAA showed strong indicators of fungal degradation, likely due to proteolytic activity associated with *Fusarium* spp. Principal Component Analysis (PCA) further supported these patterns by clearly separating LOW and FUM groups. The present results confirm that FUM contamination not only compromises the nutritional profile of corn but also promotes selective degradation of essential amino acids, which has direct implications for diet formulation in poultry production. Importantly, such compositional alterations were reflected by NIRS responses, since proteins, lipids, and carbohydrates contribute to characteristic absorption bands in the near-infrared region. This reinforces the potential of NIRS as a rapid and non-destructive screening tool for mycotoxin risk, enabling the direct detection of contamination through the nutritional changes induced by *Fusarium* spp.

Keywords: fumonisins; nutritional degradation; amino acid profile; NIRS; corn quality

T215 Near-infrared spectroscopy as a rapid tool to monitor mycotoxins in corn from different Latin American countries in 2025

Cristina T. Simoes*¹², Denize Tyska¹², Adriano Mallmann¹, Isadora F. Laber¹², Carlos A. Mallmann² ¹*Pegasus Science, Santa Maria, Rio Grande do Sul, Brazil;* ²*Federal University of Santa Maria, Department of Preventive Veterinary Medicine, Santa Maria, Brazil*

Mycotoxin monitoring is essential in poultry nutrition, where the high use of corn demands rapid and reliable analytical methods to reduce productivity and economic losses. This study investigated the mycotoxicological contamination of corn marketed in different Latin American countries, predicted by near-infrared spectroscopy (NIRS) from January 1st to October 31st, 2025. The spectra were obtained from 19,258 corn samples consumed by the livestock industry in Argentina (n=547), Bolivia (n=35), Brazil (n=17,460), Colombia (n=43), Costa Rica (n=89), El Salvador (n=114), Ecuador (n=96), Mexico (n=153), Paraguay (n=348), and Peru (n=373). Concentrations of total fumonisins (FBs=FB₁+FB₂), aflatoxin B₁ (AFB₁), deoxynivalenol (DON), zearalenone (ZEA), and ochratoxin A (OTA) were predicted by previously developed NIRS calibration curves based on partial least squares regression with cross-validation, using HPLC-MS/MS as the reference method. The correlation coefficients for FB₁, FB₂, AFB₁, DON, ZEA, and OTA were, respectively, 0.88, 0.88, 0.87, 0.81, 0.86, and 0.82. Quantification limits (in µg/kg) were 200 for FBs, 5 for AFB₁, 250 for DON, 30 for ZEA, and 10 for OTA. Descriptive statistics was conducted using the software Statgraphics Centurion. FBs were the most prevalent mycotoxins in Latin America, detected in 68% of corn samples, with an overall mean of 1,161±936 µg/kg. Results ranged from 100% in El Salvador (mean 3,264 µg/kg) to 63% in Paraguay (mean 707 µg/kg). ZEA had a 35% occurrence, with 25.6±19.3 µg/kg overall mean. Results ranged from 86% in El Salvador (mean 76.1 µg/kg) to 10% in Paraguay (mean 7.2 µg/kg). DON was detected in 30% of samples, with an overall mean of 107±102 µg/kg. Argentina had the lowest mean (7.89 µg/kg) and occurrence (3%), whereas Colombia and El Salvador presented the highest results (100% and 96%, and 365 and 500 µg/kg, respectively). AFB₁ was the least prevalent mycotoxin (17%), with an overall mean of 1.63±1.10 µg/kg. It was not detected in Costa Rica and El Salvador, whereas it had the highest occurrence in Argentina (31%, mean 3.8 µg/kg). OTA was only detected in 7 samples from Brazil. Different results among countries highlight the need for rapid and reliable tools, such as NIRS, to ensure corn safety for poultry feeding.

Keywords: feed safety; Latin America; mycotoxins; NIRS; poultry nutrition

T216 Near-infrared spectroscopy for non-invasive assessment of dietary density Impact on egg yield and adiposity in laying hens

Lieske van Eck*¹², Ines Carvalhido⁴, Maci Oelschlager³, Manuel Da Costa³, Elisangela Guaiume³ ¹*Cargill Animal Nutrition and Health, R&D, Velddriel, Netherlands;* ²*Wageningen University, Wageningen, Netherlands;* ³*Cargill, Savoy, Illinois, United States;* ⁴*Cargill Animal Nutrition and Health, Velddriel, Netherlands*

The transition from rearing pullets to laying hens is challenging and requires proper nutrition. Recent advances allow non-invasive measurement of laying hen body composition using Near-Infrared (NIR) spectroscopy. This technique was used to assess the effects of dietary energy (low, medium, high) and amino acids (AA; low, medium, high) on egg production and fat deposition during early laying. A total of 540 Dekalb White hens were allocated to 60 pens (9 hens/pen) and fed 5 diets at the Cargill Animal Nutrition

Innovation Center (Elk River, USA). Diets were provided from 17 to 30 weeks in two phases, with a change at 20 weeks. Diets included three Metabolizable Energy (ME) levels and three AA levels: low ME = 2650 kcal/kg, 0.73% Lys; medium ME = 2750 kcal/kg, 0.73% Lys; high ME = low AA = 2850 kcal/kg, 0.73% Lys; medium AA = 2850 kcal/kg, 0.78% Lys; high AA = 2850 kcal/kg, 0.93% Lys. In phase 2, all diets increased with 50 kcal/kg and +0.05% Lys. Other nutrients were standardized, and AA:Lys ratios followed breed guidelines. Eggs were collected daily; egg weights and average feed intake (ADFI) were recorded weekly. At weeks 17, 21, 25, and 30, body weight and fat pad weights were measured using the NIR tool (REVEAL™ Layer). Data were analyzed using pen as the unit and mixed models in R (v4.1.1). Linear and quadratic contrasts were applied. Increasing dietary AA linearly reduced laying rate from weeks 25 to 30 (P=0.052; -4.5%) but increased egg weights linearly across all periods (P<0.05; +1.8g). As a result, egg mass did not differ (P>0.05). From weeks 21 to 30, ADFI decreased linearly with higher ME diets (P<0.05; -3.7g), improving feed conversion ratio (P<0.05; +0.07). Laying rate and egg weights were not impacted by dietary ME level. Body weight and gain were unaffected by diet density (P>0.05). Hens fed high ME diets did show increased abdominal fat pad weights at weeks 21 and 30 (P<0.05). This might indicate energy oversupply, but could also be functional for energy storage and influence other processes through adipokines. In conclusion, dietary AA affected egg weight and laying rate, while dietary ME influenced ADFI, feed efficiency, and body composition. NIR is a useful tool to assess diet impact and guide feeding strategies to support hen productivity.

Keywords: Laying hens; nutrition; amino acids; energy; body composition

T217 Comparative evaluation of ultra-high protein, low oligosaccharide soybean meal on broiler and turkey performance in commercial production systems

Matthew Begemann*¹, Erin Zess¹, Roy Brister¹ ¹*Confluence Genetics, St. Louis, Missouri, United States*

Multiple commercial feeding trials were conducted to evaluate ultra-high protein, low oligosaccharide (UHP-LO) soybean meal (SBM) made from Confluence Genetics' ProVIA soybean varieties as a complete replacement for conventional SBM in poultry diets. The UHP-LO soybean meal contains approximately 14% higher crude protein (53% vs 46.4%) and 92% lower oligosaccharides compared to conventional SBM, with metabolizable energy credited at 176 kcal/kg above conventional SBM. In broiler trials, birds fed isocaloric and isonitrogenous diets containing UHP-LO SBM demonstrated improvements in final body weight ranging from 2.2% to 5.1% and feed conversion ratio improvements of 2.6% to 3.2% compared to conventional SBM treatments. Turkey trials showed numerically higher final body weights (22.68 vs 22.12 kgs) and improved mortality-adjusted feed conversion ratios (2.332 vs 2.426) with UHP-LO SBM, though differences were not statistically significant. Carcass characteristics, including white meat yield, were maintained or numerically improved across trials. Data from all trials were analyzed using a 1-way ANOVA for a completely randomized design and the significance value for all analyses was P<0.05. University of Arkansas metabolizable energy bioassays confirmed UHP-LO SBM contained 95-256 kcal/kg additional metabolizable energy in broilers and 133-367 kcal/kg in turkeys compared to conventional meal. Results demonstrate that UHP-LO SBM can effectively replace conventional SBM in commercial poultry diets, improving growth performance and feed efficiency while reducing production costs.

Keywords: soybean meal; metabolizable energy; broilers; turkeys; feed efficiency

T218 How broiler chicken mortality has changed in the last 15 years in the United States

Jose Otavio Sorbara*¹ ¹University of Georgia, Poultry Science, Hoschton, Georgia, United States

Broiler mortality has become an increasing challenge for U.S. poultry production. Average mortality data from the Agri Stats database, spanning from January 2010 to June 2025, were analyzed. This database encompasses nearly 150 billion birds raised in the USA over the past 15 years. The analysis included total mortality rates, weekly mortality across nine age intervals (ranging from 0 to 7 days to greater than 56 days), and mortality categorized by seven live-weight groups (3.6 to 4.4 pounds to greater than 8.5 pounds). Linear regression models were fitted for each variable, with the year serving as the predictor. All statistical analyses were conducted using JMP v19 (SAS Institute). Total mortality increased significantly over time ($Y = -369.8 + 0.1858 \times \text{Year}$; $R^2 = 0.845$; $P < 0.0001$). Weekly mortality increased in all periods except >56 d ($P = 0.2447$). Significant yearly increases were observed for 0–7 d (slope = 0.0511; $R^2 = 0.935$), 8–14 d (0.0099; $R^2 = 0.701$), 15–21 d (0.0196; $R^2 = 0.914$), 22–28 d (0.0236; $R^2 = 0.917$), 29–35 d (0.0210; $R^2 = 0.880$), 35–42 d (0.0193; $R^2 = 0.641$), 43–49 d (0.028; $R^2 = 0.493$), and 50–56 d (0.0744; $R^2 = 0.803$). Late-period mortality rose most sharply between 2020–2025. Mortality also increased with market weight ($Y = 2.1 + 0.4457 \times \text{WT}$; $R^2 = 0.402$; $P < 0.0001$) and with bird age ($Y = 1.27 + 0.07729 \times \text{Age}$; $R^2 = 0.282$; $P < 0.0001$). All weight categories showed significant yearly increases: 3.6–4.4 lb (0.1576; $R^2 = 0.818$), 4.4–5.2 lb (0.1627; $R^2 = 0.953$), 5.2–6.0 lb (0.1186; $R^2 = 0.667$), 6.0–6.8 lb (0.2201; $R^2 = 0.845$), 6.8–7.5 lb (0.0839; $R^2 = 0.474$), 7.5–8.5 lb (0.2193; $R^2 = 0.762$), and >8.5 lb (0.2435; $R^2 = 0.701$). Broiler mortality in the U.S. has increased steadily from 2010 to 2025, with consistent rises across weekly age periods and all market-weight categories. The greatest increases occurred in heavier birds and in later growth phases, indicating that higher final weights and extended grow-out ages are important contributors to the upward trend. However, these factors alone do not fully explain the industry-wide rise in mortality. Additional research is needed to better understand the biological, management, environmental, and health-related drivers behind these changes and to identify effective mitigation strategies.

Keywords: Death; Culling; Liviability

T219 Efficacy of tannin-free grain sorghum in mitigating necrotic enteritis in broilers under *Eimeria maxima* / *Clostridium perfringens* challenge

Isabella Estrada*², Brett Lumpkins¹, William Bridges³, Ahmed Ali², Mireille Arguelles-Ramos² ¹Southern Poultry Feed and Research, Inc, Athens, Georgia, United States; ²Clemson University, Animal and Veterinary Sciences, Clemson, South Carolina, United States; ³Clemson University, Mathematical and Statistical Sciences, Clemson, South Carolina, United States

A 42-day floor pen trial was conducted to test the efficacy of U.S. #2 tannin-free grain sorghum-based diets to reduce the severity of necrotic enteritis in broilers. The study used a 2×2 factorial design with 2 dietary treatments (corn-based vs. sorghum-based diets) and two challenge treatments (challenged with *Eimeria maxima* (EM) and *Clostridium perfringens* (CP) vs. no challenge). A total of 1,600 Cobb males were randomly distributed to one of four treatment combinations, with 8 replicate pens per treatment and 50 birds per pen. On d 17, birds in the challenged group were orally inoculated with ~5,000 oocysts of EM, and on d 22 and 23 birds were given a broth culture of CP with ~108 CFU/mL once daily.

On day 24, one bird per pen was scored for the degree/presence of necrotic enteritis (NE) lesions. Bird foot pads were scored for degree/presence of foot pad dermatitis (FPD) and litter moisture percentage was measured on d 14, 28, and 42. Birds and feed were weighed weekly to calculate average feed intake (FI), body weight gain (BWG), and adjusted feed conversion ratio (AdjFCR). Data were analyzed using a two-way ANOVA for diet, challenge, and their interaction. From 0–14 d, birds fed sorghum had higher FI ($P=0.006$) and BWG ($P=0.011$; $P=0.046$), but birds fed corn showed better AdjFCR from 21–42 d ($P=0.0003$; $P=0.0001$; $P<0.0001$) with unchallenged groups having better feed efficiency ($P=0.046$; $P=0.04$; $P<0.0001$). Challenged birds fed sorghum had significantly lower lesion scores ($P = 0.03$) and total oocyst counts ($P = 0.02$), though livability was higher among corn-fed birds ($P = 0.024$), regardless of challenge. Litter moisture was significantly lower in unchallenged groups fed sorghum on day 28 ($P=0.038$), while FPD scores showed no significant differences. Tannin-free grain sorghum improved intestinal integrity and reduced NE severity with minimal impact on overall performance, supporting its potential as an alternative energy source for broiler diets under enteric challenge.

Keywords: Grain sorghum; Necrotic enteritis; *Clostridium perfringens*; *Eimeria maxima*

T220 Study on effect of energy and protein level on production performance of local chicken breeds during early growth phase

Rupendra Chaulagain*¹ ¹HICAST/ Purbanchal University; Nepal, Veterinary Parasitology and Microbiology, Kathmandu, Bagamati, Nepal

The study on effect of energy and protein on the on-farm performance of local chickens during the early growth phase was conducted ILFC, HICAST, Kirtipur, Kathmandu. The farm experiment was done to evaluate the efficiency of production and its possible relationships with different nutritional factors such as diet composition, feeding strategies, and other managerial indicators. Qualitative and quantitative traits was recorded and analyze with multiple variants of two different phenotypic categories of native chicken. The growth evaluation and survivability were performed on the Sakini and Ghatikhuile based on the effect of feeding system and diet containing three diets, one containing 19% CP and 2900 Kcal ME/Kg (D1); 18% CP and 2800Kcal ME/Kg (D2)/mash feed; and diet (D3)/pellet feed which acted as the control with 23% CP and 3200 Kcal ME/Kg were fed upto 8 weeks of age. The experiment was conducted according to Randomized Complete Block Design (RCBD) and analyzed using analysis of variance (ANOVA) using R statistical software (R Core Team, 2023). The average weight was in all treatment groups were significantly different in all weeks. The maximum weight was observed in week 8 for all groups. The average weekly body weight gain (g) in all treatment groups were found to be significantly different ($p<0.05$) at week 1–8. The feed consumption (g) was significantly different ($p<0.05$) in the treatment groups during week 1–8. The maximum feed consumption for all groups was in week 8. The average FCR for the treatment groups differed significantly ($p<0.05$) in week 1–8 age. The higher mean FCR for T1 was 7.93 ± 0.03 in week 7, T2 in week 6 (5.77 ± 0.09), T3 in week 8 (3.93 ± 0.03), T4 in week 8 (6.23 ± 0.03), T5 in week 6 (10.93 ± 0.07), and T6 in week 6 (5.37 ± 0.12). There was no significant difference ($P>0.05$) in mean mortality among different diets in any age groups. Mean mortality was significantly higher ($P<0.05$) in Ghatikhuile during week 1 but Sakini had significantly higher ($P<0.05$) mortality in week 8. There was no significant difference ($p>0.05$) in mortality during any other time period. Therefore, a diet containing 18%CP and 2800 Kcal ME/kg is

sufficient for rearing local chickens in the early growth phase (0-8 weeks) on-farm.

Keywords: Plane of Nutrition; Poultry; Local Chicken Breeds; Nepal

T221 Unique strontium method for tracing and modeling calcium uptake, storage, and mobilization within the broiler breeder's system

Cole Umberson*¹⁵, Diego Martinez², Joshua Wilkes⁴, Tyler Goepfert³, Gwyneth Gordon³, Craig N. Coon⁴ ¹University of Arkansas, Poultry Science, Lincoln, Arkansas, United States; ²University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ³Arizona State University, METAL, Tempe, Arizona, United States; ⁴University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ⁵Plaza Feed and Crop Nutrition, Lincoln, Arkansas, United States

A study was conducted to evaluate the effect of Ca, NPP, NaHCO₃, and K levels on broiler breeder performance. A completely randomized design with a factorial arrangement of 2 x 2 (two genetic lines and 2 diets) was used. Data were analyzed using one-way ANOVA and means were separated using Student-t test. Two genetic broiler breeder lines were reared under two dietary treatments with four replicates per genetic x diet treatment. Total eggs and hen-day egg production were calculated for a 20-week production study. Whole body breeder composition (crude protein, ash, and fat) was determined at: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25, 27, 29, 33, 35, 37, 39, 41, 43, and 45 weeks of age using dual energy x-ray absorptiometry (DEXA). Egg quality factors (egg weight, shell wt., shell Ca. wt., shell thickness (mm), albumen wt., and yolk wt.) were determined using weekly DEXA scans from 25 to 55 weeks of age. The ratio of strontium (Sr) isotopes (⁸⁷Sr/⁸⁶Sr) was monitored for uptake efficiency of calcium (Ca) in each feeding phase. The medullary bone of breeder pullets fed 0.25% NaHCO₃ had an increased deposition of Ca and Sr at 25 weeks to support egg production compared to control hens ($p < 0.05$). Line A control treatment breeders fed Breeder 1 diet at 25 weeks mirrored the starter/grower feed phase exhibiting lower bone turnover rates as Ca mobilized from the cortical to medullary bone ($p < 0.05$). The addition of NaHCO₃ reduced body ash content in Line A but the line produced more total shell mass with increased egg production compared to the control fed breeders, a genetic difference was determined when NaHCO₃ was added to the diet as Line B increased in body ash content ($p < 0.05$). Total egg production was significantly higher in all Line A control, Line A + NaHCO₃, and Line B + NaHCO₃ compared to Line B control diet ($p < 0.05$). Reducing %NPP to 0.15 in the pre-breeder lowered egg production limiting mineral mobilization for eggshells but did not affect shell quality. In conclusion, utilizing strontium isotope ratios to monitor Ca mobilization in the breeder system showed

addition of NaHCO₃ to increased medullary bone mineralization for the purpose of egg production.

Keywords: Strontium; Calcium; Body Composition; Broiler Breeder; Isotopes

T222 Supplementation of enzyme-treated soybean meal during the starter phase improves broiler early intestinal development and performance

Megan R. Bible*¹, C.A. Ma³, Yuxin X. Li², Marisabel Caballero², Trine Friis² ¹Hamlet Protein Inc, Findlay, Ohio, United States; ²Hamlet Protein A/S, Horsens, Denmark; ³Northwest A&F University, College of Animal Science, Yangling, Shaanxi, China

The digestive tract of newly hatched poultry is in development in terms of size, structure, immune response, and microbial population. During the first weeks, digestive enzyme secretion and nutrient transport systems also develop, particularly those related to protein digestion. Young animals require highly available proteins for proper growth. Anti-nutritional factors (ANFs), as trypsin inhibitors, present in soybean meal, the most widely used source of protein for poultry, hinder this development. Enzyme-treated soybean meal (ESBM, Hamlet Protein – Horsens, Denmark & Findlay, Ohio, USA), a specialty feed ingredient with low ANFs and high available protein, was evaluated in broiler starter diets. Three hundred 1-day-old Arbor Acres broilers (as hatched) were divided into two groups with ten replicates of 15 broilers each and fed with iso-nutritious diets containing 0 or 5% ESBM from day (d) 1 to 10. Subsequently, both groups were fed the same grower (d11-21) and finisher diets (d22-42). The contents of the duodenum, jejunum, and ileum, and samples of intestinal mucosa, of one bird per repetition, were collected on d10. Blood samples were taken from the wing vein on d10, 21, and 42. The data on growth performance and apparent nutrient digestibility were assessed for statistical significance using ANOVA. The Independent-samples T-test was used for the results of free amino acids (FAA) in blood serum, digesta enzyme activity, and mucosa expression of peptide and amino acid transporters (PAAT), using IBM SPSS 26.0. The outcomes of 5% ESBM during the first 10 days were heavier body weights on d10 and 42 ($P < 0.05$), with similar feed conversion ratios compared to the control group, enhanced digestibility of dry matter and crude protein, improved proteolytic enzyme activity in the digesta, upregulation of PAAT expression in the jejunum, and FAA in blood serum ($P < 0.05$, d 10). A positive carry-over effect was observed in gut health indicators, as blood endotoxins, diamine oxidase, and malondialdehyde, at d21 and 42 ($P < 0.05$). Inclusions of consistent protein ingredients with low ANF and highly digestible protein in starter feeds can be used to promote broilers' early intestinal development and improve long-term performance.

Keywords: Early nutrition; Protein utilization; Protein kinetics; Intestinal development; Improved growth

Food Safety III

T223 Fresh litter poses a selective pressure on *E. coli* strains that harbor siderophores, ColV plasmids and antimicrobial resistance genes than reused litter

Taejung Chung*¹², Adelumola Oladeinde¹, Reed Woyda³⁴, Samiru S. Wickramasuriya³, Joshua M. Lyte⁵, Rohana Liyanage⁶, Michael Rothrock¹, Sohyun Cho¹, Xiang Li⁷, Jodie Plumlee Lawrence¹, Dinku Endale⁸, Timothy Strickland⁸, Zaid Abdo³⁴ ¹USDA-ARS, Egg and Poultry Processing Safety Unit, U.S. National Poultry Research Center, Athens, Georgia, United States; ²USDA-ARS, SCINet Program, ARS AI Center of Excellence, Beltsville, Maryland, United States; ³Colorado State University, Department of Microbiology, Immunology, and Pathology, Fort Collins,

Colorado, United States; ⁴Colorado State University, Program of Cell and Molecular Biology, Fort Collins, Colorado, United States; ⁵USDA-ARS, Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States; ⁶University of Arkansas, Department of Chemistry, Fayetteville, Arkansas, United States; ⁷University of Tennessee, Department of Animal Science, Knoxville, Tennessee, United States; ⁸USDA-ARS, Southeast Watershed Research, Tifton, Georgia, United States

Escherichia coli (*E. coli*) is a common bacterium in poultry production that can harbor antimicrobial resistance (AMR) and virulence factor (VF) genes, with potential to cause disease in

humans and chickens and to transmit these traits to other bacteria. Reuse of broiler litter introduces continuous environmental changes that may influence the survival and fitness of *E. coli*. Understanding how management factors (litter reuse, housing, and grow-out period) and environmental factors (house temperature, litter pH, and moisture content) are associated with fitness factors (AMR and VF genes) is critical for identifying the drivers of highly pathogenic *E. coli* strains on broiler farms. In this study, we characterized the genome of 217 *E. coli* isolates recovered from the litter of three successive broiler flocks from four houses using whole-genome sequencing, antimicrobial susceptibility testing, and growth experiments under metal stress. Environmental factors differed significantly by flock and grow-out stage ($p < 0.05$). Genomic analysis revealed high strain diversity with persistent sequence types such as ST10, ST212, and ST117 across successive flocks. Isolates from the first flock, which was raised on fresh peanut hulls, harbored significantly more fitness factors including siderophore biosynthesis operons (yersiniabactin, salmochelin, aerobactin), ColV plasmids, class 1 integrase gene (*intl1*), and metal resistance genes than from later flocks raised on reused peanut hulls ($p < 0.05$). Litter eluate from the first flock carried significantly lower levels of trace metals such as iron and copper than eluates from flock 2 and 3 ($p < 0.05$). These findings suggest that the fresh litter environment of the first flock favored strains with enhanced adaptive traits for metal acquisition. Growth assays using lysogeny broth showed that acidified copper sulfate supplementation (~180 ppm) caused the strongest reduction in growth for ST117 strains compared to FeCl₃ (100 μM) or CuSO₄ (100 μM), however, the carriage of the siderophore, yersiniabactin, influenced their growth potential. These findings suggest that the fresh litter used for raising the first flock in this study selected for more pathogenic *E. coli* strains than the reused litter used for later flocks.

Keywords: Broiler litter; Reused litter; *E. coli*; Fitness factors; Siderophores

T224 Recovery of *Salmonella* and indicators from poultry feed samples: a comparison of two buffered media

Elizabeth McMillan*², Anthony Pokoo-Aikins¹ ¹USDA-ARS, US National Poultry Research Center, Toxicology and Mycotoxin Research Unit, Athens, Georgia, United States; ²USDA-ARS, US National Poultry Research Center, Poultry Microbiological Safety and Processing Unit, Athens, Georgia, United States

Poultry feed can be a route of *Salmonella* exposure for broiler and layer flocks. However, detection can be difficult due to the biochemistry of the feed samples during incubation and feed is therefore an under-investigated route of exposure. The purpose of the study was to investigate a potential method of buffering feed to detect *Salmonella* by determining the prevalence of *Salmonella* and indicators of contamination in 120 poultry feed samples. Briefly, the experimental design consisted of two different buffered pre-enrichment media used to isolate *Salmonella*, *Campylobacter*, Enterobacteriaceae, Coliforms, and total aerobic organisms: a previously published media (containing phosphate, Tris pH8, and sodium bicarbonate) and neutralizing buffered peptone water (nBPW). Samples were plated to rapid aerobic count (RAC) and Enterobacteriaceae (EB) petrifilm prior to pre-enrichment. Incubated samples were used to isolate *Salmonella* and *Campylobacter*. Total aerobic organisms were detected in all samples, with both media. Quantity was not statistically different for the two media ($p=0.31$; T-test). Enterobacteriaceae was detected in 86 samples (71%); recovery was not significantly different between media ($p=0.94$; T-test). *Campylobacter* was never detected and *Salmonella* was only detected in three samples (2.5%). *Salmonella* of the same

serogroup were detected by both media for two samples, but only nBPW in a third sample. In conclusion, this analysis showed that *Salmonella* prevalence in poultry feed is low and that nBPW can be an effective buffering pre-enrichment to isolate it. However, Enterobacteriaceae was not an effective indicator of *Salmonella* presence.

Keywords: poultry feed; salmonella; campylobacter; indicators; isolation media

T225 Comparison of traditional and novel methods for detection of *Campylobacter* in commercial poultry processing plants

Caitlin Harris*³, Amber Richards¹, Quentin Read², Elizabeth McMillan³, R. J. Buhr³, Nikki Shariat¹ ¹University of Georgia, Department of Population Health, Athens, Georgia, United States; ²USDA-ARS, Office of the Southeast Area Director, Raleigh, North Carolina, United States; ³USDA-ARS, Poultry Microbiological Safety and Processing Unit, Athens, Georgia, United States

Campylobacter is a major food safety concern associated with the consumption of undercooked or mishandled poultry products. In the United States, the standard sampling method for pathogen detection during broiler processing is the whole carcass rinse (WCR) or parts rinse (PR). The objective here was to compare the rinse method to a new tool, the MicroTally Mitt, for recovery of aerobic bacteria (RAC), Enterobacteriaceae (EB), and *Campylobacter*. Three commercial processing plants were sampled, with 2 visits each, at 4 locations: hot rehang (n=150), post-chill (n=150), whole wings pre-dip (n=136), and whole wings post-dip (n=136). Three sampling methods were utilized: WCR or PR (n=233), MicroTally Mitt on one carcass or 4lbs of whole wings (n=233), and Multi-mitts (n=117), which consisted of utilizing the MicroTally Mitt on 50 whole carcasses sampled online or on 100 whole wings. Analysis was done using R software v4.5.0 (R Core Team, 2025), with significance set at $p \leq 0.05$. For RAC and EB, counts were highest for the hot rehang and pre-dip wing samples and decreased following intervention steps. WCR and PR had higher RAC and EB counts for each location, except parts pre-dip. For *Campylobacter* prevalence, there was little recovery for the post-intervention sample locations (0–4%), while prevalence was higher for hot rehang samples (55–77%). For hot-rehang *Campylobacter* enumeration, the MicroTally average counts was higher (304 CFU/mL) than the WCR or Multi-Mitt groups (119 and 19.9 CFU/mL, respectively). The MicroTally Mitt was comparable to WCR at hot-rehang, but further experimentation is needed post-intervention. This study emphasizes the importance of antimicrobial interventions in a processing plant for reducing bacterial contamination on products.

Keywords: Broilers; Campylobacter; Processing; Whole Carcass Rinse; MicroTally

T226 35,000 Campylobacter genome sequences reveal both known and previously unknown bacteriophages targeted by CRISPR spacer sequences

Richard J. Meinersmann¹, Timothy R. Satterlee², Stephen N. White*¹ ¹USDA-ARS U.S. National Poultry Research Center, Poultry Microbiological Safety & Processing, Athens, Georgia, United States; ²USDA-ARS U.S. National Poultry Research Center, Athens, Georgia, United States

Campylobacter bacteria cause an estimated 1.3-1.5 million illnesses per year in the U.S. alone, making them the largest bacterial cause of foodborne illness. Bacteriophages (phages) are being evaluated for possible use in controlling pathogens including the poultry bacteria *Campylobacter* spp. Important gaps remain in

defining 1) mechanisms of *Campylobacter* resistance to phages and 2) the range of phages infecting and possibly killing *Campylobacter* species. CRISPR-associated nucleases (guided by CRISPR spacer sequences) are a major mechanism of bacterial defense. Thus, spacer specificity can provide clues to the nature of important viruses in a population. Genome sequences for 35,784 isolates of *Campylobacter* spp. were scanned for CRISPR spacer sequences, and 10,108 unique spacer sequences were identified. The putative targets (protospacers) for 6,699 spacers were determined using National Center for Biotechnology Information nonredundant database plus four custom databases designed to emphasize differing types of *Campylobacter* mobile genetic elements. As expected, the majority of identified spacers (4,469) were for phage genes. Spacer sequences were analyzed in depth for two types of phages found in *Campylobacter* spp.: the Fletchervirus and DA10-like Caudoviricetes. The DA10-like phages were targeted by many more spacers than were the Fletchervirus phages, even though the Fletchervirus genome was about three times larger than DA10-like genomes. These data clarify common mechanisms of *Campylobacter* CRISPR-based resistance to phages and provide evidence for previously unknown phages of *Campylobacter*. Additional work will be necessary to further characterize newly identified phages and assess their potential for *Campylobacter* control.

Keywords: *Campylobacter*; CRISPR; target; bacteriophage; spacer

T227 The fitness of *Salmonella* Enteritidis in poultry is strongly shaped by interactions between environmental microbial community

Adelumola Oladeinde^{*2}, Taejung Chung², David Bradshaw⁴, Kimberly Cook¹, Zaid Abdo³, Shawn Bearson⁴, Michael Rothrock², Jodie Plumblee Lawrence² ¹*Nutrition, Food Safety/Quality, Washington, District of Columbia, United States*; ²*USDA-ARS National Poultry Research Center, Egg and Poultry Production Safety Research Unit, Athens, Georgia, United States*; ³*Colorado State University, Department of Microbiology, Immunology and Pathology, Fort Collins, Colorado, United States*; ⁴*National Animal Disease Center, Ames, Iowa, United States*

Foodborne illnesses linked to *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) strains with decreased susceptibility to quinolones including ciprofloxacin are on the rise in the United States, Canada, and Europe. In 2025, *S. Enteritidis* strains with resistance to nalidixic acid and decreased susceptibility to ciprofloxacin have been linked to four *Salmonella* outbreaks in the United States that resulted in >180 hospitalization and 3 deaths. Furthermore, it has led to the recall of millions of eggs. Yet, the ecological and genetic pressures that drive the fitness of these emerging lineages of *S. Enteritidis* in poultry environments is not understood. In this study, we combined whole genome sequencing, machine learning and broiler chicken litter microcosm

experiments, to investigate factors that shape the fitness and antimicrobial resistance development of *S. Enteritidis*. We analyzed more than 10,000 genomes to assess the role bacteriophages, plasmids, and chromosomal mutations play in driving *S. Enteritidis* fitness. We report for the first time that the gyrase A (*gyrA*) mutation that is responsible for the decreased susceptibility to quinolones in *S. Enteritidis* is significantly ($P < 0.00001$) associated with the carriage of a particular bacteriophage and plasmid group. We found that when either of the two mobile genetic elements are carried by a *S. Enteritidis* strain, the likelihood of having *gyrA* mutations was negligible. Strains of *S. Enteritidis* lacking both the phage and plasmid developed parallel mutations in genes linked to DNA topology, antimicrobial peptide resistance, and virulence. We found that reused litter significantly reduced *S. Enteritidis* survival compared to fresh litter wood shavings ($P < 0.05$) and suggest that microbiome interventions could be used to reduce the spread of antibiotic resistant *Enteritidis*. Together, our study shows that the survival and evolution of *S. Enteritidis* in poultry environments are strongly shaped by interactions between bacteriophages, plasmids, and the microbial community.

Keywords: *Salmonella* Enteritidis; Antimicrobial Resistance; Poultry; Broiler litter; Microbiome

T228 Recovery of *Salmonella* and *Campylobacter* from alternative anatomical sample sites after a challenge with *Salmonella* serotypes or *Campylobacter jejuni* in turkeys

James A. Byrd^{*1}, Denise Y. Caldwell¹, Ken J. Genovese¹, Michael H. Kogut¹, Casey Johnson¹, Ryan A. Arsenault¹ ¹*USDA, FFSRU, College Station, Texas, United States*

Investigating the on-farm status of foodborne pathogens and how it relates to post processing products in turkeys remains an interest for the control of *Salmonella* and *Campylobacter*. The current study investigated alternative anatomical sampling locations for detecting *Salmonella* and *Campylobacter* in preharvest turkeys. In this study, 36-day-old turkeys were challenged with a cocktail of *Salmonella* Hadar, *S. Typhimurium*, and *S. Schwarzengrund* or *Campylobacter jejuni* by oral gavage or transdermal approaches (skin, or footpad; N=96). The birds were sampled on Day 0-, 6-, 9- and 12-days post-challenge. Across all *Salmonella* serotypes, total *Salmonella* was significantly higher in samples taken from the cloaca, trachea crop, joint, liver-spleen, vent feathers, and footpad samples when compared to the cecal samples ($P < 0.05$). *Campylobacter* was only recovered in high numbers in the gastrointestinal tract and GI associated sites including the ceca, cloaca, and crop in orally challenged birds. Study results demonstrate that *Salmonella* can spread systemically in turkeys without demonstrated morbidity and can be recovered at other anatomical locations at higher rates than found in ceca. *Campylobacter* remains in the gastrointestinal and GI associated sites.

Keywords: *Salmonella*; *Campylobacter*; turkeys; recovery

POSTER ABSTRACTS – STUDENT COMPETITION

Environment and Management

229P Off-the-shelf accuracy of water meters used to monitor water consumption in commercial poultry and livestock systems

Savannah L. Webb*^{1UG}, Jeremiah D. Davis¹, Kelly G. Griggs¹, Jesse C. Campbell¹, Carson M. Edge¹, Joseph L. Purswell² ¹*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ²*USDA ARS, Poultry Research Unit, Starkville, Mississippi, United States*

Water consumption is a common parameter that is monitored across poultry and livestock production systems. Accurate measurements are needed to monitor health and disease, nutrition levels, and thermal homeostasis. Accuracy testing information is highly variable or missing across water meter brands. The objective of this study was to determine the accuracy of new off-the-shelf water meters used in commercial poultry and livestock production systems. Eleven meter-treatments were created from five brands (Arad, Barn Talk, Carlon, Dwyer, and Zaxe), three-meter types (multijet, oscillating piston, and ultrasonic), and three sizes [16 x 20 mm, 20 mm, and 25 mm (5/8 x 3/4 inch, 3/4 inch, and 1 inch)]. A sample size of ten meters was tested for each meter treatment. Descriptive statistics were used to evaluate the number of meters in each treatment that passed or failed three categories of maximum permissible error (MPE) for the maximum, intermediate, minimum flow rates prescribed for the type and size of each meter. Of the 50 meters tested, 95% of the mechanical meters tested passed the manufacturer-specified MPE for all flow rates. The mean absolute error for all mechanical meter treatments was better than 1.37% for all flow rates. The multijet meters performed similarly across meter sizes. Excluding one manufacturer, 95% of the 39 ultrasonic meters tested passed the manufacturer-specified MPE for all flow rates. The mean absolute error for the ultrasonic meter treatments was better than 1.58% for all flow rates. Most brands performed well across meter sizes and were within their manufacturer-reported accuracy, supporting their suitability for poultry and livestock monitoring applications.

Keywords: water meter; multijet; ultrasonic; positive displacement; accuracy

230P Evaluating the efficacy of different temperature measurement methods in neonate poultry

Alexandra Jackson*^{1GS}, Lillian Lins¹, William A. Dozier¹, Bethany I. Baker-Cook¹² ¹*Auburn University, Poultry Science, Auburn, Alabama, United States;* ²*Institut de Recerca i Tecnologia Agroalimentària (IRTA), Animal Welfare Program, Monells, Spain*

Neonate poultry lack fully developed thermoregulation, meaning it is vital to carefully manage their environment and thermal status. Reliable methods of monitoring body temperature are essential to help ensure chick comfort. This study aimed to validate commonly used and potential methods of measuring chick temperature. Twenty broiler chicks underwent 10 repeated temperature measurements, using four different methods. Measurements of surface body, subcutaneous and core (control), and vent temperature were conducted using infrared thermography (IRT), internal temperature transponder (ITT), and contact thermometry probe (CTP), respectively (in order from least to most required bird handling). Prior to sampling, an ITT was ingested by chicks, and another was subcutaneously injected between the shoulders. The IRT images were taken from four views (anterior body, AB; lateral body, LB; dorsal body, DB; dorsal head, DH) and analyzed post-collection, where the maximum (max), minimum (min), and

average (avg) temperatures from each view were recorded. To determine the efficacy between the temperature measurement methods, an ANOVA was performed using the GLIMMIX procedure (gamma distribution; SAS 9.4). For method comparison ($P < 0.01$), temperatures measured by IRT (max values only) on the LB were highest (40.03°C), followed by subcutaneous (39.68°C) and core ITT readings (39.05°C), then CTP (39.05°C), succeeded by IRT of the AB (38.85°C), then the DB (38.49°C), with lowest being the DH (37.53°C). Regarding IRT body region and values ($P < 0.01$), the higher temperatures recorded were from the max values of the LB (40.03°C), followed by the AB (38.85°C), the DB (38.49°C), and then the DH (37.53°C). These were succeeded by the avg temperatures from the LB (36.43°C), then the AB (35.84°C), DB (35.96°C), and DH (35.81°C). This was followed by the min temperatures from the DH (33.96°C), then the LB (33.66°C) and DB (33.58°C), and the lowest temperature reading was the min AB (33.33°C). Overall, the results indicate that subcutaneous temperature measurement via ITT provides the closest approximation of true core body temperature. However, the use of CTP and IRT may be valid methods of bird temperature monitoring when their limits are considered.

Keywords: chick temperature; thermal monitoring; core body temperature; infrared thermography

231P Assessing the effect of platform enrichments on broiler density, activity level, drinking, and eating in a commercial house

Jose Millan*^{2GS}, Emily Nemerovsky¹, Katy Tarrant² ¹*California State Polytechnic University, Pomona, Pomona, California, United States;* ²*Fresno State, Animal Science and Agricultural Education, Fresno, California, United States*

Welfare problems are commonly found in conventional broiler husbandry. Introducing environmental enrichments is used as a practice to encourage poultry welfare, activity and health. This study aimed to evaluate the effect of platforms in bird density, activity, drinking and eating behaviors. A total of 5,100 conventional broilers were placed per pen in two pens measuring 375 m² and 371 m² within a 1,484 m² commercial broiler house. Twelve 2.32 m² observational areas within the video were defined as plots and arranged along the center of the two pens without any physical barriers or disturbance to the birds. Plots were classified as either control or platform, depending on whether a wooden rectangular platform was present. Six controls and six platforms were observed, platforms were placed at equal distance from feed and water lines. A control and a platform plot were under each camera, giving a total of six cameras used. A short clip was captured automatically everyday at times 6:30, 9:30, 13:30, 17:30 and 21:30 using a cc-TV system and used for bird count. The number of birds inside the 2.32 m² plot and number of birds active, drinking and eating within the plot were counted in all the control and platform plots and used for a statistical analysis. The experiment was conducted in two consecutive flocks to ensure replication and reliability. Data from the two flocks were pooled, and total values from all observation days were combined to generate flock means. Flock means were compared using a Welch's t-test in RStudio. The mean bird count by plot and percent birds active within the plot was significantly larger for plots containing platforms versus plots containing no platform in both flocks, averaging 43.77 ± 0.26 versus 32.95 ± 0.25 ($P < 0.0001$) and 9.47 ± 0.13% versus 7.82 ± 0.14% ($P < 0.0001$). The means percent birds drinking and percent eating within the plots were lower in the plots containing platforms for both flocks, averaging

9.39 ± 0.11% versus 13.65 ± 0.16% ($P < 0.0001$) and 20.71 ± 0.20% versus 31.64 ± 0.31% ($P < 0.0001$), respectively. Our results suggest that the presence of a platform increases activity and bird density, while it correlates with reduced drinking and eating behaviors when a platform is in the immediate vicinity.

Keywords: environmental enrichment; broiler; production system

232P Effect of breeder hen age, strain, and incubation egg weight loss on chick weight and yield in commercial broiler hatchery

Ryan Mitchell^{*2UG}, Roselina Angel¹, Sunoh Che¹ ¹*University of Maryland, Animal and Avian Sciences, College Park, Maryland, United States;* ²*University of Maryland, College of Information, College Park, Maryland, United States*

This study investigated the effects of breeder hen age, strain, and percentage egg weight loss to 18-day of incubation on chick weight and chick yield (the ratio of chick weight to initial egg weight at set) in a commercial broiler hatchery. The research aimed to provide practical insights to optimize hatching outcomes and subsequent broiler performance, factors critical for poultry production efficiency. An observational dataset consisting of 80 hatch batches from January 2023 to September 2025 was analyzed. Key variables included breeder hen age (25–60 weeks), strain (eight commercial strains), and percentage 18-day egg weight loss (7.78–12.07%) during incubation. Chick weight (g) and chick yield were the response variables. Statistical analysis utilized multiple linear regression models with strain as a categorical variable and age and egg weight loss as continuous predictors. Statistical significance was defined as $p \leq 0.05$. Results indicated that breeder hen age positively influenced chick weight (coefficient = 0.265, $p < 0.001$) but showed a marginal negative effect on chick yield (coefficient = -0.002, $p = 0.04$). One strain exhibited higher chick weights compared to others (coefficient = 0.416, $p = 0.033$). Percentage egg weight loss during incubation demonstrated a trend for marginal negative associations with both chick weight (coefficient = -130.95, $p = 0.048$) and chick yield (coefficient = -2.43, $p = 0.057$). The models explained a modest amount of variation ($R^2 = 28\%$ for chick weight, 16% for chick yield). In conclusion, breeder hen age and strain significantly influence chick weight and yield, while controlling incubation egg weight loss within an optimal range is essential to maximize hatchery performance. These findings suggest that focused flock management and precise, hen age-specific incubation control can improve broiler production outcomes.

Keywords: Breeder hen age; Chick weight; Chick yield; Hatchery performance; Incubation weight loss

233P Effects of water deprivation on growth performance, feed efficiency, and litter moisture in broilers selected for water conversion ratio

Karen M. Rivera^{*1GS}, Rosie Whittle¹, Angela Perretti¹, Jaelen M. Cherry¹, Sara K. Orłowski-Workman¹, Maricela A. Maqueda¹, Shawna Weimer¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Water is one of the most essential nutrients influencing growth, feed efficiency, and overall welfare in broilers. This study aimed to evaluate the effects of water deprivation (WD) on production metrics and litter quality in lines selected for low (LWCR) and high (HWCR) water conversion ratio. Broilers selected for LWCR and HWCR were raised in 12 pens per line in a 2 × 3 factorial design (two genetic lines and 3 WD durations). Birds were subjected to 0h, 12h, and 24h of WD at D25 or 26 and D38 or 39 to account for sampling intervals. Body weight (BW, g), feed conversion ratio (FCR), and water conversion ratio (WCR) were

recorded weekly, and litter samples were collected on D40 from three locations in each pen to determine litter moisture content (%). Data were analyzed in R using LMM and ANOVA. HWCR broilers had greater BW than LWCR ($p < 0.05$) throughout the study, with final BW means for HWCR being 2596g and 2455g for LWCR. Feed conversion ratio (FCR) was affected by WD only in weeks when water was restricted. FCR increased incrementally as WD duration was increased on D21-28 (0h: 1.74, 12h: 1.58, 24h: 2.28, $p = 0.02$) and D35-41 (0h: 1.99, 12h: 2.17, 24h: 2.29, $p = 0.02$). In the week between WD periods (D28-35), FCR was not affected by WD duration (0h: 1.65, 12h: 1.64, 24h: 1.64). The highest litter moisture content was under the water line (26.2%), followed by feed (19.0%) and then open (15.2%, $p < 0.001$), and litter moisture was the highest for HWCR ($p = 0.01$, 34.6%) compared with the LWCR line (14.5%). There were no significant effects of WD duration on litter moisture (0h: 18.3%, 12h: 19.2%, 24h: 21.2%, $p = 0.19$). This study demonstrated that the genetic selection for WCR may influence other production traits and litter quality. Interestingly, WD had only a short-term impact on FCR, with no effects on BW, WCR, or litter moisture. This indicates that periods of up to 24h of water restriction have little effect on production parameters.

Keywords: Water deprivation; litter moisture; body weight; water conversion ratio; production

234P Parametric lighting simulation for broiler houses: validating Radiance within Rhino-based design environments

Joshua A. Etherton^{*1GS}, John E. Linhoss¹, Jeremiah D. Davis², Joseph L. Purswell³, Anna Linhoss¹ ¹*Auburn University, Biosystems Engineering, Auburn, Alabama, United States;* ²*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ³*USDA-ARS, Poultry Research Unit, Starkville, Mississippi, United States*

Light strongly influences broiler performance, welfare, and behavior, making accurate prediction of in-house lighting conditions essential for research and management. Recent advances in parametric modeling platforms such as Rhino and Grasshopper combined with the Radiance simulation engine offer unprecedented flexibility for lighting research and design. This study reports the performance statistics of the calibration and validation of Radiance within a Rhino/Grasshopper-based workflow involving the use of additional tools such as Ladybug and Honeybee for predicting light intensity in commercial broiler houses with natural light provided by windows. A comparison is made between the performance metrics of the novel model setup and those of two commercially available light modeling software, namely AGI32 and Relux. Two houses (18.3 × 186 m) with distinct fenestration layouts were modeled: one with windows on both sidewalls (2SW) and one with windows on a single sidewall (1SW). Surface reflectance and window transmissivity were measured and incorporated into the parametric model. The 2SW dataset was used for calibration and the 1SW dataset for validation. Radiance predictions were evaluated against 750 measured light intensity values using mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R^2). Validation results showed Radiance achieved MAE of 11.74 lux, RMSE of 26.15 lux, and R^2 of 0.78 comparable to traditional tools such as Relux and AGI32 with MAE of 11.64 and 14.43, RMSE of 24.61 and 31.40, and R^2 of 0.81 and 0.74, respectively. While predictive accuracy was similar across platforms, the integration of Radiance with parametric design tools enables rapid scenario testing, advanced fenestration design analysis, and potentially, complex temporal design analysis. This work establishes a validated Radiance-based workflow within Rhino using Grasshopper, Ladybug, and Honeybee, enabling reliable

predictions and unlocking advanced capabilities for optimized lighting design in poultry environments.

Keywords: Light intensity; Broilers; Natural light; Light modeling; Poultry

235P *In vitro* evaluation of sodium bisulfate-treated buffered peptone water on *Salmonella* survival at varying pH levels

Kalyn Nelson*^{1UG}, Hudson Thames¹, Haley Nabors¹, Craig Coufal², Luis Muñoz¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Jones-Hamilton Co., Maumee, Ohio, United States

Salmonella is one of the most significant foodborne pathogens worldwide and continues to cause major public health and economic challenges. Poultry is a major reservoir for *Salmonella* spp. and an important source of human infection. Water acidification has been explored as a strategy to reduce *Salmonella* colonization, and several studies suggest that inorganic acidifiers can have strong bactericidal effects against *Salmonella* spp. The objective of this study was to evaluate the effects of sodium bisulfate-treated buffered peptone water (BPW) at different pH levels on *Salmonella* spp. survival *in vitro*. The experiment included four pH levels (2.5, 3.0, 3.5, and 4.0) acidified with sodium bisulfate and an unadjusted control (pH 7.25). Each treatment used three biological replicates, and samples were collected at 4, 24, and 48 hours after inoculation with 1.12×10^3 CFU/mL of a mixed inoculum containing equal volumes of *Salmonella* Typhimurium, *Salmonella* Infantis, *Salmonella* Enteritidis, and *Salmonella* Kentucky. Samples were serially diluted and plated on XLT4 for enumeration. All CFU counts were log₁₀ transformed and analyzed using the GLIMMIX procedure in SAS version 9.4, and means were separated using Tukey's HSD. A significant treatment x time interaction was detected ($P < 0.0001$). The pH 2.5 treatment did not show any detectable *Salmonella* spp. at any sampling time. Similarly, samples acidified to pH 3.0 showed no detectable *Salmonella* spp. at 24 and 48 hours, following an initial count of 3.84 log₁₀ CFU/mL at 4 hours. The pH 3.5 treatment demonstrated a marked reduction over time, decreasing from 3.97 log₁₀ CFU/mL at 4 hours to 1.49 and 1.0 log₁₀ at 24 and 48 hours, respectively. In contrast, the pH 4.0 treatment showed an increase over time, rising from 4.02 log₁₀ CFU/mL at 4 hours to 5.46 and 7.63 log₁₀ CFU/mL at 24 and 48 hours, which was similar to the unadjusted control at 48 hours (7.75 log₁₀ CFU/mL). Overall, these *in vitro* results support that lowering pH with sodium bisulfate, particularly to 3.5 or below, can substantially reduce *Salmonella* spp. survival and may be a useful intervention to explore further in live-bird studies to determine its applicability in poultry production.

Keywords: *Salmonella* Reduction; Sodium Bisulfate; Water Treatment; Antimicrobial Activity

236P Impacts of egg storage on hatchability and chick quality

Grant G. Bennett*^{1GS}, Victoria Holliday¹, Laura Ellestad¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Fertile eggs are stored at both the broiler breeder farm and hatchery prior to incubation to slow embryo development and allow for synchronized hatches to meet production demands. Egg storage over 7 days negatively impacts hatchability and chick quality. One strategy to offset losses from extended storage is short periods of incubation during egg storage (SPIDES). This study sought to establish an objective means of determining the effectiveness of SPIDES protocols on hatchability and chick quality. Fertilized

broiler eggs were randomly assigned to fresh (FR; stored 5 days), prolonged storage (PS; stored 21 days), or SPIDES (stored 21 days with 3 incubations) ($n=360/\text{treatment}$). For SPIDES, eggs received 3 incubations (99.5°F for 4 hours) on days 6, 12, and 18 of storage. For the 21-day incubation, eggs were incubated at standard conditions, with embryo viability assessed when eggs were transferred to the hatcher. Fertility and stage of embryo mortality were determined for non-viable eggs. Chick quality was determined via the Pasgar scoring system. Body weight (BW) and residual yolk sac weight (RYS) were used to calculate yolk-free body weight (YFBW) and RYS/BW ratio. Weight parameters were analyzed using a one-way ANOVA and Fisher's LSD test. Pasgar scoring was analyzed with the Kruskal-Wallis test and Steel-Dwass method for nonparametric multiple comparisons. The hatchability for FR was 83.3%, while PS was 39.6% and SPIDES was 62.1%. When assessing early embryonic mortality through absence of an egg tooth, SPIDES (14.9%) and PS (31.5%) eggs had greater early embryo mortality than FR eggs (2.5%). Chick BW and YFBW were similar among storage conditions ($P > 0.05$); however, chicks from PS had higher RYS and RYS/BW ratio ($P \leq 0.05$). The FR and SPIDES groups had higher average chick quality scores than PS ($P \leq 0.05$). Higher RYS may indicate that yolk nutrient utilization is reduced by prolonged egg storage. Paired with lower chick quality, this could negatively affect growth performance during post-hatch rearing, and SPIDES may help circumvent these effects. By understanding how egg storage affects hatchability and chick quality, future studies can assess how prolonged egg storage and SPIDES may affect endocrine regulation of embryonic and post-hatch growth.

Keywords: SPIDES; Pasgar scoring; yolk sac; early embryonic mortality; incubation

237P Impact of feeding regimens on growth, reproductive development, and individual performance of broiler breeder males

Madison Berger*^{1GS}, Kristen Diehl², Brenda Flack³, Patricia Quino¹, Kristian J. Almendares¹, Olivia McGuire¹, Charlene Hanlon¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²USDA-ARS, Animal Biosciences & Biotechnology Laboratory, Beltsville, Maryland, United States; ³Cobb-Vantress, Siloam Springs, Arkansas, United States

Genetic selection for rapid broiler growth causes broiler breeder males to overconsume feed and exceed optimal reproductive weight. Feed restriction regimens (FR) manage body weight (BW) and maintain mating ability. This study evaluated FR effects on growth, reproductive development, and capability in broiler breeder males. At 3 weeks of age (woa), 144 Cobb males were caged and assigned one of three FR: (1) daily-fed standard diet (ED), (2) skip-a-day-fed standard diet (SKIP), or (3) daily-fed high-fiber diet (HF). FR began at 4 woa, with energy-adjusted allocations across FR. SKIP transitioned to daily feeding at 19 woa, while HF continued throughout. Birds were photostimulated at 21 woa. Weekly BW was recorded; semen was collected from 24 woa, with concentration and mobility assessed weekly. Comb size and fleshing scores were measured at 18 woa and every 10 weeks thereafter. Abdominal fat pad (AFP), liver, and testes were weighed at 6, 12, 20, 22, 26, 35, and 55 woa from a subset of birds ($n=6/\text{treatment}/\text{timepoint}$). ANOVA was performed using SAS GLIMMIX (v9.4) with age and FR as fixed effects. An age x FR interaction influenced BW ($P < 0.001$), as HF and ED males had greater BW than SKIP males from 10 and 34 woa, reflecting divergent growth patterns. Fleshing score was also affected by age x FR ($P = 0.003$), as HF had higher scores than SKIP males at 18 woa, suggesting enhanced lean tissue deposition. Comb size was

greater in HF than SKIP males ($P = 0.007$), indicating enhanced androgenic traits. Semen traits improved from 24-31 woa ($P < 0.001$), and ED demonstrated more consistent production than SKIP males ($P = 0.003$). BW threshold analysis showed 93% production at 2.6 kg and 98.6% at 4.2 kg, with no upper limit. AFP varied by age and the interaction ($P < 0.001$); SKIP males had heavier AFP than ED at 20 woa but not at 22 woa, and all males exhibited substantial depletion post-peak. Liver weight was higher in SKIP males ($P=0.002$), suggesting increased metabolic stress. Testes weight was greater in HF and ED males ($P = 0.004$), supporting the role of daily feeding in gonadal development. Overall, nutrient delivery methods affect growth, reproductive development, and semen consistency in broiler breeder males. Further research should address fertilization outcomes.

Keywords: Broiler Breeder Males; Feeding Regimens; Reproductive Development; Growth

238P Evaluating low-cost Black Globe Thermometers for thermal comfort assessment in commercial poultry housings

Nibir Kanti Roy*^{1GS}, John E. Linhoss¹, Jeremiah D. Davis¹², Joseph L. Purswell³, Mehmet Arik⁴ ¹Auburn University, Biosystems Engineering, Auburn, Alabama, United States; ²National Poultry Technology Center, Auburn University, Auburn, Alabama, United States; ³USDA-ARS Poultry Research Unit, Starkville, Mississippi, United States; ⁴Auburn University, Mechanical Engineering, Auburn, Alabama, United States

Black globe thermometers (BGT) measure a single temperature that estimates the combined effects of air temperature, radiation, and air speed on animal thermal comfort. BGTs play a critical role in assessing animal thermal comfort and are commonly used to calculate more sophisticated heat stress-indices. However, BGTs for continuous monitoring applications are expensive (~\$750) and cheaper alternatives are needed. This project employed a field study to compare the performance of the following five alternative BGT designs to a reference 152.4 mm diameter (D) copper BGT: 1) 63P (plastic, D=63.5 mm), 2) 63PP (plastic, D=63.5 mm, painted black), 3) 63C (copper, D=63.5 mm, painted black), 4) 152P (plastic, D=152.4 mm), and 5) 152PP (plastic, D=152.4 mm, painted black). The alternative plastic globes were 3D-printed using polylactic acid (PLA) filament. BGT data was collected in an outdoor location for all BGTs at 1-min intervals over 18 weeks. Mean BGT temperatures were analyzed as a one-way ANOVA in R (R Core Team, 2024). Computational fluid dynamics (CFD) simulation software (Icepack, Ansys Electronic Desktop, Canonsburg, PA) was also used to inform the preliminary design of a cheap and accurate 3D-printed BGT. The CFD model was calibrated by varying globe parameters (emissivity, absorbance) and boundary conditions and comparing predicted temperatures to the measured reference BGT data. Results indicated that the

average temperature for the reference BGT was significantly warmer (25.6 °C) than all other globes. Mean temperatures for the larger diameter plastic globes (152P & 152PP) were significantly higher than the smaller copper and plastic globes and more similar to the reference globe. CFD simulations were calibrated against reference BGT measurements and showed a mean error of 1.95 ± 1.16 °C. These results indicate that 3D-printed globes are capable of outperforming smaller copper globes under comparable conditions. Ongoing work will optimize the 3D-printed design to enhance its thermal response and achieve closer alignment with the reference globe.

Keywords: Black Globe Thermometer; Thermal Comfort; Computational Fluid Dynamics

239P Assessment of thermal variation in commercial broiler houses during spring and later summer growout periods

Olumide B. Falana*^{1GS}, John E. Linhoss¹, zhaofei Fan², Bethany I. Baker-Cook³ ¹Auburn University, Biosystems Engineering, Auburn, Alabama, United States; ²Auburn University, Forestry Wildlife and Environment, Auburn, Alabama, United States; ³Institute of Agrifood Research and Technology, Barcelona, Spain

Variations in in-house temperatures can subject broilers to heat and cold stress, which can influence performance, health, welfare, and productivity. This study assessed temperature variation in four 18.3×182.9 m tunnel-ventilated commercial broiler houses during growout conditions. In-house temperature was measured at 5-minute intervals at 77 locations in each house during spring and late summer. A Generalized Linear Mixed Model was used to determine the effects of house (H1–H4) and season (spring and late summer) on temperature coefficient of variation (CV), with day (14–49) included as a random effect. Spatial interpolation in R was also used to evaluate daily CV patterns. The result showed that the mean CV did not differ significantly ($P>0.05$) between houses. However, season had a significant effect on temperature CV ($P<0.01$), with spring exhibiting a higher mean CV (6.64%) than late summer (5.78%). Spatial analysis showed that temperature CV increased progressively with bird age during the spring growout period, showing a longitudinal gradient that increased toward the pad end. In summer, this pattern became irregular, with increased variability emerging during mid-growout. Consistent with these spatial patterns, paired t-tests indicated that CV was significantly higher at the pad end in spring ($p < 0.01$), whereas the fan end exhibited significantly higher variability in summer ($p < 0.01$). These findings underscore the importance of considering both seasonal and spatial dynamics when managing in-house thermal environments.

Keywords: Broiler; Temperature variability; Spatial thermal gradient; Thermal management; House microclimate

Food Safety

240P Chickpeas and their derived fibers as next-generation prebiotics surpassing commercial formulations

Sukhman Kaur*^{1GS}, Shreeya Sharma¹, Abigail Schneider³, Hosni Hassan², Khaled Abdelaziz¹ ¹Clemson University, Animal and Veterinary Sciences, Central, South Carolina, United States; ²North Carolina State University, Poultry Science, Chapel Hill, North Carolina, United States; ³North Carolina State University, Animal and Veterinary Sciences, Chapel Hill, North Carolina, United States

Synbiotics, combinations of probiotics and prebiotics, are increasingly recognized for their role in improving gut health and enhancing pathogen resistance in poultry. This study aimed to

investigate and compare the prebiotic potential of chickpea flour and chickpea-derived fibers with commercially available prebiotics, including fructooligosaccharides, Galactomune (a blend of galactooligosaccharides [GOS] and β -glucan), and inulin. In this study, we evaluated the effects of these formulations on promoting the growth of *Lactobacillus* spp., enhancing metabolic activity, and improving bactericidal activity against *Salmonella* Typhimurium. Data were statistically analysed using one-way ANOVA followed by Tukey's post hoc test to determine significant difference among treatments ($p < 0.05$). Our findings demonstrate that chickpea flour and its derived fibers exhibit a superior capacity to stimulate the growth of *Lactobacillus acidophilus*, *L. reuteri*, *L. crispatus*, and *L. animalis*, as well as to

increase the production of key metabolites, including acetate and lactate, compared to the commercial prebiotics tested. Furthermore, inclusion of chickpea flour, chickpea-derived dietary fibers, or commercial prebiotics in a suspension containing *L. acidophilus* and *Salmonella* completely inhibited *Salmonella* growth while promoting *Lactobacillus* growth. Ongoing research is examining the prebiotic potential of chickpeas in enhancing *Lactobacillus* effectiveness against *Salmonella* biofilm formation. Overall, these results suggest that chickpeas and their derived fibers hold promise as cost-effective prebiotics for enhancing probiotic activity, though additional *in vivo* validation is warranted.

Keywords: Synbiotics; prebiotics; probiotics; chickens; chickpeas

241P Modulation of gut microbiota by probiotics and postbiotics delivered orally or via cloaca in *Campylobacter*-infected broiler chickens

Shreya Sharma*^{1GS}, Hosni Hassan², Khaled Abdelaziz¹ ¹Clemson University, Department of Animal and Veterinary Sciences, Clemson, South Carolina, United States; ²North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States

Campylobacter jejuni is a leading cause of foodborne illness worldwide, with poultry as a major reservoir. Due to restrictions on antibiotic use, probiotics and their metabolites (postbiotics) are gaining attention as sustainable alternatives. This study compared the efficacy of oral versus cloacal administration of probiotic lactobacilli and their postbiotic in modulating the cecal microbiome in *Campylobacter*-infected broiler chickens. Day-old chicks (n = 10/group) were assigned to seven treatments receiving postbiotic (supernatant of the overnight *Lactobacillus* culture), whole probiotic cultures or probiotic cells of four *Lactobacillus* strains (*L. reuteri*, *L. acidophilus*, *L. animalis*, and *L. crispatus*), delivered orally or intracloacally, with non-treated birds serving as the negative control. All birds were challenged with *C. jejuni* 81-176 at two weeks of age, and cecal contents were collected at week five of age for enumerating *Campylobacter* and microbiome profiling using 16S rRNA gene sequencing. Statistical analyses included the Kruskal–Wallis test for alpha diversity and *Campylobacter* load, followed by Dunn’s posthoc comparison, PERMANOVA for beta diversity, and MaAsLin2 for differential abundance modeling. Both oral and cloacal delivery of live *Lactobacillus* cells significantly lowered cecal *Campylobacter* loads by 0.34- and 0.78-log₁₀, respectively, compared to the control. Probiotic and postbiotic treatments significantly enhanced microbial richness and evenness compared to the control. Community composition shifted noticeably among treatment groups relative to the control. At the phylum level, Proteobacteria, which includes *Campylobacter*, were lower in the probiotic- and postbiotic-treated groups (0.33–1.45%) compared to the control (2.10%). Opportunistic genera, including *Escherichia* and *Shigella*, were also more abundant in the control, while unclassified *Firmicutes* were enriched in treated groups. Overall, while both probiotics and postbiotics modulated the gut microbiota, probiotic cells provided additional benefits by reducing *Campylobacter* colonization.

Keywords: *Campylobacter*; probiotic; postbiotic; microbiome; broiler

242P *Salmonella* susceptibility to Peracetic Acid (PAA) is influenced by temperature, serovar, and bacterial concentration

Marco Reina*^{1GS}, Aaron R. Bodie¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

Peracetic acid (PAA) is the most commonly used antimicrobial in poultry processing due to its ability to effectively reduce foodborne pathogens, such as *Salmonella*, before breaking down into non-toxic byproducts. About 74% of processing plants report using PAA as the primary intervention during chilling. Despite widespread use, guidelines for PAA application are limited, partially due to an incomplete understanding of the factors affecting its efficacy. Therefore, the objective of this study was to evaluate how PAA efficacy against *Salmonella* is influenced by environmental and biological factors (temperature, serovar, and bacterial concentration). This study evaluated four *Salmonella* serovars (Typhimurium, Enteritidis, Newport, and Kentucky) with three strains of each. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by broth microdilution, where a spectrum of bacterial suspensions (3-8 log₁₀CFU/mL) was challenged with nine concentrations of PAA (50-600 ppm). Next, all strains were independently subjected to three temperature conditions (4, 26, and 37°C) for 24 h. Data were analyzed using Cumulative Linked Mixed Models and effects were considered significant at *p*<0.05. Across all strains and conditions, the constructed models estimated a theoretical MIC of 67 ppm and MBC of 104 ppm when determined at 5 log₁₀CFU/mL. However, PAA efficacy decreased in response to increments in bacterial concentration. For each 1 log₁₀CFU/mL increment in bacterial concentration, MIC values increased 32 ppm, and MBC values increased 39 ppm. Temperature was also influential, samples that were held at 4°C had higher MBC values than those held 26°C and 37°C; on average, they were 78 ppm higher. Additionally, serovar showed to be significant, yet was context specific. As an example, Newport had comparatively higher MIC and MBC values (12 ppm and 30 ppm higher, respectively) than other serovars at 37°C, but this trend was not sustained at 4 or 26°C. Overall, PAA efficacy against *Salmonella* is influenced by both environmental and biological factors, which highlights the importance of tailoring pathogen control strategies that account for application temperature and target high-risk serovars at probable bacterial concentrations.

Keywords: peroxyacetic acid; antimicrobial; susceptibility; pathogen control; foodborne

243P The combination of antimicrobial blue light and peracetic acid is effective in reducing *Campylobacter jejuni* on poultry meat

Osian Nzoyisenga*^{1GS}, Issmat Kassem¹ ¹University of Georgia, Center for Food Safety, Food Science and Technology, Griffin, Georgia, United States

C. jejuni is a leading cause of bacterial foodborne infections in the USA. *Campylobacteriosis* is often sporadic and frequently linked to consuming raw or undercooked poultry. It is well established that controlling *C. jejuni* throughout the poultry production chain is challenging. To support the poultry industry and enhance food safety, we evaluated the impact of novel interventions, namely antimicrobial blue light (aBL) with and without traditional antimicrobials, on the reduction of *Campylobacter* on chicken meat. For this purpose, chicken breast pieces (10-11g) were inoculated with 8log CFU (colony-forming units)/mL *C. jejuni*. The inoculated pieces were then subjected to aBL with and without Peracetic acid (PAA). Two scenarios were investigated, 1) tandem treatments (PAA followed by aBL and vice versa) and 2) simultaneous treatments (PAA and aBL applied together), using different PAA concentrations (200 and 400 ppm) and aBL doses (60 and 120 J/cm²). After treatments, reductions in *C. jejuni* CFUs were calculated and reported as log reduction CFU/g. Differences in mean logCFU/g between treatments and controls were analyzed

using one-way ANOVA followed by the Tukey-Kramer HSD test ($\alpha=5\%$). The experiments were repeated at least three times, and each sample was tested in duplicate. Exposure to aBL alone caused 0.3-1.5 logCFU/g reduction in *C. jejuni* counts under different dose and temperature conditions, while PAA alone resulted in 0.7-1.3 logCFU/g reduction. In comparison, tandem and simultaneous treatments resulted in reductions of 1.7-2.4 and 2.0-2.2 logCFU/g, respectively. Exposure to 400 ppm PAA for 30 min followed by 120 J/cm² aBL, or vice versa, resulted in the highest reduction of ~2.4 logCFU/g ($p<0.05$). However, the reductions associated with simultaneous treatments were achieved in 15 min of total exposure. Therefore, the simultaneous treatments were considered more adequate for achieving a 2-log reduction in a relatively shorter time. We also showed that aBL had a negligible impact on the sensory properties of the meat at lower doses. Overall, these findings indicate that combining aBL with PAA enhances *C. jejuni* reduction on poultry meat. aBL is a user-friendly and affordable technology that can lead to improved post-harvest intervention strategies.

Keywords: Campylobacter; antimicrobial blue light; peracetic acid; chicken meat; food safety

244P Microbial presence of poultry feed and ingredient samples acquired from commercial and integrated feed mills

Tara Grant*^{1GS}, Emily Magee¹, Victoria Ayers², Li Zhang¹, Timothy Boltz¹ ¹Mississippi State University, Mississippi State, Mississippi, United States; ²Tennessee Tech, Cookeville, Tennessee, United States

Microbial contamination in the feed mill poses a significant threat to biosecurity and food safety. Understanding where pathogens naturally occur and how contamination plays a role in the feed mill is critical to minimize contamination and produce hygienic feed. The objective of this study is to determine the presence of *Salmonella*, *Campylobacter*, and *Escherichia coli* (*E. coli*) in raw ingredients and finished feed from different feed mills across the Southeastern region of the United States. A total of 180 samples were obtained from 14 commercial and integrated feed mills, labeled A-N, in February of 2024 and 2025. Samples were collected aseptically and analyzed for microbial prevalence. Two grams of each samples were placed in a filtered Whirl-Pak bag with 10 mL of buffered peptone water (BPW), homogenized, and incubated for 24 h at 37°C. Traditional culture methods were followed to analyze the prevalence of *Salmonella*, *Campylobacter*, and *E. coli* in all samples, and culture-positive (C+) samples were confirmed by Polymerase Chain Reaction (PCR). Compact Dry Plates evaluated the presence of *E. coli* (EC), Total Counts Rapid (TCR), and Coliforms (CF) after being diluted. PROC GLIMMIX was utilized in SAS 9.4 to analyze feed mill facility x prevalence, sample type x prevalence, and year received x prevalence. Results from this study indicated that EC, TCR, and CF were prevalent in all samples and feed mills. All C+ *Salmonella* samples were confirmed by PCR. In total, 43 samples were C+ for *Campylobacter* (23.8%), but after PCR, only 28 samples were confirmed positive (15.56%). These findings emphasize the persistent risk of pathogen contamination in poultry feed and environments within the feed mill, indicating the necessity for sanitation and monitoring protocols in both commercial and integrated feed mills. This study emphasizes that a comprehensive assessment of multiple pathogens, including less routinely tested organisms such as *Campylobacter*, is essential for identifying potential vehicles of contamination in poultry feed and reducing the risk of foodborne illness in humans.

Keywords: Feed; Hygiene; Salmonella; Campylobacter; Feed Microbiology

245P Electron beam irradiation techniques can alter the microbiome and extend the shelf life of chicken tenders

Blesseth McDonald*^{1GS}, Pheron A. Collie², Oliva Hawkins², Marco Reina², Gabriela Barszcz³, Aaron R. Bodie¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States; ³Reveam, McAllen, Texas, United States

Fresh chicken has a shelf life of two days after purchase when stored at proper refrigeration temperatures. Primarily, due to its moisture and nutrient content, this small window supports microbial growth. The perishability of chicken meat poses a challenge for poultry processors. Currently, irradiation is an alternative method for food preservation; however, consumers tend to reject this option due to the association with radioactivity (Cobalt-60, used in gamma irradiation). Alternatively, E-beam irradiation uses ionizing radiation that applies electrons to effectively reduce spoilage loads, thereby extending the shelf life of food. This study evaluated the effects of E-beam irradiation on the microbiome of chicken tenders over 27 days of storage at 4°C. Microbial communities were assessed by exposing chicken tenders to five irradiation doses: control (no treatment), 1, 1.5, 2.5, and 3.5 kGy. Each treatment was evaluated on day 3 and 27. Microbial profiling was performed by DNA extraction, polymerase chain reaction, and 16S rRNA gene sequencing using Nanopore's MinION. Data obtained was analyzed using EPI2me. On Day 3, the control samples reach a *Pseudomonas* taxa abundance of 49.70%. By day 27, control samples displayed *Pseudomonas* genera reduced to 29.00%, while members of *Serratia* at 33.01% emerged as the dominant genera. This indicated that the shelf life of the controls reached a full spoilage community. While 3.5 kGy (highest E-beam dose used in this study) on day 27 showed an early-stage microbiome spoilage with *Pseudomonas* abundance at 37.92% as the highest genera, numerically lower than the day 3 control samples. Higher irradiation doses suppressed the emergence of key spoilage taxa and significantly reduced alpha diversity ($P < 0.05$), indicating a delayed or inhibited microbial progression during storage. These compositional shifts demonstrate that E-beam treatment disrupted normal spoilage ecology and extended the microbial stability of chicken tenders under refrigeration. These results suggest that E-beam is a promising intervention for poultry processing to enhance the shelf life of chicken meat.

Keywords: Irradiation; spoilage; microbiome; shelf-life; ionizing radiation

246P Salmonella serovar transmission patterns across broiler production

Carlos D. Ayala*^{1GS}, Nikki Shariat¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

One out of five cases of salmonellosis are attributed to chicken consumption. *Salmonella* serovars associated with salmonellosis have been found throughout the production chain, with vertical transmission from broiler-breeders considered a significant source of *Salmonella* in broiler chickens. However, serovars identified in broiler-breeders do not align with those identified at processing, suggesting additional sources. Further, it has been demonstrated that multiple serovars exist at each production and processing stage. Therefore, studying the movement of individual serovars across multiple production stages should be considered within this context. The objective of this study was to determine whether there are serovar-specific transmission patterns within broiler complexes. Across six complexes in four states, bootsock samples were collected from breeder flocks (n=53) 2-3 days after egg were set at the hatchery, followed by swabs (n=45) of chick boxes (n=720) containing chicks from these breeders. Bootsocks from

the broiler houses prior to placement (n=44) and 7 days after placement (n=45) were collected. *Salmonella* was cultured following the National Poultry Improvement Plan protocol. Serovar populations were determined by CRISPR-SeroSeq and statistical analyses performed on *R. Salmonella* prevalence differed across samples (Fisher's exact test, $p < 0.05$) and was highest at 7-days (100%) and lowest at pre-placement (27.3%). Serovar complexity was higher at 7-days, compared to other production points (Fisher's exact test, $p < 0.01$). Serovar Infantis was observed only at broiler farms pre- and post-chick placement, suggesting horizontal transmission and on-farm persistence. Serovars Enteritidis and Mbandaka were identified in every stage but pre-placement, suggesting transmission from broiler-breeder flocks. This study highlights differences in serovar transmission across broiler production stages. Understanding the transmission patterns of *Salmonella* serovars of concern is key for targeting and controlling these specific serovars at live stages, which in turn is required to reduce their incidence in poultry products.

Keywords: Salmonella; Serovars; Broilers; Transmission; Environment

247P Impact of broiler processing interventions on *Salmonella* prevalence and enumeration on different chicken parts

Amber Richards*^{1GS}, Caitlin Harris², Elizabeth McMillan², Nikki Shariat¹ ¹University of Georgia, Population Health, Athens, Georgia, United States; ²USDA-ARS, Poultry Microbiological Safety and Processing Unit, Athens, Georgia, United States

Over the past few years, salmonellosis cases linked to the consumption of chicken products have remained constant, despite a reduction in contamination of final products within the same timeframe. Previous studies have demonstrated that antimicrobial interventions, specifically peracetic acid, effectively reduce *Salmonella* from post-defeathering to post-chilling while there is an increase after carcasses are cut into parts. The study objective was to assess *Salmonella* prevalence and quantity in broiler processing, specifically comparing skin-on, bone-in parts (whole and cut wings) with skinless, boneless parts (whole breast or tenders). Four-pound parts rinse samples were collected from 20 processing plants in five states, from both pre- and post-intervention as well as at the beginning and end of shifts (n = 2523). *Salmonella* prevalence and enumeration were performed with the Gene-Up assay (bioMérieux). In breast (tenders = 19 plants, or whole = 1 plant), 26.8% (169/631) of pre-intervention samples were *Salmonella*-positive, which was significantly reduced to 12.4% (78/631) post-intervention ($X^2(1, N = 1262) = 40.774, p < 0.05$). Wings were lower with 19.0% (120/630) pre-intervention and 8.9% (56/631) post-intervention ($X^2(1, N = 1261) = 26.325, < 0.05$). Enumeration data from 16 plants showed a decrease in the average *Salmonella* load in tenders and wings following antimicrobial application. In breasts load decreased from 187.31 to 80.85 CFU/mL after antimicrobial application, although this difference was not statistically significant (Wilcoxon rank-sum test, $W = 4763, p = 0.167$). The average *Salmonella* load

in wings decreased significantly after antimicrobial application, from 55.92 to 8.80 CFU/mL (Wilcoxon rank-sum test, $W = 2339.5, p\text{-value} = 0.019$). These data demonstrate that in-plant processing antimicrobial interventions reduce *Salmonella* prevalence in both tenders and wings. However, there is a difference in load reduction between the two parts, suggesting that certain mechanisms of tender processing may pose an increased cross-contamination risk.

Keywords: Broilers; Salmonella; Processing; Tenders; Wings

248P Effect of vinegar water on chicken carcasses contaminated with *Enterococcus faecium* as a *Salmonella* surrogate

Carly G. Long*^{1GS}, Md Shafiu Islam Rion¹, Lucas E. Knarr¹, Elizabeth A. Lynch², Emily B. Estanich¹, Joseph Moritz¹, Cangliang Shen¹, Timothy Boltz³, Reuben Adejumo⁴ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²West Virginia University, Nutritional and Food Science, Martinsburg, West Virginia, United States; ³Mississippi State University, Poultry, Starkville, Mississippi, United States; ⁴West University, Animal, Food and Nutritional Sciences, Morgantown, West Virginia, United States

As the demand for locally grown products increases, pastured poultry production and on-farm mobile poultry harvest become more prevalent. Recently, some Mid-Atlantic states, including Kentucky, Pennsylvania, and Ohio started offering Mobile Poultry Processing Units (MPPUs) for farmers. Applying vinegar water to inactivate foodborne pathogens in pastured poultry is an attractive method to West Virginia local small broiler growers, as it is viewed as an organic process. Therefore, the objective of this study is to evaluate the anti-bacterial efficacy of vinegar water of 0, 50, and 75% vinegar during the chilling process of broiler samples grown at the West Virginia University poultry farm and processed at the enclosed MPPU facility. The freshly processed carcasses were surface inoculated with *E. faecium*, followed by dip-chilling in refrigerated water for 24 hours. The broiler carcasses were rinsed with D/E neutralizing solution in a standard poultry sampling bag for 30 seconds, followed by a 10-fold serial dilution and spread plating onto Bile Esculin agar, respectively. The recovered *Salmonella* surrogate *E. faecium* on inoculated but unchilled broiler carcasses was $6.02 \pm 0.29 \log_{10} \text{CFU/ml}$. Chilling for 24 h in water and 5 ppm of chlorine solution significantly decreased ($P < 0.05$) the surrogate bacterial counts to 5.13 ± 0.08 and $5.10 \pm 0.16 \log_{10} \text{CFU/ml}$, respectively. Applying 2.5% lactic and citric acid blender, 50% and 75% vinegar water in the chilling tank further reduced ($P < 0.05$) the surrogate counts to 3.45 ± 0.04 , 3.64 ± 0.15 , $3.29 \pm 0.17 \log_{10} \text{CFU/ml}$, respectively. Microbial analysis was evaluated using least squares means (LS means) and analyzed statistically through ANOVA. Results illustrated a reduction in microbial growth, further validating vinegar water as a potential antimicrobial treatment. Further MPPU studies are needed to verify more effective chilling and processing strategies.

Keywords: Vinegar Water; Chilling; Surrogate; *E. faecium*

Machine/Deep/AI Learning & Modeling

249P PoultryTalk: A multi-modal RAG framework providing science-driven insights for poultry management

Kapalik Khanal*^{1GS}, Biswash Khatiwada¹, Stephen Afrifa¹, Sanjay Shah¹, Frank Bai¹, Ramesh B. Bist¹ ¹North Carolina State University, Department of Biological and Agricultural Engineering, Raleigh, North Carolina, United States

The poultry industry is essential to global food security. However, many small- and medium-scale farmers lack easy access to expert advice on disease, nutrition, housing, and management. Challenges like climate change, fluctuating feed prices, and ongoing disease risks make quick, informed decisions difficult. This study introduces PoultryTalk, a new system that uses advanced AI to provide real-time expert guidance via text and images. PoultryTalk draws from 462 open-access research papers

and extension publications. It offers more than 2,500 pages of practical information on housing, health, production, welfare, and biosecurity. The system uses OpenAI's text-embedding-3-small model to find relevant information. GPT-4o generates helpful responses. Users can describe problems, show images, or ask questions to get tailored advice. We tested the system with 200 expert-verified questions and invited over 100 participants. These included students, professors, extension specialists, farmers, and industry professionals. Of these, 34 people actively used the system and submitted 267 questions. The statistical analysis comparing GPT-4o and the PoultryTalk RAG model uses a one-way ANOVA. Results showed strong technical performance. PoultryTalk achieved 84.0% semantic similarity and an average response time of 3.6 seconds. Compared to OpenAI's GPT-4o, PoultryTalk gave more accurate and reliable poultry-related information. Participants rated the system's response accuracy at 89.9%. About 9.1% of answers were marked as incorrect, mostly when questions were unclear or when several users used the system at once. A follow-up survey showed high satisfaction: 95.6% said the chatbot was "always correct" or "mostly correct." Another 82.6% would recommend it, while 17.4% were unsure. Overall, PoultryTalk provides accurate, relevant information and is well-received by users. By combining multi-modal interaction with advanced AI, PoultryTalk helps bring research knowledge directly to farmers. This supports more innovative and sustainable poultry production.

Keywords: Artificial Intelligence; Natural Language Processing; OpenAI; Poultry Extension; Precision Poultry Farming

250P Computer vision approaches for assessing feather coverage in cage-free laying hens

Samin Dahal*^{1GS}, Bidur Paneru¹, Anjan Dhungana¹, Lilong Chai¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

The Feather condition is an indicator of productivity and welfare status in poultry. Conventional manual feather scoring procedures are laborious, time-consuming, subjective, and stressful to the hens. Alternatively, thermal imaging aims to challenge those shortcomings. Featherless areas radiate more heat to the environment, leading to higher feed consumption. However, previous studies have not been consistent in establishing a standard temperature range correlated with specific featherless areas. This observational, cross-sectional study utilized deep learning techniques to automatically assess feather scores of the dorsal body using thermal imaging. Thermal images (n=1222) of the dorsal body of hens were captured from cage-free hens with varying degrees of feather damage. Manual feather scoring was performed, classifying the hens into one of 0, 1, or 2 according to the increasing severity of feather loss to train the models. The dataset was split into training (80%), validation (10%), and testing (10%) for evaluation. A custom convolutional neural network was trained to classify thermal images into feather score categories. In addition, we trained and optimized You Only Look Once (YOLO) models to detect areas of feather damage and classify them into one of the feather scores. Python programming language was used to train models and analyze their performance. Model performance was evaluated using standard metrics, including accuracy, precision, recall and mean average precision at 0.5 intersection over union (mAP@0.5). Confusion matrices were also generated to look into class-wise performance of the models. The classification model achieved an overall accuracy of 81%, with high precision for severe feather loss. The YOLO-based object detection model showed optimum performance with YOLO11n, which achieved a precision of 71%, a recall of 61%, and a mAP@0.5 of 71%. Results show the potential of combining

thermal imaging with deep learning techniques to perform objective, automatic, and scalable feather scoring procedures. Future studies should focus on data diversity, multiple-part scoring, and semantic segmentation for robust performance.

Keywords: cage-free; computer vision; deep learning; poultry welfare; thermal imaging

251P Using aerial imagery and deep learning to identify Alabama poultry farms and evaluate rainfall capture potential

Md Min-Ha-Zul Abedin*^{1GS}, John E. Linhoss¹, Tanzeel Rehman¹, Carson M. Edge² ¹*Auburn University, Biosystems Engineering, Auburn, Alabama, United States;* ²*Auburn University, Biosystems Engineering, Huntsville, Alabama, United States*

Datasets containing the location of poultry farms are not readily available as a result of industry efforts to safeguard grower privacy. However, anonymized datasets containing geospatial information of poultry farms could be used for planning and response to animal health outbreaks, environmental monitoring, and resource conservation strategies like rainwater harvesting. The objectives of this study were to combine remote sensing imagery and deep learning techniques to create an anonymized dataset of poultry farms in Alabama and to calculate rainfall capture potential (RFCP) for the houses. 2,190 aerial images (30 cm resolution) of Alabama were obtained from the USDA National Agriculture Imagery Program and annotated using the Computer Vision Annotation Tool (CVAT). 8,918 poultry houses were annotated and used to train an object detection model (YOLOv11). 70%, 20%, and 10% of the images were used for model training, evaluation, and testing, respectively. Statistics including, Mean Average Precision (mAP), precision and recall curves, and F1 scores were used to evaluate the performance of the object detection model. mAP was 0.982 at an Intersection over Union (IoU) threshold of 0.5. Precision and recall curves indicated reliability across confidence thresholds, with the optimal F1 score reaching 0.96 at a confidence level of 0.525. Results from a Segmentation Anything Model (SAM) were used to estimate broiler house roof areas and estimate RFCP at the county level. In broiler-producing counties, RFCP ranged from 816 to 682,691 m³ per year. Errors in roof-area estimates averaged 14.71% and were typically due to overestimation. These findings demonstrate the feasibility of combining high-resolution remote sensing and deep learning methods to generate accurate, anonymized geospatial datasets of poultry farms that can support data-driven planning and resource management efforts across the poultry industry.

Keywords: Yolo; SAM; environmental monitoring; RFCP; resource conservation

252P Cassette2Vec-EC: island-aware, explainable machine learning for early prediction of pathogenic *Enterococcus cecorum* from NGS genomes

Rushikesh R. Lagad*^{1GS} ¹*University of Arkansas, Animal Sciences, Fayetteville, Arkansas, United States*

Enterococcus cecorum (EC) causes substantial morbidity and economic loss in poultry, yet distinguishing pathogenic from commensal isolates remains difficult with conventional culture or gene-list approaches. We developed Cassette2Vec-EC, an island-aware, explainable machine-learning framework that uses next-generation sequencing (NGS) genomes and genomic-island (GI) context to generate calibrated pathogenicity scores. EC genomes from pathogenic (n = 54) and commensal (n = 96) cohorts were analyzed to derive (i) antimicrobial-resistance (AMR) gene calls with ABRicate against curated databases, (ii) GI coordinates with IslandViewer, and (iii) functional and operon annotations with Prokka and eggNOG. Feature engineering captured mechanisms

of horizontal gene transfer and cassette structure, including the fraction of AMR loci embedded within GIs, integrase/replicon markers, recurrent cassette co-occurrence (for example, tet(L)/tet(M) and mef(A)/msr(D)), and islanded operon blocks. Group differences were assessed using the Wilcoxon rank-sum test, and model performance was estimated with stratified repeated cross-validation. L1-regularized logistic regression and class-weighted gradient boosting (XGBoost) models were trained with probability calibration and SHAP-based interpretability. Pathogenic isolates exhibited a higher AMR-in-GI burden than commensal isolates and more frequent integrase-linked cassette pairings. Calibrated XGBoost models achieved strong discrimination (ROC-AUC = 0.976, PR-AUC = 0.862) with good

probability calibration and low Brier loss, while L1-logistic regression provided a sparse, interpretable baseline. SHAP attributions mapped predictions to specific islands, operons, and plasmid contexts that remained stable across resamples. Conclusion: Cassette2Vec-EC moves beyond raw AMR counts to mechanism-aware, explainable prediction of EC pathogenicity from NGS data, supporting early flock-risk assessment, antibiotic-sparing decisions, and prioritization of candidate genomic targets for surveillance or vaccine design.

Keywords: Enterococcus cecorum; Genomic islands; Antimicrobial resistance; Poultry health; Machine learning

Metabolism and Nutrition: Amino Acids

253P Bone morphology and mineralization responses to the manipulation of dietary protein and amino acids in broiler diets

Gabrielle Lum^{*1UG}, Adeleye Ajao¹, Bhargavi Kasireddy¹, Oluyinka A. Olukosi¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

The effect of reducing dietary protein and supplementing with amino acids is well documented, but possible influence on bone characterized is less known. This experiment investigated the effect of a reduced crude protein (RCP) diet with variable levels of proteogenic amino acids lysine (Lys) and branched-chain amino acids (BCAA) on morphology and mineralization of femur and tibia in 21-d-old broiler chickens. There were five treatments: 1) positive control with standard crude protein (PC) level, 2) an RCP diet (NC), 3) RCP + 110% BCAA and 90% Lys (NC+BCAA1), 4) RCP + 120% BCAA and 80% Lys (NC+BCAA2), and 5) RCP + 130% BCAA + 70% Lys (NC+BCAA3). At 21 d, the tibia and femurs were collected from the birds. The proximal epiphysis length and width, distal epiphysis length and depth, diaphysis, and total length were measured. Percent ash and P were determined for the bone samples. The data was analyzed using a one-way ANOVA test on JMPPro18. The femur for the NC+BCAA3 group was significantly shorter than all other groups (P=0.0008). The femur proximal epiphysis width was narrower for the NC+BCAA2 group compared to the PC, NC, and NC+BCAA1 groups (P<0.001). Femur proximal epiphysis width was narrower for the NC+BCAA3 group when compared to all other treatment groups. The femur distal epiphysis depth was shallower for the NC+BCAA3 group than the NC, NC+BCAA1, and NC+BCAA2 groups (P=0.0013). The tibia proximal epiphysis width was narrower in the NC+BCAA2 group compared to the NC (P=0.0001). The tibia proximal epiphysis width was narrower in the NC+BCAA3 group than in the PC, NC, and NC+BCAA1 groups (P=0.0001), but there were no treatment effects on the length of the diaphysis. There was no observed effect on the P content and the ash percentage of the femur and tibia between treatment groups. The data suggests that reduced crude protein diets supplemented with BCAA in place of Lys negatively affected the morphology of the femur and tibia, primarily the fast-growing sections, but had no effect on the mineralization of the bones. Consequently, the treatment influenced whole-body nutrient accretion rather than having a specific impact on the rate of mineral accretion in the bones.

Keywords: crude protein; amino acids; bone morphology; broiler chickens; mineralization

254P The standardized ileal digestibility of soybean meal increased in a stepwise manner with increasing levels of dietary lard inclusion in the assay diet

Sujitha Veeraganti^{*1GS}, Olanrewaju Ayinde¹, June Hyeok Yoon¹, Bhargavi Kasireddy¹, Oluyinka A. Olukosi¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

In previous studies, we reported that the type of fat used in the assay diets had a marginal effect on the standardized ileal amino acid digestibility (SIAAD) of soybean meal (SBM). In the current experiment, we examined how the dietary inclusion level of fat influences the SIAAD of SBM. A total of 160 male broiler chicks were assigned to 32 metabolism cages to assess the effect of the graded inclusion level of lard in assay diets on SIAAD of SBM. There were four semi-purified diets (L10, L20, L30, and L40), with 10, 20, 30, or 40 g/kg of lard, respectively, each with eight replicates. One nitrogen-free diet with lard as a fat source was fed to measure basal endogenous amino acid flow (BEL). All birds received a basal corn-soybean meal diet from day 0 to 16, followed by experimental diets from day 16 to 21. On day 21, all birds were euthanized, and digesta were collected from the distal ileum to determine SIAAD. Data were analyzed using one-way ANOVA, and orthogonal polynomial contrasts were used to ascertain linear and quadratic responses among treatment means. Differences were considered statistically significant at P < 0.05. The SIAAD for all amino acids (AA) increased in a quadratic manner as the level of lard increased (P < 0.05). Except for the L20 group, there was a stepwise increase in SIAAD of AA with increasing dietary lard inclusion, with Cys being the most increased and Pro being the least increased. In conclusion, increasing the dietary lard content in the assay diets increased SIAAD of SBM. Therefore, it is important to consider the level of dietary fat used in the semi-purified diets when comparing SIAAD values across studies.

Keywords: standardized digestibility; SBM; lard; graded levels; amino acid

255P Comparative effects of soybean meal and corn distillers dried grains-based diets on amino acid digestibility, performance, and egg quality in laying hens

Randy N. Domer^{*1GS}, Jillian Gillum¹, Pratima Adhikari¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States

Soybean meal (SBM) provides a balanced branched-chain amino acid (BCAA) profile, whereas corn distillers' dried grains with solubles (cDDGS) exhibit elevated Leu:Ile and Leu:Val ratios (>2.6; 15–20 points), potentially inducing BCAA antagonism in layers. This study investigated the effects of SBM and graded cDDGS inclusion levels on performance, egg quality, egg solids, and nutrient digestibility were evaluated in Hy-Line W-36 hens (27 to 47 wk of age). A total of 560 hens were randomly assigned to seven diets (20 replicates/treatment) in a randomized complete block design: T1 = corn-SBM (Met + Lys); T2 = corn-SBM (Met + Lys + Thr); T3 = corn-SBM (Met + Lys + Thr + Val); T4 = corn-

SBM + 7.5% cDDGS (Met, Lys, Thr, Val, Ile); T5 = corn-SBM + 15% cDDGS (Met, Lys, Thr, Val, Arg, Trp); T6 = corn-SBM + 22.5% cDDGS (Met, Lys, Thr, Val, Arg, Ile, Trp); T7 = corn-SBM + 30% cDDGS (Met, Lys, Thr, Val, Arg, Ile, Trp). Data were analyzed as a one-way ANOVA using the GLM procedure of SAS 9.4. Hens were housed in A-frame cages at industry-standard density and provided ad libitum feed. Performance was recorded daily and weekly; ileal digesta and excreta were collected for digestibility analysis at trial end. Hen-day egg production was highest in control diets and declined with increasing cDDGS, lowest in (T1–T3 = 91%; T7 = 78%; $P < 0.0001$). Feed intake (g/b/d) and FCR (lb/doz) were highest in T3 (125.82 g; 3.64, respectively, both $P < 0.0001$) and lowest in T7 (101.63 g; 3.54, respectively, both $P < 0.0001$). Egg weight (62.36 g; $P < 0.0001$) and eggshell weight were highest in T3 (5.99 g; $P = 0.0007$). Apparent ileal digestibility of nitrogen and crude protein improved in T2–T4 (74.94%, 75.90%, 75.28% respectively; $P = 0.003$). These findings indicate that moderate inclusion of cDDGS (≤ 7.5 –15%) with crystalline amino acid supplementation in SBM-based diets supported optimal egg production and N and CP utilization, whereas higher inclusions (22.5 and 30%) reduced feed efficiency and egg production. Despite higher inclusion of crystalline amino acids in the high-cDDGS diets, optimal performance parameters were not achieved.

Keywords: Corn distillers' dried grains; Soybean meal; Amino acids; Laying hens; Egg quality

256P Assessing a simplified indicator amino acid oxidation procedure to evaluate essential amino acid utilization in broiler breeder pullets

Oscar H. Aguilar^{*1GS}, Diego Martinez¹, Lindsey Conaway², Erik Pollock², Carol Ponce-de-Leon³, Craig N. Coon¹ ¹University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ²University of Arkansas, Stable Isotope Laboratory, Fayetteville, Arkansas, United States; ³Independent Researcher, Fayetteville, Arkansas, United States

The experiment examined whether a simplified sampling approach within the Indicator Amino Acid Oxidation (IAAO) technique could reliably estimate essential amino acid (AA) utilization in

broiler breeder pullets, twelve pre-breeder CDP5 pullets were housed in individual respiratory chambers and given a 2-day adaptation period on a control diet before testing. To evaluate each digestible AA (LYS, TSAA, ARG, ILE, THR, VAL, TRP, and CP), a mixed-model Latin-square-type structure (six diets \times six pullets per AA) was used, with two AAs assessed concurrently. For each AA, birds received six diets ranging from deficient to excessive concentrations, one per day, completing the full series in 6 days. The daily ration was distributed into thirteen meals delivered at 30-min intervals. Meals 1–4 and 6 contained no isotope, meal 5 supplied a priming dose of 4.5 mg L-[1-¹³C]phenylalanine (¹³C-PHE) per kg BW, and meals 7–13 each provided 1 mg/kg BW. Air samples were taken every 30 min (13 per pullet per day), starting before meal 2, and analyzed for ¹³CO₂ enrichment using isotope-ratio mass spectrometry. A $\delta^{13}\text{C}$ profile was generated for each pullet/day, from which baseline and plateau $\delta^{13}\text{C}$ values were derived using two calculation schemes: Approach A (full set of 13 samples) and Approach B (three predetermined baseline and three plateau samples). $\Delta\delta^{13}\text{C}$ was obtained as the difference between plateau and baseline for each method. Data ($n = 288$ per method; 36 per AA) were evaluated in JMP Pro 16 using a mixed model with calculation approach, AA tested, and their interaction as fixed effects, and the order of AA testing as a random effect. A regression analysis compared values from Approach B (predicted) against those from Approach A (observed). Model performance was assessed through the approach effect, prediction error, and R^2 . Results showed no detectable difference between approaches in $\Delta\delta^{13}\text{C}$ ($P > 0.05$), no AA \times approach interaction ($P > 0.05$), prediction error $< 12\%$, and $R^2 > 0.78$. SD of the minimum dietary concentration achieving maximal utilization were also similar ($P > 0.05$). In conclusion, the simplified IAAO calculation approach can be applied in breeder pullets to estimate digestible AA utilization while also providing confidence intervals that reflect biological variability.

Keywords: Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; Isotope; Pullets

Metabolism and Nutrition: Enzymes

257P Effects of protease supplementation, alone or in combination with multi-carbohydrase, on growth performance, amino acid digestibility, and volatile fatty acids in turkey poults fed a reduced amino acid diet

Ashir F. Atoo^{*1GS}, Jorge Y. Perez Palencia², Rob Patterson², Crystal L. Levesque¹ ¹South Dakota State University, Animal Science, Brookings, South Dakota, United States; ²CBS Bio Platforms Inc, Calgary, Alberta, Canada

This study aimed to determine the effects of dietary protease supplementation, alone or combined with multi-carbohydrase, on growth performance, amino acids (AA) digestibility, and volatile fatty acids (VFA) of turkey poults fed a low-AA density diet. A total of 600 one-day-old turkey poults with an initial body weight (BW) of 55.9 g were randomly allotted to one of five dietary treatments (40 pens; 8 replicate pens/treatment; 15 birds/pen). The study lasted for 42 days and was divided into 2 phases. Experimental diets were a corn-SBM-based diet (positive control [PC]), a corn-SBM-based diet with 5% less AA (negative control [NC]), the NC diet with protease (250 ppm; NC+P), the NC with multi-carbohydrase (250 ppm; NC+M), and the NC diet with both enzymes (NC+P+M). Growth performance: BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were assessed on days 14, 28, and 42. On

day 28, two birds per pen were euthanized to collect ileal digesta (analyzed for AA, and apparent ileal digestibility [AID] of AA was calculated) and cecal digesta (analyzed for volatile fatty acids). The data were analyzed using the MIXED procedure in SAS, and preplanned contrasts were used to compare NC with the NC+ diets. There was no difference ($P > 0.05$) in BW, ADG, and FCR at d14, d28, d42, or during the overall period when PC was compared with NC, NC with NC+P, NC+M, and NC+P+M. The NC+P had higher ($P < 0.05$) ADFI than the other treatments at day 14; however, there were no differences ($P > 0.05$) between PC and NC, or between the other diet comparisons. The AID of AA was, on average, 12% greater ($P > 0.05$) in NC+M than in the other treatments without enzymes, and 5% greater ($P > 0.05$) than in the treatments with enzymes. Also, there were no differences ($P > 0.05$) in cecal volatile fatty acids among dietary treatments, between PC and NC, or among the other diet comparisons. In conclusion, a 5% reduction had no effect on growth performance, AA digestibility, or cecal VFA; therefore, this reduction was likely insufficient to cause any adverse changes in growth or AA digestibility, allowing for little to no potential for improvement with enzyme supplementation.

Keywords: amino acids; digestibility; enzymes; performance; turkey

Metabolism and Nutrition: Feed Additives

258P Utilizing an apical-out chicken enteroids model to screen monoglyceride and plant bioactive compounds against oxidative stress and tight junction disruption

Isabella Ferraro*^{1GS}, Zhaoyan Zhu¹, Jundi Liu², Yihang Li¹ ¹University of Delaware, Animal and Food Science, Newark, Delaware, United States; ²Eastman Chemical Company, Animal Nutrition BU, Kingsport, Tennessee, United States

Monoglycerides of short- and medium-chain fatty acids and plant bioactives are some of the most studied antibiotic alternatives that offer promising results for their antioxidant, anti-inflammatory, and antimicrobial functions. The current study investigates the impact of short-chain fatty acid monoglyceride (monobutylin) and plant bioactives (turmeric, olive, yellow tea, rosemary), alone or in combination, on tight junction integrity and oxidative stress mitigation in broiler chickens. To mimic the broiler intestine *in vitro*, an apical-out enteroids model was utilized to determine the efficacy of testing active compounds against tight junction barrier challenges by lipopolysaccharide (LPS), oxidative stress challenge by menadione (MD), and on the molecular level by gene expression analysis of antioxidant genes. Before the enteroids were challenged, the viability of the testing compounds was determined by assessing the enteroids quality and number at both 24 and 48 hours after incubation with the testing compounds. The active ingredients concentration used during the challenges was determined by viability testing. The student's t-test was utilized to identify the significance between the positive control and the compounds treated enteroids co-cultured with the stressor. All the plant bioactive treatments alone, and in the combination with Monobutylin, effectively reduced the amount of reactive oxygen species (ROS) under 400 μ M MD challenge, excluding olive extract alone, under varying concentrations ($P < 0.05$). Yellow tea, rosemary, monobutylin, and turmeric blend reduced the tight junction damage caused by LPS ($P < 0.05$). The expression of SOD1 was upregulated compared to MD after 24 hour exposure to yellow tea, turmeric, and monobutylin ($P < 0.05$), indicating activation of antioxidant enzymes, whereas SOD2 expression was downregulated compared to MD in response to all plant bioactives ($P < 0.05$). These findings demonstrate that selected plant bioactives alone and in combination with monobutylin can be promising candidates to mitigate the consequences of oxidative stress and inflammatory challenges in broiler chickens.

Keywords: monobutylin; plant bioactives; oxidative stress; gut barrier; broiler chickens

259P Effects of guanidinoacetic acid supplementation on growth performance and the incidence and gene expression of spaghetti meat and woody breast in broilers

Bishal Bhattachan*^{1GS}, Sarai Celestine¹, Randolph Mitchell², Sunoh Che¹ ¹University of Maryland, Department of Animal and Avian Sciences, College Park, Maryland, United States; ²Perdue Foods LLC, Salisbury, Maryland, United States

This study investigated the effects of guanidinoacetic acid (GAA) supplementation on growth performance, myopathy incidence, and gene expression associated with broiler breast myopathies, specifically spaghetti meat (SM) and woody breast (WB). We hypothesized that GAA, a precursor of creatine, influences muscle energy metabolism and susceptibility to myopathies. A controlled trial was conducted using Ross 708 broilers (42 birds \times 36 pens, total $n=1512$) assigned to two dietary treatments: control (18 pens) and GAA supplementation at 0.08% (18 pens) under commercial-like conditions. Body weight and feed conversion ratio (FCR) were measured at four production stages, and myopathy scores were assessed using visual and tactile examination at 42 and 48

days. *Pectoralis major* samples from affected and normal tissues underwent transcriptomic analyses, including hierarchical clustering and principal component analysis (PCA), to evaluate gene expression differences related to treatment and myopathy severity. Results showed that GAA supplementation significantly increased body weight and weight gain at 48 days ($p=0.03$), while FCR and myopathy incidence did not differ significantly between groups (SM: $p=0.089$; WB moderate: $p=0.901$; WB severe: $p=0.105$). Differentially expressed genes in transcriptomic analysis revealed upregulation of *ADAMTS5* and downregulation of *CD69L*, *CRYBG3*, *TMEM200A*, and *PTCHD1* genes in response to the treatment. Hierarchical clustering indicated greater upregulation of inflammation and repair genes (*MDK*, *PTX3*, *MYH1G*) in WB muscles overall, regardless of treatment. PCA and PERMANOVA showed that the age, myopathy phenotype, and their interaction significantly influenced gene expression patterns ($F=18.18$, $p=0.01$; $F=44.3$, $p=0.001$; and $F=16.9$, $p=0.01$, respectively), but treatment alone did not significantly alter global gene expression. These complementary findings suggest that GAA may modulate muscle energy and tissue remodelling process, potentially affecting myopathy development, even in the absence of measurable prevalence changes. This study highlights the complex molecular effects of nutritional supplementation and identifies gene expression changes that may help develop future mitigation strategies to reduce broiler breast myopathies.

Keywords: breast myopathy; transcriptomic analysis; nutritional supplementation; growth performance; broiler chicken

260P Impact of dietary sodium bisulfate supplementation on intestinal sulfated mucin content during a broiler coccidiosis infection

Cameron S. White*^{1GS}, Craig Coufal², Ryan N. Dilger¹ ¹University of Illinois, Animal Sciences, Urbana, Illinois, United States; ²Jones Hamilton Co., Maumee, Ohio, United States

Prior literature indicates exogenous sulfate supplementation increases levels of sulfated-mucin expression by gut goblet cells. Increased sulfation of mucins may better protect these compounds from degradation, thus supporting the intestinal barrier. In this study, a total of 84 male Ross 308 chicks at 2 d post-hatch were supplemented with a dietary sulfate source [sodium bisulfate (SBS)] and allotted to 1 of 6 treatment groups with 14 birds per treatment. This 21-d study was conducted in an incomplete factorial arrangement with two levels including: 1) *Eimeria* coccidiosis vaccine challenge (non-challenged or challenged) and 2) SBS supplementation (0.0, 0.2, 0.3, or 0.4% of the diet). Each *Eimeria*-challenged (EC) group received one of 4 SBS inclusion levels, whereas non-challenged (NC) groups received only 0.0 or 0.4% SBS. In all diets, SBS replaced sodium bicarbonate and sand filler such that diets were balanced for Na and Cl. On study d 15, EC groups were orally gavaged with a commercial coccidiosis vaccine at 5 \times the recommended dose while NC birds received a sham gavage. To increase enteric stress, EC groups were housed on used litter from a prior coccidiosis study. At 6 d post inoculation, jejunal and ileal tissue samples were collected from 8 birds per treatment and stained using the high iron diamine-Alcian blue technique. QuPath v0.5.1 software was used to quantify sulfated-mucin-positive cells within randomly selected fields of view. Data were analyzed by ANOVA using the MIXED procedure of SAS. NC birds fed 0.0% SBS had the lowest ($P < 0.01$) number of total sulfated-mucin-positive jejunal cells, whereas EC birds fed 0.4% SBS had the highest ($P < 0.01$). Relative to total cells in a given area, groups fed the highest SBS inclusion had a greater ($P < 0.01$) percentage of sulfated-mucin-

positive cells compared with treatments fed no SBS. In the ileum, NC birds fed 0.0% SBS had the fewest ($P < 0.05$) sulfated-mucin-positive cells, however relative percentages did not differ ($P > 0.05$) among treatments. These results suggest dietary SBS increases levels of sulfated-mucin-expressing goblet cells in the gut, though additional research is warranted to determine if these increased levels help maintain gut integrity and health during an infection.

Keywords: broiler; coccidiosis; health; intestine; mucin

261P The effect of feeding a supplemental sodium gluconate on growth performance of commercial male broilers

Colby Allensworth^{*1GS}, Daisuke Matsui², Hiroya Kondo³, Shiori Iino³, Mari Haneta³, Tsuyoshi Tonoue³, Izuru Shinzato³, Janet Snow⁴, Hilary O. Pavlidis⁴, Gregory Archer¹ ¹Texas A&M University, Bryan, Texas, United States; ²Valent BioSciences LLC, Animal Nutrition, Libertyville, Illinois, United States; ³Sumitomo Chemical Co., Ltd., Bioscience Research Laboratory, Osaka, Osaka, Japan; ⁴CSA Animal Nutrition, Dayton, Ohio, United States

The study objective was to evaluate the sodium gluconate (SG) effects on the performance of commercial male broilers. 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. Performance parameters including body weight (BW), feed intake (FI), feed conversion ratio (FCR), and livability (LIVE) were measured from D1–42. On D5 and D10, 2 birds from each pen were randomly selected for measurement of coccidia lesion scoring and oocysts per gram (OPG) counts. Data were analyzed using the MIXED and GLIMMIX procedures of SAS[®]. Differences ($P < 0.05$) were observed for D42 BW, with PC birds the heaviest (3099g) and NC the lightest (2832g) with SG1 (2962g) and SG2 (2965g) being statistically intermediate. Cumulative FCR to D42 were poorest for NC (1.631) while the PC (1.556) and SG2 (1.567), the lowest FCR with SG1 (1.608), being intermediate ($P < 0.05$). Significant differences were observed for D42 cumulative FI with NC (4328g) and SG2 (4391g) have the lowest FI with the PC (4517g) the highest and SG1 (4418g) being statistically intermediate. On D42 no significant differences ($P > 0.05$) were observed for cumulative LIVE, BW CV% nor OPG counts or coccidia lesion scores on D5 and D10. These results indicate that SG supplementation improved production performance relative to the challenged NC, suggesting that SG may serve as an effective alternative to coccidiostats and provide potential benefits under commercial conditions.

Keywords: Sodium Gluconate; Performance; Broiler; Growth; Paraprobiotic

262P Effect of direct-fed microbials on the cecal microbiome diversity in tom turkeys reared under commercial-like conditions

Annabelle Biggins^{*1UG}, Ana Fonseca¹, Samantha Seibel¹, Karah Cramer¹, José Ferronato¹, Erika Ganda¹, Brian Dirks², John W. Boney¹ ¹Pennsylvania State University, Animal Science, Gettysburg, Pennsylvania, United States; ²United Animal Health, Norristown, Pennsylvania, United States

Direct-fed microbials (DFM) are possible alternatives to antimicrobial growth promoters (AGP) but their potential to support the microbiome and improve poultry performance is not fully understood. Turkey production stressors such as housing,

litter, and diet changes may affect the cecal microbiome. This study evaluated the effects of Novela[®] ECL inclusion on the cecal microbiome of turkeys reared under commercial-like conditions. Tom turkeys (720) were randomly assigned to one of three treatments: control (CON), CON+0.0125% DFM, or CON+0.025% DFM. Birds were brooded in pens with fresh shavings (d0–36) and then finished in grow out pens with used litter (d37–126). Treatments were arranged in a RCBD with eight replicate pens (30 birds/pen) per treatment. Litter (L) samples were collected weekly, and cecal (C) content samples were taken from one bird/pen on d38, 49, 97, and 126 targeting the V4 region of the 16S rRNA gene using an Illumina platform. Alpha diversity (Kruskal-Wallis), beta diversity (PERMANOVA) and differential relative abundance (ALDEX2) were analyzed. The most abundant phyla were Firmicutes (C-87.5%; L-42.1%), Actinobacteriota (C-6.4%; L-56.6%), Bacteroidota (C-3.7%; L-0.1%), and Proteobacteria (C-1.2%; L-1.2%). The most abundant genera were Faecalibacterium (C-14.9%; L-3.9%), Brachybacterium (L-21.8%), Yaniella (L-9.1%), Lactobacillus (C-6.8%; L-5.2%), and Escherichia-Shigella (C-1.2%; L-1.2%). Age affected Shannon Diversity for cecal ($P < 0.01$) but not for litter ($P = 0.46$) samples. Changes in beta diversity were observed between age (C- $P < 0.01$; L- $P < 0.01$) but not treatment (C- $P = 0.89$; L- $P = 0.99$). However, age and treatment interacted for cecal samples from d38 to 49 (C- $P < 0.01$; L- $P < 0.01$) where 0.0125% DFM (C- $P < 0.01$; L- $P < 0.01$) and 0.025% DFM (C- $P < 0.01$; L- $P < 0.01$) contributed to the ecological succession. Faecalibacterium and Lactobacillus were the differentially abundant taxa identified when d38 and 126 were compared. While age is the major driver of microbiome composition changes, DFM inclusion also contributed to succession when housing, litter, and diet changed. Overall, this study brings insight into how DFMs may be a valuable tool to enhance gut health and used as an alternative to AGPs.

Keywords: microbiome; turkey; alpha diversity; beta diversity

263P Effects of repeated exposure to Fumonisin, Deoxynivalenol, and Zearalenone on egg quality in broiler breeder hens and the multiprotect products

Laharika Kappari^{*1GS}, Clarisse Techer², Matthew K. Jones³, Anthony Pokoo-Aikins⁴, Todd Applegate¹, Ramesh Selvaraj¹, Anthony Glenn⁴, Charles L. Hofacre⁵, Revathi Shanmugasundaram⁴ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²miXscience, Bruz, France; ³Southern Poultry Research Group, Watkinsville, Georgia, United States; ⁴US Department of Agriculture, Athens, Georgia, United States; ⁵Southern Poultry Research Center, Watkinsville, Georgia, United States

Fusarium mycotoxins are common feed contaminants, and their chronic presence in broiler breeder diets may influence eggshell quality and hatchability. The objective of this study was to evaluate the effects of repeated exposure to mycotoxin-contaminated diets on egg quality and the ability of binder feed additives (Multiprotect Y and Multiprotect Up) to mitigate those effects. Challenged groups were exposed to mycotoxins (4 mg/kg of deoxynivalenol, 3 mg/kg of fumonisins, and 1 mg/kg of zearalenone) in the diet for 1 week, followed by 4 weeks of unchallenged feed from 14 weeks onward. A total of 160 pullets and 24 roosters were assigned to four treatments: 1. Control, 2. Mycotoxin, 3. Mycotoxin + Multiprotect Y at 2 kg/MT, and 4. Mycotoxin + Multiprotect Up at 1.5 kg/MT. Egg quality parameters (egg weight, albumen height, Haugh unit, yolk color, height, diameter, index, eggshell breaking strength, thickness, and weight) were measured every week (WK) from WK 24 to 34 using a digital egg tester. The data was analysed using Kruskal-Wallis test. Eggshell breaking strength was reduced significantly in the mycotoxin treatment at

WK 24 and 32 by 4.4% and 14%, respectively ($P < 0.05$) compared to the control. Multiprotect Y improved strength at WK 24 by 14% and at WK 32 by 4% ($P < 0.05$) compared to the mycotoxin group. Egg shell thickness was reduced in the mycotoxin group at WK 24 by 2.4% ($P < 0.05$), when compared to the control, and Multiprotect Y supplementation improved the thickness at WK 24 by 12% when compared to the mycotoxin group. Shell weight also decreased in the mycotoxin group at WK 24 and 26, by 3.7% and 4% ($P < 0.05$) compared to the control, but supplementation with Multiprotect Y increased shell weight by 9% at WK 24 and 6% at WK 26 ($P < 0.05$). Multiprotect Up at 1.5 kg/MT had no significant effect on egg quality parameters. In this experiment, repeated exposure to mycotoxins significantly altered eggshell characteristics but had no significant effect on internal egg quality parameters. To conclude, Multiprotect Y at 2 kg/MT mitigated the effects of mycotoxin exposure, and supplementation in broiler breeders can effectively preserve shell quality under repeated mycotoxin challenge.

Keywords: Mycotoxins; Broiler breeder; Egg quality

264P Comparative evaluation of the wheat bran and soyhull alone or in combination with stimbiotic on ileal nutrient digestibility and relative digesta oligosaccharide profile in broiler chickens

Bhargavi Kasireddy*^{1GS}, Oluyinka A. Olukosi¹ ¹University of Georgia, Athens, Georgia, United States

Supplementation of stimbiotic (STB- xylanase and xylo-oligosaccharide) with either wheat bran (WB) or soyhull (SH) facilitates the breakdown of complex non-starch polysaccharides into fermentable oligosaccharides, enhancing microbial enzyme activity and nutrient release, and improving the overall digestibility in broilers. This 35-day study compared WB and SH, alone or in combination with STB, or STB supplementation alone, on dry matter, amino acid digestibility and relative oligosaccharide profile in broilers. For soyhull inclusion, diets were formulated with low-protein soybean meal (LPSBM). The LPSBM was produced by adding SH to the standard soybean meal (SSBM) at a 56 g/kg rate to make an LPSBM of 440g/kg crude protein. A total of 924 day-old male broiler chicks were allocated to six treatments in a randomized complete block design: 1) SSBM; 2) SSBM + 0.1 g/kg STB; 3) SSBM + 50 g/kg WB; 4) SSBM + 50g/kg WB + 0.1g/kg STB; 5) LPSBM diet; and 6) LPSBM + 0.1g/kg STB. On day 28, ileal content was collected and analyzed for dry matter (DM), amino acid digestibility, and the oligosaccharide profile. The data were analyzed in a 2 × 3 factorial arrangement (diet type and STB supplementation) using JMP Pro 18. There were no significant interactions or main effects of diet type and STB on DM, dispensable and indispensable amino acid digestibility ($P > 0.05$), except Thr. A significant main effect of diet type was observed for Thr, with digestibility significantly higher in CSBM and CSBM+WB than in LPSBM ($P = 0.007$). There were no significant interactions or main effects observed for relative abundance of hexoses (Hex) and pentoses (Pent) oligosaccharides ((Hex)3, (Hex)4, (Hex)5, (Hex)6, (Pent)3, (Pent)4, (Pent)5, and (Pent)6). In conclusion, neither WB nor SH inclusion nor STB supplementation influenced the amino acid digestibility and the ileal oligosaccharide profile in broilers. The lack of response to STB may be related to the moderate dietary level of the fiber, which could have limited substrate availability for enzyme activity.

Keywords: Wheat bran; soy hulls; nutrient digestibility; stimbiotic; oligosaccharide profile

265P The potential of natural adsorbents to mitigate Clostridium perfringens-induced oxidative stress in chicken intestinal epithelial cells

Jayton Glenn*^{1UG}, Samuel E. Agada², Iyabo Oluseyifunmi², Samson Oladokun² ¹Texas A&M University, Veterinary Medicine and Biomedical Sciences, College Station, Texas, United States; ²Texas A&M University, Poultry Sciences, College Station, Texas, United States

Poultry remains an affordable and accessible source of protein for the growing global population. However, the productivity of the industry is increasingly constrained by consumer demand for reduced antibiotic use and the need for sustainable disease control alternatives. Natural adsorbents such as clays and biochar have shown promise as alternatives for managing diseases like necrotic enteritis, which is caused by the Gram-positive bacterium, Clostridium perfringens, a pathogen of major economic importance in the poultry industry known for inducing oxidative stress in the chicken intestines causing intestinal lesions and depression in growth performance. As a cost-effective and practical validation approach, this study employed an in vitro cellular assay, 2',7'-Dichlorodihydrofluorescein diacetate (H2DCFDA /DCFDA) cellular ROS Assay to evaluate the potential of two natural adsorbents; clay material (kaolinite) and Agricultural waste biochar in mitigating oxidative stress in chicken intestinal epithelial cells (IECs) of duodenal and ileal tissue origin, infected with Clostridium perfringens. The concentrations of the adsorbents tested include 3.125, 6.25, 12.5, 25, 50, 100 mg/mL. Results showed that kaolinite exhibited a clear linear dose-dependent positive ($P < 0.05$) effect from concentrations 12.5 to 100 mg/mL in duodenal IECs. In contrast, only biochar concentrations of 50 and 100 mg/mL were effective ($P < 0.05$) in reducing oxidative stress in duodenal IECs. Similarly, in ileal IECs, oxidative stress reduction was observed only at the 50 mg/mL dose for both biochar and kaolinite treatments. In conclusion, kaolinite was the more potent adsorbent, reducing oxidative stress in duodenal IECs at lower concentrations (≥ 12.5 mg/mL), while biochar was effective only at higher doses (50–100 mg/mL). Further research is recommended to validate the oxidative stress-mitigating potential of this natural product through in vivo studies.

Keywords: clostridium perfringens; oxidative stress; intestinal epithelial cells; adsorbents; chicken

266P Evaluation of an Lippia organoides essential oil and fructooligosaccharide prebiotic on growth performance and energy utilization in broilers to 21 d post-hatch

Matias Moreno*^{1GS}, Jaime A. Ángel Isaza², Mariana Parra Cerezo², Samuel J. Rochell¹, Gustavo Quintana Ospina¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Promitec, Bucaramanga, Colombia

The search for effective alternatives to antibiotic growth promoters has intensified interest in phyto-genic and prebiotic additives that may improve gut health and performance in broilers. The objective of this study was to evaluate the effects of a *Lippia organoides* essential oil-based product and a fructooligosaccharide (FOS) prebiotic on growth performance and nitrogen-corrected apparent metabolizable energy (AMEn) in broilers from 0 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 organoides* essential oil (EO), and a FOS prebiotic (PRE). Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were evaluated at 7, 14, and 21 d, and AMEn was determined during a balance assay from 18 to 21 d. Data were analyzed using a one-way ANOVA, and means

were separated using Fisher's LSD test; significance was declared at $P < 0.05$. From 0-7 d, birds fed EO (FCR = 1.045) and PRE (FCR = 1.027) had lower FCR compared with CTL (FCR = 1.074; $P < 0.05$), indicating improved early feed efficiency. Body weight gain from 0-14 d was higher ($P < 0.05$) for EO (0.491 kg) than CTL (0.473 kg), with PRE being intermediate (0.488 kg). Cumulative results to 21 d showed that birds fed EO and PRE achieved higher ($P < 0.05$) final BWG (EO = 1.110 kg, PRE = 1.099 kg) than CTL (1.054 kg). Cumulative FI (0-21 d) was highest for EO (1.362 kg), intermediate for PRE (1.337 kg), and lowest for CTL (1.306 kg; $P < 0.05$). Mortality and dietary AMEn did not differ among treatments ($P > 0.05$). In summary, supplementation with a *Lippia origanoides* essential oil and FOS prebiotic improved early feed efficiency and increased cumulative BWG to 21 d compared with the control diet. Although no changes in AMEn were observed, additional research is warranted to determine if performance response was related to dietary nutrient or energy utilization.

Keywords: Lippia origanoides; Fructooligosaccharides; Apparent metabolizable energy; Essential oils; Prebiotic

267P Effects of dietary bio-clay on organ weight, serum biochemistry, and immune response in aflatoxin-challenged broiler chickens

Haley R. Blades^{*21UG}, Iyabo Oluseyifunmi², Samson Oladokun² ¹Texas A&M University, Department of Animal Science, Kingsbury, Texas, United States; ²Texas A&M University, Department of Poultry Science, College Station, Texas, United States

This study investigated the efficacy of dietary Bio-clay product, a sodium-rich montmorillonite conjugated with ZnO in mitigating the toxic effects of aflatoxin (AF) in broiler chickens. A total of 192 day-old off-sex Cobb-500 broilers were allocated to 8 replicate cages of 6 chicks each and assigned to 4 treatments from hatch to day 28: (1) control (corn-soy diet), (2) control + clay (8 g/kg), (3) AF challenge (2 mg/kg), and (4) AF challenge + clay (2 mg/kg AF + 8 g/kg clay). Growth performance was monitored and recorded on a weekly basis. On day 28, blood samples were collected for serum biochemical analysis, and relative organ weights were measured. The results showed that relative bursa weight was greater ($P = 0.052$) in the aflatoxin challenge + clay group compared to other groups, except for aflatoxin-only group. The relative spleen and liver weights were greater in the challenged group with or without clay. Nonetheless, a 5.77% reduction ($P > 0.05$) in liver weight was found in aflatoxin challenge + clay group relative to aflatoxin-challenge group, suggestive of reduced inflammation. Serum biochemistry revealed that the glucose and cholesterol were lower ($P < 0.05$) in aflatoxin-exposed groups (with or without clay) than in the control and clay-only groups and reverse is the case for chloride, an indication of alteration in lipid and glucose metabolism. Alkaline phosphatase (ALKP) was lower ($P = 0.002$) in aflatoxin-exposed groups (with or without clay) relative to control except clay-only group. Aspartate aminotransferase (AST) was lower ($P = 0.049$) in AFB + clay group relative to control but comparable to others. Glutamate dehydrogenase (GLDH) a marker of liver damage was reduced ($P = 0.012$) in aflatoxin challenge + clay group relative to aflatoxin challenge group by 23%. The serum immunoglobulin G level was unaffected ($P = 0.477$). Dietary Bio-clay partially mitigates aflatoxin-induced liver damage and inflammation in broilers but does not fully prevent metabolic disturbances or affect humoral immunity.

Keywords: Aflatoxin; Organ weight; Bio-clay; Serum biochemistry; Broiler chickens

268P Evaluation of non-formaldehyde liquid antimicrobial feed sanitation on broiler breeder performance

Beverly McLendon^{*1GS}, Tatyany Mani¹, Emmillie Boot¹, Rebecca Wysocky¹, Yun-mei Amy Lin², Ramon Malheiros¹ ¹North Carolina State University, Prestage department of Poultry Science, Raleigh, North Carolina, United States; ²Anitox Corporation, Lawrenceville, Georgia, United States

Improving broiler-breeder (BB) performance remains a priority for the poultry industry, particularly in relation to reducing bacterial contamination of fertile eggs to safeguard hatchability and chick quality. Older BB flocks are known contributors to vertical transmission of bacteria, underscoring the need for effective interventions. Antimicrobial feed treatments represent a promising but under-researched approach. This study evaluated the efficacy of a non-formaldehyde liquid feed treatment on the performance and egg microbial load of older broiler-breeder hens. A total of 128 Ross 708 hens were housed with Yield Plus males and randomly assigned to control or treated diets across four replicates per treatment. Birds received their respective diets from 23 to 65 weeks of age. From 40 to 65 weeks, egg-shell contamination, hatchability, chick quality, and broiler livability were assessed. Feed samples and egg-shell washings were analyzed for total aerobic bacteria, yeasts, molds, and *Salmonella* spp. The feed treatment did not negatively affect egg production, hatchability, chick quality, or livability. At 50 weeks of age, treated hens produced eggs with reduced mold contamination compared with controls (control: 2.80 log mold/egg; treated: 2.59 log mold/egg; $p = 0.0004$). Hatchability improved in treated birds at 40 and 55 weeks (control: 74.91%; treated: 81.89%; $p = 0.0546$). Microbial enumeration values were log-transformed prior to statistical analysis, and all data were analyzed using JMP Pro 18 with $\alpha = 0.05$. These results demonstrate that a non-formaldehyde liquid feed sanitizer can reduce certain microbial contaminants without compromising productivity, suggesting its potential value as an antimicrobial feed additive for older broiler-breeder flocks.

Keywords: Antimicrobial; Broiler Breeders; Feed Additive; Hatch; Chick Quality

269P Dietary bio-clay product modulates organ weights and serum biochemical responses in broiler chickens challenged with aflatoxin

Eilyn G. Caceres^{*2UG}, Iyabo Oluseyifunmi¹, Timothy Phillips⁴, Thomas Isakeit⁵, Youjun Deng³, Samson Oladokun¹ ¹Texas A&M University, Poultry Science, College Station, Texas, United States; ²Texas A&M University, Animal Science, College Station, Texas, United States; ³Texas A&M University, Soil and Crop Sciences, College Station, Texas, United States; ⁴Texas A&M, Veterinary Integrative Biosciences, College Station, Texas, United States; ⁵Texas A&M University, Plant Pathology, College Station, Texas, United States

A total of 192 day-old off-sex Cobb-500 broiler chickens were used to evaluate the potential ameliorative effects of dietary Bio-clay product, a sodium-rich montmorillonite conjugated with ZnO, on aflatoxin (AF) toxicity in a 21-day study. Birds were randomly assigned to four treatments with eight replicates per treatment in a completely randomized design: (1) corn-soybean meal-based control diet, (2) control + clay minerals (8 g/kg), (3) aflatoxin challenge (2 mg/kg AF), and (4) aflatoxin challenge + clay minerals (2 mg/kg AF + 8 g/kg clay). Birds and feed were weighed on days 0, 7, 14, and 21 to calculate feed intake (FI), weight gain (WG), and feed efficiency. Blood samples and relative organ weights were collected on day 21. Relative bursa ($P = 0.014$) and spleen ($P < 0.001$) weights were higher in AF-challenged birds than control but comparable among other treatments. Liver weight was markedly lower ($P < 0.001$) in AF + clay birds compared to

AFB₁ alone, indicating reduced hepatic stress. Serum glucose and cholesterol were lower ($P < 0.05$) in AF-challenged birds with or without clay, while alkaline phosphatase (ALKP) was higher ($P = 0.002$) in clay-only birds but comparable to control. Aspartate aminotransferase (AST) was lower ($P = 0.028$) in AF-challenged birds relative to control. Uric acid was higher in clay-only birds than aflatoxin-challenged group. Serum Na⁺ and Na⁺: K⁺ ratio was higher ($P < 0.05$) in AF + clay birds than clay-only but comparable to other groups, while Cl⁻ increased ($P < 0.05$) with AF (with or without clay). Total antioxidant capacity (TAC), superoxide dismutase (SOD), and immunoglobulin Y (IgY) were not significantly affected ($P > 0.05$) by treatments. Bio-clay product recorded hepatoprotective effect and alleviated the adverse effects of aflatoxin exposure on serum biochemical parameters, whereas its effect on antioxidant and immunological markers was limited under these conditions.

Keywords: Bio-clay; Aflatoxin; Relative organ weights; Serum biochemistry; Broiler chickens

270P **Invitro screening of antimicrobial and cytoprotective effects of natural adsorbents as therapeutic candidates for the control of Necrotic Enteritis in poultry**

Samuel E. Agada*^{1GS}, Morgan Farnell¹, Youjun Deng², Audrey McElroy¹, Denise Y. Caldwell³, James A. Byrd³, Timothy Phillips⁴, Samson Oladokun¹ ¹Texas A&M University, Poultry Sciences, College Station, Texas, United States; ²Texas A&M AgriLife Research, Soil and Crop Sciences, College Station, Texas, United States; ³U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center., College Station, Texas, United States; ⁴Texas A&M University, Department of Veterinary Physiology and Pharmacology, College Station, Texas, United States

Necrotic enteritis (NE), caused by *Clostridium perfringens*, continues to pose a major threat to poultry health, particularly under antibiotic-free production systems. Natural adsorbents such as biochar, clay minerals, and graphite are promising antibiotic alternatives due to their potential to bind bacterial toxins and modulate oxidative stress. This study investigated the direct antimicrobial and cytoprotective and antioxidant effects of seven natural adsorbents: clays (kaolinite, bentonite, Wyoming clay, sodium montmorillonite), graphite, wood biochar, and agricultural waste biochar. Samples (n=6) of each natural adsorbent in 3 independent experiments were used for all assays. Antibacterial activity against *Clostridium perfringens* was evaluated using microbroth dilution and agar diffusion assays, while cell viability was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay and quantification of intracellular reactive oxygen species (ROS) levels using the DCFDA/H2DCFDA (2',7'-dichlorofluorescein diacetate/2',7'-dichlorodihydrofluorescein diacetate) assay using primary chicken intestinal epithelial cells derived from the duodenum, jejunum, and ileum. Agar diffusion showed that sodium montmorillonite, graphite, kaolinite, bentonite, and both biochar types inhibited ($P < 0.05$) *Clostridium perfringens* growth in a dose-dependent manner. Sodium montmorillonite, hardwood biochar, and agricultural-waste biochar also exhibited the strongest ($p < 0.05$) antibacterial activity at 100, 50, and 25 mg/mL using agar well diffusion. A marked reduction ($p < 0.05$) in ROS generation in adsorbent-treated cells compared to negative controls was also observed, with the most pronounced antioxidant effect observed in

graphite, bentonite, and wood biochar treatments across all intestinal segments. The MTT data indicates that kaolinite, sodium montmorillonite and wood biochar maintained high cell viability at the lowest concentrations of tested concentration range (3.125–100 mg/mL). Collectively, these findings suggest that specific natural adsorbents particularly graphite, sodium montmorillonite, bentonite, and hardwood biochar based on demonstrated antimicrobial and cytoprotective effects could be potential therapeutic agents against NE in poultry.

Keywords: Natural Adsorbents; Clostridium Perfringens; Necrotic Enteritis; Intestinal epithelial Cells; Antimicrobial activity

271P **Effects of dietary propolis supplementation on growth performance and gut integrity in broilers challenged with Eimeria spp.**

Hanseon Ko*^{1GS}, Jihwan Lee², Doyun Goo¹, Venkata Sesha Reddy Choppa¹, Woo Kyun Kim¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Jeonbuk National University, Animal Science, Jeonju-si, Jeonbuk-do, Korea (the Republic of)

The objective of this study was to evaluate the effect of dietary propolis supplementation on growth performance, intestinal lesions, and gut integrity in broilers challenged with *Eimeria spp.* The experiment was designed as a 2x2 factorial arrangement with *Eimeria* challenge (non-challenged vs. challenged) and dietary propolis supplementation (0 vs. 250 ppm). A total of 336 one-d-old broilers were randomly allocated into 28 cages (12 birds each) for 28 d. Propolis groups received diets containing propolis at 250 ppm from d 0 to 28. On d 14, all birds in the *Eimeria* challenged groups were orally challenged with 1 mL of *Eimeria* oocyst suspension containing 62,500 (*E. acervulina*), 12,500 (*E. maxima*), and 12,500 (*E. tenella*) sporulated oocysts. Growth performance, oocyst shedding, gut permeability, intestinal lesions, histology, and jejunal gene expression of tight junction proteins and cytokines were measured. The data generated from d 0 to 14 were analyzed using a t-test to compare the propolis supplementation effect. The data from d 14 to 28 were analyzed using two-way ANOVA, and the model included the *Eimeria* challenge and propolis supplementation as the main effects and their interaction effects. Interaction effects were observed in BW on d 20 and 28, both BWG and FCR from d 14 to 20 and d 14 to 28; *Eimeria* challenge control showed the lowest BW and BWG and the highest FCR compared to the other treatments ($P < 0.05$). Propolis supplementation increased FI from d 14 to 20 and improved BWG and FCR from d 20 to 28 ($P < 0.05$). Reduced gut permeability and lowered lesion scores were observed in propolis groups on d 20 and 21, respectively ($P < 0.05$). *Eimeria* challenge downregulated gene expression of claudin (CLD) 2, CLD4, occludin (OCLD), and junctional adhesion molecule (JAM) 2 in the jejunum on d 20 ($P < 0.05$), whereas propolis supplementation upregulated gene expression of CLD2, OCLD, ZO2, and JAM2 ($P < 0.05$). Overall, dietary propolis supplementation improved growth performance, reduced intestinal lesions, and modulated gut integrity-related gene expression in broilers challenged with *Eimeria*. These findings suggest that dietary propolis may serve as a potential nutritional strategy to mitigate gut damage associated with coccidiosis.

Keywords: Propolis; Coccidiosis; Feed supplement; Gut health; Broiler

Metabolism and Nutrition: General Nutrition

272P Supplementation of standard and higher doses of phytase enzyme on alfalfa and wheat midds-based diets fed to 66 wk old laying hens

Macy Keeton-Reddix*^{1UG}, Craig Wyatt², Pratima Adhikari¹ ¹Mississippi State University, Poultry Science Department, Mississippi State, Mississippi, United States; ²AB Vista Feed Ingredients, Plantation, Florida, United States

Phytase supplementation in poultry diets has been shown to improve nutrient absorption, such as calcium, phosphorus and amino acids. Alfalfa meal, a natural source of beta-carotene and xanthophyll, can be incorporated into poultry diets to improve yolk and skin pigmentation. However, a study combining higher phytase supplementation and alfalfa meal on pigmentation levels in layers has not been completed, hence this trial was conducted to investigate this interaction. A total of 120 Hy-Line 66-week-old hens were randomly placed in cages (2 hens/cage) with 15 replicates/treatment. The factorial design consisted of 2 levels of phytase and 2 fiber sources: TRT 1—corn—2% wheat middling (WM) with 400 FTU phytase; TRT 2—corn—2% alfalfa meal (AM) with 400 FTU; TRT 3—corn—WM with 2,000 FTU; and TRT 4—corn—AM with 2,000 FTU. All diets were fed for 6 weeks as mash.

Feed intake and feed conversion were calculated at 3 and 6 weeks. Bird weights were recorded at the beginning and end of the trial. One egg from each pen was collected at 0, 3, and 6 weeks for egg quality. Blood samples were collected for plasma inositol, and liver weights and fatty acid profiles were determined at the end of the study. Data were analyzed using Proc GLM, and means were separated using Fisher's protected LSD. Yolk color was significantly higher ($P < 0.001$) in TRT 2 (7.46) and TRT 4 (7.53). At week 6, feed intake showed a trend ($P = 0.0872$), with TRT 3 (118.55 g/day) and TRT 4 (115.13 g/day) highest. Inositol concentrations differed among treatments ($P < 0.0001$), with TRT 3 highest (21.679) followed by TRT 4 (19.147), while TRT 1 (17.981) and TRT 2 (17.818) were lower. Fatty acid analysis showed lower w-3 values in alfalfa diets (0.218 and 0.234) compared with non-alfalfa diets (0.236 and 0.242), while w-6 values did not differ among treatments. These findings suggest that alfalfa meal improves yolk pigmentation and increases inositol values, while increasing phytase above standard inclusion does not further enhance pigmentation, fatty acids, or inositol response.

Keywords: alfalfa; phytase; layers

Metabolism and Nutrition: Vitamins and Minerals

273P Performance, intestinal morphology and tibia morphometry of broilers of 21-days of age fed diets supplemented with 25-hydroxycholecalciferol and probiotics

Fatima Y. Araujo Caldas*^{1UG}, Carlos N. Vilchez Perales¹ ¹La Molina Agrarian University, Lima, Peru, Animal Science, Lima, Peru

This study investigated the effects of dietary supplementation with 25-hydroxycholecalciferol and probiotics (*Bacillus amyloliquefaciens* CECT 5940) 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 supplemented with probiotics (T2), a basal diet supplemented with 25-hydroxycholecalciferol (T3), and a basal diet supplemented with a combination of both (T4). At 21 days of age, the chickens in the T4 group exhibited a significantly higher total weight gain (1359.0 ± 117.84 g) compared to the other treatments ($p < 0.05$). The registered data were submitted to ANOVA under a Completely Randomized Design and for the means separation the Duncan Test was used. The results also revealed positive effects on intestinal health, with the T4 treatment showing a significantly greater ileal villus height-to-crypt depth ratio (8.4 ± 1.4 ; $p < 0.05$) than T1, T2, and T3. This indicates a larger surface area for nutrient absorption and improved intestinal integrity. Furthermore, significant differences were observed in bone morphometry, specifically in the total weight, head width, and diaphysis width of the tibia, where the T4 group showed superior results. In conclusion, the combined supplementation of 25-hydroxycholecalciferol and probiotics acts synergistically to significantly improve productive performance, intestinal morphology, and bone morphometry in broiler chickens at 21 days of age. These findings highlight the combination of these two additives as a promising strategy for optimizing growth and health in poultry.

Keywords: 25-hydroxycholecalciferol; Broilers; intestinal morphology; bone morphometry; *Bacillus amyloliquefaciens*

274P Influence of phosphorus level in the pre-experimental diet on endogenous losses and standardized phosphorus digestibility of copra meal for broiler chickens

Vitor Santos Haetinger*^{1GS}, Olayiwola Adeola¹ ¹Purdue University, Department of Animal Science, West Lafayette, Indiana, United States

Broiler nutrition in the starter period may affect digestive capacity later in life, however it is unclear how phosphorus (P) nutrition may impact endogenous losses and P digestibility of feed ingredients. The objective of this study was to determine the effect of pre-experimental diet non-phytate P (nPP) concentration on endogenous losses of P and the standardized P digestibility of copra meal with or without phytase supplementation for broiler chickens. The treatments were organized in a 2 x 3 factorial arrangement in a split plot experiment with two pre-experimental starter diets with low or recommended level of nPP (3 or 4.5 g/kg) fed for 18 days (24 replications with 8 birds per cage), and three experimental grower diets fed for the subsequent 3 days, two with copra meal as the sole source of P, with or without 1,000 FYT/kg of phytase, and one P-free diet formulated to determine endogenous losses of P (8 replications with 6 or 10 birds per cage). Statistical analysis was conducted as a randomized complete block in a split-plot design, with the starter diets as whole plots and grower diets as subplots, and the P-free diets analyzed separately. Ileal digesta and excreta samples were collected at the end of the experiment. No significant interaction was observed for growth performance or digestibility metrics, birds fed the low nPP starter presented higher ($P < 0.05$) standardized P and Ca digestibility, phytase supplementation increased ($P < 0.05$) standardized P digestibility. The basal endogenous losses of P and Ca were estimated at 118 and 208 mg/kg of dry matter intake, respectively, and they were not affected by the starter diet. The standardized P digestibility of copra meal was 80% in the low P starter and 77% in the adequate P starter ($P < 0.05$), and with phytase supplementation these were increased to 88 and 83%, respectively. In conclusion, the nPP level fed during the pre-experimental period affected P digestibility estimates and thus should be considered when evaluating ingredients, in addition copra meal presented considerably high P digestibility which was enhanced with phytase supplementation.

Keywords: digestibility; phosphorus; endogenous loss

275P Effects of inorganic, HMTBa-chelated trace mineral, or blended sources of zinc, copper, and manganese on the egg quality of laying hens

Ruth L. Wallace*^{1GS}, Timothy Boltz¹, Jarred Oxford², Melanie Roux³, Pratima Adhikari¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Cargill Inc., Wayzata, Minnesota, United States; ³Novus International Inc., Chesterfield, Missouri, United States

Optimizing trace mineral nutrition is essential for sustaining productivity and egg quality in modern laying hens. Inconsistent absorption of inorganic trace minerals has heightened interest in organic sources with potentially improved bioavailability and cost efficiency. This study evaluated replacing inorganic trace minerals (ITM) with HMTBa-chelated minerals (MMHAC), or varying blends, on egg quality in laying hens. The trial focused on zinc (Zn), copper (Cu), and manganese (Mn) source supplementation during the peak phase (19-40 wks of age). Five hundred Lohmann LSL Lite hens were randomly assigned to one of five dietary treatments (TRTs): TRT1 100% ITM, TRT2 75%ITM:25%MMHAC, TRT3 50%ITM:50%MMHAC, TRT4 25%ITM:75%MMHAC, or TRT5 100% MMHAC (four hens per cage, 25 replicates per TRT). Reduced mineral inclusion levels were used to account for the higher bioavailability expected from

MMHAC sources. All diets were iso-methionine and iso-caloric. Egg quality was assessed every 4 wks by collecting one egg per pen, candling for translucency, and analyzing with the Egg Tester Ultimate (ORKA Food Technology). Data were analyzed using a mixed procedure of JMP, where TRT, time, and TRT*time interactions were considered fixed effects, replicates were considered a random effect. Least squares means were compared using student's t-test after two-way ANOVA analysis; significance levels were based on $P \leq 0.05$. Translucency scores were higher ($P=0.02$) in TRT5 (2.57) compared to TRT1 (2.36), TRT2 (2.42), TRT4 (2.41), but not different from TRT3 (2.55). Egg weight was higher ($P=0.01$) in TRT2 (60.40g) compared to TRT1 (59.17g) and TRT3 (59.81g); intermediate values were observed for TRT4 (59.53g) and TRT5 (60.11g). Yolk weight was higher ($P=0.03$) in TRT2 (15.74g) compared to TRT1 (15.44g) and TRT4 (15.30g); intermediate values were observed for TRT3 (15.51g) and TRT5 (15.49g). In conclusion, partial or complete replacement of ITM with MMHAC had measurable effects on egg quality in laying hens at peak phase. These findings indicate that MMHAC may enhance egg quality, though long-term studies are needed to assess its overall efficacy.

Keywords: egg quality; laying hens; trace minerals; peak; performance

Pathology

276P Characterization of chicken innate and adaptive immune cells by flow cytometry-based assay

Vinicius Apolloni*^{1GS}, James Krehling¹, Miria Ferreira Criado¹ ¹Auburn University, Pathobiology, Auburn, Alabama, United States

Chicken immune system is constituted by innate and adaptive components, displaying unique and homologous features compared to mammals. Due to limited availability of specific immunological tools, it remains poorly characterized. This study aimed to standardize a flow cytometry-based assay to phenotype immune cells from healthy chickens of various ages, aiming for further animal experiments. Understanding the chicken immune system is essential for improving animal welfare, enhancing poultry productivity and safety, ensuring food security, preventing disease outbreaks, and developing effective vaccination strategies against infectious agents. To isolate peripheral blood mononuclear cells (PBMCs), fresh blood was collected from 6 and 8-week-old broiler chickens from Miller Center, Auburn University. PBMCs were obtained through Ficoll-Paque PLUS Medium, using a standardized protocol. Isolated cells were adjusted to 1×10^6 cells/sample for flow cytometry analysis in Beckman Coulter CytoFLEX LX at Flow Cytometry Laboratory, Auburn University. Cells were evaluated based on size, granularity, and fluorescence intensity within the viable cell gate. We defined flow cytometry panels according to the commercial availability of antibodies recognizing chicken immune cell surface markers. To optimize the staining performance, we tested the fluorochrome combinations and performed antibody titrations in triplicate. This assay enabled the identification of circulating classical T cells ($CD4^+$, $CD8^+$, and $CD4^+CD8^+$), non-classical T lymphocytes ($CD3^+TCR-1^+$ and $CD3^+TCR-1^+CD8^+$), B cells ($BU-1^+$), natural killer cells ($CD3^+CD8^+$), thrombocytes ($CD41/61^+$), and monocytes/macrophages ($KUL01^+$) under steady-state conditions in 6- and 8-week-old broiler chickens. Moreover, we found age-dependent changes on frequency of thrombocytes, classical T lymphocytes, non-classical T lymphocytes, and B cells between 6- and 8-week-old broiler chickens. In this study, we established a multi-parameter flow cytometry panels to assess age-dependent

immune cells distribution in chickens at steady-state, supporting future studies in chickens under various viral infection contexts.

Keywords: innate cells; lymphocytes; immune system; chicken

277P Effect of Hypochlorous Acid (HOCl) application on respiratory tissue integrity in broiler chicks during the first week of life

Leticia Orellana*^{1GS}, Cassidy Morris¹, Kenneth S. Macklin¹ ¹Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States

Broiler house sanitation is essential for reducing disease incidence. Hypochlorous acid (HOCl) is widely recognized as an effective sanitizer against pathogenic bacteria; however, little is known about its health effects when applied in the presence of birds. This study evaluated the effects of HOCl application, by fogging and/or through drinking water, on the health and potential risk factors of broiler chicks. A total of 150 one-day-old chicks were randomly assigned to three treatments and reared under standard conditions of temperature, feed, and stocking density. Treatments were as follow: (A) control, receiving saline fog; (B) HOCl fog; and (C) HOCl fog combined with HOCl-supplemented drinking water. Fogging was applied for 8 minutes twice daily for seven days. On day 7, 25 chicks per treatment were evaluated for tracheal and lung microscopic lesions, and body weight, spleen weight, and bursa size were recorded. Data were analyzed using a General Linear Model, and significant differences ($P < 0.05$) among means were separated using Tukey's test. The results showed no significant differences in tracheal lesions, body weight gain, spleen weight, or bursa size among treatments ($P > 0.05$). However, fogging with HOCl (Treatment B) reduced lung damage in 7-day-old chicks compared with the control ($P=0.016$). In conclusion, short-term exposure to HOCl through fogging may help minimize lung tissue damage in broiler chicks without adversely affecting growth or immune organ development.

Keywords: Hypochlorous acid (HOCl); Respiratory health; Sanitation; Broiler

278P Comparative genomic analysis of *Enterococcus faecalis* isolates from broiler chicken ceca

Manhong Wang*^{1GS}, Chuan-yu Hsu², Mark A Arick II², Xue Zhang³, Pratima Adhikari¹, Peixin Fan³, Katie Elliot⁴, Kenneth S. Macklin¹, Li Zhang¹ ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²Mississippi State University, Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States; ³Mississippi State University, Department of Animal and Dairy Sciences, Mississippi State, Mississippi, United States; ⁴USDA, Agriculture Research Service, Poultry Research Unit, Mississippi State, Mississippi, United States

Enterococcus faecalis is a commensal bacterium in the gastrointestinal tract, with certain strains exhibiting probiotic potential, while others are known as opportunistic pathogens. To explore the potential functional roles of two *E. faecalis* strains, MS8004 and MS8005, isolated from the healthy ceca of broiler chickens, we performed a comparative genomic analysis with eight reference *E. faecalis* genomes from the NCBI database, including two marketed probiotics, three non-pathogenic non-probiotic (NPNP) strains, and three pathogenic strains. Whole-genome sequencing of MS8004 and MS8005 was performed using the Oxford Nanopore MinION platform. Reads were filtered with FilTlong, assembled with Flye, and annotated through the Prokaryotic Genome Annotation Pipeline. Sequence types (ST) were assigned by the PubMLST database. Antimicrobial resistance (AMR) genes were identified using the CARD database. Predicted functions of protein-coding genes was performed with eggNOG-mapper to assign Clusters of Orthologous Groups (COG) categories. Statistical differences among groups were evaluated using the Kruskal-Wallis test ($P < 0.05$). The assembled genomes of MS8004 and MS8005 were 2.7 Mb and 3.2 Mb, with GC contents of 37.8% and 37.1%, and containing 2,494 and 2,980 protein-coding sequences, respectively. MLST analysis assigned MS8005 to ST82, while MS8004 lacked matches for three housekeeping genes, indicating that further analysis is required to determine whether it represents a new ST. AMR genes *efrA*, *lsaA*, *dfrE*, and *emeA* were present in all strains. MS8004 and MS8005 exhibited the similar AMR profiles with probiotic and NPNP strains and lacked the vancomycin resistance genes found in pathogenic strains. Functional classification based on COG revealed that genes related to carbohydrate transport and metabolism (G) and transcription (K) were abundant across all strains. However, no significant differences were observed among groups ($P > 0.05$), likely due to the limited number of strains analyzed. As a result, it remains unclear whether MS8004 and MS8005 are more closely related to any specific group. Future studies incorporating a broader range of strains will be necessary to clarify their phylogenetic relationships.

Keywords: Probiotics; Pathogens; Antimicrobial resistance; Functional classification; Clusters of Orthologous Groups

Physiology, Endocrinology and Reproduction

280P Optimizing the utilization of fertile eggs in broiler breeder flocks

Tatyani Mani*^{1GS}, Beverly McLendon¹, Emmillie Boot¹, Rebecca Wysocky¹, Mike Wineland¹, Ramon Malheiros¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States

In broiler breeder production, the shortage of fertile eggs and declining flock fertility highlight the need to maximize the utilization of all available eggs. Dirty or floor eggs are typically excluded from incubation due to the risk of microbial contamination. This study evaluated the effectiveness of an egg-

279P Chicken embryo lethality assay to determine the pathogenicity of clinical *Enterococcus cecorum* isolates under two inoculation routes

Ashton R. Ramirez*^{1UG}, Linan Jia¹, Kalyn Nelson², Haley Nabors¹, Chris Magee³, Luis Muñoz¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Mississippi State University, Poultry Science, Trinity, Alabama, United States; ³USDA Agricultural Research Service (ARS), Starkville, Mississippi, United States

Enterococcus cecorum (EC) has emerged as an important pathogen in broiler chickens, associated with vertebral osteomyelitis, femoral head necrosis, and increased mortality. The objective of this study was to evaluate the effects of two inoculation routes and nine EC isolates on embryonic development and mortality using an embryo lethality assay. A total of 552 fertile broiler eggs were randomly assigned to 23 treatment groups representing two inoculation routes (albumen or allantoic cavity) and nine EC isolates (9A-C, 10A-C, 16A-C), along with one unpunched control and route-specific dry-punch and negative (PBS) controls. Isolates were recovered from clinical cases in a commercial flock and originated from lesions of the free thoracic vertebrae (9A-C and 10A-C) and femoral head (16A-C). Eggs were incubated at 37.5°C with 50–55% relative humidity and turned hourly at a 45° angle. On day of incubation (DOI) 12, eggs were inoculated with approximately 100 CFU/egg via the albumen or allantoic cavity. Embryos were monitored daily for mortality through DOI 19, and bacteriological confirmation was performed on CNA with 5% sheep blood. Survival data were analyzed using Cox proportional hazards models and log-rank tests in RStudio. Albumen-inoculated embryos challenged with isolates EC-10A, EC-9A, and EC-9B showed the highest mortality (87.5%, 21/24) with a mean survival of 4.46 days, whereas albumen EC-16A and two control treatments (allantoic cavity negative control and unpunched eggs) showed the lowest mortality (8–12.5%, 2–3/24) with survival of 6.0–6.83 days. Cox models identified EC-10A as the most virulent isolate in the albumen route, while EC-16A and control treatments showed low hazard and prolonged survival, indicating marked strain-dependent differences in virulence. Log-rank tests demonstrated significantly higher mortality in albumen inoculated embryos compared with the allantoic cavity route ($P = 0.026$) and unpunched controls ($P = 0.006$), highlighting a strong effect of inoculation route on EC pathogenicity. These results indicate that EC virulence varies among isolates and is strongly influenced by the inoculation route, and they support the embryo lethality assay as a practical model to characterize EC pathogenicity in broilers.

Keywords: *Enterococcus cecorum*; embryo lethality; broiler; inoculation route; virulence

washing procedure using a disinfectant solution and the Mach-C washing system (Mach-C Solutions, The Netherlands) in reducing contamination and improving hatchability. A total of 3,240 eggs were collected and divided into three groups: clean nest eggs ($n = 1,080$), dirty unwashed eggs ($n = 1,080$), and dirty washed eggs ($n = 1,080$). Washed eggs were processed using the Mach-C washing protocol, and each group was incubated separately under identical conditions to prevent cross-contamination. At 18 days, eggs were candled to assess embryonic development, and viable eggs were transferred to the hatcher. The proportion of contaminated eggs was significantly lower in the washed group compared with the dirty group (0.64% vs. 1.68%; $P = 0.0048$). Hatchability of fertile

eggs was numerically highest in the clean nest group (94.60%), followed by the washed (93.08%) and dirty groups (92.94%; $P = 0.4204$). These results indicate that washing dirty broiler breeder eggs using the Mach-C system effectively reduces bacterial contamination by 1.04% and provides a modest improvement in hatchability compared to unwashed dirty eggs. The technique offers a potential means to increase the overall utilization of fertile eggs in breeder operations facing limited egg availability.

Keywords: Egg-washing; Incubation; Hatchability

281P Evaluating the effects acute and chronic heat stress and antibiotic growth promoter supplementation on jejunal nutrient transporter expression in modern and legacy broilers

Colin A. Barcelo*^{1GS}, Katarina Glassman¹, Virginia Jacobs¹, Sidona Rao¹, Anneliese Amukamara¹, Laura Ellestad¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

Efficient intestinal nutrient uptake is vital in maintaining optimal broiler production. Genetic selection for improved feed efficiency and robust growth has left broilers susceptible to the negative effects of heat stress (HS). Poor performance during HS has been shown to improve with supplementation of antibiotic growth promoters (AGPs); however, their potential mechanisms of action remain uncertain. This study evaluated the effects of bacitracin methylene disalicylate (BMD) at AGP levels on the expression of jejunal carbohydrate and amino acid transporters in modern and legacy broilers subjected to acute and chronic HS. Day (D) of hatch male chicks were randomly assigned into 8 groups (n=6 pens/group) with two lines [Ross 708 (ROSS) or Athens-Canadian Random Bred (ACRB)], two diets [antibiotic-free (ABF) or BMD-supplemented], and two temperatures [thermoneutral (75°F) or HS (95°F for 8h/D)]. Treatment diets were fed for the entire experiment, and birds were subjected to temperature treatments from D32-D38. On D32 and D38, jejunal mucosa was collected from one bird per pen to assess the effects of acute and chronic HS, respectively. Jejunal mucosa was analyzed for mRNA levels of glucose (*GLUT1* & 2; *SGLT1*) and amino acid (*EAAT3*; *b(0,+)**ATI*; *CAT2*) transporters via RT-qPCR. Data were analyzed by ANOVA and Fisher's LSD test when ANOVA indicated significance. Line-by-diet-by-temperature interactions were observed for several genes. Expression of *GLUT2* and *SGLT1* during acute HS and *GLUT2* during chronic HS was unchanged in ACRB and decreased in ROSS; however, BMD-ROSS had greater expression than ABF-ROSS ($p \leq 0.05$). Expression of *CAT2* was elevated in ABF-ROSS during chronic HS, with other groups expressing similar levels ($p \leq 0.05$). Line-by-temperature interactions were present for *GLUT1*, *EAAT3*, and *b(0,+)**ATI*, where expression was

decreased in ROSS during acute HS with no changes in ACRB ($p \leq 0.05$). The opposite was true for *CAT2*, where expression was increased in ROSS during HS and unchanged in ACRB ($p \leq 0.05$). Taken together, genetic selection has diminished the capacity to cope with HS, and BMD-supplementation during HS likely improves broiler performance in part by mitigating the negative effects of HS on jejunal nutrient transporter expression during AGP absence.

Keywords: Glucose transporter; Sodium glucose linked transporter; cationic amino acid transporter; excitatory amino acid transporter; Bacitracin methylene disalicylate

282P Keel bone fractures show no significant effect on the mRNA abundance of heat shock proteins in the cecal tonsils of *Salmonella*-challenged layers

Aden Smith*^{1UG}, Asghar Sedaghat², Rami A. Dalloul¹ ¹University of Georgia, Poultry Science, Jefferson, Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States

Keel bone fractures (KBF) have become increasingly common in the layer industry as the proportion of commercial cage-free layer houses has risen. KBF can have severe effects on layer performance, well-being, and immune competence. An analysis of the cecal tonsils in a flock of commercial laying hens found to have KBF was carried out with a specific focus on the effects on heat shock proteins (HSP) in response to the non-thermal stressor, *Salmonella*. The study experimental design was CRD with KBF groups split between mild and severe fractures post euthanasia and prior to sampling. Fourty laying hens were randomly selected from a 54-week-old cage-free commercial flock. The first 20 hens were euthanized and cecal tonsil samples collected. The remaining 20 hens were challenged through inoculation with 1mL of 10^7 wild *Salmonella* Enteritidis and cecal tonsil samples were collected 5-days post challenge. RNA were extracted and the resulting cDNA were subjected to qPCR using Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene. Data were analyzed by a one-way ANOVA between mild and severe groups with significance ($P \leq 0.05$) and Fisher's LSD test. The mRNA abundance observed were HSP60, HSP70, and HSP90, none of which yielded any statistical significance. The results of this study indicate that KBF does not have a substantial effect on the HSP response to *Salmonella* Enteritidis in cecal tonsils. Further research is warranted on the effects of KBF and *Salmonella* Enteritidis on the stress response in cecal tonsils and other immune tissues.

Keywords: keel bone fracture; *Salmonella* Enteritidis; cage-free hens; heat shock protein; cecal tonsils

Processing and Products

283P Cooking methods alter intermolecular interactions and gastronomic properties in the egg protein and yolk matrix

Rudro Deb Sardar¹, Harleen Kaur¹, Thiago Belem*^{4GS}, Chinelo S. Onah-Kachiside², Elavarasan Krishnamoorthy³, Amit Morey¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Auburn University, Poultry Science, Auburn, Alabama, United States; ³Auburn University, Poultry Science, Auburn, Alabama, United States; ⁴Auburn University, Poultry Science, Auburn, Alabama, United States

Considering the growing evidence relating to the processing and consumer health, and egg-being a high-quality reference source of proteins, understanding the changes at the molecular level and associated gastronomical properties of egg proteins is of paramount importance. In the present investigation, whole egg

(WE), egg white (EW), egg yolk (EY), and the graded level of EW/EY mixtures (80/20, 60/40, 40/60, 20/80) were studied (2x7x5 factorial design) for gastric buffering capacity (GBC), formation of intermolecular forces (IMF-ionic bond, hydrogen bond, hydrophobic interaction and disulfides) and proteins participation profile in aggregation through various IMF using electrophoresis (SDS-PAGE) under water bath and microwave cooking. The data collected were analyzed using two-way ANOVA and mean values were compared using Tukey's HSD test ($P < 0.05$). GBC found to be higher for EY than the EW while the lowest was recorded for WE. Water bath did not alter the GBC of the egg mixtures significantly. Microwaved whole eggs ($130.87 \pm 34.18 \mu\text{Mol/pH.g}$) and egg yolk ($189.50 \pm 64 \mu\text{Mol/pH.g}$) showed significantly higher GBC. IMF estimation through protein solubility and SDS-PAGE analysis revealed that the hydrophobic interaction and disulfide

bond is responsible for the formation of high molecular weight protein aggregates (HPA) in all the egg protein matrix cooked in waterbath. Specifically, inclusion of yolk contributed greatly to the formation of HPA (approx. 225-85 kDa). Ovotransferrin (76 kDa) and ovalbumin (45 kDa) molecules of egg white interacted mainly through ionic and hydrogen bonds. Whereas the EW protein-lysozymes (14.3 kDa), and EY proteins – cystatin (12 kDa) and flavoproteins (32 kDa) are held in the matrix mainly through hydrophobic (10-32 mg/g) and disulfide bonds (23-37 mg/g). Microwave cooking resulted in totally different intermolecular interactions among the protein species compared to waterbath and dominated significantly by the ionic and hydrogen bonds. From the results, it is evident that the mode of heating affects intermolecular interactions of proteins in egg components. Further investigation will span to connect and understand the implications of these changes in digestibility, bio-functionalities, and health outcomes.

Keywords: Egg proteins; Intermolecular interactions; Gastric buffering capacity; Thermal processing; Chemical forces

284P Effect of storage temperature and duration on egg quality

Emily E. Shin*^{1UG}, Deependra Paneru¹, Woo Kyun Kim¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

Egg quality begins to decline immediately after laying, and maintaining freshness during storage and transport remains a major challenge in the global egg supply chain. Therefore, a study was conducted to investigate the interactive effects of storage temperature and duration on external and internal quality parameters in table eggs. A total of 260 freshly laid eggs (mean weight 53.9 ± 0.2 g) from 24-week-old Hy-Line W36 hens were allocated to a baseline group (day 0; n = 20) and three temperature regimes: 4.4°C (refrigerated), 12.7°C (cool ambient), and 22.2°C (room temperature), with 80 eggs per treatment stored for up to 28 days. At 7, 14, 21, and 28 days, 20 eggs per treatment were evaluated for egg weight, shell weight and thickness, albumen height and weight, yolk weight and Haugh unit (HU). Data were subjected to two-way ANOVA followed by Tukey's HSD post-hoc test ($P < 0.05$). Storage duration and temperature significantly affected most external and internal egg quality parameters. Egg weight declined progressively with longer storage and higher temperatures ($P < 0.001$). Eggs refrigerated at 4.4°C showed negligible weight loss over 28 days, whereas those at 12.7°C remained stable until day 21, and the eggs at 22.2°C showed significant deterioration after day 14. Albumen weight followed a similar trend, decreasing significantly with longer storage and higher temperatures ($P < 0.001$). This reduction in albumen weight led to a corresponding decrease in albumen percentage and an increase in yolk percentage ($P < 0.05$). Albumen height and HU also declined significantly with increasing storage duration and temperature ($P < 0.001$), reaching the lowest values at 22.2°C by day 28; even the refrigerated eggs showed reduced albumen height after day 14. However, eggshell thickness remains unaffected by storage duration ($P = 0.410$) and temperature ($P = 0.863$). In

conclusion, refrigeration at 4.4°C effectively slowed internal egg quality deterioration, whereas storage at 12.7°C and 22.2°C accelerated declines in freshness and internal egg quality. Future research should investigate the underlying molecular mechanisms and evaluate novel preservation technologies to further extend functional shelf life of table eggs.

Keywords: Egg quality; Storage temperature; Storage duration; albumen degradation; Haugh unit

285P Effects of dietary supplementation of organic acids and their derivatives on broiler breast meat quality

Rishav Kumar*^{1GS}, Shijinaraj Manjankattil¹, Zahidul Tushar¹, Muhammad Naeem¹, Matthew B. Hughes¹, Dianna Bourassa¹, Sabin Poudel¹, Jundi Liu², Jinquan Wang¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Eastman Chemical Company, Animal Nutrition BU, Kingsport, Tennessee, United States*

Short- and medium-chain fatty acids and their derivatives have been used in poultry diets by enhancing immunomodulation ability, nutrient utilization, and antioxidant capacity. However, there is lack of research on whether the benefits in live production could transfer to an improvement in meat quality. Therefore, the aim of this study was to evaluate the effects of dietary organic acids and their derivatives on the proximate composition and physicochemical properties of broiler breast meat. A total of 600 male broilers (Ross 708) were assigned to three treatments with 25 birds per pen and 8 replicates per treatment: control (basal diet), formic acid based blend (FB) at 6 kg/mt, and short-/medium-chain monoglycerides blend (MB) at 3 kg/mt for 42 days. Electric stunning was conducted, with post-stunning procedures adhering to standard industry practices, and carcasses were subsequently chilled using the water immersion method. Breast fillets were collected and analyzed for pH, lipid oxidation, color attributes (L^* , a^* , b^* , hue, and chroma), shear force, and proximate analysis 24 h postmortem. Data was analyzed using one-way ANOVA in SAS for proximate analysis and in R Studio for physicochemical properties. Means were separated using Tukey's method with significance deemed at 0.05. Results showed that no significant differences were found in protein, moisture, fat content in the FB and MB treatments compared to the control. The FB treatment increased salt content in breast fillet compared to the control ($P < 0.05$). Dietary supplementation of FB and MB reduced insoluble collagen levels in breast fillet compared to the control ($P = 0.05$). Lipid oxidation was lesser ($P < 0.01$) in the breast fillets of both the FB (0.031 mg MDA/kg) and MB (0.029 mg MDA/kg) as compared to the control (0.059 mg MDA/kg). No significant differences were detected in pH, lightness, redness, yellowness, or chroma, as well as shear force with the product treatments. Our findings indicated that dietary supplementation of FB and MB enhances oxidative stability without any adverse effect on meat quality attributes, which suggests that organic acids and their derivatives can be an effective measure to extend shelf life of chicken meat.

Keywords: Formic acid; monoglycerides; broiler; meat quality; oxidative stability

SCAD

286P Evaluating the effects of genetically distinct avian pathogenic *Escherichia coli* strains on embryonic development and mortality using an embryo lethality assay

Keaura Mearday*^{2UG}, Huanling Xing¹, Jeffrey D. Evans³, Kelsy Robinson³, Pratima Adhikari¹, Li Zhang¹, Linan Jia¹ ¹*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States*; ²*Mississippi State University, Animal and Dairy*

Sciences, Mississippi State, Mississippi, United States; ³*USDA-ARS, Mississippi State, Mississippi, United States*

Avian Pathogenic *Escherichia coli* (APEC) is a major bacterial pathogen in poultry that can significantly reduce embryo survival and overall hatchery success. The goal of this study was to evaluate how genetically distinct APEC strains influence the development

and survival of chicken embryos. A total of 576 fertilized broiler eggs were incubated at 37.5 °C and 50-55% relative humidity with automatic turning. On day 12 of incubation, eggs were assigned to 24 treatments, including negative control (NC, sterile saline), unpunched eggs, dry-punched eggs, and 21 *E. coli* isolates representing diverse genetic backgrounds based on serogroup and whole-genome sequence. The experiment followed a randomized complete block design, with incubator levels considered as blocks. A total of six blocks were used, and within each block, four eggs were assigned to each treatment, resulting in 24 eggs per treatment. Each egg received a 0.1 mL inoculation (around 100-500 CFU of *E. coli*) into the allantoic cavity, and embryo mortality were monitored daily through day 18. Embryos that did not survive were cultured on MacConkey agar and confirmed for *E. coli* infection. On day 18 of incubation, egg and embryo weights were recorded for all surviving embryos. The 7-day mortality data were analyzed using two-proportion *z*-test against the NC with Holm adjustment, and survival embryo weight were compared by one-way ANOVA; statistical significance was set at $P \leq 0.05$. Among the 21 tested isolates, 5 showed no significant difference (*P*-values range from 0.110-0.336) from NC in embryo mortality, ranging from 8.3% to 16.7%, whereas 16 isolates exhibited significantly higher mortality rates, ranging from 20.8% to 83.3% ($P < 0.05$). Embryo weight analysis revealed that 15 isolates caused significantly lower embryo weights compared with the NC group ($P < 0.05$), indicating a consistent reduction in embryo growth among virulent strains. Early findings confirm that the virulence level varies substantially among APEC strains, with isolates have o-serogroups 2, 4, 17, 86, and 109 causing markedly higher embryo mortality and growth impairment than others. These results enhance our understanding of APEC strain diversity and their differential impacts on chicken embryo viability, and developmental outcomes.

Keywords: Embryonic health; Avian Pathogenic *Escherichia coli*; Disease Prevention; Poultry health

287P Immune response evaluation during necrotic enteritis in broiler chickens and identifying genes possibly associated with disease resistance

Matthew Browning^{*1GS}, Pok Man Chan², Carissa Gaghan², Raveendra Kulkarni² ¹North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina, United States; ²North Carolina State University, Raleigh, North Carolina, United States

Necrotic enteritis (NE) is an enteric disease of poultry caused by the bacterium *Clostridium perfringens*. This is a gram-positive, anaerobic, and toxin-producing bacteria that negatively impacts the broiler industry due to decreased growth performance in both its clinical and subclinical forms. Despite the large economic impact of NE, no effective vaccines or antibiotic alternatives are currently available for its treatment or prevention. This can largely be attributed to the poor understanding of the host immune responses during NE. In the present study, we used a NE predisposing model (dietary + *Coccidia*) to evaluate the mucosal (duodenum and jejunum) and lymphoid (cecal tonsil "CT" and bursa of Fabricius "Bursa") immune gene expression in broilers during NE disease using a virulent strain of *Clostridium perfringens* (Cp44). Additionally, birds were separated into NE "Resistant" and "Susceptible" groups based on gross lesions at necropsy and histological evidence of NE. Immune gene expression was then evaluated in birds that demonstrated resistance or susceptibility to further characterize the immune genes involved in NE pathogenesis. Statistical analysis revealed that birds that were resistant to NE had increased ($P < 0.05$) expression of IFN γ , iNOS, IL-10, and TGF β in the duodenum

compared to birds that received *Coccidia* alone. Interestingly, birds that showed susceptibility to NE also had increased ($P < 0.05$) duodenal expression of IL-10 compared to birds that received *coccidia* alone. In the CT, NE resistant birds had increased ($P < 0.05$) expression of IFN γ , iNOS, IL-10, and TGF β compared to the *coccidia* alone group. Additionally, resistant birds also had increased ($P < 0.05$) cecal tonsil expression of IFN γ compared to the birds that showed susceptibility. Collectively, these findings demonstrate that birds showing resistance to NE development had increased expression of cytokines (IFN γ , iNOS) involved in T cell and macrophage activation and immunomodulatory molecules (IL-10, TGF β) controlling inflammation in both the mucosal and lymphoid organs. These findings help further characterise the immune response of birds during NE infection as well as identify biomarkers of resistance and susceptibility.

Keywords: Necrotic Enteritis; Chickens; Immune Response; *Clostridium perfringens*; *Eimeria*

288P Efficacy of bacitracin methylene disalicylate antibiotic in ameliorating effects of necrotic enteritis disease in broiler chickens

Kahlil King^{*1UG}, Tunde E. Ogundare¹, Oluteru Orimaye¹, Safiu A. Suberu¹, Lekan Elegbede¹, Yewande Fasina¹ ¹North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States

This study investigated the effect of dietary bacitracin methylene disalicylate supplementation on necrotic enteritis (NE), an economically important disease in broiler chickens. In a 28-day experiment, day-old broilers (Ross 708 male, $n = 400$) were randomly allotted into four treatments, each consisting five replicates of 20 birds each, in a completely randomized design. Birds in the control treatment (CON) were fed an unmedicated corn-soybean meal (SBM) basal diet, while birds in the BMD treatment were given the corn-SBM basal diet that was supplemented with 55 mg/Kg bacitracin methylene disalicylate antibiotic. The ACON and ABMD treatments represented NE-challenged birds given diets similar to CON and BMD birds, respectively. On d 13, chicks in ACON and ABMD groups received 1 mL (10X dose) *coccidia* vaccine, followed by 3 mL *Clostridium perfringens* (CP, 3.2×10^8 CFU/mL) on d 18 and 19, while CON and BMD chicks were mock challenged with sterile fluid thioglycolate media. Growth performance parameters including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded throughout the experiment. On d 3 and d 9 post-challenge (d 20 and d 26 of the experiment), two chicks per pen were randomly euthanized and aseptically necropsied for the removal of the small intestine segments (duodenum, jejunum and Ileum) for lesion scoring. All data were analyzed using One-way ANOVA. Results indicated that CON and BMD had the highest BWG ($P < 0.05$; 1.23 and 1.28 kg/bird respectively) and a superior FCR ($P < 0.05$; 1.501 and 1.457, respectively) compared to ACON and ABMD (BWG, 0.907 and 0.939, respectively; FCR, 4.752 and 4.329, respectively). Mortality was higher ($P < 0.05$) in ACON and ABMD birds compared to CON and BMD groups. There were no intestinal lesions observed in CON and BMD birds when examined on d 20 and d 26. On the other hand, among NE-challenged birds, ABMD birds had lower lesions ($P < 0.05$) on d 20 compared to ACON birds. However, by d 26, the improvement observed in ABMD birds was no longer apparent. It was concluded that dietary supplementation of bacitracin methylene disalicylate did not improve BWG nor reduced mortality during the early phase of NE disease. However, it transiently reduced intestinal lesion scores.

Keywords: Necrotic enteritis; Broiler chickens; *Clostridium perfringens*; Lesion score

289P Dietary inclusion of *Saccharomyces cerevisiae* components, saponins, and an essential oil may reduce the severity of cecal and liver lesions of poult directly challenged with *Histomonas meleagridis*

Kasey A. Matusik*^{1GS}, Theresia Lavergne², Charlie Elrod², Aaron Forga¹, Danielle Graham¹ ¹University of Arkansas, Fayetteville, Arkansas, United States; ²Natural Biologics, Inc., Stephenville, Texas, United States

This pilot trial was conducted to evaluate the effect of Naverde® Plus (yeast cell wall plus hydrolyzed yeast and saponins; Natural Biologics, Newfield, NY), along with an essential oil (EO), on *Histomonas meleagridis* transmission and severity of lesions in turkey poults. At day-of-hatch, 200 turkey poult hens were allotted to two treatment groups: 1) Challenged control and 2) Challenged + Naverde Plus with EO. There were 10 replicate cages/treatment with 10 poults/cage. On day 13, density was reduced to 8 poults/cage. Poults were fed a standard turkey starter diet from day 0 to 6, and an amino acid restricted (56%) soybean meal-based diet from day 6 to 27. On day 13, seeder poults (2 poults/cage) were intraocally challenged with 100,000 *H. meleagridis* cells (PHL strain)/0.5mL and wax paper was placed in all cages and replaced as needed until the termination of the trial. All mortalities and remaining seeder poults (day 27) or contact poults (day 33) were necropsied for evaluation of liver and cecal lesions. Lesions were scored on a scale of 0 to 3, with 0 = no lesions and 3 = severe lesions associated with *H. meleagridis*. ANOVA was used to determine differences in lesion scores (LS) and means were separated using Student's t test. When seeder poults were fed Naverde Plus with EO (liver LS 1.65 ± 0.28; cecal LS 2.25 ± 0.24), the average liver lesion score was significantly reduced (P=0.0105) and the cecal lesion score was numerically reduced (P>0.05) compared to challenge control seeder poults (liver LS 2.60 ± 0.21; cecal LS 2.60 ± 0.21). For both seeders and contacts, mortality was not affected (P>0.05) by treatment but was numerically lower in seeder poults fed Naverde Plus with EO (35%) compared to seeder poults in the challenged control (55%) group. Naverde Plus with EO had no significant effect (P>0.05) on horizontal transmission, as there were no significant difference in the transmission rate, which was lower than expected (<50%), between treatment groups. Thus, feeding Naverde Plus with EO to poults directly infected with *H. meleagridis* may reduce the severity of disease. A more robust horizontal transmission study is underway to elucidate any effects of Naverde Plus with EO on *H. meleagridis* transmission in turkey poults.

Keywords: poults; *Histomonas meleagridis*; *Saccharomyces cerevisiae*; saponins; essential oil

290P Coccidiosis vaccination on the day of hatch modulates gut integrity markers

Abhisek Niraula*^{1GS}, Davis A. Fenster¹, Ryan C. Rhodes¹, Paniz Zarghami Dastjerdi¹, Gabriella C. Ribeiro¹, Rami A. Dalloul¹ ¹University of Georgia, Avian Immunobiology Laboratory, Department of Poultry Science, Athens, Georgia, United States

Live coccidiosis vaccines are widely used in broiler production to promote early immunity through controlled *Eimeria* spp. exposure. The resulting oocysts cycling during the first few weeks might influence intestinal integrity and barrier function. A 49-day (d) study was conducted to evaluate the effects of a commercial live coccidiosis vaccine consisting of *E. acervulina*, *E. maxima*, and *E. tenella* on gut integrity markers in the jejunum of broiler chickens. Day-old Ross 708 broiler chicks (n=500) were assigned to a non-vaccinated control group (NC) or vaccinated group (VG; spray vaccinated on day of hatch), with 10 replicate pens/group

and 25 birds/pen. Fresh fecal droppings were collected from all the pens on days 6, 7, 8, 14, 15, and 16 to assess oocyst shedding. Jejunal tissues were collected from one bird/pen on days 7 and 14 to quantify mRNA abundance of tight junction proteins, including claudin (CLDN)1, CLDN2, CLDN3, occludin (OCLN), and intestinal health and function marker [mucin-2 (MUC2)] by qPCR using the 2^{-ΔΔCt} method. Data were analyzed using t-test with statistical differences considered significant at P ≤ 0.05. Oocyst shedding of all three *Eimeria* spp. confirmed that parasites were actively cycling during the first two weeks. On d 7, the VC group had significantly greater mRNA abundance of CLDN1 and OCLN and reduced abundance of CLDN2 (P = 0.04) compared to NC. On d 14, the mRNA abundance of CLDN1 was significantly reduced, and CLDN2 showed an increasing trend, in VC compared to NC. These findings suggest that vaccination caused modulation of jejunal tight junction proteins during the early oocyst cycling, followed by a reduction in tight junction stability during the second cycle. These changes at d 14 coincide with the onset of clinical necrotic enteritis outbreak in the vaccinated flock around d 15-16, indicating that the epithelial stress associated with *Eimeria* cycling might have caused increased susceptibility. Therefore, strategies to support gut barrier function during the first few weeks after coccidiosis vaccination could be beneficial.

Keywords: coccidiosis; vaccine; tight junction protein; gut integrity; broilers

291P Impact of day of hatch coccidiosis vaccination on performance parameters and immune markers

Ryan C. Rhodes*^{1UG}, Abhisek Niraula¹, Davis A. Fenster¹, Paniz Zarghami Dastjerdi¹, Gabriella C. Ribeiro¹, Rami A. Dalloul¹ ¹University of Georgia, Avian Immunobiology Laboratory, Department of Poultry Science, Athens, Georgia, United States

Coccidiosis, an enteric disease caused by *Eimeria* species, remains a major economic burden to the broiler industry. Live coccidiosis vaccination is routinely used in the poultry industry, which introduces a regulated exposure to *Eimeria* to promote immunity, but the subsequent oocyst cycling can affect early performance. This study evaluated the effects of a commercial coccidiosis vaccine on performance parameters and immune markers in broiler chickens. Day (d)-old Ross 708 chicks (n = 500) were assigned to two treatment groups: non-vaccinated (NC) and vaccinated (VG; spray vaccine containing *E. acervulina*, *E. maxima*, and *E. tenella* on day of hatch). Each group consisted of 10 replicate floor pens with 25 birds per pen and followed the same dietary program throughout the study. Body weight and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Jejunal tissues were collected from one bird per pen on d 7 and 14 to assess the mRNA abundance of immune markers, including interleukin (IL)-1β, IL-10, IL-12B, and interferon-gamma (IFN-γ) quantified by qPCR using the 2^{-ΔΔCt} method. Data were analyzed using a t-test with significance set at P ≤ 0.05. During d 7-14 and 0-14, ADG and ADFI were significantly higher in VG compared to NC. However, during d 0-28, FCR significantly increased in VG. On d 7, mRNA abundance of IL-1β was significantly greater in VG compared to NC, whereas no other immune markers differed statistically. Greater ADG and ADFI observed in VG during the first two weeks showed that although the parasites were cycling, there was no negative impact on performance. The elevated levels of IL-1β at d 7 might reflect epithelial irritation and inflammation triggered by early *Eimeria* cycling. Concurrent necrotic enteritis (NE) outbreak occurred only in the VG, evident by NE lesions observed in the small intestine during the third week despite the absence of *Clostridium perfringens* challenge. This observation

correlated with greater FCR in birds of this group. These findings suggest that vaccine-associated intestinal stress and epithelial disruption may have increased susceptibility to opportunistic *C. perfringens* overgrowth in vaccinated birds.

Keywords: coccidiosis; vaccine; interleukins; broilers; mRNA abundance

292P Development of a molecular diagnostics tool for avian metapneumovirus

Anish Sapkota*^{1GS}, Suman Bhattarai¹, Erich Linnemann¹, Maricarmen Garcia¹, Lok Raj Joshi¹² ¹University of Georgia, Department of Population Health, Athens, Georgia, United States; ²University of Georgia, Precision one health Initiative, Athens, Georgia, United States

Avian metapneumovirus (AMPV) is a viral pathogen that causes respiratory tract infection in poultry characterized by sinusitis, swollen heads and increased mortality. This virus belongs to genus *Metapneumoviridae* under family *Pneumoviridae* and is classified into four subtypes: A, B, C and D. While AMPV-C has been known to exist sporadically in the US, subtypes A and B had not previously been identified in US poultry until late 2023 and early 2024. The AMPV genome is a non-segmented, single stranded RNA molecule containing eight genes that encode nine proteins. Out of which, glycoprotein G has roles in viral attachment, entry and immunomodulation as well as functions as a major antigenic site. Mutations in the glycoprotein gene are associated with altered cellular affinity and viral fitness. With increasing AMPV cases of subtype A and B in the United States, we developed a PCR-based diagnostic assay for AMPV-A and B. Briefly, viral RNA was extracted followed by cDNA synthesis using random hexamer primer. AMPV subtype specific primer sets were designed to amplify full-length glycoprotein G of AMPV-A and B. The amplicons were sequenced by conventional Sanger method and Nanopore long-read sequencing method and reads were aligned with reference strains to determine mutations. The AMPV primers amplified the G gene with high specificity with no cross-reactivity between the subtypes. Sequencing and alignment results showed that the sequences we characterized were closer to other AMPV strains circulating in the United States, but distinct from strains circulating in Europe and Asia. Overall, we developed a molecular diagnostic tool for identifying AMPV subtypes and determining the amino-acid sequence of the G protein, which can help in understanding of virus evolution and vaccine matching.

Keywords: Avian Metapneumovirus; Diagnostic tools; Virus evolution

293P Antimicrobial and immunological characterization of chicken Lactobacillus strains

Abbey M. Brake*^{1GS}, Carissa Gaghan², Aakriti Tyagi², Raveendra Kulkarni² ¹North Carolina State University College of Veterinary Medicine, Raleigh, North Carolina, United States; ²North Carolina State University College of Veterinary Medicine, Department of Population Health and Pathobiology, Raleigh, North Carolina, United States

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an economically important bacterial disease of chickens. In the current era of growing poultry without antibiotics, NE incidences in chicken flocks are on the rise. To develop novel Lactobacillus-based probiotics for NE control in poultry as antibiotic alternative, this work was aimed at isolation, identification and characterization of lactobacilli possessing anti-*C. perfringens* and anti-inflammatory properties in-vitro. A total of 60 fecal/intestinal samples collected from 3-week-old healthy broilers were analyzed to isolate Lactobacillus colonies followed by 16s rRNA

sequencing. About 53 sequences had a definitive Lactobacillus species ID and 13 Lactobacillus strains, representing all the species, were further tested for their anti-*C. perfringens* property using agar-well diffusion assay. The pH-neutralized cell-free supernatants from all 13 strains displayed zones of inhibition but with varying degrees of antimicrobial effects. Additionally, a bacterial coculture assay to determine the direct *C. perfringens* inhibitory effect of selected strains, NCKL-11C, 45C and 49B, showed marked antimicrobial effects. Furthermore, avian macrophages stimulated with these 3 strains showed that Str. 45C and 49B induced anti-inflammatory responses, as determined by downregulated IL-1 β and upregulated IL-10 cytokine expression coupled with increased cellular production of Nitric Oxide. Collectively, our work showed that in-vitro isolation, identification and screening methods employed for chicken lactobacilli can yield selection of certain Lactobacillus strains possessing superior anti-inflammatory and antimicrobial effect against *C. perfringens*.

Keywords: Immunology; Probiotic

294P Effects of different Eimeria maxima infection doses on growth performance, gut and reproductive health in dual-infection model of necrotic enteritis in layer pullets (17-19 weeks)

Hemanth Reddy Katha*^{1GS}, Venkata Sesha Reddy Choppa¹, Hamid Reza Rafieian Naeimi¹, Seshidhar Reddy Gudidoddi¹, Doyun Goo¹, Venkata Prathap Reddy Keshavareddy¹, Ishwari Gyawali¹, Woo Kyun Kim¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Necrotic enteritis (NE) causes major economic losses in the poultry industry. Although most research focuses on broilers, studies in laying hens are limited despite increasing reported cases. Along with the predisposing effects of *Eimeria maxima* (EM), physiological changes during the transition from the non-laying to laying phase can induce stress and increase susceptibility to NE. The objective of this study was to investigate the effects of two doses of EM with or without *Clostridium perfringens* (CP) challenge on growth performance, gut and reproductive health in a dual infection NE model in layer pullets. A total of 70 pullets at 17 weeks of age were assigned to 5 treatments with 7 replicates. Each replicated cage contained two birds. The treatments were T1: Non-challenged control (NC); T2: 20,000 EM oocysts; T3: 40,000 EM oocysts; T4: 20,000 EM oocysts + CP at 10⁹ Colony Forming Units (CFU)/mL; and T5: 40,000 EM oocysts + CP at 10⁹ CFU/mL. Birds were challenged with EM immediately after allocation (0 days post-inoculation (DPI)). CP was administered twice on 4 and 5 DPI. Growth performance was monitored for 21 days following EM inoculation. The data was analysed using one-way ANOVA. On 7 DPI, birds in the T2 and T5 showed reduced body weight ($P \leq 0.05$), while all the challenge groups had reduced body weight gain from 0 to 7 DPI compared to the NC ($P \leq 0.05$), with the T5 having the lowest gain. The challenged groups showed decreased feed intake (FI) compared to the NC ($P \leq 0.05$) from 0 to 7 DPI, whereas there were no significant differences on FI from 0 to 4 DPI among the treatments. In addition, the challenge groups had higher the jejunal and cecal colony counts of CP on 7 DPI compared to the NC group ($P \leq 0.05$). The reproductive tract length and weight and ovary weight decreased significantly in the challenge groups ($P \leq 0.05$), indicating the severe impact of NE on reproductive tract development. In conclusion, challenging with EM potentiated NE proving the susceptibility of laying hens. The transition stage in laying hen cycle is important that it influences the egg laying capacity of the hen over their life-time egg-laying period. Hence, this study provides insight on the disease dynamics which help in finding potential mitigation strategies.

Keywords: Necrotic enteritis; Laying hens; Reproductive health; Transition phase

295P Differences in cytokine mRNA expression at the site of primary and secondary vaccinations with herpesvirus of turkeys (HVT) in egg-type chickens

Allie K. Bowerman*^{1UG}, Jossie M. Santamaria¹, Chrysta N. Beck², Stephanie Shouse¹, Gisela Erf¹ ¹University of Arkansas, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States; ²Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States

Marek's disease, a lymphoma causing virus, remains a major concern in poultry production systems. Although vaccination with live herpesvirus of turkeys (HVT) is crucial for controlling this disease, information on how the vaccine drives local/tissue immune responses that lead to protective immunity is limited. Using the growing feather (GF) in vivo bioassay, we assessed local cytokine mRNA expression following primary and booster intradermal (i.d.) HVT vaccinations in egg-type chickens. For this, six 9-week-old and six 19-week-old Light-brown Leghorn (LBL) pullets were i.d. injected into the pulp of growing feathers (GF; 10 μ L/GF; 16 GFs/bird) with a primary (V1) or secondary (V2) live HVT vaccine, respectively. GFs were collected before (0 d), 0.25, 1, 2, 3, 5, 7, and 10 d post GF-pulp injection (p.i.) for gene expression analysis by qRT-PCR. Data were subjected to 2-way ANOVA to evaluate the effects of vaccine, time, and their interactions, and followed by Bonferroni multiple means comparison as appropriate. Significance was set at $P < 0.05$. A marginal interaction ($P = 0.052$) of vaccine by time was observed for IL-1 β mRNA levels. Following V1 vaccination, IL-1 β remained near pre-injection levels (0 d) up to 3 d p.i., declined at 5 d, and returned to 0 d levels at 7 d, before decreasing again on 10 d p.i. In contrast, V2 vaccination increased IL-1 β expression from 0 d to the highest levels at 0.25 d and 1 d p.i., after which IL-1 β returned to baseline levels. IL-1 β expression was higher at 0 d, 3 d, and 7 d p.i. with V1 than V2. There was a main effect of time for the expression of iNOS ($P = 0.033$), IFN- α ($P = 0.016$), and IFN- β ($P = 0.002$), whereby levels declined post-vaccination, increased to near baseline levels on 7 d, and then dropped again below baseline levels at 10 d p.i. The cyclical drops in iNOS and type I interferons in both vaccination groups, and in IL-1 β in V1 pullets, likely reflect the HVT infection-mediated suppression of

inflammatory and antiviral pathways. The positive IL-1 β response profile following the secondary vaccination of live HVT-vaccine suggests a protective effect by the first HVT vaccination in preventing suppression of this inflammatory pathway, which plays an important role in signaling tissue infection and/or injury.

Keywords: Marek's disease; HVT vaccine; Cytokine expression; Primary response; Secondary response

296P Antibiotic resistance and genetic characterization of *Mycoplasma synoviae* strains from recent outbreaks in the United States

Michael R. Davis*^{1GS}, Eniope Oluwayinka¹, Naole Ferguson-Noel¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

Mycoplasma synoviae (MS) is a major poultry pathogen that, although usually resulting in subclinical infection, may also cause respiratory disease, infectious synovitis, and eggshell abnormalities, leading to significant economic losses. MS spreads both horizontally and vertically through transovarian transmission. While long-term control strategies like vaccination and eradication exist, antibiotics provide short-term relief from clinical effects, reduce shedding of MS, and limit horizontal and vertical transmission. Previous studies have linked genetic mutations and single nucleotide polymorphisms to macrolide and tetracycline resistance in MS isolates. This research focused on comparing recent MS strains from the U.S. to identify genetic variations and assess antibiotic resistance. *In vitro* antibiotic resistance tests were conducted on isolates to determine the minimum inhibitory concentrations (MICs) for tylosin and tetracycline. Genome libraries for each isolate were generated using Illumina technology for comprehensive genetic analysis. Nonsynonymous mutations were identified among the isolates; however, a direct correlation between these variations and antibiotic resistance has not yet been determined. Further investigations will focus on the significance of these variations and explore the potential role of other genetic factors that may influence MS pathogenicity, including genes linked to transmissibility, colonization efficiency, and immune response. Understanding these genetic factors is crucial for developing more effective strategies to control MS infections in poultry populations.

Keywords: Mycoplasma; Antibiotic

Welfare and Behavior

297P Automated counting of the perching frequency of cage-free laying hens using a YOLOv8-based deep learning approach

Bidur Paneru*^{1GS}, Ramesh B. Bist¹, Xiao Yang¹, Anjan Dhungana¹, Samin Dahal¹, Lilong Chai¹ ¹University of Georgia, Athens, Georgia, United States

Accurate quantification of perching frequency is critical for evaluating the welfare and behavioral development of laying hens in a cage-free (CF) housing system. While previous work demonstrated high precision (> 94%) for detecting perching behavior, automated counting of perching events across different growth phases remains unexplored. This study aimed to (1) develop and implement a perching frequency counting algorithm using the previously optimized YOLOv8x-PB model and (2) evaluate its accuracy against manual observations across growth phases of laying hens in a CF facility. The same datasets used for perching behavior detection were utilized, encompassing multiple growth phases (starter, grower, developer, prelay, peaking, and layer) and recorded from four CF rooms (200 hens/room). The automated perching counts were validated against manually

annotated data to assess precision, recall, and mean absolute error (MAE) of the algorithm. Statistical analysis was done using one-way ANOVA to compare counting accuracy between different variants of the model and between different ages at a significance level of $p < 0.05$. The YOLOv8x-PB-based counting system achieved an overall accuracy of over 88% across all phases, with the highest correspondence to manual counts during the prelay and peaking phases and slightly reduced accuracy during the starter phase due to occlusion and overlapping perching events. These results demonstrate that integrating detection and counting algorithms provides a robust, scalable solution for continuous monitoring of perching behavior in the CF system, reducing labor demands while improving the reliability of welfare assessments.

Keywords: Behavior counting; Computer vision; Laying hens; Perching; Precision poultry farming

298P Detour test performance in Japanese quail divergently selected for high and low-corticosterone response to immobilization

Gabriella S. Pryde*^{1UG}, Rosie Whittle², Sara K. Orlowski-Workman², Maricela A. Maqueda², Shawna Weimer² ¹*University of Arkansas, Animal Science, Fayetteville, Arkansas, United States;* ²*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Stress can impair cognition, and chronic stress contributes significantly to long-term cognitive decline. The detour test is commonly used to assess problem-solving and cognitive flexibility in animals. Although Japanese quail are not typically studied for cognition or behavior, lines bred for high and low corticosterone response to immobilization offer a valuable model for investigating the relationship between stress reactivity, cognition, and social behavior. This study examined how selectively bred high-stress (H), low-stress (L), and randomly bred (RB) quail lines performed in a detour task. We predicted that low-stress birds would complete the task more quickly and that there would be sex differences. The quail were raised in mixed sex floor pens (48 pens, 16 per line). At 6 weeks of age, 3 quail per pen (N=72) were tested in a detour pen. Each bird was placed behind a clear divider, allowing visual access to two conspecifics from the same pen. Video recordings of each detour test were analyzed using EthoVision tracking software, which tracked movement patterns including distance traveled, velocity, total activity, and latency to complete the task. A split-plot design was used with line and sex as fixed factors. Data were analyzed using linear mixed models. Males (M) generally had higher velocity (4.79cm/s, $p=0.007$) and more active (0.0011%, $p=0.01$) than females (F) (3.36cm/s, 0.0004%). HM moved a greater distance (6.31cm/s) than HF (3.42cm/s, $p=0.05$), and LM tended to travel a greater distance (7.21cm/s) than the LF (3.77cm/s, $p=0.07$). F had a higher velocity (3.36cm/s) than M (4.79 cm/s, $p=0.007$). M had higher activity (0.0012%) than F (0.0004%, $p=0.01$). HF was less active (0.0002%) than HM (0.001%, $p=0.02$), and LM were more active (0.002%) than LF (0.0003%, $p=0.02$). HF took longer to complete the task (371s) than HM (201s, $p=0.04$), and a similar trend was observed between LM (191s) and LF (348s, $p=0.09$). Our results indicate that sex-related factors have a greater influence on detour test performance than selection for corticosterone in quail. The findings emphasize the importance of considering sex differences in behavioral and cognitive studies of poultry and may provide

new insight into how stress physiology relates to problem-solving ability.

Keywords: Japanese quail; corticosterone; cognition; detour test; behavior

299P The effects of feeding supplemental tryptophan or probiotic on egg production, stress and fear response in laying hens

Emily Jiral*^{1GS}, Gregory S. Fraley², Melanie Bergman², Gregory Archer¹ ¹*Texas A&M University, Poultry Science, College Station, Texas, United States;* ²*Purdue University, West Lafayette, Indiana, United States*

This study evaluated the effects of adding L-tryptophan or a probiotic to the diet on welfare, fear response and production parameters in laying hens. Three treatments were tested: 1) standard layer diet (CON), 2) CON with L-tryptophan at 1 lb/ton (Trp), 3) CON with probiotic at 5 lb/ton (PB). Birds were raised for 45 days in conventional cages with 30 hens per treatment. Fear responses of all birds were assessed via tonic immobility (TI), inversion (INV) and pencil test (PT). Body weights, egg weights, albumen height, haugh unit, eggshell thickness, egg breaking strength, egg puncture, and heterophil/lymphocyte ratios were determined. All measurements were taken on day 0, 14 and 28. On day 0 and 28, brain tissues from the caudal, rostral mesencephalon and diencephalon (CM, RM, DI) were analyzed for static serotonin and dopamine as well as turnover. All data were analyzed using a one-way ANOVA test. Fisher's LSD was used for mean separation. On d14, Trp had the longest latency to right (227.1 s) followed by CON (200.3 s) and PB taking the least time to right compared to the others (133.4 s, $P<0.05$). There were no differences in TI on d28 ($P>0.05$). CON had the highest concentration (1.10, $P<0.05$) of DOPAC in the RM region of the brain compared to all other treatments and baseline measurements (0.269). There were no other differences in any of the egg quality, fear measurements, stress parameters or production measurements ($P>0.05$). These results indicate that feeding tryptophan or a probiotic in the diet does not influence static or activity levels of serotonin or a negative impact on egg production.

Keywords: Layer; Serotonin; Tryptophan; Welfare

POSTER ABSTRACTS – NON-COMPETITION

Environment and Management

300P Effect of Yeast Cell Wall on duck performances under challenging rearing conditions: large-scale field trial

Erwan Laudrin*², Mathieu Castex¹, Pierre Lebreton¹, Audrey Sacy¹ ¹Lallemand SAS, Animal Nutrition, Blagnac (31), Midi Pyrénées, France; ²Biochenevert, Arzacq Arraziguat, France

Duck farming systems are increasingly facing complex sanitary challenges, including the emergence and persistence of pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Campylobacter*, as well as heightened risks of mycotoxin contamination. The winter season poses additional risks for ducks raised in free-range systems, particularly when ponds are present. This study aimed to evaluate the effects of a yeast cell wall (YCW) product on zootechnical performance and slaughter outcomes in ducks fattened under challenging winter conditions. Eleven “Mulard” duck farms located in southwestern France, all using complete feed and outdoor rearing from 2 to 84 days of age, were divided into two treatment groups. One group received a YCW supplement (AGRIMOS at 1 kg/ton of feed), while the other received a conventional additive (a blend of essential oils and organic acids). Data were analyzed according to 3 levels of sanitary risk during winter, based on outdoor environmental criteria: Good Conditions (GC), Medium Conditions (MC), and Low Conditions (LC). Survival rates, performance metrics, fecal microbial loads, and slaughter results were all correlated with sanitary conditions, confirming clear distinctions between farms. Overall, YCW-supplemented ducks maintained growth performance while reducing feed intake (per duck per cycle: -1 kg in GC and -2 kg in LC), resulting in improved feed conversion ratios. Body weight uniformity improved under higher sanitary risk conditions (MC: +5 points; LC: +15 points). Total coliform and *E. coli* populations were significantly reduced across all groups (by 1 log in GC and 2 logs in MC and LC). Ducks in all groups met the cooperative’s expected performance standards, including those in LC, with higher yields of fatty liver and breast muscle observed in the YCW-treated MC group. This trial highlights the critical role of outdoor rearing conditions in achieving optimal performance and minimizing pathogen pressure in duck production. Despite the adverse effects of winter-related sanitary risks, YCW supplementation provided notable benefits in supporting production and enhancing bird resilience against opportunistic bacterial contamination, particularly under challenging environmental conditions.

Keywords: Duck; microbiota; Yeast cell wall; *E. coli*

301P Environmental enrichment using perches and hanging bottles improves welfare and feed efficiency in broiler chickens

Abdul Ghani*¹², Shahid Mehmood¹, Faisal Hussnain¹, Dr. Saima² ¹University of Veterinary and Animal Sciences, Lahore, Pakistan, Poultry Production, Lahore, Pakistan; ²University of Veterinary and Animal Sciences, Lahore, Pakistan, Animal Nutrition, Lahore, Pakistan

The provision of environmental enrichment (EE) is a promising strategy to address welfare concerns in intensive broiler production. This study evaluated the effects of specific EE tools (perches, pecking balls, hanging bottles, and laser lights) on growth performance, behavior, welfare indicators, and meat quality. A total of 300 commercial broiler chickens were randomly assigned to one of five treatments: four EE groups (each with a different tool) and a control group with no enrichment. Compared to the control, birds provided with perches and hanging bottles exhibited significantly lower feed intake and an improved feed

conversion ratio. Behavioral observations revealed that enriched birds were more active, engaging in more walking, jumping, running, wing flapping, and aggressive interactions, while control birds spent a greater proportion of time feeding and drinking. Furthermore, EE groups demonstrated superior welfare outcomes, with a significantly reduced incidence of toe injuries and footpad dermatitis. While meat quality was influenced by enrichment, the effects were tool-specific; birds with perches produced meat with higher lightness (L*), whereas meat from the control group exhibited lower cooking loss and shear force. In conclusion, environmental enrichment, particularly perches and hanging bottles, enhances activity, improves key welfare metrics, and optimizes feed efficiency without compromising final body weight. These findings provide concrete evidence for the implementation of specific EE tools to improve broiler production systems.

Keywords: Environmental Enrichment; Broiler Chickens; Animal Welfare; Behavior; Feed Efficiency

302P The CORAX Light Spectrum enhances growth performance, immunity, and meat quality in broiler chickens

Abdul Ghani*¹, Shahid Mehmood¹, Faisal Hussnain¹, Saima Saima² ¹University of Veterinary and Animal Sciences, Lahore, Pakistan, Poultry Production, Lahore, Pakistan; ²University of Veterinary and Animal Science, Lahore Pakistan, Animal Nutrition, Lahore, Punjab, Pakistan

Light spectrum is a critical environmental factor in modern poultry production, with potential to influence physiology and behavior. This study compared the effects of three light sources (CORAX (HATO), Light Emitting Diode (LED), and an Energy Saver (control)) on broiler chickens. A total of 180 birds were randomly allocated to the three treatments, with five replicates of twelve birds each. Broilers reared under CORAX light exhibited significantly superior ($P < 0.05$) overall growth performance, achieving the highest final body weight, feed intake, feed conversion ratio (FCR), and livability. Behaviorally, the CORAX group was more active, while the control group was more inactive, with the LED group intermediate. Carcass and meat quality were also significantly enhanced under CORAX lighting, evidenced by greater carcass and liver weights, improved intestinal morphology, and superior meat attributes, including higher initial pH, improved color, and greater tenderness. Immunologically, both the CORAX and LED groups demonstrated strengthened humoral immunity, with higher antibody titers against infectious bronchitis and Newcastle disease. Critically, all welfare indicators (hock burn, footpad dermatitis, toe injuries, gait, and symmetry) were comparable across treatments, with only feather condition being better in the control group. In conclusion, the CORAX light spectrum offers a multifaceted advantage, simultaneously optimizing productivity, meat quality, and immune status in commercial broilers without adversely affecting animal welfare.

Keywords: Light Spectrum; Broilers; CORAX; Growth performance; Animal welfare

303P Extended periods of feed and water deprivation after hatch can reduce the performance of broiler chickens in later stages of growth

Hector Leyva-Jimenez*¹, Katherine A. McCormick¹, Julie Simmons¹, Brian Dirks¹ ¹United Animal Health, Sheridan, Indiana, United States

Newly hatched chicks are often left without access to feed and water for extended periods due to management and transportation. Chicks will rely on their yolk sac reserves to supply water and nutrients until they have access to feed and water. It is uncertain if the yolk sac reserves are enough to sustain optimal performance. Therefore, the objective of the present study was to develop an experimental model to evaluate the impact of extended periods of feed and water deprivation (FWD) after hatching on the growth of broiler chickens, allowing for future testing of strategies that could ameliorate the negative impact of FWD. The study consisted of a complete randomized block design of 6 treatments: positive control (PC), no FWD, and 5 increasing times of FWD (12h, H12; 24h, H24; 36h, H36; 48h, H48; and 60h, H60), starting from the time of arrival at the experimental facility. Each treatment included 9 replicate cages with 9 male broilers allocated to battery cages. The feeding program consisted of 1 dietary starter phase, fed for 18 days. Performance was evaluated at 7 and 18 days. On day 7, one bird per pen was selected to evaluate the relative organ weight of liver, proventriculus, gizzard, and spleen. Additionally, intestinal samples were collected from the jejunum and ileum to evaluate villi height, crypt depth, and their ratio (V:C) for PC, H12, and H24. Data were subjected to ANOVA ($P < 0.05$), and means were compared using the Student's *t*-test of JMP 17.1 software. At 7 days, the BW of H12, H24, H36, H48, and H60 was reduced ($P < 0.001$) by 4.45%, 13.49%, 20.77%, 29.49%, and 37.97% compared to the PC, while FCR increased ($P < 0.001$) by 0.69%, 0.78%, 1.18%, 3.92%, and 9.70%, respectively. The relative weight of the gizzard of H60 was higher ($P = 0.001$) compared to all other groups. Jejunum and ileum V:C were numerically decreased as FWD increased. After 18 days, all FWD groups had lower ($P < 0.001$) BW compared to the PC. FCR was higher ($P = 0.028$) for all groups except for H36 compared to the PC. In conclusion, the extended periods of FWD were effective in reducing the performance of broiler chickens after hatch, and the effect was sustained up to 18 days of age. Additional studies are recommended to study other response variables to FWD, such as blood biomarkers.

Keywords: management; deprivation; broiler; performance; dehydration

304P Evaluating the impact of enrichment introduction date on usage in broiler chickens

Chelsea S. Castillo Najera^{*1}, Emily Nemerovsky², Katy Tarrant¹ ¹California State University, Fresno, Animal Sciences and Agricultural Education, Fresno, California, United States; ²California State Polytechnic University, Pomona, Pomona, California, United States

Consumer demand for enhanced welfare practices has driven the implementation of environmental enrichment in commercial broiler facilities. Commonly, structural enrichments, such as platforms, can be introduced to add complexity to birds' environments and stimulate natural behaviors. In the industry, there is no single standard for implementing environmental enrichments. Factors such as the bird's age and the design of the enrichment may affect the bird's use of a platform, as does the age at introduction. Prior literature indicates that, in commercial facilities, birds use enrichments at different rates throughout their lives. Thus, the evaluation of the age of introduction may affect the novel use and activity of birds on the platforms. This study aims to compare how the day of introduction affects platform usage in a commercial facility. On day 0, three platforms were placed. Additional platforms were added on days 21 and 35, resulting in a total of nine by day 35. For each replicate, platforms were added under the same camera, placed at equal distances from the feed and water lines, and arranged north of the previously placed platforms.

Usage of the platforms during the last 10 days of the flock's grow-out period (days 35-44) was recorded and pooled for analysis. For each observation, counts of 4 metrics were recorded: # of birds active on, inactive on, using, and around the platform. Data were evaluated using Tukey's HSD in JMP ($P < 0.05$). Significant differences in platform use were observed among the platforms introduced on days 0, 21, and 35: 5.65 ± 0.17 , 4.38 ± 0.22 , and 2.46 ± 0.31 ; $P < 0.05$. Birds inactive on the platform showed significant differences at 4.32 ± 0.14 , 3.50 ± 0.18 , and 1.90 ± 0.26 ; $P < 0.05$. Birds around the perimeter showed significant differences at 11.63 ± 0.31 , 10.34 ± 0.22 , and 9.35 ± 0.17 ; $P < 0.05$. Active birds showed a significantly greater difference when introduced at day 0 (1.30 ± 0.06 ; $P < 0.05$). These results indicate that the timing of introduction is a critical factor impacting bird engagement. Findings suggest that with earlier exposure, platforms maintain their novelty with greater and more sustained use over time. Thus, highlighting the need to refine enrichment guidelines to optimize introduction age and support continued welfare improvements.

Keywords: enrichment; broiler; platform; commercial

305P Summarization of microbial environmental surveillance in targeted hatchery areas in 2025

Ellie L. Wollett^{*1}, Benjamin N. Alig², Joshua Deines² ¹Zoetis, US Poultry Diagnostics, Raleigh, North Carolina, United States; ²Zoetis, US Poultry, Durham, North Carolina, United States

Environmental and microbial surveillance is crucial for the effective management of bacterial and fungal contamination in hatcheries. When embryos are exposed to fungal spores or bacteria, it may lead to embryonic death or failure to hatch. Hatchery contamination can also lead to higher first week mortality, lung lesions or yolk sac infections. Routine environmental surveys are crucial to target problem areas within the hatchery so that cleaning methods and biosecurity protocols can be improved. This study evaluated microbial loads across various hatchery sites, including egg rooms, hatchers, vaccine preparation laboratories, ventilation systems, hatch baskets, tray washers, egg transfers, and *in-ovo* vaccination rooms. The objective was to identify potential problem areas that are consistent across multiple hatcheries. Routine surveys were performed at 9 facilities varying in age and hatcher systems (single- versus multi-stage). Dry swabs were plated onto Sabouraud Dextrose Agar (SAB) plates to isolate fungi and yeast and Trypticase soy agar (TSA) plates were used to evaluate overall bacterial growth. After collection, plates were sent to our diagnostic lab, and incubated for 24-48 hours at 37 °C for TSA or 42 °C for SAB plates. Colony forming units (CFU) were recorded and analyzed to identify consistently high contamination levels in specific hatchery zones, particularly *in-ovo* vaccination and transfer areas. Surveys showed aspergillus contamination on the setter fan in 6 hatcheries, the rooftop HVAC intake in 5, and the setter wall in 4. TSA plate analysis showed bacterial contamination from egg touches in all 9 hatcheries, with 7 positive results from clean wet hatch baskets and 6 from clean dry baskets. Additionally, 6 hatcheries had bacterial contamination on the lab counter, water spigot, transport cooler, ice packs, inovoject alcohol, and DI water container spigots. This data shows that a greater focus in cleanliness can be applied to the hatch baskets, setters and lab environment as these areas were the most contaminated. By focusing on these critical areas, hatcheries can effectively minimize disease risks and promote optimal outcomes for chick livability and overall flock health.

Keywords: bacteria; fungus; hatchery; environmental survey; biosecurity

306P A case study of a commercial broiler farm in Cullman County, Alabama utilizing a rainwater harvesting system to offset rising water costs

Carson M. Edge*¹, Jeremiah D. Davis¹, Jesse C. Campbell¹, Eugene Simpson¹, Joseph L. Purswell² ¹*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ²*USDA-ARS, Starkville, Mississippi, United States*

Broiler producers must have access to sufficient water supplies to maintain the health and well-being of broilers. For some producers who rely on municipal water sources, increased water rates have begun to affect their bottom line. This has been the experience for broiler producers in Cullman County, Alabama, where the county water department announced customers would see increased water rates beginning in 2015. Water rates have since increased by 69% in 10-yrs (\$2.51 per 1,000 L in 2015 to \$4.25 per 1,000 L in 2025). In anticipation of these water rate increases, a pilot study was initiated in 2015 to evaluate the performance of a rainwater harvesting (RWH) system (storage capacity of 378,541 L) in offsetting rising water costs for a four-house broiler farm in Cullman, Alabama. While the producer has observed a reduction in their monthly water bill, the system did not include an effective way of measuring rainwater use (RWU) and municipal water use (MWU). Therefore, the purpose of this study was to quantify farm RWU and MWU to better understand the overall performance of the RWH system. Two wireless ultrasonic meters were installed and monitored RWU and MWU over six flocks reared from 13 Jan. 2024 to 3 Jan. 2025. Average flock age during the test period was 45 d with an average flock placement of 27,517 birds. Descriptive statistics were used to evaluate total water use (TWU) for each flock and the proportion of RWU and MWU to TWU was presented. A simple economic analysis of water bill savings based on water use, water rate, taxes, and fees during the study period was also performed. Total water use for the farm during the 12-month study period was 7,267,625 L with a mean flock TWU of 1,211,271 L, ranging from 1,005,634 to 1,593,661 L. Municipal water use represented 45% of TWU and RWU represented 55% of TWU over the entire study period for the farm. The producer paid \$14,307 for water with an estimated water bill savings of \$17,017 had the producer not been able to utilize RWH. While results from this study suggest water bill savings can be achieved using RWH, continuous monitoring of this system should occur to understand the long-term use of RWH on this commercial broiler farm.

Keywords: Broiler water consumption; Water conservation; Rainwater Harvesting

307P Effectiveness of an aluminum based litter amendment on re-used broiler litter for ammonia emissions, litter moisture and litter bacteria

Charles L. Hofacre*¹, Matthew K. Jones², Jennie Baxter², Ziv Raviv³, Amy Syester⁴, Delana Peralta⁴, Scott McKenzie⁴ ¹*Southern Poultry Research Center, Watkinsville, Georgia, United States;* ²*Southern Poultry Research Group, Watkinsville, Georgia, United States;* ³*Poultry PathoScience Solutions Inc, Fort Lauderdale, Florida, United States;* ⁴*Chemtrade Logistics Inc, Parsippany, New Jersey, United States*

U.S. broiler farms re-use litter for multiple flocks. Bacteria action can result in higher ammonia. Ammonia gas can cause cornea and trachea damage in the broilers. Two controlled studies with re-used litter from previous N.E. studies and broiler chicks were performed. Study 1 were 2 environmental rooms each 11m² with 120 Ross male chicks/room. The litter in room 1 was treated with an aluminum product (Al-Clear) at 100 pounds/ 1000 square feet, room 2 was not treated. Study 2 evaluated an acidified clay (T1), an aluminum product T2 (Al+Clear Plus) and a sodium bisulfate (T3) in 3 larger rooms (each 62m²). All products were spread dry

at 100 pounds/1000 square feet 24 hours prior to chick placement. Study 2 had 16 replicate pens in each room with 27 Ross male chicks/pen to enable statistical analysis of results. Statistical evaluation used ANOVA with a comparison of means using LSD t-test at P_≤0.05. Both studies measured room ammonia, air humidity and litter moisture from DO-35. Coliform and Clostridium bacteria counts as well as litter pH were measured on day -1, 0, 7, 14, and 35. Footpad lesions and histology of eyes were evaluated on day 35. Study 1 at one hour post treatment ammonia reduced from 27 to 9 ppm in treated room. The mean ammonia level from day 0 to 35 was 7.1 ppm (treated) and 16.6 ppm (control). Bacteria counts in litter as well as footpad lesions were lower in treated room. Study 2 had immediate reduction in ammonia in all 3 treatments. T1 from 50 ppm to 1.0 ppm; T2 from 70 ppm to 7.0 ppm and T3 55 ppm to 2.0 ppm. Room ammonia, humidity, litter moisture and pH were not significantly different between treatments throughout the 35 day study. T1 had significantly heaviest 35 day body weight (2.180 kg^A), followed by T3 (2.089 kg^B) and aluminum product (T2) 2.069 kg^B. Since re-used litter was from an N.E. study, competitor T1 had 2.78%^A N.E. mortality, aluminum (T2) had 0.23%^A and T3 was 1.16%^A. On day 35 there were no significant differences in footpad lesion scores or histologic eye lesions. Overall, the aluminum litter treatment safely lowered air ammonia levels and in Study 2 performed as well as competitor litter treatment products in 35 day broiler body weight and FCR. In addition, had lower overall mortality and N.E. mortality.

Keywords: Re-used litter; ammonia; aluminum litter; litter treatment; necrotic enteritis

308P Impact of geographic location and flock age on hatching egg bacterial abundance

Maryam Mohammadi-Aragh¹, Dilber Annageldiyeva¹, Jessica Drewry*¹, Katie Elliot² ¹*Mississippi State University, Agricultural and Biological Engineering, Mississippi State, Mississippi, United States;* ²*USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi, United States*

Published data on eggshell bacterial loads prior to sanitation vary in methodologies and results from 0.85 to 5.4 log₁₀CFU/mL in the literature. With concerns of optimizing broiler chick quality and immunity through commercial practices such as fogging eggs during storage, updating the knowledge base with current bacterial loads on the surface of clean settable broiler eggs warrants further studies on the impact of eggshell bacteria on the chick and potential novel intervention strategies. The objectives of this study were to evaluate the impact of flock age and geographic location on total aerobic bacteria present on hatching eggshell surfaces. Total aerobic bacteria were enumerated from eggs collected at two commercial hatcheries in adjacent states in the Southeastern US. Two flock age groups were included, with eggs originating from either peak-age hens (30 and 35 weeks) or post-peak hens (46 and 50 weeks). A random sample of approximately thirty eggs clean, settable eggs were evaluated for each location and flock age (n = 128), and total aerobic bacterial counts were determined using Aerobic Count Plate Petrifilms. A two-way ANOVA revealed significant effects of location (P < .0001), flock age (P < .0001), and their interaction (P < .0001) on bacterial counts (log₁₀CFU/mL). Mean bacterial loads were 3.5 and 4.1 log₁₀CFU/mL for Location A and B, respectively. Across locations, post-peak hens averaged 3.3 log₁₀CFU/mL compared to peak hens at 4.3 log₁₀CFU/mL. Within Location A, there was a greater variation in bacterial loads between flock ages (1.61 log₁₀CFU/mL) compared to Location B (0.18 log₁₀CFU/mL). Values fall within the range of published literature values over the past several decades. The results suggest that breeder flock

management, hatchery management, hen age, and environmental conditions continue to significantly influence eggshell bacterial loads. Continued data collection, including additional hatchery/farm sites, will guide future studies related to background eggshell bacterial loads.

Keywords: aerobic bacteria; eggshell; flock age

309P Effects of nitrate-contaminated drinking water on early broiler performance, methemoglobin levels, and amino acid digestibility

Faija S. Pory*^{1GS}, Md Raihanul Hoque¹, Biswajit Kumar Biswas², Thyneice Taylor-Bowden², Samuel Nahashon², Pramir Maharjan¹ ¹Texas A&M University, Department of Poultry Science, College Station, Texas, United States; ²Tennessee State University, Department of Food & Animal Science, Nashville, Tennessee, United States

Nitrate contamination of groundwater is an emerging concern in poultry production, as elevated levels in drinking water may pose risks to bird health and physiological function. Two controlled trials were conducted to evaluate the impact of nitrate contaminated drinking water on early broiler performance, with nitrate levels prepared using ammonium nitrate. In the first trial, 240 broilers were allocated to four nitrate concentrations (0, 10, 30, and 50 ppm) in six replicates of ten birds each, whereas the second trial involved 180 broilers assigned to three nitrate levels (0, 100, and 200 ppm), also arranged in six replicates, and both experiments were conducted for 14 days. The following parameters were measured for all treatment diets: body weights, weight gain, feed intake, feed utilization, water intake, mortality %, relative organ (liver, heart, spleen) weight, amount of methemoglobin, and amino acid digestibility. Statistical analysis was conducted using one-way ANOVA followed by Tukey's HSD test. No significant differences were observed among treatments in body weight, weight gain, feed intake, feed conversion, water intake, mortality percentage, or relative organ weights (liver, heart, spleen) in either experiment. However, methemoglobin concentration (ng/mL) increased significantly ($P < 0.05$) with rising nitrate levels in both experiments, with the highest value in the first trial observed at 50 ppm (0.429 ng/mL) and the highest value in the second trial observed at 200 ppm (0.682 ng/mL). In the first trial, some amino acids (Lysine, Methionine, Tryptophan, Arginine, and Serine) digestibility values were higher in birds receiving 50 ppm nitrate compared with the other treatment groups, whereas no significant differences in amino acid digestibility were detected among treatments in the second trial. Overall, these findings indicate that early-age broilers can tolerate moderate nitrate concentrations in drinking water without major impacts on growth performance or organ development, although elevated nitrate levels do increase methemoglobin formation. Further studies are warranted to understand impacts of chronic exposure of higher nitrate levels on bird health and performance.

Keywords: Nitrate Contamination; Broiler; Methemoglobin; Amino acid digestibility; Water quality

310P Longitudinal metagenomic analysis of poultry litter reveals climate- and event-driven community shifts

Elena G. Olson*¹⁴, Haley Nabors², Kalyn Nelson², Luis Muñoz², Cassie L. Ettinger³, Michael B. Rhys³, Casey E. McGinley³, Jake K. Byrnes⁵ ¹University of Wisconsin, Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States; ²Mississippi State University, Starkville, Mississippi, United States; ³Barnwell Bio, New York, New York, United States; ⁴Supreme EGO LLC, Middleton, Wisconsin, United States; ⁵Barnwell Bio, San Diego, California, United States

Effects of climate and flock events on pathogen abundance remain unclear. We conducted a four-month longitudinal study in two broiler houses to track litter microbial succession using weekly boot-cover sampling and shotgun metagenomics. Environmental temperature (ET), humidity (EH), and in-house temperature (HT) and humidity (HH) were recorded. Relative abundances of *E. coli*, *E. faecium*, *E. cecorum*, *S. aureus*, and *S. hyicus* were quantified as indicator taxa based on their persistent, high-ranking abundance and epidemiologic relevance on this farm. Samples were assigned to the nearest flock event, and pre-, post-, and event differences were tested using Kruskal–Wallis with Dunn's post hoc. Spearman correlations were computed for placement and mortality. Linear mixed-effects models assessed environmental prediction of barn microclimate. ET and EH significantly predicted HT and HH ($P < 0.001$). HH strongly influenced total pathogen abundance ($P = 0.013$), whereas HT effects were marginal ($P = 0.07$). Climate responses varied by taxon. *E. coli* correlated with HH ($\rho = -0.37$, $P < 0.001$); *E. faecium* with HT ($\rho = 0.36$, $P = 0.001$) and HH ($\rho = -0.52$, $P < 0.0001$); and *S. hyicus* with HT ($\rho = 0.26$, $P = 0.02$). *E. cecorum* correlated only with environmental factors, while *S. aureus* showed none. We then evaluated how relationships between taxa themselves shifted across flock events, independent of microclimate. *E. coli*, *E. faecium*, and *S. hyicus* significantly fluctuated around mortality ($P < 0.05$), and *E. coli*, *S. aureus*, and *S. hyicus* differentiated around placement ($P < 0.05$). Correlation structures shifted sharply across events. *E. coli* and *E. faecium* were strongly positive during placement and pre-/post-mortality ($\rho > 0.5$) but became strongly negative during mortality ($\rho = -1$). *E. cecorum* and *E. faecium* shifted from negative (pre/post) to strongly positive during mortality ($\rho = 1.00$). Staphylococcal correlations reversed from positive at placement ($\rho = 0.74$) to negative at mortality ($\rho = -0.63$). Overall, pathogen dynamics reflected interacting effects of environmental forcing, moisture-driven microhabitats, and event-aligned instability. Integrating climate-informed and event-based monitoring may improve early detection of pathogen shifts in broiler production systems.

Keywords: poultry litter; metagenomics; pathogen dynamics; microclimate; event-driven shifts

311P Combined sprinkler and evaporative cooling system effects on water usage, bird performance and litter microenvironments of broiler houses

Jonathan Moon*¹, Jessica Drewry², Joseph L. Purswell³, Matthew Rowland³, Tom Tabler⁴ ¹Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States; ²Mississippi State University, Mississippi State, Mississippi, United States; ³USDA, ARS, Starkville, Mississippi, United States; ⁴University of Tennessee, Animal Science, Spring Hill, Tennessee, United States

Poultry production is a top U.S. agricultural commodity but faces challenges from rising protein demand and climate-related stressors. Heat, limited water, and high humidity reduce broiler performance and welfare. Conventional evaporative cool-cell (CC) systems manage heat but use large amounts of water and often raise humidity above 80%, limiting birds' ability to cool through respiration. Sprinkler systems (SS) cool birds directly, save water, and reduce house humidity, but there is limited research on SSCC combinations under commercial conditions. This study compared a combined SSCC strategy with a conventional CC system across two summer flocks at Mississippi State University using commercial-sized research houses with reversed treatments. The combination system used activity-promotion sprinkling from day 21 and sprinkler cooling from day 28, with CC activation delayed until 31°C. The CC treatment followed standard evaporative thresholds. Cooling and drinking

water usage, litter nutrient and microbial measures, ammonia levels, body weights, paw scores, soiled plumage, mortality, and core body temperatures were recorded. Data were analyzed using GLM (SPSS v29) with Tukey HSD. The combination treatment trended with a 39% reduction in cooling-water usage compared to the CC treatment (63,696 vs. 105,091 gallons, $P = 0.112$). The SSCC treatment exhibited higher average plumage soiling scores (.61 vs. 1.77, $P < 0.001$). The off-white plumage color of SSCC birds is due to dust interacting with water droplets, which remains after evaporation. Overall core temperatures were not significantly different except at two time points within a 72-hour window, where SSCC values were significantly lower: TP14-4 pm (41.42 °C vs. 41.6 °C, $P = 0.026$) and TP17-4 am (41.4 °C vs. 41.7 °C, $P = 0.006$). There were no significant differences in drinking water intake, body weight, paw quality, mortality, or litter microbial or nutrient metrics. Results indicate that combining sprinklers with evaporative cooling can maintain broiler comfort and performance while drastically reducing cooling-water consumption in real-world applications, without creating adverse effects on the house environment.

Keywords: Sprinkler; Water conservation; Welfare; Performance; Litter Micro environment

312P Modeling the relationship between environmental conditions and egg quality in laying hens

Ayodeji Aderibigbe*¹ *Ohio State University, Animal Sciences, Wooster, Ohio, United States*

In this study, a pasture-based layer flock was utilized as a model to investigate how natural environmental fluctuations influence egg quality traits. A total of 150 Rhode Island Red hens were monitored over 18 weeks (August–December) on a commercial pasture farm in North Florida. Twenty-four eggs were collected weekly, and internal and external quality parameters including yolk color, yolk height, Haugh units, shell thickness, peak shell force, and egg weight were measured with an automated egg tester. In addition, real-time microclimate variables including temperature, relative humidity, heat index, dew point, density altitude, and station pressure were monitored daily at 10 minutes intervals using a Kestrel Drop-3. Average weekly temperature ranged from 52 – 82°F and relative humidity ranged from 62–87%. Time-series regression (PROC AUTOREG, SAS 9.4) identified temperature as the primary determinant of internal egg quality. Haugh units declined linearly with rising temperature ($\beta = -0.64$; $P = 0.001$), while relative humidity was not significant ($P = 0.18$). Yolk height also decreased with temperature ($\beta = -0.15$; $P = 0.02$). Canonical correlation analysis revealed a strong multivariate association between egg and environmental variables (canonical $r = 0.97$; $P = 0.006$). Cooler, higher-pressure conditions were associated with thicker shells, greater shell strength, and heavier eggs, whereas higher heat load reduced internal quality ($P < 0.05$). These findings provide a predictive framework linking environmental variability to egg quality responses, supporting the implementation of climate-adaptive management strategies in commercial and alternative layer operations.

Keywords: egg quality; temperature; environmental variation; time-series analysis; laying hens

Food Safety

313P Effects of a monoglyceride, natural extract, and organic acid-based feed mitigant on antimicrobial activity against *Salmonella* Typhimurium and pH modulation in different water sources

Janghan Choi*¹, Aubrey Tambe¹, Woongbi Kwon², Fredrik Sandberg², Steve England² ¹*Texas Tech University, Department of Animal and Food Sciences, Lubbock, Texas, United States*; ²*Furst-McNess Company, Rockford, Illinois, United States*

Feed can serve as an important route for pathogen transmission, including *Salmonella*, to livestock. Increasing regulatory and consumer pressure, under the One Health framework, emphasizes the need for effective alternatives to formaldehyde-based feed sanitizers currently used in the poultry industries. FeedArmor (FA; Furst McNess Company, Rockford, Illinois) is a monoglyceride, natural extract and organic acid-based feed mitigant with strong antimicrobial properties and pH-modulating capacity, making it a promising candidate to provide an alternative to formaldehyde-based products. This study investigated the *in vitro* antimicrobial activity of FA against *Salmonella* Typhimurium and assessed its pH modulation properties in water sourced from Western Texas (WTX) and Georgia (GA), which differ in baseline pH and hardness. Minimum inhibitory concentrations (MIC) were determined using microdilution methods, and bacterial counts were quantified at sub-MIC concentrations. To assess pH variability, FA (1%) was dissolved in distilled water and water collected from both regions. Moreover, feed pH was measured in diets containing 0.5, 1.0, 2.0, and 3.0 mg/mL FA prepared with each water source. One-way ANOVA with Tukey's post hoc test was performed for statistical analysis. FA exhibited an MIC of 3 mg/mL against *Salmonella* Typhimurium. At sub-MIC levels, FA at 2 mg/mL and 2.5 mg/mL significantly reduced bacterial counts by 1.11-log and 1.48-log, respectively compared to the control (0 mg/mL FA) ($P < 0.05$). The pH of 1% FA dissolved in distilled

water was 2.35. The pH of 1% FA dissolved in WTX was significantly higher than that observed in GA water ($P < 0.05$). Feed prepared with WTX water maintained a higher pH across all FA inclusion levels (upto 0.3%) compared with feed prepared using GA ($P < 0.05$). However, FA inclusion up to 3 mg/mL did not significantly alter feed pH ($P > 0.05$). Therefore, these findings demonstrate that FA has potent *in vitro* antimicrobial activity against *Salmonella* as well as a strong capacity to modulate pH. Nevertheless, antimicrobial efficacy and pH response may vary depending on water quality characteristics including baseline pH and mineral hardness.

Keywords: *Salmonella* Typhimurium; Feed mitigant; pH modulation; Water hardness; Antimicrobial effects

314P Early *Campylobacter jejuni* exposure in chicks: Tissue gene expression and potential immunization biomarkers

Xin Ye*¹, Hailey Fugate¹, Chrysta N. Beck¹, Li Zhang¹, Linan Jia¹ ¹*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States*

Campylobacter jejuni is a leading foodborne pathogen, and extensive cecal colonization in broilers driver of carcass contamination during processing. Although clinically silent in chickens, it activates immunity and defining systemic adaptive signals (spleen) and B-cell programs (bursa) can inform vaccine design. We profiled tissue-specific, time-resolved responses to guide vaccine formulation and timing, aiming to identify transcript markers of early systemic immunity in spleen and B-cell programming in bursa after inoculation. 72 one-day-old male broilers were randomized to three groups: negative control, 10^4 CFU/mL, 10^6 CFU/mL (24 birds per group in 6 cages of 4). At day 7, birds received 0.1 mL by oral gavage of sterile BHI (control) or a 1:1:1 cocktail of *C. jejuni* strains CJ901, CJ153, and CJ273 at

the assigned dose. At 7 and 14 days post inoculation (DPI), one bird per cage was sampled for spleen and bursa. RT-qPCR quantified *IFN- γ* , *IL-6*, *IL-1 β* , *TNF- α* , *SOCS3*, *IL-10*, *TGF- β 1*, *AvBD1*, *BAFF*, *CD40*, and *TLR-21* expression with *18S rRNA* as the reference gene. Δ Ct values were analyzed by two-way ANOVA (group, DPI, interaction) with Tukey's HSD for within-DPI pairwise comparisons. In the spleen, most responsive genes increased from 7 to 14 DPI similarly across groups with little dose separation, while *IL-10*, *IL-6*, and *TGF- β 1* were not different for any factor. At 14 DPI, only *BAFF* and *TLR-21* were lower ($p < 0.05$) and *CD40* was marginally lower ($p = 0.051$) in the low-dose group than in the control ($p < 0.05$), while gene expressions in the high-dose- and control-groups were similar. In the bursa, most targets showed group \times DPI interactions ($p < 0.05$), and *TNF- α* and *IL-1 β* had group main effects ($p < 0.05$). By 14 DPI, the high-dose group exceeded control for *IFN- γ* , *IL-1 β* , *TNF- α* , *IL-10*, *TGF- β 1*, *CD40*, and *TLR-21* expression ($p < 0.05$), indicating that dose effects emerge later in this tissue and align with enhanced B-cell activation and antigen presentation, whereas spleen responses were largely time-driven and dose-insensitive. Bursal gene markers at 14 DPI, particularly in the high-dose group, serve as candidate indicators for vaccine screening, optimization of immunization timing and dose, and on-farm monitoring strategies to reduce flock carriage and carcass contamination.

Keywords: *Campylobacter jejuni*; Gene expression; Dose-response; Immunization biomarkers; Spleen and Bursa of Fabricius

315P Colonization of *Salmonella* Enteritidis (SE) in ceca and ovaries, after SE challenge, in *Salmonella* vaccinated commercial layer hens fed *Saccharomyces cerevisiae* components

Theresia Lavergne¹, Mikayla Baxter², Charlie Elrod¹ ¹Natural Biologics, Inc., Stephenville, Texas, United States; ²Ozark Avian Research, LLC, Gravette, Arkansas, United States

This trial was conducted to evaluate the effect of Cascade (hydrolyzed yeast plus yeast culture; HY/YC; Natural Biologics, Newfield, NY) and NaverdeTM (yeast cell wall plus hydrolyzed yeast; YCW/HY; Natural Biologics, Newfield, NY) on the colonization of *Salmonella* Enteritidis (SE) in the ceca and ovaries of SE challenged layers that were vaccinated (Avipro[®] Megan Egg[®], Elanco) for *Salmonella*. Seventy-two, 25-week-old Hy-line brown layers were allotted to three treatments: Challenged, control (C), Challenged, HY/YC (100 g/ton), or Challenged, YCW/HY (100 g/ton). There were 12 replicates per treatment with two layers per cage. All birds were vaccinated at day of hatch and at eight weeks of age. Layers were fed a non-medicated commercial-type diet that met the requirements for Hy-line brown layers. After a four-week adaptation to treatments, layers were orally challenged with nalidixic acid resistant SE (10^9 cfu/mL). Seven-days post challenge, layers were necropsied to remove ceca and ovaries for analysis of *Salmonella* prevalence and load with the BAX[®] System SalQuant[®] Real-Time PCR Assay. Egg production and feed intake were measured for calculation of FCR. Also, layers were orally gavaged with FITC-d (8.32 mg/kg of body weight). One-hour post-gavage, blood was collected, and FITC-d was measured to evaluate gut permeability. Treatment did not affect ($P > 0.05$) egg production, feed intake, or FCR. *Salmonella* was not detectable in any ovaries. Cecal *Salmonella* prevalence and load were numerically ($P > 0.05$) higher in the C than both the YCW/HY or HY/YC. *Salmonella* prevalence was 95.8%, 91.7%, and 83.3% for the C, HY/YC, and YCW/HY, respectively. While *Salmonella* load was 94.8, 10.5, and 10.5 CFU/g and 0.441, 0.170, and 0.133 log CFU/g for the C, HY/YC, and YCW/HY, respectively. There was no difference in FITC-d between

treatments (34.15, 38.08, and 36.40 ng/ml, for C, HY/YC, and YCW/HY, respectively). *Salmonella* challenge did not affect gut permeability. Feeding vaccinated laying hens either YCW/HY or HY/YC numerically reduced their cecal *Salmonella* load post-infection. Thus, yeast derived components as a feed intervention, along with *Salmonella* vaccination of laying hens, can be combined to help prevent *Salmonella* contamination of shell eggs.

Keywords: layers; *Salmonella* Enteritidis; yeast; food safety; gut health

316P Evaluation of the in vitro efficacy of disinfectant types against *salmonella* senftenberg and *salmonella* mbandaka on different contact surfaces used in animal feed mills

Liris Kindlein^{*1}, Adrieli Biava² ¹UFRGS, Preventive Medicine, Porto Alegre, RS, Brazil; ²UFRGS, Post-graduate Program Animal Food, Porto Alegre, RS, Brazil

In animal feed mills, contamination by *Salmonella* is a constant challenge, making the implementation of a rigorous biosecurity chain essential to ensure food safety and animal health. *Salmonella* forms biofilms on inert surfaces. These biofilms provide a protective barrier that makes the bacteria more resistant to disinfectants and cleaning procedures. This study evaluated, through *in vitro* analyses, the efficacy of chemical compounds with antimicrobial action in reducing *Salmonella* serotypes (*S. Senftenberg* and *S. Mbandaka*) on plastic, metal, or concrete surfaces. Five treatments were tested: T1 - control, T2 - products based on formaldehyde and organic acids, T3 - organic acids, T4 - quaternary ammonium and T5 - oxidizing compounds. Statistical analysis (ANOVA and Tukey HSD) indicated that strain concentration was the most determining factor for logarithmic reduction ($F = 8.090$; $p < 0.001$), while group and surface had a marginal influence, and serotype was not significant. The Tukey HSD test revealed variation between groups and surfaces, with the greatest reduction in T2 on concrete (1.5 log) and the lowest in T3 on the same surface (0.62 log). Overall, values ranged from 0.62 to 1.5 log, highlighting concrete as the most variable surface, metal with intermediate performance, and plastic as the most stable. The interaction between microbial concentration and surface type showed that, at a low microbial load (10^3 CFU), plastic and metal surfaces exhibited greater efficacy (≈ 1.2 log reduction), while concrete showed lower efficacy (≈ 0.8 log). At an intermediate concentration (10^4 CFU), all surfaces demonstrated a decrease in efficacy, with concrete reaching approximately 0.6 log. At a high concentration (10^5 CFU), a partial recovery was observed, with plastic and metal achieving around 1.1 log reduction and concrete approximately 1.0 log. Thus, the efficacy of chemical compounds depends on the initial contamination of the strain and the surface, with concrete being more sensitive to variation. These findings reinforce the need to consider surface characteristics and microbial load when defining hygiene protocols and sanitizing products in feed mills.

Keywords: Organic Acids; *Salmonella*; quaternary ammonium; contact surfaces; formaldehyde

317P Evaluation of PhytoCare water treatment on *Salmonella* Enteritidis colonization in broilers at processing age

Matthew K. Jones^{*1}, Charles L. Hofacre¹, Jennie Baxter¹, Roy Berghaus², Conrad Schmidt³, Robert Tims³, Daryl Hammer³ ¹Southern Poultry Research Group, Watkinsville, Georgia, United States; ²University of Georgia, Department of Population Health, College of Veterinary Medicine, Athens, Georgia, United States; ³Precision Health Technologies, Worthington, Minnesota, United States

As a critical part of food safety programs, producers work to reduce *Salmonella* on broiler carcasses. Utilization of water treatments close to termination is one of the means to reduce the *Salmonella* load coming into the processing plant. The current study evaluated PhytoCare, a patented plant extract solution, at 0.11% in drinking water for broilers near processing age. Treatments included a 1) challenge control, 2) PhytoCare in the drinking water six days and 3) three days before processing. Each of the three treatments were represented by three replicate floor pens with ten broilers. The study began when male Ross broilers were 42 days of age at which time the litter was sprayed with 6.0×10^8 CFU/mL of *Salmonella* Enteritidis (SE). After a brief feed withdrawal on day 49, crop and ceca samples were collected aseptically from three birds per pen for *Salmonella* prevalence and enumeration. Environmental samples were collected with boot sock swabs on day 42 and 49. All boot sock swabs were positive for SE at both time points. Cecal samples were numerically lower in SE prevalence and enumeration by Most Probable Number (MPN)/gram in treated groups compared to the challenge control. Utilizing both prevalence and enumeration in a Tobit regression, the two-log separation between treated and untreated groups neared significance ($P=0.14$). Similar to ceca data, crop SE enumeration was numerically lower in water treatments. When enumeration and prevalence were combined using Tobit regression, the 6-day treatment had significantly lower crop SE \log_{10} MPN/g than the challenge control. The shorter duration was statistically intermediate. Although the number of replicates and birds in this pilot trial were limited, the observed outcomes of the birds supplemented with plant extract were compelling. The longer duration of product usage had a greater reduction in crop *Salmonella* levels, however, both water applications had a functional impact on cecal colonization based on the numerical two-log separation.

Keywords: Salmonella; Water Treatment; PhytoGenic; Food Safety; MPN

318P Machine learning-powered paper-based sensor for *Salmonella* serotype surveillance on chicken meat

Zhen Jia*¹, Boce Zhang³, Wei-shinn Ku², Xian Gao², Stephen N. White⁴ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Auburn University, Computer Science and Software Engineering, Auburn, Alabama, United States; ³University of Florida, Food Science and Human Nutrition, Gainesville, Florida, United States; ⁴USDA, Agricultural Research Service, US National Poultry Research center, Athens, Georgia, United States

Rapid and accurate detection of *Salmonella* serotypes associated with foodborne illnesses is critical to ensuring the safety of poultry products. In this study, we developed a smart, nondestructive, machine learning-powered paper-based sensor (ML-PS) approach for continuous detection and differentiation of *Salmonella* serotypes *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) on chicken breast, even in the interference of native microflora. The sensor contained an array of 9 chemical dyes that react with serotype-specific volatile organic compounds to generate distinguishable colorimetric patterns. The sensor was exposed to chicken meat spiked with and without SE and ST and stored at room temperature and 4°C. During storage, PS images and corresponding “ground truth” labels (temperatures, time points, bacterial species/serotypes, and bacterial population) were collected to generate a database. The database was split into training and testing datasets at a ratio of 90:10. The training dataset was used to develop ML models, while the testing dataset was used to assess model performance. ML models, including support vector machines (SVM) and k-nearest neighbors (KNN), were

developed to recognize these PS images for bacterial identification. Model input was PS image data, and output was the associated “ground truth” labels. During training, both PS image data and labels were available to the models to optimize model architecture and parameters. During testing, only PS image data was input to the trained model for predicting the labels, while the true “ground truth” labels were withheld. The predicted labels were matched with the true “ground truth” to evaluate the testing accuracy. Both SVM and KNN models achieved good performance in detecting and differentiating SE and ST and in distinguishing them from native microflora under both storage conditions, with over 80% accuracy. The ML-PS requires no enrichment, sample preparation, or complex instruments, offering a low-cost, non-destructive, culture-free, smart toolkit for pathogen detection on food. This approach shows strong potential for extension to detect other pathogen species, serotypes, and food matrices.

Keywords: Machine learning; Paper-based sensor; Salmonella; Serotypes; Poultry meat

319P *Salmonella enteritidis* control on broiler transport cages using a cinnamon product

Virginia Baxter*¹, Charles L. Hofacre², Matthew K. Jones³, Roy Berghaus⁵, Lanny Weaver⁴, Neil Teevan⁴, Earnie Porta⁴ ¹Southern Poultry Research Group, Danielsville, Georgia, United States; ²Southern Poultry Research Center, Watkinsville, Georgia, United States; ³Southern Poultry Research Group, Watkinsville, Georgia, United States; ⁴Arkion Life Sciences, Newcastle, Delaware, United States; ⁵University of Georgia, Athens, Georgia, United States

Washing and disinfecting live haul cages without proper drying may increase *Salmonella* exposure on the feathers and skin of broilers during transport from farm to the processing plant. A practical intervention could be spraying the cage floors with a natural cinnamon-based product. The Cinnamon product was shown to be effective in reducing *S. enteritidis* (S.E.) growth in an in vitro antimicrobial inhibition assay inhibiting the growth of *Salmonella enteritidis* within 2 hours. Three broiler live haul cage units from a broiler producer had the floors contaminated with S.E., following the method described by Berrang et.al, 2011. The S.E. culture was grown in brain heart infusion broth to a concentration of 10^9 CFU/ml. Broiler intestinal content were mixed with the S.E. and used to inoculate the floor surfaces of the three live haul cages. To assess the level of S.E. on the floors, sterile sponge swabs were collected at pre-treatment (30-minute post contamination) and 15 minutes, 2 hours and 24 hours post spray application. A sterile 2-kilogram weight was placed on the sponge to simulate a broiler resting on the floor of the cage. T1 (S.E. infected) no treatment, T2 (Cinnamon 19 ml/50 feet²) single spray treatment, T3 (Cinnamon 19 ml/50 feet²) two spray treatments 4 hours apart. Prior to treatment, all sponges were 100% S.E. positive. At 15 minutes and 2 hours post treatment, the untreated cages remained 100% S.E. positive, while the treated cages showed a significant reduction in S.E. prevalence (T2 – 20%; T3 – 10%). S.E. numbers were evaluated as MPN (Most Probable Number)/per sponge. The MPN/sponge were log transformed prior to analysis and $P < 0.05$ was considered significant. Statistical analysis using Tobit regression indicated that, the level of S.E. by most probable number/sponge (MPN/sponge) demonstrated a significant reduction at 15 minutes and at 2 hours in the cages treated with the cinnamon product. Overall, the cinnamon product quickly and significantly reduced the S.E. level on the surface of the broiler live haul cage floors.

Keywords: Salmonella; Live Haul Cages; Cinnamon; Disinfection; Spray

320P Compatibility of a multi-strain probiotic and a live *Salmonella* vaccine in broiler chicken

Charles L. Hofacre*¹, Matthew K. Jones², Maria Terra-Long³, Alberto Conde-Aguilera³, Charlene Dazeur³, Agnes Mori³ ¹*Southern Poultry Research Center, Watkinsville, Georgia, United States*; ²*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ³*Phileo-Lesaffre, Milwaukee, Wisconsin, United States*

Salmonellosis remains a significant challenge in broiler live production. For broilers and breeders live vaccines are a common tool to reduce *Salmonella* on the farm. As some probiotics can modulate gut health and immunity, a probiotic feed additive may impact live vaccine colonization or efficacy. The effect of a probiotic containing *B. amyloquefaciens*, *B. licheniformis*, and *B. pumilus* was evaluated for (1) colonization of the live *Salmonella* vaccine (MeganVac1ä), and (2) the vaccine's ability to reduce *Salmonella* Enteritidis (SE) colonization in a controlled challenge model. Treatments were unvaccinated control (T1), vaccine only (T2), and vaccine + probiotic (T3; 0.25 lb/ton) with 3 replicate pens/treatment and 25 birds/pen. The vaccine (MeganVac1) was applied by coarse spray at day 0. Then SE (4.3×10^7 CFU/bird) was administered by gavage on day 8 to all birds. Vaccine recovery (ceca, liver/spleen) was assessed at day 3 and SE colonization (ceca prevalence and number (MPN/g), liver/spleen prevalence) on day 21. *Salmonella* culture enriched in tetrathionate broth overnight (42°C) and then struck onto XLT-4. Then micro MPN/g was assessed per Berghaus et al., 2013. *Salmonella* prevalences were compared between treatment groups using Fisher's exact test, $P < 0.05$. On day 3, vaccinated groups, with and without probiotic, had 100% liver/spleen vaccine recovery ($P < 0.001$) while ceca vaccine prevalence was 33%^{ab} for birds treated with the probiotic and 78%^b on the vaccine only group. At day 21, there were no significant differences between vaccinated treatments for SE prevalence in ceca ($P = 0.77$). The prevalence of SE from liver and spleen was significantly reduced in both vaccinated groups (11%^a for vaccine only, 6%^a for vaccine plus probiotic) compared to control (67%^b; $P < 0.001$). This reduction in both vaccinated groups suggests the probiotic did not affect the immunity development against SE. The probiotic plus vaccine group had numerically fewer positive samples, including two pens entirely negative. The inclusion of the multi-strain probiotic in broiler feed did not impair colonization of the live *Salmonella* vaccine (MeganVac1) and may limit SE translocation to internal organs, as indicated by the numerically lower liver/spleen prevalence in the probiotic group.

Keywords: *Salmonella*; broilers; probiotic; live vaccine; S.E.

321P Synergistic activities of a blend of essential oils and organic acid for in vitro control of *Salmonella* spp.

Clarisse Techer*¹ ¹*miXscience, Bruz, France*

Salmonella spp. remains a critical challenge in poultry production, threatening food safety and animal health. Effective control relies on integrated strategies combining biosecurity, farm management, health practices, and feed additives such as organic acids and essential oils, which have proven to be valuable complementary tools. This study assessed the efficacy and synergy of a blend of essential oils and organic acid against *Salmonella* using MIC determination and adhesion inhibition assays. Tested compounds included a phenolic nature-identical compounds mixture (EO), organic acid (OA), and their combination (Lumigard TCB, Mixscience, France). MICs were measured for seven poultry isolates (*S. typhimurium* [2 strains], *S. enteritidis*, *S. heidelberg*, *S. senftenberg*, *S. idikan*, and *S. typhimurium* variant). Adhesion assays were performed on HT-29 cells with *S. typhimurium* ATCC 14028 (poultry origin) under

competition and displacement models, with triplicate tests. Statistical analysis used two-tailed Student's t-tests ($p < 0.05$ significant; $p < 0.01$ highly significant). To assess synergy, MICs values of the combination were compared to a theoretical "no-interaction" prediction, calculated as the linear combination of the residual effects of EO and OA at their respective proportions in Lumigard TCB using Loewe additivity model. TCB consistently lowered MICs compared to single OA and EO, achieving 1000–2000 ppm versus OA (2000–4000 ppm) and near EO levels (125–250 ppm). Predicted additive MICs (1930–2970 ppm) exceeded observed TCB values, confirming synergy beyond simple additivity[SFI], notably in *S. typhimurium* and *S. senftenberg*. Adhesion was significantly reduced: residual adhesion with TCB was 7.2% in competition and 38.4% in displacement ($p < 0.01$). Compared to EO, TCB reduced adhesion by 33.9% ($p < 0.01$) in competition and 36.9% ($p = 0.06$) in displacement; versus OA, reductions were 72.8% and 39.8% ($p < 0.05$), respectively. In conclusion, Lumigard TCB demonstrates dual action, antibacterial and anti-adhesion against *Salmonella*, with clear synergistic effects between essential oils and organic acid. These findings support its application as a feed additive to mitigate *Salmonella* contamination in poultry production.

Keywords: *Salmonella*; synergy; essential oils; organic acid; Lumigard TCB

322P Evaluation of water acidification with sodium bisulfate as a *Salmonella* intervention for broilers prior to processing

Craig Coufal*¹, Dana Didde¹, Juan Suarez¹, Charles L. Hofacre², Matthew K. Jones², Chris Tate², Roy Berghaus³ ¹*Jones-Hamilton Co., Maumee, Ohio, United States*; ²*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ³*University of Georgia, Department of Population Health, Athens, Georgia, United States*

While drinking water acidification during feed withdrawal of broilers prior to processing is widely used in the poultry industry, little research has been published regarding the optimum water pH and duration of administration to achieve maximum *Salmonella* reduction. This study utilized 10 water treatments for broilers prior to harvest for processing: an untreated control (tap water, pH=7.6) and 9 treatment combinations of water at pH 3, 4 or 5 provided for the last 3, 5 or 7 d of grow-out. Sodium bisulfate (PWT®) was added to tap water in predetermined amounts to achieve each level of water pH. The study utilized 120 Ross 708 43-day old broilers that had served as the challenged control birds in a previous *Salmonella* challenge study. Birds were randomly assigned to pens with used litter (4 birds/pen; 3 pens/treatment), and the litter was sprayed with 100 mL of 10^8 CFU/mL *Salmonella enteritidis* culture. Birds were fed a common broiler finisher ration throughout. After 7 d (50 d of age), administration of acidified water began for the 7 d treatments, followed by the 5 d treatments on d 52 and the 3 d treatments on d 54. Water consumption was recorded daily. At 57 d of age, feed was withdrawn from all birds for approximately 12 h. Birds were then weighed, euthanized, and crop and ceca samples collected from 3 birds per pen to determine *Salmonella* prevalence and MPN/g. MPN values were log-transformed prior to statistical analysis, and culture-positive samples were compared using linear mixed models. Tobit regression models with pen as a random effect were also used to compare *Salmonella* MPNs while considering culture-negative samples censored at $-0.5 \log_{10}$ MPN/g. No statistical differences were found between the treatments for crop or ceca prevalence. However, the challenged control birds were 67 and 78% *Salmonella*-positive for the crop and ceca, respectively, whereas the pH 4 water for 3 d treatment had the numerically lowest prevalence of all the treatments at 44% positive for both the

crop and ceca. The pH 4 water for 3 d treatment was also numerically lower for MPN/g for both the crop and ceca compared to the control. This study demonstrated that water acidification with sodium bisulfate can reduce the *Salmonella* load of broilers prior processing.

Keywords: Salmonella; broilers; water acidification; sodium bisulfate; feed withdrawal

323P Direct detection of *Salmonella* from carcass rinsates using fiber-optic surface enhanced Raman spectroscopy (SERS) sensor in 20 minutes

Nivin Nasser*¹, Mahamoud Almasri², Amit Morey¹, Kate Trout², Haitao Li³, Azlin Mustapha², Hoa Hoang², Tatijana Fisher⁴, Kamran Bashir Taas², Amjad Hasan², Mai Abuhelwa², Mohammed Almalaysha², Md Asaduzzaman Chunn², Adheesha Bandara² ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²University of Missouri, Columbia, Missouri, United States; ³University of Missouri, St. Louis, Missouri, United States; ⁴Lincoln University of Missouri, Jefferson, Missouri, United States

Rapid and accurate detection of foodborne pathogens is critical for improving food safety in poultry processing. This study validates a fiber-optic based surface enhanced Raman spectroscopy (SERS) label-free sensor for detection of *Salmonella* directly from carcass rinsates with no enrichment and sample processing. A CRD was used to evaluate sensor performance across different *Salmonella* concentrations and serovars. Raw poultry rinsates were filtered (70 µm) and spiked with serial dilutions of several *Salmonella* serovars at concentrations ranging from 1 to 10⁸ CFU/mL. Each treatment was replicated three times. SERS spectra were recorded before and after sample loading, and background signals were subtracted to isolate *Salmonella* fingerprints. *Salmonella* cultures treated with peracetic acid and/or heated for 30 minutes were used to differentiate between live and dead *Salmonella* based on the variation in spectral signal. SERS spectra were processed by subtracting background signals from total intensity to isolate *Salmonella* fingerprints. Data are shown as mean relative Raman intensity (± SD) for characteristic peaks following standard SERS Raman protocol for peak normalization and baseline correction. Representative spectra will be shown for each concentration and serovar. Data was analyzed using ANOVA to compare mean SERS signal intensities across concentrations and serovars. Similarly, mean intensities were compared between live and dead *Salmonella* cells. Tukey's HSD test was applied for pairwise comparisons. Significance was declared at P < 0.05. Based on preliminary testing, we detected SERS spectral peaks corresponding to *Salmonella* molecular fingerprints at ~20 min without enrichment. Significant differences in Raman intensity were observed among concentration levels. For *Salmonella* Typhimurium, significant Raman peaks were detected at 510 ± 2 nm, 612 ± 12 nm, 821 ± 15 nm, and 1071 ± 21 nm, confirming molecular fingerprint specificity. Our findings show a significant potential to improve operating efficiency at poultry processing facilities by reducing the reliance on time-consuming conventional detection methods. Detecting *Salmonella*, before the poultry products are released into the market, provides huge potential for food safety applications.

Keywords: fiber optics; surface enhanced Raman spectroscopy (SERS); Salmonella; rapid detection; raw poultry

324P Comparative transcriptomic profiling of *Campylobacter coli* under aerobic and microaerobic conditions

Farazi Abinash Rahman*¹, Dianna Bourassa¹, Sabin Poudel¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States

Campylobacter coli (*C. coli*) is an important foodborne pathogen. Consumption of contaminated chicken meat is the major contributor to human *Campylobacteriosis*. *C. coli* is a microaerophilic but recent studies shows its increasing ability to adapt in aerobic conditions. Therefore, the aim of this study was to compare the transcriptional adaptation of *C. coli* under aerobic and microaerobic conditions grown in BHI broth. Samples were collected after 24h of incubation either on aerobic or microaerobic condition and total RNA were extracted, quality-checked, rRNA-depleted, and sequenced on the Illumina NovaSeq platform. Reads were aligned to the reference genome using HISAT2. Gene counts were generated with HTSeq. Differential expression was analyzed using DESeq2 using |log₂FC| ≥ 1 and p < 0.05. COG functional categorization (eggNOG) was performed using an e-value cutoff of 0.001, seed ortholog score ≥ 80, and ≥ 60% query/subject coverage to determine functional distributions of differentially expressed genes. Among 1896 genes analyzed, 944 were significantly regulated (p < 0.05), with 479 upregulated and 465 downregulated in aerobic conditions. Aerobic exposure upregulated key stress-response genes (*clpB*, *dnaK*, *groEL*, *dnaJ*, *grpE*) and *radA* that are involved in heat-shock chaperone activity, DNA-repair pathways and protection from oxygen-induced protein damage. In contrast, microaerobic conditions upregulated energy-metabolism genes (*aspA*, *sucA*, *aceE*, *fdhA*, *frdA*), enhancing TCA cycle activity and supporting metabolic adaptation to low-oxygen environments. COG analysis showed aerobic conditions activated chaperone and protein-repair, while microaerobic conditions mainly enhanced metabolic functions, ion transport, and membrane biogenesis. Aerobic conditions upregulated motility- and chemotaxis-related genes (*fliS*, *fliY*, *fliF*, *flgG*, *fliQ*, *cheY*, *motA*), while microaerobic conditions showed higher expression of flagellar assembly genes (*fliP*, *flgH*, *fliL*, *flgL*). These findings show that *C. coli* adapts metabolically and structurally when shifting from microaerobic to aerobic conditions, revealing survival mechanisms that can inform strategies to reduce carcass contamination and control campylobacteriosis in poultry production.

Keywords: Transcriptomics; Microaerobic; Aerobic; Campylobacteriosis

325P In-depth genomic characterization of *Campylobacter* Spp. isolated from retail poultry meat products Georgia, USA

Nivin Nasser¹, Jouman W. Hassan*¹, Issmat Kassem¹ ¹University of Georgia, Center for Food Safety, Griffin, Georgia, United States

Campylobacter is a leading cause of foodborne gastroenteritis and is highly associated with the consumption of poultry products. Here, we aimed to determine the prevalence, antimicrobial resistance, and virulence of *Campylobacter* isolated from chicken breasts in Georgia, USA. Chicken breast samples (n=122) were collected from two major locations in Georgia. *Campylobacter* isolation was performed according to ISO-10272-2017 with modifications, and enriched samples were inoculated onto *Campylobacter* Blood-Free Selective Agar (mCCDA) with selective supplement. The plates were incubated under microaerobic conditions for 48 h at 41°C. Confirmation of identity and speciation were performed by multiplex PCR analysis. The isolates were also screened for the presence of eight pathogenic genes: *cdtA*, *cdtB*, *cdtC*, *cadF*, *flaA*, *virB*, *wlaN*, and *tam*. Whole-genome sequencing was performed to identify antibiotic resistance genes, clonal complexes, and sequence types (STs). Antibiotic resistance phenotypes were determined using the broth microdilution method. The prevalence of *Campylobacter* was 40.2% (49/122). *Campylobacter jejuni* was the predominant species (p < 0.05), accounting for 85.7% of the isolates. The most prevalent *C. jejuni* CC was ST-353 CC (52.5%), followed by ST-

48 CC (12.5%), while most *C. coli* isolates (77.8%) belonged to the ST-828 CC. *cdtABC* and *cadF* were detected in all *C. jejuni*, while *flaA* and *iam* were found in 85.7% and 34.7% of the isolates, respectively. Analysis using ResFinder v.4.4.2 database showed that 51% of the isolates carried β -lactamase encoding genes with *bla_{OXA-61}* being the most prevalent ($p < 0.05$), while 20.4% carried the *tet(O)* gene and 8.2% had mutations in *gyrA*. Phenotypically, the highest resistance was observed against ampicillin (63.3%), followed by resistance to tetracycline (20.4%), nalidixic acid (18.4%), and ciprofloxacin (14.3%). Interestingly, some of the strains showed potential tolerance to 400 ppm of peracetic acid and chlorine, respectively. Our findings suggest that current processing interventions might need to be bolstered to eliminate problematic and processing-tolerant *Campylobacter* strains on chicken meat.

Keywords: Campylobacter; Poultry; Antibiotic resistance; Whole genome sequencing; Virulence

326P Case Study: Natural antioxidant and antimicrobial interventions improve quality and shelf life of fresh chicken meat

Poholsky Courtney*¹, Nicholas Howes¹ ¹*Camlin Fine Sciences, Jeannette, Pennsylvania, United States*

Fresh poultry meat is highly susceptible to quality deterioration due to microbial activity and oxidative processes that begin immediately after harvest. Spoilage bacteria such as *Pseudomonas* spp. are largely responsible for off-odors, discoloration, and loss of freshness. In parallel, lipid and pigment oxidation contributes to

declines in color, flavor, and overall sensory quality. These spoilage mechanisms contribute substantially to retail and consumer waste of fresh products. Natural antioxidants and antimicrobials offer potential strategies to mitigate deterioration pathways. This case study aimed to evaluate whether a natural antioxidant and a natural antimicrobial could extend the shelf life of fresh chicken products. Two product types, marinated blacked chicken legs and umami-flavored chicken tenders, were examined. A box of 40 pounds of meat served as the experimental unit. For each product type, one box was untreated (negative control), and one box was treated with 0.2% rosemary extract-based liquid antioxidant (NaSure 06) plus 1.2% liquid vinegar. Samples were stored at 4°C for a duration of 25 days, during which individually packaged samples were analyzed for hexanal concentration and aerobic plate counts. All analytical measurements were performed in duplicate, and results are reported as descriptive averages. Lipid oxidation results demonstrated that the combination of NaSure 06 and vinegar improved oxidative stability, with lower hexanal values observed in treated blackened chicken legs between days 16 and 22. Similar reductions in lipid oxidation were observed for the treated umami chicken tenders. Microbial results showed that shelf life, defined by an aerobic plate count threshold of log 7 CFU/g, was extended by 8 days for blacked chicken legs and by 19 days for the umami chicken tenders when treated with NaSure 06 and vinegar. In conclusion, the combined use of rosemary extract and vinegar helped reduce oxidative degradation and microbial growth, thereby enhancing shelf life of fresh chicken products and supporting strategies to reduce grocery store waste.

Keywords: antioxidant; antimicrobial; chicken meat; shelf life

Machine/Deep/AI Learning & Modeling

327P Novel individual approach to infectious laryngotracheitis virus vaccination

John Ngunjiri*¹, Mark Sokolowski¹, James Titus McQuillan¹, Meagan Maxwell¹, Jenna Siwan¹, Andrew Smith¹ ¹*Targan, Bio Process, Raleigh, North Carolina, United States*

Background: Infectious laryngotracheitis (ILT) remains endemic in poultry, requiring new mitigation strategies. Use of highly immunogenic chick embryo origin (CEO) live vaccines for day of hatch (DOH) vaccination often leads to notable post-vaccination reactions due to imprecise dosing from mass application. TARGAN has developed an AI vision-based high-throughput individualized vaccination system (IVS) to deliver precise ocular doses to each chick. This study aimed to identify a CEO ILT vaccine dose for delivery via TARGAN's IVS to achieve high vaccine take/replication with minimal clinical signs. Methods: Two experiments were conducted to assess different doses of CEO ILT vaccine. Experiment 1 tested 1X (n = 30), 0.5X (n=30), and 0.25X (n=30), 0X (n=30) doses; Experiment 2 tested 0.25X (n = 45), 0.125X (n = 45), 0.0625X (n=30), 0.03125X (n=30), 0.01X (n=30), 0X (n=30) doses. Oropharyngeal (OP) swabs were collected at 4 days-post-vaccination (dpv) and tracheal (TR) swabs at 7 dpv. Vaccine take/replication was measured by qPCR. At different time points, body weight (BW) measurements were taken and clinical scores (CS) recorded. ANOVA and correlations by Pearson R were used for statistical analysis. Results: CS peaked at 6 dpv. CS prevalence increased with doses $\leq 0.125X$, peaked at 0.125X, then consistently remained 80% – 97% at higher doses. Mean CS at 6 dpv increased with dose from 0.23 at 0.01X to 1.90 [JT1] at 1X. At 0.01X, qPCR positivity was 50% in OP at 4 dpv and 90% in TR at 7 dpv; all higher doses were 100% for both time points. OP and TR virus outputs for 0.01X differed from other doses ($p < 0.05$). Mean Cq value at 0.01X was 35.73 in OP [JT2] swabs and 32.44 in TR

swabs [JT3], compared with <27 and <26 , respectively, at higher doses. Average BW at 0.01X matched unvaccinated chicks at 4-7 dpv, while other groups lost up to 20% BW by 7 dpv. Mean BW and CS were strongly negatively correlated ($r, -0.57$) at 6 dpv. Conclusions: TARGAN's IVS enables precise ocular CEO ILT vaccination at DOH. Low doses near 0.01X maintained high vaccine take, with minimal induction of CS and BW suppression. These findings show individualized high-throughput systems like TARGAN's IVS can allow early ILT vaccination. Challenge studies will confirm protection at reduced doses.

Keywords: Vaccination; Infectious laryngotracheitis; Chicken embryo origin

328P Developing a disease model to understand the effects of Newcastle Disease vaccination in gamefowl populations in southern California

Maurice Pitesky¹, Alec Michael*¹, Joseph Gendreau¹, Myrna Cadena¹ ¹*University of California, Davis, Poultry Health and Reproduction, Davis, California, United States*

Outbreaks of the virulent strains of Newcastle Disease (vND) pose a continuous threat to the poultry industry as well as non-commercial poultry owners. Over the last 50 years, 3 major outbreaks of vND in Southern California resulted in the loss of millions of birds and hundreds of millions of dollars of economic damage. Understanding various interventions via the creation of a modified Susceptible-Exposed-Infected-Recovered (SEIR) based spatial disease model would allow the investigation of potential effects of several outbreak mitigation strategies, including vaccinations of specific populations of poultry. Here, we present the results of a modified SEIR based vND disease model designed to investigate the effect of vaccination against ND in game fowl with respect to the length of the outbreak and total infections in

non-commercial and commercial flocks in Southern California. The model included various spatio-temporal and vND based variables including farm locations, contact rate, probability of transmission, attempted vaccination rates, improper vaccination rates, and depopulation rates. To understand the effect of ND vaccination in gamefowl populations, 50 iterations of an outbreak were run under 11 different gamefowl vaccination scenarios ranging from 0-100%. Results showed a significant increase in both the length of the outbreak and the number of premises infected at the highest vaccination rates versus the lower vaccination rates. These results suggest that the best way to mitigate an outbreak of vND in the complex environment of Southern California with high spatial proximity of commercial and non-commercial (e.g. backyard poultry and gamefowl) poultry is good biosecurity, ND vaccination in commercial birds and no vaccination in GF. Specifically, because gamefowl are highly likely to attend various events and hence interact with otherwise healthy birds, the potential for asymptomatic spread of infected birds that are vaccinated and infected is a significant contributor to disease transmission.

Keywords: Newcastle Disease; Poultry; Food security; Disease Modeling; SEIR (Susceptible-Exposed-Infected-Recovered)

329P Evaluation of a mechanistic broiler growth model

Matheus Reis*², Ana Isabel Garcia-Ruiz³, Neil Jaworski¹, Neil Ferguson¹ ¹*Trouw Nutrition, GSS, Toledo, Toledo, Spain;* ²*Trouw Nutrition, GSS, Amersfoort, Netherlands;* ³*Trouw Nutrition, R&D, El Viso de San Juan, Toledo, Spain*

A mechanistic broiler growth model was evaluated to determine its predictive performance for body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). A database was compiled from eight controlled research trials and three published studies, representing two commercial strains (75% Ross 308; 25% Cobb 500) and yielding 199 phase level pairs of

observed and model predicted outcomes. Each study was simulated using the same diet formulations, ingredient profiles, and rearing conditions described in the original experiments, and predictions were compared to observations by phase. Adjacent diets within each study were paired, and differences in performance (Δ Observed and Δ Predicted) were calculated to assess whether the model correctly reproduced the direction and magnitude of responses to changes in standardized ileal digestible (SID) lysine and metabolizable energy (ME). Precision was quantified by the coefficient of determination (R²) from simple linear regression of observed on predicted values, and accuracy was summarized by the mean absolute percentage error (MAPE). To establish a reference threshold for accuracy, baseline biological variation was estimated from the same database. Across the full dataset, the model closely matched observed performance: BW MAPE 5.39% with R²=0.993; BWG 6.55% with R²=0.988; FI 10.0% with R²=0.990; and FCR 7.01% with R²=0.909. Delta analyses confirmed that the model reproduced both direction and magnitude of responses to diet changes. For SID Lys (n=126), MAPE and R² were: BW 4.17%, 0.99; BWG 8.02%, 0.96; FI 10.44%, 0.98; FCR 6.94%, 0.90. For ME (n=81), results were: BW 4.4%, R²=0.999; BWG 5.58%, 0.997; FI 9.06%, 0.991; FCR 5.9%, 0.954. As a benchmark, the natural between trial variation under standardized conditions averaged MAPE \approx 10.6% for BW, 9.56% for BWG, 10.8% for FI, and 15.4% for FCR, indicating that the reported model errors were within typical biological variability. Overall, the model reproduced growth performance and nutrient response patterns with high precision and satisfactory accuracy across diverse datasets. These findings support its application for comparing feeding strategies, projecting performance, and conducting “what if” nutrition scenarios in broiler production.

Keywords: Performance; Estimation; Prediction; Simulation; Feed intake

Metabolism and Nutrition: Amino Acids

330P Development of the indicator amino acid oxidation technique to assess amino acid utilization in broiler breeders

Diego Martinez¹, Carol Ponce-de-Leon², Cole Umberson¹, Lindsey Conaway³, Erik Pollock³, Oscar H. Aguilar¹, Craig N. Coon*¹ ¹*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States;* ²*Independent Researcher, Fayetteville, Arkansas, United States;* ³*University of Arkansas, Stable Isotope Laboratory, Fayetteville, Arkansas, United States*

This study developed an equipment setup and feeding procedure for administering an isotope to broiler breeders under the conditions (baseline, plateau; no under-/over-priming) required to determine oxidation-driven ¹³C enrichment rates from a labeled amino acid for the Indicator Amino Acid Oxidation (IAAO) technique. Twelve female broiler breeders of a new genetic line (CDP5) were placed in respiratory chambers equipped with a system built up for periodic breath sampling. Birds were adapted to a nutritionally balanced control mash diet for 2 days before the assay. A lysine-deficient diet (0.23% dig. LYS) was then used for 1 day, with three approaches tested (A, B, C; n = 4 each). Daily feed allocation was split into meals offered every 30 min. Approaches A and B had 13 meals (meal 5 = 11 g; others = 10 g), whereas C used 18 meals (meal 1 = 9 g; meal 5 = 10 g; others = 7 g). The first four meals were isotope-free. Subsequent meals contained L-[1-¹³C]phenylalanine (¹³C-PHE) replacing cellulose: priming meal 5 (A and C = 4.5 mg/kg BW; B = 3.5 mg/kg BW), meal 6 (A and C = isotope-free; B = 1 mg/kg BW), and remaining meals (A, B, C = 1 mg/kg BW per meal). Gas samples were collected every 30 min (18 intervals), starting right before meal

2. ¹³CO₂ enrichment was analyzed by isotope-ratio mass spectrometry (IRMS), converted to atom-percent excess, and used to calculate the rate of ¹³CO₂ release and $\delta^{13}\text{C}$ (deviation of sample ¹³C/¹²C from the reference standard). The $\delta^{13}\text{C}$ enrichment curve was obtained, and a regression was fitted between the post-priming $\delta^{13}\text{C}$ peak and the steady-state plateau. The approach with the slope closest to zero indicated optimal stabilization. Using JMP Pro 16, the slopes for A and C did not differ (P>0.05) and were both closer to zero than B (P<0.05), indicating that A and C reached steady state earlier. Their equivalent $\delta^{13}\text{C}$ plateau values indicated that \leq 13 meals were sufficient to construct a reliable $\delta^{13}\text{C}$ enrichment curve. Moreover, feeding the full daily allocation during those meals was unnecessary, as the remainder could follow afterward. In conclusion, an effective breeder-housing system and robust feeding protocol were established to generate accurate $\delta^{13}\text{C}$ enrichment curves for studying amino acid utilization in broiler breeders.

Keywords: Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; Isotope; Nutritional requirements

331P Modeling isotopic enrichment dynamics in the indicator amino acid oxidation technique for broiler breeders

Diego Martinez*¹, Carol Ponce-de-Leon², Oscar H. Aguilar¹, Erik Pollock³, Lindsey Conaway³, Craig N. Coon¹ ¹*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States;* ²*Independent Researcher, Fayetteville, Arkansas,*

United States; ³University of Arkansas, Stable Isotope Laboratory, Fayetteville, Arkansas, United States

This study modeled the $\delta^{13}\text{C}$ enrichment curve in broiler breeders fed a labeled amino acid as part of developing a simplified Indicator Amino Acid Oxidation (IAAO) procedure to determine mean $\delta^{13}\text{C}$ values at baseline and plateau phases. Twelve female breeders of a new genetic line (CDP5) were housed in respiratory chambers equipped with a system built up for periodic breath sampling. Birds were adapted to a balanced control diet for 2 days before the assay. A double Latin square design with 6 dietary treatments (0.23, 0.33, 0.42, 0.89, 0.99, and 1.09% dig. LYS) and 12 hens was used. Each hen received 1 treatment per day, completing all diets over 6 days. Fifty percent of the daily feed was divided into thirteen 5-g meals offered every 30 min; the remainder was fed after the IAAO procedure. Meals 1–4 and 6 were isotope-free, meal 5 provided 4.5 mg of L-[1- ^{13}C]phenylalanine (^{13}C -PHE) per kg BW (priming dose), and meals 7–13 contained 1 mg/kg BW each. Gas samples were collected every 30 min (13 per hen per day), starting before meal 2, and analyzed by isotope-ratio mass spectrometry (IRMS; 936 samples). Seventy-two $\delta^{13}\text{C}$ enrichment curves (one per hen per day) were obtained. Gompertz, logistic,

piecewise, and non-linear multiphasic models were evaluated to represent baseline, rising, declining, and plateau phases. Data were fitted in JMP Pro 16 using training and validation subsets. The Gompertz and logistic models failed to capture over- or under-priming effects. The multiphasic logistic-difference model achieved the lowest AICc and BIC and the highest adjusted R^2 (>0.86), with lower RMSE and absolute percentage error ($<0\%$) than simpler models. It accurately captured the transient post-priming peak and steady-state plateau, with the smallest drop in R^2 ($<15\%$) between training and validation datasets. The piecewise model generated abrupt transitions, limiting interpretability and predictive stability. Cross-validation confirmed the superior performance of the multiphasic form. Testing alternative sampling schemes showed that three baseline and three plateau samples provided equivalent precision ($P>0.05$) to four-sample sets, while two per phase reduced accuracy ($P<0.05$). In conclusion, amino acid utilization can be reliably assessed using 6 samples rather than 13.

Keywords: Indicator Amino Acid Oxidation technique; Breeders; Amino acid requirements; Modeling; Isotope

Metabolism and Nutrition: Enzymes

332P Evaluation of multi-carbohydrase enzyme complex and single-activity carbohydrase enzyme products on performance, blood glucose, and short-chain fatty acids of broiler chickens

Hector Leyva-Jimenez¹, Carlos Soto*¹, Katherine A. McCormick¹, Brian Dirks¹ ¹United Animal Health, Sheridan, Indiana, United States

The objective of the present study was to compare the efficacy of a multi-carbohydrase enzyme complex (MCE) and a single-activity carbohydrase enzyme (SCE) on performance, blood glucose, and short-chain fatty acids (SCFA) of broiler chickens fed energy-reduced diets. Commercially available MCE (xylanase, cellulase, beta-glucanase, mannanase, and alpha-galactosidase) or SCE (xylanase) products were used. The study consisted of a complete randomized block design of 4 treatments: positive control (PC), negative control (NC; -132 kcal/kg of ME), NC + MCE at 125 ppm (ENZ1), and NC + SCE at 20 ppm (ENZ2). Each treatment included 13 replicate cages with 8 male broilers allocated to battery cages inside an environmentally controlled room. The feeding program consisted of 2 dietary phases (starter 0-14 d; grower 14-29 d). Phase and cumulative performance were evaluated. On days 14, 21, and 28, blood glucose was evaluated on 3 birds per cage using a drop of blood and a glucose meter. On day 29, 1 bird per cage was selected to collect the contents of the ceca and immediately frozen until SCFA analysis. Data were subjected to ANOVA ($P<0.05$), and means were compared using the Student's t-test of JMP 17.1 software. Trends were declared when $0.05 \leq P \leq 0.10$. Cumulatively (0-28 d), the reduction of 132 kcal/kg numerically reduced ($P=0.154$) BW by 2.96% and trended to increase ($P=0.075$) FCR by 3.8%. SCE and MCE supplementation recovered 7.09% and 20.60% (BW), and 37.10% and 58.15% (FCR), respectively. On day 14, blood glucose trended ($P=0.085$) to be higher in ENZ1 and ENZ2 compared to NC. On day 21, ENZ2 had higher ($P=0.013$) glucose (219.05 mg/dL) compared to the NC (206.59 mg/dL), while the PC (213.51 mg/dL) and ENZ1 (213.64 mg/dL) were intermediate. No significant glucose effects were observed on day 28. Numerically, the total SCFA profile was higher for both ENZ1 and ENZ2 compared to the NC, and the acetate to propionate ratio was lower for the same treatments. In conclusion, the dietary energy reduction negatively affected performance; MCE was more effective in maintaining performance compared to SCE. Further research is required to

better understand glucose dynamics and SCFA to supplemental exogenous enzymes.

Keywords: exogenous enzyme; performance; broiler; glucose; xylanase

333P Impacts of protease sources on growth and carcass response, gut health, nutrient digestibility, and cecal microbiota profiles in broilers fed poultry-by-product-meal-based diets

Muhammad Shahbaz Zafar*^{1,2} ¹University of Sargodha, Interdisciplinary Research for One Health, Sargodha, Pakistan; ²University of Veterinary & Animal Sciences Lahore, Animal Nutrition, Lahore, Punjab, Pakistan

Background: The current study aimed to evaluate the effects of the supplementation of protease sources on growth and carcass response, gut health, nutrient digestibility, and cecal microbiota profiles in broilers fed poultry-by-product-meal (PBM)-containing diets. **Methods:** In total, 800 one-day-old mixed-sex broilers (Arbor Acres) were weighed and allocated to one of the four dietary treatments in a completely randomized design, with eight replicates and 25 birds each per replicate. The treatments were as follows: (1) T0, control diet (without protease supplementation and 3% PBM); (2) T1, control diet supplemented with acidic protease at 100 g/ton (50,000 U/g); (3) T2, control diet supplemented with alkaline protease at 200 g/ton (25,000 U/g); (4) T3, control diet supplemented with neutral protease at 200 g/ton (25,000 U/g). **Results:** Protease supplementation enhanced ($p < 0.05$) body weight gain and the feed conversion ratio, predominantly in broilers fed PBM-based diets containing alkaline protease. Alkaline protease supplementation increased ($p < 0.05$) the apparent ileal digestibility of proteins (AIDP) by 4.3% and the apparent ileal digestibility of amino acids (AIDAA) by up to 5.8%, except for ornithine. Increments ($p < 0.05$) in carcass, breast, and leg quarter yields due to protease supplementation were evident, particularly in broilers fed diets containing alkaline protease. Alkaline protease improved ($p < 0.05$) the duodenal villus height (VH), reduced the crypt depth (CD), and increased the villus height to crypt depth ratio (VCR). Alkaline protease supplementation reduced ($p < 0.05$) cecal counts of Salmonella, Escherichia coli, and Clostridium in the broilers, whereas it increased ($p < 0.05$) the Lactobacillus counts. **Conclusions:** the supplemented alkaline

protease resulted in improved growth performance and carcass traits, better gut health, as well as improved ileal digestibility of nutrients, including crude protein (CP) and acid insoluble ash (AIA), with a more balanced cecal microbial composition in broilers.

Metabolism and Nutrition: Feed Additives

334P Effects of increasing levels of *Acacia mearnsii* tannins on growth performance and intestinal morphometrics of broiler chickens challenged with *Salmonella Heidelberg*

Danielle D. Brutti¹, Everton L. Albigo², Guilherme L. Godoy², Catarina Stefanello*² ¹SETA S.A., Estancia Velha, RS, Brazil; ²Federal University of Santa Maria, Santa Maria, RS, Brazil

Tannins are polyphenolic compounds recognized for their antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities, and have been explored to enhance growth performance, gut health, and meat quality in broiler chickens. This study aimed to evaluate the effects of increasing levels of supplemental tannins on growth performance, intestinal morphology, litter moisture, and cecal *Salmonella* occurrence in broiler chickens up to 42 days of age. A total of 1,400 Cobb 500 male chicks were allocated to 40 floor pens, distributed in a completely randomized design, and fed five dietary treatments, each with eight replicates (35 birds per pen). Treatments consisted of *Salmonella Heidelberg*-challenged groups receiving 0, 300, 500, 700, or 900 mg/kg of *A. mearnsii* with a 73.5 g/kg minimum guarantee of condensed tannins. Birds were fed a four-phase program comprising pre-starter, starter, grower, and finisher diets. At 3 days of age, all birds were orally inoculated with a field strain of *S. Heidelberg* (1.0×10^6 CFU/mL). Feed intake, body weight gain (BWG), and feed conversion ratio (FCR) were determined through day 42. Morphometric evaluations of the duodenum, jejunum, and ileum were conducted at 7 and 42 days. The effect of dietary treatments on response variables was assessed using linear and quadratic regressions of SAS ($P \leq 0.05$). Contrasts were used to compare the occurrence of *S. Heidelberg* between the non-supplemented control and the tannin-supplemented diets. Mortality and litter moisture were not affected by either the challenge or dietary tannin supplementation throughout the study. From days 1 to 28, 1 to 35, and 1 to 42, tannin supplementation resulted in quadratic improvements in BWG, with estimated optimal inclusion levels of 265, 412, and 456 mg/kg, respectively ($P < 0.05$). Tannins had no significant effect on FCR across all periods. Ileal villus height increased with 600 mg/kg tannins ($P = 0.0100$). At 42 days, the occurrence of *S. Heidelberg* reached 88% in the non-supplemented control, decreasing to 38% with 300 mg/kg tannins and to 13% with 500 mg/kg. In conclusion, *Acacia mearnsii* tannins improved body weight gain and intestinal morphology of broilers exposed to an *S. Heidelberg* challenge.

Keywords: broiler; feed efficiency; litter moisture; phytogenic additive

335P Mineralisation and bone resistance: the importance of organic selenium for the prevention of bone pathology in poultry

Monika Leukert¹, Stéphane Poulain², Romain Bouvet³, Christophe Alleno³, Hannah Elliott¹, Florence Barbe¹, Pierre Lebreton¹, Audrey Sacy*¹ ¹Lallemand SAS, Animal Nutrition, Blagnac (31), Midi Pyrénées, France; ²Aveyron Labo, Rodez, France; ³Zootests, Ploufragan, France

Bone health is a major concern in poultry production, with conditions such as osteoporosis in laying hens and locomotor disorders in broilers leading to fractures, lameness, and welfare

Keywords: protease; growth performance; gut health; amino acids digestibility; broiler

issues. Selenium (Se), widely recognized for its antioxidant and immune functions, has recently been linked to bone health in humans. The objective was to evaluate the impact of different selenium sources on bone mineralization and mechanical strength in broilers. A benchmark study compared tibial selenium (Se) deposition in broilers supplemented with sodium Se (SS), three commercially available Se-enriched yeasts (SY1, SY2 and SY3 (Alkosel)), Se proteinate (SePro), and three synthetic organic selenium products (Zn-SM, SM1 and SM2) equally incorporated at 0.2ppm Se. 104 ROSS 308 day-old chicks were raised on floor-pens (13 chicks/pen) receiving dedicated feed per pen. Se concentration in the feed was also analyzed to calculate transfer rate into bone tissues. Results demonstrate better enrichment of the bone matrix with Se from SY3 compared to any other Se sources at D14. In this study, SY3 is the only organic Se source able to significantly enrich Se in the bones of broilers, with some organic Se sources resulting in lower tibial Se concentration than SS. The transfer rate of Se in the tibia (calculated as the quantity of Se in the tibia divided by the Se intake) following supplementation with different sources of Se was improved for SY3 (+77%), SY1 (+28%) and SM1 (+47%), equivalent to sodium selenite for Zn-SM (+3%) and SM2 (+1%) and even reduced compared with sodium selenite for SY2 (-56%) and Se Pro (-29%). Additionally, broiler tibia of SS and SY3 were analyzed for breaking strength resistance parameters. Data shows significant improvement for SY3 birds: +4%, +5%, +6% at D14 and +24%, +14%, +20% at D32 for rupture force, maximum force and stiffness, respectively. Supplementation with SY3 resulted in superior selenium deposition in bone tissue and significantly improved tibial mechanical strength compared to other sources. These findings highlight the potential of high-quality organic selenium to support skeletal development and bone robustness in broilers, while emphasizing the importance of source selection to optimize benefits in modern poultry production.

Keywords: Selenium yeast; bone health; transfer rate

336P Dose response of a new *Bacillus* strain from marine origin on broiler performance and gut health

Jose Arce Menocal², Pierre Lebreton*¹, Eric Chevaux¹ ¹Lallemand SAS, Animal Nutrition, Blagnac (31), France, France; ²Indepesa, Michoacán, Mexico

The trial evaluated the dose-effects of a *Bacillus pumilus* (NCIMB 30362) strain of marine origin supplementation on broiler health and performance. Birds were housed in floor-pens of 35 chicks with 14 pens per treatment group: Control (0) vs 3×10^8 vs 5×10^8 vs 8×10^8 cfu/kg feed. At day 42, broilers fed Probiotic doses 2 (5×10^8 CFU/kg) and 3 (8×10^8 cfu/kg) showed significantly higher body weights than the control group. Feed conversion ratio (FCR) improved notably with Probiotic dose 3, while feed and water intake remained similar across treatments. Mortality rates were unaffected by probiotic inclusion. Spleen relative weight was heavier for Probiotic dose 3 while no difference was depicted for the Bursa of Fabricius and the thymus. Litter quality was generally good, with Probiotic dose 3 achieving the best score at day 41. Litter dry matter was slightly higher in probiotic groups, especially dose 3. Feces dry matter was significantly higher in Probiotic dose 1 at day 20, indicating better gut function, while a tendency for dryer feces was reported in probiotic dose 3 at the end of the study.

Intestinal morphology revealed enhanced villus height, crypt depth, and absorption area in the ileum for Probiotic doses 2 and 3, suggesting improved nutrient absorption. These findings suggest that the *B. pumilus* NCIM 30362 probiotic strain can enhance intestinal health and overall performance in broilers.

Keywords: Probiotic; *Bacillus pumilus*; broiler; dose response

337P Investigation of gut health and feed efficiency with a new *Bacillus* probiotic strain from marine origin

Pauline Belloir², Charlotte Pais², Eric Chevaux¹, Anais Nozeran¹, Pierre Lebreton¹, Audrey Sacy*¹ ¹Lallemand SAS, Animal Nutrition, Blagnac (31), Midi Pyrénées, France; ²EIP Purpan, Purpan, France

In broiler production, *Bacillus*-based probiotics are increasingly recognized for their ability to enhance protein digestion and gut health. Through the secretion of proteolytic enzymes, these beneficial bacteria improve nutrient absorption, reduce nitrogen excretion, and help mitigate digestive disorders such as necrotic enteritis, ultimately contributing to better performance and sustainability. Each probiotic strain possesses a unique mode of action and specific enzymatic features, making comparative evaluation essential to identify the most effective solution for enhancing nutrient utilization and gut health in broiler production. This study aimed to evaluate the impact of a marine-derived *Bacillus pumilus* NCIMB 30362 (EffiXience) on nitrogen retention and overall performance in broiler chickens. The trial involved 216 Ross 308 chicks over 35 days, divided into two groups: one receiving *Bacillus subtilis* at 9.10^8 CFU/kg of complete feed (BS) and the other *Bacillus pumilus* at 5.10^8 CFU/kg of complete feed (BP) in a 3-phase feeding program (Starter, Grower, Finisher). Nitrogen balance was assessed between days 25 and 27 on 6 cages per group, using indicators such as nitrogen intake, retention, excretion, and fecal nitrogen. Cloaca cleanliness was evaluated at day 10 as an indirect parameter of gut health. Results shown that BP supplementation led to +5.4% higher final body weight (D35) compared to BS. BS improved both economic FCR reduced by 15 pts and technical FCR by 11 pts ($p < 0.05$). Mortality was also reduced with BS (-7.64 pts). Finally with a fecal nitrogen reduction reduced by 5.36pt, (BS: 34.57% vs BP 29.21%), nitrogen efficiency was significantly increased by 1.1%. Interestingly, cloaca cleanliness score shown with BP higher score 1 (clean cloaca: 88.8% vs 81.3%) and reduced score 3 (dirty cloaca: 7.1% vs 8.8%). No significant differences in individual feed intake were observed. Improved protein digestion and retention contributed to reduced nitrogen waste and enhanced gut health. In conclusion, the new probiotic BS significantly improves nitrogen utilization and growth performance in broilers, supporting sustainable poultry production by reducing feed costs, nitrogen emissions, and mortality rates.

Keywords: Gut Health; Feed Efficiency; *Bacillus*; probiotic; nitrogen efficiency

338P Effects of Quillaja Extracts on Growth Performance and Immune Response in Broilers During Disease Challenges

Sebastian P. Delbene¹, Diego Gonzalez*¹, Fernando Kuhlmann¹, Rosa Navarro¹, In Ho Kim², Hans Konsens¹ ¹Plantae Labs SpA, Santiago, Chile; ²Dankook University, Animal Biotechnology, Cheonan, Choongnam, Korea (the Republic of

Coccidiosis is one of the most important diseases in poultry, and accounts for several losses. Drug resistance microorganisms and antibiotic residues, has increased the interest in phytochemicals, like Quillaja saponaria (QS), a tree rich in triterpene saponins, able to decrease microorganisms load and improve growth performance. In this work, QS extracts were supplemented to broiler chickens

(Ross 308) challenged with *Eimeria* spp. and Dextran Sulfate Sodium (DSS), to evaluate its effects on growth performance, intestinal integrity, immune modulation, and oxidative balance. In vivo study was conducted in Dankook University, with 360 broilers (9 replicates) in a 42-day trial, distributed into 4 treatments, challenged control with vaccine + DSS (T1), negative control without vaccine and DSS (T2) and diets supplemented with QS at two levels, 30 g/T and 60 g/T; T3 and T4. On day 14, coccidiosis vaccine was administered via oral gavage, at 10x higher than standard dose to all broiler chickens, except T2. DSS challenge was administered between days 22-28 and growth and immune parameters were measured along the trial. All data was processed by Duncan's multiple range test using the General Linear Model procedure of SAS to test the significance between the means ($p < 0.05$). In overall growth performance, T4 achieved results comparable to the unchallenged group ($p > 0.05$), outperforming T1 ($p < 0.05$) in BWG and FCR. Regarding intestinal lesions, T3 and T4 displayed significantly reduced lesion scores in duodenum, jejunum and ileum when compared to T1, even equaling the T2, suggesting a protective effect against different *Eimeria* species damage. Flow cytometry analysis showed that T4 increased lymphocytes compared to T2 ($p < 0.05$), as well as the CD4/CD8 ratio, surpassing T1 and reflecting improved immune function. Oocyst counts in feces were also significantly lower in T4 compared with T1. In blood antioxidant profile, T2 and T4 presented higher levels of SOD, GPx, and CAT, together with reduced levels of MDA, compared to T1 ($p < 0.05$). In conclusion, QS at 60 g/T, improved growth performance, intestinal health, immune function, and oxidative balance in broiler chickens under *Eimeria* spp challenge, highlighting its potential as a natural alternative for coccidiosis management.

Keywords: Saponins; Gut health; Immunity; Antioxidant

339P Efficacy of a liquid solution based on grape and olive extracts: evaluation of oxidative stress and antioxidant activity against *Salmonella* spp. and *E. coli* in an *in vitro* intestinal model (Caco-2)

Ana M. Delgado Valencia¹, Eva León¹, Raquel Codina¹, Oscar Castro¹, Annabel Prats¹, Giuseppe Meca De Caro², Insaf Riahi*¹ ¹Bionte, Technical service, Reus, Cataluña, Spain; ²University of Valencia, Preventive Medicine, Nutrition and Food Science area, Burjassot, Valencia, Spain

The objective of the present study was to evaluate the modulation of oxidative stress and the protective antioxidant activity of a liquid solution, based on grape and olive extracts, on the intestinal Caco-2 cell model exposed to *Salmonella* spp. and *E. coli*. In the experimental design, a Caco-2 cell line was utilized as an *in vitro* model to simulate the intestinal epithelial barrier, used for evaluating metabolite bio-accessibility and bioavailability. The Caco-2 cell models were divided into two groups, both exposed to *Salmonella* spp. and *E. coli* at an inoculum of 102 CFU/mL, and incubated at 37°C for 48 hours; the second group was simultaneously treated with different percentages of the liquid solution. Oxidative stress was analyzed using flow cytometry to measure the activity of intracellular Reactive Oxygen Species (ROS) via the fluorescent conversion of specific compounds. Both samples analyzed oxidative stress markers and antioxidant activity. Statistical analysis employed One-way Analysis of Variance (ANOVA) and Linear Regression Analysis. Flow cytometry analysis of intracellular ROS confirmed an inflammatory oxidative response in the Caco-2 cell model exposed to both pathogens. Conversely, the cell model exposed to both pathogens and treated with the liquid solution demonstrated a statistically significant decrease in oxidative stress. The Caco-2 cell model exposed to both pathogens showed no observable antioxidant activity. In

contrast, the treated model with the liquid solution, exhibited statistically significant antioxidant activity: the Caco-2 cell model treated exposed to *E. coli* demonstrated a statistically significant antioxidant effect which varied between 15.3 and 45.9 $\mu\text{mol Trolox eq/g}$. Meanwhile, the Caco-2 cell model exposed to *Salmonella* spp. demonstrated an effect that was directly proportional to the percentage of the liquid solution applied, and varied between 12.4 and 49.1 $\mu\text{mol Trolox eq/g}$. The liquid solution decreased oxidative stress and increased antioxidant activity in the treated group. As a conclusion, the liquid solution, based on grape and olive extracts, demonstrates a protective antioxidant effect on the Caco-2 intestinal cells, evidenced by the modulation of oxidative stress, even in the presence of pathogenic microorganisms.

Keywords: Oxidative stress; Antioxidant activity; *Salmonella* spp; *E. coli*; Caco-2

340P The supplementation of a *Bacillus*-based direct-fed microbial and *Yucca schidigera* extract can improve the performance of broiler chickens

Hector Leyva-Jimenez^{*1}, Katherine A. McCormick¹, Julie Simmons¹, Brian Dirks¹ ¹*United Animal Health, Sheridan, Indiana, United States*

The objective of the present study was to evaluate the effect of supplementing a *Bacillus*-based direct-fed microbial (DFM) and *Yucca schidigera* extract (YUC) alone or together on the performance of growing broiler chickens. The study consisted of a complete randomized block design of 7 treatments: control (CON), basal diet with no additives, CON + YUC at 125 ppm (YC), CON + DFM (7.4×10^4 cfu/ g feed; DM), and CON + DFM (7.4×10^4 cfu/ g feed) and YUC at different inclusion levels 125 ppm (DY100), 94 ppm (DY75), 62.5 ppm (DY50), and 31.25 ppm (DY25). Each treatment included 12 replicate floor pens (used litter) with 40 male broilers per pen inside a commercial-type tunnel-ventilated house. All experimental diets were formulated based on corn, soybean meal, and DDGs. The diets were pelleted and offered ad libitum. The feeding program consisted of 3 dietary phases (starter 0-14 d; grower 14-28 d; finisher 28-42 d). Cumulative feed intake, body weight (BW), body weight gain (BWG), FCR (corrected for mortality), and FCR common weight adjusted (CW-FCR, 2600 g) were determined at the end of each feeding phase. On day 42 of the trial, blood samples were collected from 1 bird per pen to evaluate the serum concentration of uric acid (UA) and ovotransferrin (OVT) using commercial ELISA kits. Moreover, footpad lesion scores were determined from 10 birds per pen using a 0-4 scale (0= no lesions 4=severe lesions on all footpad area). Data were subjected to ANOVA ($P < 0.05$), and means were compared using the Student's t-test of JMP 17.1 software. No difference ($P > 0.05$) in performance was observed among treatments at 14 days. On day 28, YC, DY100, and DY75 treatments improved BW ($P = 0.013$) and FCR ($P = 0.008$) compared to the CON. While the rest of the treatments (DY50, DY25, and DM) had an intermediate response. After 42 days, DY100, DY75, DY50, and DM had higher ($P = 0.002$) BW compared to the CON. CW-FCR was improved for DY100, DY75, and DY50 compared to the CON. No differences were detected ($P > 0.05$) for FI, UA, OVT, or footpad lesion scores between treatments. In conclusion, feeding both DFM at 7.4×10^4 cfu/ g feed and YUC at 125, 94, and 62.5 ppm can improve the performance of broiler chickens.

Keywords: direct-fed microbial; yucca; broiler; performance

341P Case study: PHYTOZEN® Liquid improves flock performance and plant condemnations in broilers

Stephanie Cottee^{*1}, Candice E. Blue¹, Bertrand Medina¹, Stephanie Kamalanathan², Ivan Girard¹ ¹*Probiotech International, Saint-Hyacinthe, Quebec, Canada;* ²*Maple Leaf Foods, Mississauga, Ontario, Canada*

Encouraging sufficient water intake during the first days post-hatch is critical for broiler chick growth, health, and overall flock performance. This study evaluated whether a short-term water administration of PHYTOZEN® Liquid (a blend of neuromodulating monoterpenes with stress reduction effects) could stimulate drinking behavior and support a strong start in commercial broiler flocks. PHYTOZEN® Liquid was applied at 200 ml/1000 L of drinking water for the first 7 days post-hatch. Three consecutive flocks (Ross 708, mixed sex) each raised in the same barn, received the treatment (average $N = 23,315$ birds/flock; Sept 2024–Feb 2025), and data were compared with three previous consecutive untreated control flocks (average $N = 25,245$ birds/flock; Mar–Aug 2024). Performance metrics and processing outcomes were recorded. Data were analyzed using ANOVA (JMP Pro 18), with significance ($P \leq 0.05$) determined by the Tukey test. Although there were no statistical differences in overall flock FCR, ADG, flock mortality and plant DOAs, PHYTOZEN® Liquid supplementation immediately increased water consumption (WI). From Day (D) 1 to 7, WI was 17% higher ($P < 0.05$) compared to control birds. Treated flocks exhibited 23% higher D7 weights ($p < 0.05$) and 77% reduced grower condemnations at processing ($P < 0.05$). In conclusion, a brief, targeted water supplementation with PHYTOZEN® Liquid, during the first week post-hatch, enhances early drinking behavior, supports initial growth, and improves downstream processing outcomes. It is hypothesized that the treatment diverted metabolic resources away from fighting environmental stressors and put toward health, setting up a positive cascade of developmental and behavioral responses that compound over time, resulting in some measurable improvements at end of flock. Extending PHYTOZEN® Liquid use throughout the flock cycle may provide additional performance benefits, but more research is needed to explore this hypothesis.

Keywords: Phytonics; Broilers; Chicks; Condemnations; Water Consumption

342P Field evaluation of Entero-V Poultry, a natural botanical water additive, for reducing Necrotic Enteritis in commercial broilers

Stephanie Cottee^{*1}, Candice E. Blue¹, Bertrand Medina¹, Stephanie Kamalanathan², Ivan Girard¹ ¹*Probiotech International, Saint-Hyacinthe, Quebec, Canada;* ²*Maple Leaf Foods, Mississauga, Ontario, Canada*

Necrotic enteritis (NE) remains a major cause of economic loss and welfare concern in broiler production, particularly in antibiotic-free programs. This study evaluated whether short-term water supplementation with Entero-V Poultry (a synergistic blend of essential oils with broad-spectrum antibacterial and anticoccidial properties) could mitigate NE outbreaks and support improved flock performance in a "Raised Without Antibiotics" (RWA) commercial farm that was naturally susceptible and continuously challenged with NE. Entero-V Poultry was applied at 250 ml/1,000 L of drinking water from day 9 to 21, with a doubled dosage (500 ml/1,000 L) administered on days 15 and 16, to coincide with the producer's typical NE break period. Three consecutive flocks (Ross 708, cocci-vaccinated, mixed sex), each raised in the same barn, received the treatment (average $N = 22,633$ birds/flock; Jan 2025–Aug 2025), and data were compared with three previous consecutive untreated control flocks (average $N = 22,660$ birds/flock; Jul 2024–Dec 2024). Farm and processing plant performance metrics were recorded. Data were analyzed using ANOVA (JMP Pro 18), with significance ($P \leq 0.05$)

determined by the Tukey test. Treated flocks exhibited a statistically improved 2.7% gain in ADG (62.18 g/day) compared to controls (60.56 g/day). There were no statistical differences in FCR, condemnations, and DOAs and overall flock mortality. However, all three control flocks experienced NE outbreaks, with mortality due to NE ranging from 0.83 – 1.60% and overall mortality averaging 7.66%. In contrast, Entero-V Poultry treated flocks showed no NE outbreaks and 0% NE-related mortality, with overall mortality reduced to 6.23%, which is total 18.7% reduction. In conclusion, a targeted 13-day Entero-V Poultry water supplementation program effectively prevented necrotic enteritis and improved some flock outcomes. Applying Entero-V Poultry during periods of NE susceptibility offers a practical, natural strategy for producers seeking to maintain performance and gut health without relying on antibiotics.

Keywords: PhytoGENICS; Broilers; Essential Oils; Necrotic Enteritis; Raised-Without-Antibiotics

343P Modulation of inflammation and preservation of respiratory function in broiler chickens treated with a phytoGENIC additive based on essential oils

Rafael Antonio Casarin Penha Filho*²⁴, Gabriela Romanzini¹³, Maria Carolina Toth Tonelli¹, Laura da Silva Costa², Akash Singhvi⁵ ¹*Mcassab, Animal Health, São Paulo, São Paulo, Brazil;* ²*Laboratório Safe Saude Animal, Jaboticabal, São Paulo, Brazil;* ³*Federal University of Paraná, Animal Science, Palotina, Paraná, Brazil;* ⁴*São Paulo State University, Department of Veterinary Pathology, Jaboticabal, São Paulo, Brazil;* ⁵*Vessure Animal Health, Nova Delhi, India*

This study evaluated the safety and efficacy of Bronk Clean®, a liquid phytoGENIC additive composed of essential oils of mint, eucalyptus, and camphor, with expectorant and bronchodilator properties, administered via drinking water to attenuate clinical signs and respiratory lesions caused by pathogenic *Escherichia coli* (APEC) in broiler chickens. Seventy-five one-day-old chicks were distributed into three groups (n = 25): negative control (NC, non-inoculated), positive control (PC, inoculated and untreated), and Bronk Clean® (BC, 500 mL/1,000 L). Birds were intratracheally inoculated at 10 days of age and treated for five consecutive days after the appearance of the first sneezing symptoms (12–16 days of age). Data were analyzed by ANOVA followed by Tukey's multiple comparison test, adopting a 5% significance level (P < 0.05) using GraphPad Prism, version 8. Body weight gain and feed conversion ratio (FCR) were improved (p < 0.05) in the treated group compared with the PC group and were statistically similar to the NC group. This improvement reflected the rapid clinical recovery and maintenance of feed intake, preventing the catabolic effects observed in untreated birds. Severe macroscopic lesions (fibrinopurulent airsacculitis, hepatitis, and pericarditis) occurred only in the PC group. The ciliostasis score was significantly lower (p < 0.05) in treated birds, with complete restoration of ciliary activity by day 31. Bronk Clean® also promoted significant immune modulation, reducing the expression of pro-inflammatory cytokines (IL-1β, TNF-α, and IFN-γ) and increasing TGF-β (p < 0.05), favoring the return to homeostasis and epithelial regeneration in the trachea. Stable serum levels of haptoglobin and transferrin further supported the systemic control of the inflammatory response. In conclusion, Bronk Clean® was safe and effective in controlling respiratory inflammation, promoting immune modulation and preservation of ciliary function, leading to improved zootechnical performance and clinical recovery in broilers challenged with APEC.

Keywords: broiler chickens; *Escherichia coli*; phytoGENIC additive; respiratory inflammation; immune modulation

344P Effects of nano phytoGENIC feed additive as an alternative to synthetic anticoccidial on growth performance and skin pigmentation in broiler chickens challenged with *Eimeria spp.*

Jheinson A. Almanza Hernandez¹², Jose J. Yanquen Velasquez*¹, Yuliana M. Medina Sandoval¹, Angee M. Ramirez Ruiz¹, Lina M. Peñuela Sierra², Jimena A. Yepes Ramirez² ¹*Nutrexcol S.A.S., Research, Development and Innovation, Armenia, Quindio, Colombia;* ²*Universidad del Tolima, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia*

Eimeria spp. infection is a common and costly disease in poultry. Due to the risk of resistant microorganisms and the difficulty of developing new drugs, exploring innovative strategies has become essential as alternatives to synthetic anticoccidials and rotation programs, increasing the interest in natural additives. Therefore, this study aims to investigate the effects of three dosages (T3:300, T4:400 and T5:500 ppm) of a nano additive (Coxiout Pro®), compared with a negative control (NC: basal diet without anticoccidials) and positive control (PC: starter - 3.75 ppm maduramicin + 40 ppm nicarbazin and grower - 60 ppm salinomycin), on growth performance and skin pigmentation in broiler chickens challenged with 7x10⁴ *Eimeria spp.* A total of 600 male-chicks were randomly allocated in 60 floor pens (10 chicks/pen). Body weight (BW) and feed intake (FI) were measured at 14, 28 and 40 days, and the feed conversion ratio (FCR) was calculated on a per-pen basis. Data were analyzed using one-way ANOVA in a completely randomized design and mean separation was performed using Tukey's test. Results obtained at 14 days showed statistical differences (p < 0.05) between NC and PC in BW (565 vs 538 g), the highest FI was found in NC group (621.67g) and differences in FCR were observed between PC, T3 and T4 (1.11 vs 1.08 vs 1.07) showing an improvement of nearly 4 points with the additive. At 28 days, statistical differences (p < 0.05) in FI were observed among PC, T3 and T4 (2,542 vs 2,429 vs 2,426 g). At 40 days, no statistical differences were observed, but the 400 ppm nano phytoGENIC group reduced the FCR by 5 and 7 points compared with PC and NC respectively. On the other hand, the skin pigmentation was measured with CIE L*a*b* scale and showed statistical differences (p < 0.05) between NC and PC in b* value (yellowness/blueness) at 28 days (10.53 vs 13.13). Although no statistical differences were found at 42 days, the mean result with nano additive treatments was higher than both control groups, with b* color values above 18. In conclusion, a 400 ppm dose of nano phytoGENIC feed additive could be used as an alternative to synthetic anticoccidials, improving the growth performance and maintaining the skin pigmentation in broiler chickens challenged with *Eimeria spp.*

Keywords: nano phytoGENIC feed additive; performance; CIE L*a*b* scale; skin pigmentation

345P Enhanced efficacy of chemical anticoccidials with tannin-based feed additive fed to commercial turkey poults infected with a mixture of *Eimeria spp.* field isolates

Elizabeth Santin*¹, Brett Lumpkins³, Benny McClure², Karyl Hedden², Olivier Desrués¹, Mojca Osredkar Mergole¹, Zaker Rehan Mohammed¹ ¹*Silvateam S.p.A. Italy, BU Feed, Studenec, Slovenia;* ²*Silvateam USA, New York, New York, United States;* ³*Southern Poultry Feed & Research Inc., Athens, Georgia, United States*

Continuous use of anticoccidial drugs in turkey production has reduced efficacy and contributed to resistance, emphasizing the need for alternative or complementary solutions. This study investigated the efficacy of a feed additive Silvafeed® Nutri Cox containing plant extracts with tannins and saponins (908 g/t), in combination with five anticoccidial drugs Lasalocid (90 g/t),

Monensin (60 g/t), Clopidol (113.5 g/t), Diclazuril (0.91 g/t), and Zoalene (113.5 g/t) in commercial turkey poults experimentally challenged with *Eimeria meleagridis*, *E. gallopavonis*, and *E. adenoides*. Four hundred and thirty-two turkey poults were allocated into 12 treatment groups (6 replicates each), randomized within groups including unchallenged and challenged controls receiving a mash diet ad libitum for 28 days. On day 14 all birds in positive control and treatment groups received 1 ml of coccidial inoculum mixture of *E. meleagridis* (50,000 oocysts/ bird), *E. gallopavonis* (50,000 oocysts/ bird), and *E. adenoides* (50,000 oocysts/ bird) field isolates. Parameters assessed included feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), oocyst per gram (OPG), and dropping score. Data was analyzed with Statistix 10.0 using ANOVA and Tukey's test (5%). Birds in challenged control showed significant deterioration in all parameters due to *Eimeria* challenge. Lasalocid + Nutri Cox combination improved FI and BWG by up to 4%, with OPG reduced by 32% relative to lasalocid alone. Monensin + Nutri Cox combination lowered OPG by 19%, and improved FCR by 7%, and improved faecal score ($P < 0.05$) showing strong intestinal protection and performance recovery compared to monensin alone. Clopidol + Nutri Cox combination enhanced BWG by 5% and improved FCR ($P < 0.05$) compared to clopidol alone. Diclazuril + Nutri Cox combination yielded the strongest benefits as the most effective combination improving performance BWG by 10–11% and FCR for 7% compared to diclazuril alone. Nutri Cox + Zoalene combination led to a 20% BWG increase and 14% improvement in FCR compared to zoalene alone. These results indicate that Silvafeed® Nutri Cox can be an effective complementary strategy alongside traditional coccidiostats to improve anticoccidial control and maintain bird productivity.

Keywords: coccidiosis; turkey; anticoccidials; plant extracts; complementary

346P Utilization of essential oils as a replacement of antibiotics and their impact on growth performance, carcass characteristics and intestinal microflora in broilers

Bilal Qasim Ali¹GS, Irfan Ahmed², Muhammad Shoaib³, Muhammad Yousaf⁴, Muhammad Ilyas⁵ ¹Charoen Pokphand Pakistan Pvt. Ltd, Feed Technology Office, Lahore, Pakistan; ²The Islamia University of Bahawalpur-Pakistan, Faculty of Veterinary & Animal Sciences, Bahawalpur, Pakistan; ³Pakistan Kisan Feeds & Hateem Corn Products, Gujranwala-Pakistan, Gujranwala, Punjab, Pakistan; ⁴University of Veterinary and Animal Sciences, Lahore-Pakistan, Faculty of Animal Production & Technology, Lahore, Pakistan; ⁵NutriAgrow-Pakistan, Lahore, Pakistan

The experiment was carried out to evaluate the effect of oregano essential oil and a phyto-genic commercial product of oregano plant OREGO-STEM® on growth performance, carcass characteristics, gut development and intestinal microflora as antibiotic replacers. Total 588 broiler chicks were divided into 7 groups and 3 replicates of each with 28 birds per replicate. Two levels of oregano essential oil at 75 mg/kg and 150 mg/kg, oregano-stem at 300 mg/kg and 450 mg/kg and zinc bacitracin at 500 mg/kg and 750 mg/kg were given in basal diet with a control group. Feed and water were provided *ad libitum*. Starter feed was offered from 0 to 14 day and then grower feed was offered till the end of the experiment. Body weight gain, feed intake, FCR and FE was observed weekly. Birds were slaughtered at 35th day of the experiment for the measurements of the carcass traits and ileal digesta was collected for the evaluation of intestinal microflora. Data were analysed by one way ANOVA with $P < 0.05$ significance level. Results declared that oregano essential oil at 150 mg/kg improved ($P < 0.05$) the body weight gain, FCR and FE significantly in broilers compared to zinc bacitracin and control group while no effects were observed on

feed intake ($P > 0.05$). Breast yield, breast percentage and carcass percentage were improved significantly ($P < 0.05$) in the group fed OREGO-STEM® at 450 mg/kg. Length of total intestine was also increased ($P < 0.05$) by the same group. No remarkable improvement was found on the weight of heart, liver and spleen by any group. Colony forming units for *Lactobacillus* bacteria were found higher ($P < 0.05$) in the group fed oregano essential oil at 150 mg/kg while *E. coli* and clostridium were found lower in the same group than control and other groups. *Salmonella* spp. was not detected in any group. According to these results oregano essential oil may replace the antibiotics in broiler feed in order to achieve best growth.

Keywords: essential oils; growth performance; carcass characteristics; intestinal microflora; broilers

347P Effects of an essential oil–organic acid blend and a precision biotic on performance and intestinal health of broilers under cyclic heat stress

Irem Bozdogan^{1,2}, Ahmet Ceylan³, M. Shazaib Ramay¹, Pinar Sacakli¹, Ali Calik^{*1} ¹Ankara University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Ankara, Turkey; ²Graduate School of Health Sciences, Ankara University, Ankara, Turkey; ³Ankara University, Faculty of Veterinary Medicine, Department of Histology Embryology, Ankara, Turkey

High ambient temperature is a major stressor in commercial poultry, causing performance losses and impairing intestinal health and functionality. Therefore, modulation of the antioxidant response and intestinal health through various nutritional strategies has gained scientific interest. This study evaluated the effects of a blend of essential oils and organic acids (EO) and a glycan-based precision biotic (PB) on broiler performance, intestinal histomorphology, and immune response under cyclic heat stress (HS). A total of 162 one-day-old male Ross 308 chicks were randomly allocated into three groups with 6 replicates of 9 birds each. The control group was fed a corn-soy basal diet, while treatments received the same basal diet supplemented with either 300 mg/kg EO or 900 mg/kg PB. Birds were kept under recommended temperature during the starter and grower periods (d 0–24). In the finisher period (d 25–42), temperature was raised and maintained at $34 \pm 1^\circ\text{C}$ from 9 AM to 5 PM, then reduced again to recommended levels. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were recorded on days 0, 10, 24, and 42. On d 42, two birds per replicate were selected for intestinal tissue sampling. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Results showed no significant differences in BWG, FI, and FCR among groups during d 0–24. Both EO and PB supplementation significantly increased BWG during d 25–42 compared to control ($P < 0.05$). Although FI was not significantly affected, both EO and PB groups tended to show improved FCR compared to the control during d 25–42 and over the entire experimental period. Jejunal and ileal villus height and crypt depth were significantly greater in the EO and PB groups compared with the control group ($P < 0.05$). Jejunal mRNA abundance of IL-1 β , TNF α , heat-shock protein (HSP)70, and HSP90 was significantly lower in both treatment groups ($P < 0.05$). Moreover, the EO group exhibited significantly greater jejunal GPx mRNA abundance than both the PB and control groups ($P < 0.05$). In conclusion, dietary EO or PB supplementation improved performance and intestinal histomorphology in broilers under HS, while mitigating associated cellular and inflammatory responses.

Keywords: Broiler; cyclic heat stress; performance; intestinal health; immune response

348P Resolving a piece of the puzzle via untargeted metabolomics: how does a standardized dry grape extract improve bone quality in pre-laying pullets?

Fatima Z. Aberkane*^{1,2}, Paul Engler², Severine Boisard¹, Dominique Chavatte³, David Guilet¹, Mohammed El Amine Benarbia² ¹University of Angers, Sonas, Angers, France; ²Nor-Feed SAS, Beaucoz , France; ³DLJConsultant, Saint-Brieuc, France

Despite extensive evidence of plant-based additives' effects, the mystery of how they work is still largely unanswered. For instance, dietary grape extracts have long been shown to benefit bone health in both humans and animals. The present study confirmed the beneficial impact of a standardized dry grape extract (SDGE) on pullets' bone quality prior to laying. The investigation therefore focused on the initial steps in unraveling the puzzle of how this particular SDGE promotes their ossification, through untargeted metabolomics, the results of which are presented here. One-day-old pullets were reared under commercial conditions. A total of 36300 birds were randomly allocated between two barns on the same farm site. Both received the same diet, with the addition of 30 mg/kg of Nor-Grape[®] (NG) (Nor-Feed, France) in the feed of the supplemented group (NG, n=18150) from D1 until week 17. On week 17, blood and bone samples were drawn from 8 pullets per group. Untargeted metabolomics was performed, beginning with a two-step metabolites extraction protocol. UHPLC-MS/MS was followed by data treatment using Progenesis QI allowing statistical analysis based on ANOVA and OPLS-DA (P-value < 0.05, VIP > 1) and metabolites annotation. MetaboAnalyst was employed for metabolic pathway analysis. Untargeted metabolomics results revealed that the supplementation modulated several metabolic pathways in both plasma and bone tissues of pullets. In plasma, enrichment pathway analysis using MetaboAnalyst revealed that supplementation prior to the laying period notably affected pathways involved in fatty acids biosynthesis (P-value = 2.5×10^{-7}). In bone tissue, dietary intervention influenced pathways associated with estrogen signaling (P-value = 4.53×10^{-5}) and sphingolipid metabolism (P-value = 6.44×10^{-5}). This present study confirmed beneficial effects on bone quality of pullets prior to laying, thereby promoting improved skeletal conditions for efficient laying performance. The integration of untargeted metabolomics exploration with insights from the literature enabled the proposal of a novel hypothetical mechanism elucidating how this particular SDGE may improve bone quality in pullets, offering a piece of the puzzle that will be further illustrated in the poster.

Keywords: dry grape extracts; ossification; laying hens; metabolomics

349P Tributyrin as a modulator of the inflammatory response in broilers under an enteric challenge model

Gabriel R. Braga¹, Gabriel Cedrola¹, Bruno T. Ramos¹, Ana L. Barros¹, Lukas Bauer², Henrique G. Brand³, Melissa I. Hannas*⁴ ¹Universidade Federal de Viosa, Animal Science, Viosa, Minas Gerais, Brazil; ²Evonik Operations GmbH, Hanau-Wolfgang, Germany; ³Evonik Brasil LTDA, So Paulo, Brazil; ⁴Universidade Federal de Viosa, Animal Science, Viosa, Minas Gerais, Brazil

The present study aimed to evaluate the effect of tributyrin in alleviating enteric-induced inflammation in broilers. A total of 1,200 one-day-old male Cobb500 broilers were distributed in a CRD with 5 treatments, 12 replicates, and 20 birds per unit. The treatments consisted of: PC (positive control; no challenge with dextran sodium sulfate (DSS) and basal diet), NC (negative control; challenge with DSS and basal diet), TBA0.1 (NC + 0.1% dietary supplementation of tributyrin A), TBA0.05 (NC + 0.05%

TBA), and TBB0.05 (NC + 0.05% tributyrin B). Intestinal inflammation was induced by oral gavage with a 3.5% DSS solution on days 8-13 and 21-26. At d 8, 13, 21, 26, and 40, the diets and birds were weighed to measure growth performance, FI and FCR. Samples from intestinal tissue were collected at d 13, 21, 26, and 40 (1 bird/replicate) for histological and morphometric analyses. The concentration of SCFA in the cecal digesta was measured at d 26 and 40. The variables were subjected to the Kruskal-Wallis test and ANOVA, and the means compared by the SNK test. From d 1-8, broilers in the TBA0.1 had the highest BW (P<0.05). From d 1-13, broilers in the NC had the lowest FI (P<0.05). From d 1-26, broilers in the TBA0.05 had higher BW, WG, and FI compared to the NC (P<0.05). Tributyrin supplementation, especially in the TBA0.05, increased lamina propria and epithelial thickness, enterocyte proliferation, and the number of goblet cells in segments evaluated compared to the others treatments (P<0.05). At d 13, 21, and 26 and in all intestinal segments evaluated, birds in the NC showed decreased villus height, increased crypt depth, and a worsening of the villus height to crypt depth ratio compared to the PC (P<0.05), while broilers in the tributyrin supplemented treatments recovered and, in the case of the TBA0.1, even improved these parameters. At d 40, birds in the PC showed a higher acetate concentration (P<0.05) in cecum. The results indicate that dietary supplementation of tributyrin at 0.05% is capable of positively modulating the intestinal morphology of broilers during an intestinal inflammation challenge, reinforcing the potential of tributyrin as a nutritional tool for improving the recovery of inflammatory lesions and for promoting a better productive result.

Keywords: butyrate; enteritis; poultry; challenge; DSS

350P In Vitro assessment of Bacillus spore germination under simulated poultry digestive conditions

Marius Da Costa¹, Isabelle Pelissie¹, Jean-Franois Hupe¹, Anais Nozeran*¹, Vanessa Demey¹ ¹Lallemand SAS, Animal Nutrition, Blagnac (31), France, France

Bacillus strains are widely used as poultry probiotics due to their spore-forming ability, which ensures resilience and stability during intensive feed processing (eg. pelletization) and enables activity in the host's gastrointestinal tract (GIT) once germinated into vegetative cells. Understanding spore germination kinetics under GIT conditions is essential for predicting probiotic efficacy, as transit time in the poultry GIT is very short (less than 6 hours). This study aimed to develop an *in vitro* model simulating poultry digestive conditions (temperature, pH, and humidity) to evaluate Bacillus spore germination kinetics of 2 Bacillus-based probiotics: *Bacillus pumilus* NCIMB 30362 (BP) and a three-strain Bacillus mix (BM) recognized for its rapid germination. Six replicates per probiotic were prepared by inoculating 100 mL PBS with 1 g of either BP or BM. Aliquots of 1.5 mL were added to 13.5 mL TSA (diluted 1/10) to reach 10e7 spores/g. Tubes were incubated at 40 °C and pH 5.5. Samples were collected at 0, 30, 60, and 90 min. Germination was quantified at each time point using the traditional plate count method (CFU/g): total counts (spores + vegetative cells) without heat treatment, and spore counts after heat treatment (80 °C, 10 min). Total Bacillus counts and spore counts were log-transformed prior to analysis using a generalized linear mixed model (GLMM) with treatment (TRT), time, and their interaction specified as fixed effects. Total counts remained stable over time (P = 0.266) but were significantly higher for BP compared to BM (P < 0.01). Spore counts decreased over time (P < 0.01). At T0, BP had a higher spore count (100% versus 81% for BM). The survivability of BM vegetative cells at T0 (19%) may be challenged during pelletization. From 30 min onward, spore counts were significantly lower for BP compared to

BM ($P < 0.01$). The developed *in vitro* model effectively simulates poultry digestive conditions for evaluating *Bacillus* spore germination kinetics. Findings suggest strain-specific differences, with BP demonstrating enhanced germination kinetics following heat treatment, underscoring its potential as an efficient poultry probiotic. Further investigations should assess enzyme and antimicrobial peptide production by *Bacillus* probiotics.

Keywords: Probiotic; *in vitro* model; *Bacillus*; germination; spore

351P Influence of a symbiotic additive *Bacillus sp.*-based probiotic and yeast cell wall-derived prebiotic on production performance and egg quality in laying hens

Ange M. Ramirez Ruiz^{*1}, Jheinson A. Almanza Hernandez¹, Yuliana M. Medina Sandoval¹, Jose J. Yanquen Velasquez¹, Diana C. Mogollon², Mayra Diaz Vargas³ ¹Nutrexcol S.A.S., Research, Development and Innovation, Armenia, Quindio, Colombia; ²Universidad del Tolima, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia; ³Universidad Cooperativa de Colombia, Veterinary Medicine and Animal Science, Ibaguè, Tolima, Colombia

The laying hens face multiple challenges that negatively affect their performance throughout their life production. Intestinal dysbiosis and gut diseases are among the main causes of reduced productivity. For decades, antibiotics have been used as growth promoters (GPA); however, despite the risk of bacterial resistance, GPA are still the main strategy to enhance animal performance in several Latin American countries. Therefore, alternative feed additives such as symbiotics are being introduced for sustainable and safe poultry production. The objective of this study was to evaluate the influence of a symbiotic additive *bacillus sp.*-based probiotic and yeast cell wall-derived prebiotic (Modubiot Pro[®]), compared with a negative control (without GPA) and a positive control (with GPA), on production performance and egg quality of laying hens between 32 to 38 weeks of age. At 35 weeks, the animals were challenged with a high dose of medication to induce dysbiosis. A total of 192 laying hens at 32 weeks were randomly allocated into 12 floor pens (16 hens/pen). Data were analyzed using one-way ANOVA in a completely randomized design and mean separation was performed using Tukey's test. No statistical differences were obtained from 32 to 38 weeks in egg production (EP) and feed conversion ratio (FCR) per dozen. Regarding egg quality, yolk color showed statistical differences ($p < 0.05$) throughout the experiment and the supplementation of symbiotic additive maintained the best yolk pigmentation (> 12 Roche color scale). At 32 weeks, statistical differences ($p = 0.0063$) were observed in the yolk index (YI) between NC and the symbiotic additive (0.445 vs 0.419), however, in subsequent weeks the values were similar among groups. At 38 weeks, albumen height (AH) values were 6.27 mm for both PC and symbiotic additive and showed significant differences with NC ($p = 0.0328$). In the same way, the Haugh unit (HU) was comparable for PC and symbiotic additive (79 vs 80), both significantly higher than NC group (76) ($p = 0.0156$). In conclusion, the symbiotic additive exhibited similar production performance to PC with GPA and showed slightly improved egg quality parameters such as AH, HU and YC.

Keywords: probiotic; prebiotic; performance; egg quality

352P From composition to mode of action: Why Red Brazilian Propolis excels against *Candida albicans*

Sandra Vaudaux^{*1}, Nathalie Bonal¹, Claire Girard¹, Sylvain Kerros¹, Andresa A. Berretta² ¹Phytosynthese, Mozac, France; ²Apis Flora Industrial e Comercial Ltda, Ribeirão Preto, Brazil

The aim of this study was to assess the composition of green and red Brazilian propolis (Apis Flora) and to compare their antimicrobial activity to a cranberry extract and a Manuka honey powder (natural antimicrobial references). The chemical analysis were performed by HPLC/DAD. The antimicrobial activity was determined by MBC and MFB (minimum bactericidal/fungicidal concentration: lowest concentration of substance killing bacteria/fongic strains at 37°C for 24h). MBC and MFB were determined using broth dilution method following NCCLS protocols. Molecular docking analyses (AutoDock Vina) were performed to evaluate the interactions between main Brazilian propolis compounds and *Candida albicans* lanosterol 14 α -demethylase (ERG11). The ligands 3D structures were obtained from PubChem database. Crystal structure of the target protein was retrieved from UniProt, PDB 5T21. Files were prepared using Chimera 1.19. The best conformations were selected based on binding affinity (kcal/mol). Chromatograms showed that green propolis contains Artepillin C (40.48 \pm 0.14 mg/g) and baccharin (5.98 \pm 0.01). Red propolis contains vestitol (35.04 \pm 0.38), medicarpin (23.71 \pm 0.22) and guttiferone (17.77 \pm 0.43). Red propolis has the lowest MFC against *Candida albicans* (1.563 \pm 0.0 mg/mL). Green and red propolis have the lowest MBC against *Staphylococcus aureus* (1.563 \pm 0.0 mg/mL) vs 31.25 \pm 0.0 for the cranberry extract and >250 for the Manuka honey. Red propolis has the lowest MFC against *E. coli* (1.563 \pm 0.0). Both green and red propolis therefore exhibited a highest antifungal activity against *Candida albicans*. Molecular docking analyses revealed that several major constituents — including artepillin C, baccharin, guttiferone E and neovestitol— show strong binding affinities towards lanosterol 14 α -demethylase (CYP51), a key enzyme in the ergosterol biosynthetic pathway. Inhibition of CYP51 is expected to impair ergosterol synthesis, leading to membrane destabilization and fungal cell death, consistent with the *in vitro* fungicidal activity observed for both extracts. Brazilian propolis showed promising antimicrobial activity. Their diversity of its bioactive compounds may explain why red propolis was identified as the best candidate for further *in vivo* studies targeting *Candida albicans*.

Keywords: Brazilian propolis; *Candida albicans*; *Staphylococcus aureus*; *Escherichia coli*; Molecular docking

353P Basil (*Ocimum basilicum L.*) essential oil as a phytogetic supplement for heat-stressed broilers: Impacts on intestinal histomorphology, cecal fermentation, and production efficiency

Erinc Yuksel Calik¹, Ahmet Ceylan², M. Shazaib Ramay¹, Ali Calik^{*1}, Sakine Yalcin¹ ¹Ankara University Faculty of Veterinary Medicine, Animal Nutrition and Nutritional Diseases, Ankara, Turkey; ²Ankara University Faculty of Veterinary Medicine, Department of Histology Embryology, Ankara, Turkey

Heat stress (HS) challenges modern poultry production by suppressing growth and increasing mortality. Phytogetic compounds have emerged as promising dietary strategies to modulate stress responses and sustain productivity under high temperatures. This study evaluated the effects of dietary basil essential oil (BEO) on broiler performance, intestinal histomorphology, and cecal short-chain fatty acid (SCFA) concentrations under cyclic HS. A total of 216 one-day-old Ross 308 male chicks were randomly assigned to four groups with 6 replicates of 9 birds each. A corn-soybean meal based basal diet was supplemented with 0, 50, 100 and 200 mg/kg BEO for 42 days. From day 0-24, birds were maintained under standard temperature conditions, followed by cyclic HS (34 \pm 1°C, 50 \pm 5% relative humidity, 8 h/day, 0900-1700 h) from day 25-42. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were

calculated for pre-HS, HS and overall periods. European Production Efficiency Factor (EPEF) was also determined for days 0-42. On d 42, two birds per replicate were sampled for jejunal and ileal histomorphology and cecal SCFA analysis. Data were analyzed by one-way ANOVA followed by Duncan's test, with polynomial contrasts used to assess linear and quadratic effects. BEO had no significant effect on BWG, however, FI and FCR decreased linearly ($P = 0.018, 0.021$) during pre-HS phase. No significant effects were observed for BWG, FI or FCR during HS or the overall period, though EPEF improved linearly ($P = 0.017$) with BEO supplementation. With BEO, villus height (VH) and crypt depth (CD) increased significantly in both jejunum and ileum compared to control ($P < 0.05$), while ileal VH:CD ratio improved ($P = 0.018$), with 200 mg/kg dose showing the greatest effects. Linear and quadratic dose-responses were detected for jejunal and ileal VH and CD ($P < 0.05$), and a linear increase in ileal VH:CD ratio ($P = 0.042$) was observed. BEO also enhanced total SCFA production ($P \leq 0.001$), driven mainly by increases in acetic acid ($P \leq 0.001$) and moderate rises in butyric and propionic acids ($P < 0.05$). In conclusion, dietary BEO improved gut morphology and cecal fermentation, enhancing intestinal resilience and overall production efficiency in broilers under heat stress.

Keywords: Essential oil; heat stress; short-chain fatty acids; gut morphology; feed efficiency

354P Mitigating age-related decline in layer performance with calcium pidolate supplementation

Abdelhacib Kihal^{*1}, Alvaro Del Real¹, Elsa Fernandez², Juan Jose Mallo¹ ¹Norel Animal Nutrition, Barcelona, Spain; ²Fergasa nusa S.L, Valencia, Valencia, Spain

Laying hence performance exhibits a decrease in productivity and egg quality after 60 weeks of age, due to a combination of factors, including age-related changes in metabolism, hormone levels, intestinal absorption, and bone health. This study aimed to evaluate the effect of supplementing calcium pidolate in the diet of old layers on egg production performance and quality on a commercially relevant scale. A total of 66,352 68-week-old Lohmman hens were housed in two separate commercial layer houses within the same farm, randomly assigned into two treatment groups: control (CTR) and treatment (CP). The CTR group included 34,352 layers fed a standard diet following FEDNA nutritional recommendations. The CP group included 32,000 layers fed the control diet + 450gr of calcium pidolate (Calpid[®], Norel Animal Nutrition). The study was carried out at the same time and under the same production conditions in both houses. Laying production performance parameters were recorded weekly for each treatment group. Recorded parameters were: Egg production rate, egg weight, broken eggs and dirty eggs. The trial was conducted between 68 and 88 weeks. Data were analyzed by one-way ANOVA with Tukey's post hoc test of SAS. Performance analysis results showed a higher egg production rate for the CP group than the CTR group (78.4 vs. 71.4%, $P < 0.05$). For egg quality parameters, broken egg rate was lower for the CP group than the CTR group (0.75 vs. 2.45%, $P < 0.05$). Dirty eggs were numerically lower for the CP group (2.81 vs. 3.02%, $P > 0.05$), and egg weight was similar between both groups. Overall mortality during the study was lower for the CP group than the CTR group (0.34 vs. 0.76%, $P < 0.05$). In conclusion, the results of this study indicated that calcium pidolate supplementation improved the production performance of old laying hens. This effect is suggested to be related to the effect of calcium pidolate on calcium and phosphorus metabolism of birds, where it enhances their utilization efficiency on egg productivity in old layers.

Keywords: Calcium pidolate; layers; calcium; phosphorus; egg production

355P Efficacy and application of Termin-8[®] Pathenix[™] for microbial control in animal feed

Yun-mei Amy Lin¹, Andra Powell^{*1}, Nicole Holcombe¹, Cheryl Schaeffer¹ ¹Anitox, Lawrenceville, Georgia, United States

Feed and feed ingredients serve as established vectors for microbial transmission in animal production. Variable pathogen loads in high-protein ingredients such as soybean meal, rapeseed meal, and meat and bone meal pose significant biosecurity and food safety risks. Common contaminants include Salmonella spp., E. coli, Clostridium perfringens, molds, and yeasts. Heat-tolerant and spore-forming bacteria survive thermal processing, necessitating chemical intervention. While formaldehyde-based sanitizers remain the gold standard, increasing regulatory pressures have driven development of formaldehyde-free alternatives.

Organic acid (OA) blends are widely utilized in animal feeds as antimicrobial agents to control enteric pathogens by acidifying feed materials and creating hostile conditions for bacterial proliferation, thereby reducing pathogenic load while maximizing nutritional value. This study evaluates the efficacy and dose-response relationship of Termin-8[®] Pathenix[™], a next-generation formaldehyde-free feed pathogen control solution designed to synergistically amplify organic acid antimicrobial activity, representing an advancement in feed safety technology. Commercial layer feed (16% crude protein) was ground to 1-mm particle size and inoculated with Salmonella Typhimurium (ATCC 14028) at 2.73×10^6 CFU/g. Contaminated feed (1 kg) was treated with Termin-8[®] Pathenix[™] at 2, 4, and 8 kg/MT using an air atomizing application system. Post-treatment, feed was mixed, stored at room temperature for 24 hours, and analyzed by plating on XLT4 selective media after serial dilution. Four treatments were evaluated using one production lot per treatment. From each lot, 10 subsamples were collected and enumerated using 3 replicate plates per subsample (total plates = 120). Counts were log₁₀-transformed and summarized at the subsample level (n = 10 per treatment). Termin-8[®] Pathenix[™] demonstrated dose-dependent Salmonella Typhimurium control (>1-log reduction in feed; up to 1.6-log in meat and bone meal), outperforming conventional organic acid blends at equivalent dosages (2 to 8 kg/MT). This formaldehyde-free alternative meets evolving biosecurity and regulatory requirements while maintaining operational compatibility and feed quality.

Keywords: feed safety; formaldehyde-free; Salmonella control; organic acids; animal feed pathogen control

356P The effect of different doses of phytobiotics on immune response and ileal microbial counts in broiler chickens infected with Clostridium perfringens

Ala E. Abudabos^{*1} ¹Tennessee State University, Food and Animal Science, Nashville, Tennessee, United States

The current investigation aimed to evaluate the effect of different doses of Isoquinoline Alkaloids (IQs)-based phytobiotics on immunoglobulins, cytokines, and microbial counts of broilers recovering from necrotic enteritis (NE). Three doses of IQs supplementation (0.06, 0.09, and 0.12 g/kg) were compared to a reference antibiotic in broilers challenged with *Clostridium Perfringens* (*C. Perfringens*). In a CRBD, a total of 360 0-day-old Ross-308 chicks were randomly distributed to one of the six treatments as follows: T1, control, no additive or challenge (-ve control); T2, control + bacterial challenge (+ve control), T3, T2 + 0.10 g/kg Maxus, T4, T2 + 0.06 g/kg IQ, T5, T2 + 0.09 g/kg IQ; and T6, T2 + 0.12 g/kg IQ. Each group was assigned to 10 replicates with six birds per replicate. On day 7, all birds except the control group (T1) were orally inoculated with *C. perfringens* as oral gavage using a cocktail containing *C.*

perfringens at the rate of 4×10^8 CFU/ml. On day 35, samples were obtained to determine immunoglobulins, cytokines, and ileal bacterial counts. The data revealed a significant increase in IgY ($P < 0.01$) and IgA ($P < 0.001$) for T4, T5, and T6 compared to T2, with no significant difference when compared to T1. IL-1 β , IL-2, IL-6, and IFN- γ decreased significantly for T4, T5, and T6 ($P < 0.001$) compared to T2. The IQ doses showed no effect on different cytokines except for IL1, which was significantly lower for T6 compared to T4 and T5. The ileal *C. Perfringens* count was significantly lower for the unchallenged group (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. Probiotic bacteria, *Lactobacillus*, improved due to IQ supplementation, which could be attributed to an improved gut barrier function mediated by microbiota enriched in *Lactobacillus* spp. and depleting harmful Clostridia. Serum cytokine marker analysis highlights the immunomodulatory capacity of IQ groups, which exhibited significant reductions in key pro-inflammatory cytokines IL-1 β and TNF- α compared to T2. Based on all previous results, it can be concluded that the IQ can be used as an effective suppression of the initial inflammatory trigger elicited by bacterial challenge.

Keywords: Broilers; Clostridium Perfringens; bacterial challenge; immune responses; microbial count

357P Assessment of a blend of plant extracts, vitamins and highly bioavailable minerals supplementation on productive performance and hatching egg quality in Cobb broiler breeders

Clarisse Techer*¹ ¹miXscience, Bruz, France

Persistency and performance in poultry breeding decline with age, impacting both laying rate and hatchability. Functional feed additives may help mitigate these declines. The present study aimed to evaluate the efficacy of Tecnoshell (Tecnoshell by Enviup, Mixscience, France), a feed supplement formulated with highly bioavailable calcium and synergistic micronutrients, in enhancing productive performance and hatching egg selection rates in Cobb broiler breeders during the post-peak production phase. A controlled feeding trial was conducted in a Broiler Breeder Farm, in Philippines, involving 3,220 Cobb broiler breeders aged 58 weeks. Birds received active Vitamin D3 supplementation from week 55 to 57–58, followed by Tecnoshell at 2 kg/ton of feed from week 58 onward. Zootechnical parameters measured included laying rate and hatching rate. Data were analyzed using Student tests (t test/ two-tailed distribution, Microsoft Excel) to assess the impact of each supplementation strategy. Prior to Tecnoshell administration, the flock exhibited an average weekly decline in hen day egg production of 2.28%. Introduction of Tecnoshell mitigated this trend to 0.5% per week, corresponding to a net improvement of 1.78% in the weekly production rate. Additionally, hatching egg selection rates increased by 0.69% ($p < 0.05$). This could be related on the improvement of eggs and eggshell quality which consequently result in a higher number of settable eggs. A positive return economic analysis projected an average gain of 1.3 reliable eggs per bird and a net return of investment of 0.3USD per broiler breeder over the 28-days trial. Tecnoshell supplementation at 2 kg per ton of feed demonstrably improved both productive performance and hatching egg selection in post-peak Cobb broiler breeders. These findings highlight the interest of a blend of multiple active ingredients, as a robust alternative to active Vitamin D3, for optimizing productivity and economic returns in commercial broiler breeder operations.

Keywords: Eggshell quality; Tecnoshell; laying hens; hatchability; breeders

358P Evaluation of a phytogetic combination product during a coccidiosis challenge in broilers

Maci Oelschlager*¹, Manuel Da Costa¹, Yann Fournis¹, Bradley Turner² ¹Cargill, Wayzata, Minnesota, United States; ²Poultry Research Partners, LLC, Athens, Georgia, United States

Broiler producers are increasingly using phytogetic products due to their plant-derived makeup, gut health support, and impact on bird performance and health. Coccidiosis, a protozoan disease caused by *Eimeria* species, continues to impose an economic burden to the broiler industry by negatively impacting productivity and health. The objective of this trial was to evaluate a phytogetic combination product on broiler performance and health during a coccidiosis challenge. A total of 840 day-old male Cobb 500 by-product chicks were placed on used shavings in 4 x 8 ft floor pens (0.76 sq ft/bird) and randomly assigned to one of two treatments (10 replicates/treatment, 42 birds/replicate): (1) coccidiosis challenge control or (2) coccidiosis challenge + phytogetic combination product (0.6 lb/ton). All birds were challenged on d 14 with 2,500 *E. maxima* oocysts/bird via the feed and were fed a standard three-phase feeding program consisting of a starter phase (d 0 – 14), grower phase (d 14 – 28), and finisher phase (d 28 – 42). The diets were corn-soybean meal-based, provided ad libitum throughout the trial, met nutrient requirements, and contained no coccidiostats. Performance data were collected on d 0, 14, 28, and 42 and on d 21, two birds per pen were randomly selected for coccidiosis lesion scoring. Data were analyzed using treatment as the main factor. Final bodyweight at the end of the grower phase (d 28) and finisher phase (d 42) was increased ($P < 0.034$) for birds fed the phytogetic combination product treatment compared to the birds in the control treatment by 0.10 lb and 0.16 lb, respectively. For the overall trial, livability trended toward significant improvement ($P < 0.067$) for birds fed the phytogetic combination product treatment relative to the birds in the control treatment. The phytogetic combination product treatment also numerically reduced the number of birds reporting *E. maxima* lesion scores 2 and 3 compared to the birds in the control treatment. These results indicate that a phytogetic combination product may support broiler performance and health during a coccidiosis challenge, contributing to more resilient flocks under enteric stress conditions.

Keywords: broiler; phytogetic; coccidiosis; feed additive; gut health

359P Efficacy of natural choline (BioCholine) as a complete replacement for synthetic choline chloride on performance, egg quality and yolk choline deposition in BV300 layers

Shivi Maini*¹, S Yogisha² ¹Indian Herbs Specialties, Chandigarh, Chandigarh, India; ²SKANDA Life Sciences, R&D, Chandigarh, Chandigarh, India

A 20-week controlled study was conducted to evaluate the comparative efficacy of a natural choline supplement (BioCholine, M/S Indian Herbs) vis-à-vis synthetic choline chloride on performance, egg quality and yolk choline deposition in BV300 layers. A total of 150 birds were allotted to three dietary groups (50 birds/group; 5 replicates of 10). Group T1 received a basal ration without supplemental choline; T2 received basal ration supplemented with synthetic choline chloride 60% at 1 kg/ton; T3 received basal ration & BioCholine at a substantially lower dosage designed to completely replace synthetic choline. The experiment was conducted from 16–35 weeks of age at an accredited CRO in Bengaluru. Weekly performance parameters (egg production, HHE, feed intake and feed efficiency) and egg quality traits at weeks 27 and 35 were evaluated. Data were statistically analyzed following Snedecor and Cochran procedures ($p < 0.05$). BioCholine supplementation produced a marked performance

advantage, with mean egg production (weeks 25–35) significantly higher in T3 (95.84%) than T2 (90.93%) and T1 (83.81%). Yolk choline content at 35 weeks was highest in T3 (8.24 mg/g) compared with T2 (7.86 mg/g) and T1 (5.78 mg/g). This elevation is nutritionally important because higher yolk choline enhances embryonic development, supports hepatic lipid metabolism in the developing chick and improves the functional nutritional value of table eggs for human consumers. Egg quality traits including albumen and yolk index, yolk colour, Haugh unit and shape index varied significantly across treatments, with BioCholine consistently superior. Natural choline offers multiple practical and nutritional advantages over synthetic choline chloride. Unlike SCC, which is hygroscopic, unstable and prone to degrading vitamins in premixes, BioCholine is non-corrosive, stable and easy to handle. Its phytogetic matrix supports better bioavailability, resulting in superior deposition of choline in egg yolk and improved metabolic efficiency. In conclusion, low-dose BioCholine effectively replaced synthetic choline chloride and enhanced performance, egg quality, yolk antioxidant status and choline deposition, underscoring its value as a potent, stable and efficient phytogetic choline source for layers.

Keywords: yolk choline deposition; natural choline; layer nutrition; egg productivity; choline bioavailability

360P Mixer-added water and larger pellet die thicknesses may dilute and liberate essential nutrients, respectively, for Ross 308 male broilers fed diets manufactured with low-moisture corn

Lucas E. Knarr*¹, Kristina Bowen¹, Elizabeth Lynch¹, Emily B. Estanich¹, Alexis Renner¹, Reuben Adejumo¹, Jon Ferrel², Joseph Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²Azomite Mineral Products, Nephi, Utah, United States

Previous research has highlighted the effect of pellet die thickness (PDT) and Azomite® (AZM) on bird performance and nutrient digestibility. Furthermore, PDT, mixer-added water (MAW), and AZM were recently evaluated for their influence on feed manufacturing responses of feed manufactured with low-moisture corn. However, further testing is required to understand the effect of these factors on broiler production. Therefore, the objective of this experiment was to evaluate the impact of PDT (32, 38, or 45 mm), AZM (0.0% or 0.25%), and MAW (0.0%, 1.50%) on Ross 308 male broiler performance and tibia mineralization from 7 to 21 days of age. Experimentation was completed utilizing a randomized complete block design with eight blocks of the twelve treatments created from the previously mentioned factorial arrangement. Interactional, main effects, and overall treatment comparisons were evaluated, with Tukey's HSD. The interaction of PDT, AZM, and MAW affected FCR ($P = 0.049$), wherein the 45 mm Control treatment had a 5-point decrease in mortality-corrected feed conversion ratio relative to the 45 mm + MAW treatment ($P < 0.05$). However, this difference was not seen in the 32- and 38-mm PDT ($P > 0.05$). The interaction of AZM and MAW influenced feed intake per bird (FI; $P = 0.010$), wherein including MAW in the control feed increased FI by 32 g ($P < 0.05$); however, including AZM, regardless of MAW inclusion rate, provided intermediate results ($P > 0.05$). Additionally, increasing PDT from 32 to 38 mm decreased FI by 31 g ($P < 0.05$), while birds consuming feed manufactured with the 45 mm PDT resulted in an intermediate FI similar to the other PDTs ($P > 0.05$). Lastly, including 0.25% AZM tended to increase tibia mineralization by 34.43 mg/chick compared to no AZM inclusion ($P = 0.052$). In summary, incorporating 1.50% MAW likely decreased bird performance due to a simple nutrient dilution, utilizing larger PDT, in the absence of throughput agents, increased broiler productive

efficiency due to nutrient liberation when consuming feed manufactured with low-moisture corn, and including AZM at 0.25% may increase Ca and P digestibility.

Keywords: Pellet Die Thickness; Azomite; Mixer-added Water

361P The addition of 1.50% mixer-added water as a pelleting aid to feed manufactured with low-moisture corn does not lead to an increase in total aflatoxin when stored under simulated summer and fall conditions

Lucas E. Knarr*¹, Corey Coe¹, Elizabeth Lynch¹, Carly G. Long¹, Emily B. Estanich¹, Md S. Rion¹, Jon Ferrel², Joseph Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²Azomite Mineral Products, Nephi, Utah, United States

While hundreds of mycotoxins have been identified, only a few are routinely monitored in feed and feed ingredients. One such mycotoxin, Aflatoxin, has raised concerns for poultry producers for over 50 years, as it can lead to acute and chronic ailments at levels as low as 20 ppb. Mixer-added water (MAW), used to enhance pelleting productive capacity and quality, could inadvertently create a substrate suitable for Aflatoxin-producing species, posing a microbiologically derived health risk to poultry as well as other species. Therefore, an experiment was conducted to evaluate the effect of dietary treatment (DT; Control, 1.50% MAW, and 1.50% MAW + 0.25% Azomite) and simulated storage season (Summer or Fall) on total aflatoxin (TAF) content, water activity (A_w), and colony counts of feed inoculated with *A. flavus* over a 6-day storage period. A randomized complete block design with six blocks was utilized. A 3 (DT) x 2 (Season) factorial arrangement was used to create the six observational treatments. Later, the four-level factor of Time Point (D0 before inoculation, D0 after inoculation, D3 post-inoculation, and D6 post-inoculation) was incorporated to observe the change in response across simulated storage. All multiple comparisons were evaluated using Tukey's HSD, and significance was determined to be $P < 0.05$. The interaction of Season and Time Point influenced A_w ($P = 0.005$), where Summer storage conditions decreased A_w relative to Fall storage conditions on Day 6 post-inoculation, likely due to water evaporation. Total aflatoxin increased with the 6-day storage period but did not exceed critical levels within the confines of this experiment. In summary, utilizing 1.50% MAW as a pelleting aid may not pose a microbiological risk to poultry when incorporated into feed manufactured with low-moisture corn and stored for less than 7 days. However, incorporating MAW to feed manufactured with a relatively high moisture content or incorporating a higher concentration of MAW may result in microbial proliferation or toxin production, which could ultimately impact animal performance.

Keywords: Aflatoxin Production; Mixer-added Water; Feed Storage

362P Sodium bisulfate improves pellet manufacture, calcium digestibility, and Ross 308 male broiler performance

Emily B. Estanich*¹, Alexis Renner¹, Craig Coufal², Dana Didde², Joseph Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²Jones-Hamilton Co., Maumee, Ohio, United States

Sodium bisulfate (SBS) is used in poultry feed as a mineral acid that may improve feed manufacture as well as enzyme function and nutrient absorption. Four different starter, grower, and finisher diets were formulated according to Ross 308 specifications: positive control (PC), 0.2 less total calcium and available phosphorus negative control (NC), NC + phytase, and NC + phytase + SBS. Formulations containing phytase (1500 FTU) in

each diet phase were steam conditioned and pelleted in triplicate to obtain feed manufacture data. All starter diets were crumbled and fed to 12 replicate raised wire cages of eight Ross 308 male broilers for 18 days in a randomized complete block design to obtain performance, tibia ash, and nutrient digestibility data. All data were analyzed using one-way analysis of variance (ANOVA) on SAS software (version 9.4). Starter and finisher pelleted diets decreased in pH due to SBS inclusion ($P < 0.05$). Pellet mill motor load and hot pellet temperature decreased with SBS in starter and grower diets, respectively ($P < 0.05$). Pellet durability increased with SBS in finisher diets ($P < 0.05$). Day 14 FCR was lower for chicks fed SBS compared to all other treatments ($P < 0.05$). Day 18 LWG increased for chicks fed PC, NC + phytase, and NC + phytase + SBS compared to chicks fed NC ($P < 0.05$). Tibia ash differed as expected between chicks fed PC and NC diets, with diets containing phytase or phytase + SBS being intermediate ($P < 0.05$). Chicks fed NC + phytase + SBS demonstrated the highest calcium digestibility compared to all other treatments ($P < 0.05$). The SBS product utilized in this study enhanced feed manufacture and pellet quality as well as D14 FCR and D18 calcium digestibility, suggesting a benefit to SBS use in both feed manufacture and bird production settings.

Keywords: Sodium bisulfate; Enzyme function; Calcium digestibility; Feed manufacture

363P Protective effects of a Bio-clay product on growth, jejunal histomorphology, bacteriome, and metabolites in aflatoxin-challenged broilers

Iyabo W. Oluseyifunmi^{*1}, Timothy Phillips², Thomas S. Isakeit³, Youjun Deng⁴, Samson Oladokun¹ ¹Texas A&M University, Poultry Science, College Station, Texas, United States; ²Texas A&M University, Department of Veterinary Physiology and Pharmacology, College Station, Texas, United States; ³Texas A&M University, Department of Plant Pathology and Microbiology, College Station, Texas, United States; ⁴Texas A&M University, Department of Soil and Crop Sciences, College Station, Texas, United States

Aflatoxin (AF) remains a major threat to poultry health and performance, prompting growing interest in natural adsorbents such as clay minerals to mitigate its toxic effects. In a 28-day study, 240 day-old off-sex Cobb-500 chicks were used to evaluate the ameliorative effects of dietary Bio-clay (a sodium-rich montmorillonite conjugated with ZnO) against the toxic impacts of aflatoxin (AF). Birds were assigned to 4 treatments with 8 replicate cages each: (1) control (corn-soy diet), (2) control + clay (8 g/kg), (3) AF challenge (2 mg/kg), and (4) AF challenge + clay (2 mg/kg AF + 8 g/kg clay). Growth performance was assessed on days 0, 7, 14, 21, and 28. Jejunal tissues and contents were collected on day 21 for histomorphology and full-length 16S rRNA sequencing, while cecal samples were collected on days 21 and 28 for short-chain fatty acid (SCFA) analysis. From days 0–7, the weight gain (WG), final weight gain (FBW), and feed conversion ratio (FCR) were not significantly ($P > 0.05$) influenced by treatments. From days 7–14, birds in the control and unchallenged clay groups had greater ($P < 0.05$) WG and FBW and lower FCR than other groups, although AF-challenged birds receiving clay achieved FI and FBW comparable to both unchallenged groups. Across days 14–21, 21–28, and cumulatively from 0–28, WG and FBW remained higher ($P < 0.05$) and FCR lower in the control and unchallenged clay-fed birds, while challenged birds fed clay-maintained FI similar to the control. SCFA concentrations at day 21 were not significantly affected; however, branched-chain fatty acids (isovalerate, isobutyrate) were higher ($P = 0.001$) in the unchallenged birds fed dietary clay at day 28. Jejunal histomorphology and bacterial diversity (alpha & beta) were not

significantly ($P > 0.05$) altered. Linear discriminant analysis effect size (LEfSe) indicated a tendency ($P = 0.068$) for clay to increase family *Streptococcaceae* abundance under unchallenged conditions. Overall, these findings indicate that while Bio-clay did not fully counteract AF-induced growth depression, it was able to sustain feed intake, maintain gut integrity, and microbial diversity in AF-challenged birds.

Keywords: Aflatoxin; Bio-clay; Growth performance; bacteriome; short-chain fatty acids

364P Foot pad dermatitis: Is there a role for a feed additive mainly based on carvacrol?

Juliette Foglio^{1,2}, Jean François Gabarrou^{*1} ¹Laboratoires Phodé, AC, TERSSAC, France; ²INPT Agro Toulouse, Animal Science, Toulouse, France

Food pad dermatitis is a multifactorial issue in broiler production, influenced by litter conditions and the quality of droppings. Phodé has developed a feed additive mainly based on carvacrol. The aim of this trial is to evaluate the effect of this dietary supplementation on foot pad dermatitis. This trial was conducted at the NKP Research Farm (Thailand). 336 chicks (Ross 308) were randomly placed in 24 cages ($n=14/\text{cage}$) and assigned to two groups: 12 cages for control group (CTL) fed by a corn-soybean meal diet meeting recommendations for Ross 308, and 12 cages for a treated group (CAR) fed with the same diet with 125 ppm of the Phodé feed additive replacing ground corn in the CTL formulation. Litter amounts were reduced from 6 kg to 3.5 kg/m². Animals and feed were weighted the first day and at the end of each phase to estimate feed intake, final body weight and feed conversion ratio (FCR). Foot pad dermatitis was evaluated as a health indicator using a 0–2 score (0 = healthy footpads; 1 = mild superficial lesions; 2 = severe lesions with haemorrhage and swelling). Data were analyzed using ANOVA in a randomized complete block design (RCBD) using SAS software. P-value inferior to 0.05 was considered significant. The starter and grower phase results showed the same trend as those observed at the end of the finisher phase. Feed intake significantly ($p=0.018$) increased by 2.7% in the CAR group compared to the CTL group, respectively 3.68 ± 0.031 vs 3.58 ± 0.032 kg. Final body weight was also significantly ($p=0.002$) increased by 3 % in the CAR group compared to the CTL group, respectively 2.59 ± 0.016 vs 2.51 ± 0.019 kg. No significant differences were observed between control and treated groups for FCR. This could be explained by the higher feed intake, observed in both groups compared with the Ross 308 standards. Regarding health indicator, the number of birds showing foot pad dermatitis was significantly ($p=0.036$) reduced by 34% in the CAR group compared to the CTL group, respectively 0.59 ± 0.080 vs 0.79 ± 0.067 . Dietary supplementation with carvacrol-based additive improved broiler growth and reduce foot pad dermatitis, suggesting a beneficial impact on animal welfare. More studies are needed to better understand the mechanisms.

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

365P Antioxidant supplementation (Elife®): effects on the reproduction of Rhode Island Red Roosters and the embryonic development of brown egg-laying hens

Alexandre P. Rosa^{*1}, Amanda Dani¹, Cassiane Ditadi¹, Rafaela Barden¹, Marcela Arruda¹, Julia Schneider¹, Daniele Escrobo², Guilherme Deda² ¹Federal University of Santa Maria, Animal Science, Santa Maria, RS, Brazil; ²Impextraco, São Paulo, Brazil

The objective of this study was to evaluate the effects of an antioxidant additive (Elife®) on the productive performance and semen quality of Rhode Island Red roosters, as well as embryonic

mortality and chick weight at hatch in brown egg-laying hens from 54 to 70 weeks of age. Trial 1: Thirty-two roosters were assigned to 10 replicates (one bird per replicate). Every 28 days, body weight, feed intake, and semen quality were assessed. Semen samples were analyzed for sperm vigor, motility (%), ejaculate volume (mL), and the percentage of normal spermatozoa. Trial 2: Two hundred and ten White Plymouth Rock hens were allocated into 10 replicates with seven hens per experimental unit. Every 28 days, body weight, feed intake, laying rate, embryonic mortality, and chick weight at hatch were recorded. Both trials followed a completely randomized design with three dietary treatments: control (no supplementation), 0.5 kg Elife®/ton of feed, and 1.0 kg Elife®/ton of feed, evaluated across four 4-week periods. Data were analyzed by ANOVA, and means were compared using Tukey's test ($P < 0.05$). In males, body weight and feed intake did not differ among treatments. Antioxidant supplementation improved sperm motility ($P = 0.01$), with the 0.5 and 1.0 kg/ton groups showing higher motility (91.05% and 96.30%, respectively) than the control (83.67%). Ejaculate volume increased ($P = 0.04$) in roosters supplemented with 0.5 kg/ton (0.63 mL vs. 0.40 mL in the control). The percentage of normal spermatozoa was also higher in the 1.0 kg/ton group (96.30%) compared with the control (87.50%). In females, feed intake was reduced in birds receiving 1.0 kg/ton. Elife® supplementation increased chick weight at hatch ($P < 0.01$) and reduced early embryonic mortality ($P = 0.03$) in the 1.0 kg/ton treatment. Overall, Elife® supplementation represents a promising nutritional strategy to enhance semen quality, embryonic viability, and chick quality in brown egg-laying breeders.

Keywords: brown egg; polyphenols; reproductive; roosters

366P A proprietary blend of botanical extracts optimizes cocci-preventive strategies and maintain growth performance in *Eimeria*-challenged broilers

Bertrand Medina^{*1}, Ivan Girard¹ ¹*Probiotech International Inc., Innovation and Development, Saint-Hyacinthe, Quebec, Canada*

Two consecutive trials evaluated one proprietary botanical extract blends combined or not with a cocci-vaccine in *Eimeria*-challenged broilers. Trial#1 involved 2,475 Ross 308 males (55 birds/pen, 9 replicates) assigned to: 1) non-infected (NI); 2) infected (IF); 3) IF+decoquinat/zoalene shuttle program (DZ); 4) IF+500 ppm (SYN500). In trial#2, 1,980 *Eimeria*-vaccinated (IMMUCox3®, CEVA) birds were assigned to: 1) NI; 2) IF; 3) SYN250. *Eimeria* infection was induced by an oral administration at d14 of 2×10^5 sporulated oocysts of mixed *Eimeria* species. Data were analyzed by a mixed model including treatment and barn section as fixed effects and by Kruskal-Wallis tests. In trial#1, IF reduced body weight (BW) at d20 (-40 g, -4.9%, $P=0.0042$), d34 (-106 g, -4.5%, $P=0.0032$), worsened feed conversion ratio (FCR) during 10-20d (+0.06, +3.3%, $P=0.0154$), decreased liveweight per pen at d34 (-12.7 kg, -13.3%, $P=0.0028$) increased mortality rate overall (+8.6%, $P=0.0395$), and increased levels of oocysts per gram of feces (OPG) at d20 (+4.2 log₁₀, $P<0.0001$) and intestinal *E. acervulina* (+1.1, $P<0.0001$) and *E. tenella* (+1.2, $P<0.0001$) scores at d21, compared to NI. DZ decreased these *E. spp* inoculation. SYN500 increased BW at d34 (+82 g, +3.6%, $P=0.0195$) and SYN250 showed a trend (+58 g, +2.6%, $P=0.0904$), decreased mortality rate overall (-9%, $P<0.05$), and increased liveweight per pen at d34 (+12.4 kg, +15.0%, $P=0.0033$), without affecting FCR, compared to IF. In trial#2, IF reduced BW 6 dpi (d20) (-47 g, -6.0%, $P=0.0456$), worsened FCR (10-20d) (+0.13, +9.6%, $P=0.0478$), levels of OPG at d20 (+0.7 log₁₀, $P<0.0001$) and intestinal *E. tenella* score at d21 (+1.1, $P=0.0002$), compared to NI. SYN250 increased BW at d10 (+9 g, +3.9%, $P=0.0012$) and at d20 (+56 g, +7.6%, $P=0.0443$),

and improved FCR during 0-10d (-0.07, -5.3%, $P=0.0001$) and 10-20d (-0.15, -10.0%, $P=0.0261$), compared to IF. SYN250 did not affect OPG or intestinal lesions compared to IF. Overall, SYN - designed for standard production - supports coccidiosis vaccination and should be introduced to any coccidiosis control strategies without anticoccidial drugs.

Keywords: Broilers; standard; botanicals; botanicals

367P Effect of phytase on nutrient digestibility and productive performance in broilers fed reduced metabolizable energy diets

Mayra Diaz-Vargas^{*1}, Juan A. Javierre², Andrés F. Gutiérrez Jiménez³, Carlos F. Duque-Ramírez¹ ¹*Universidad Cooperativa de Colombia, Medicina Veterinaria y Zootecnia, Ibagué, TOLIMA, Colombia;* ²*Tekzol SAS, Bogotá, D.C., Bogotá DC, Colombia;* ³*Universidad Nacional Abierta y a Distancia - UNAD, Especialización en Nutrición y Alimentación Animal Sostenible, Bogotá, D.C., Bogotá DC, Colombia*

The objective of this study was to evaluate the effect of phytase inclusion in reduced metabolizable energy (ME) diets on growth performance, nutrient digestibility, and economic returns in broiler chickens. Four treatments were evaluated: a positive control (PC) containing 1,000 FTU/kg of phytase and three experimental diets (ED1, ED2, ED3) with ME reductions of -20, -40, and -60 kcal/kg, respectively. Each treatment consisted of 60 replicates. Birds were reared under controlled environmental conditions with continuous monitoring of temperature and humidity. During the starter phase, ED2 resulted in the greatest body weight gain (BWG) and best feed conversion ratio (FCR). Across the entire period, FCR improved linearly as ME decreased ($p = 0.0017$), with ED3 demonstrating the highest feed efficiency and outperforming PC. At 21 days, apparent ileal digestibility showed a positive linear response for crude protein ($p = 0.0189$) and a significant quadratic response for ether extract ($p = 0.0060$), with an optimum estimated at 2,970.8 kcal/kg ME. At 42 days, ether extract digestibility again showed a quadratic response ($p = 0.0109$), with an optimum at 3,134.6 kcal/kg. Gross energy digestibility also improved significantly across reduced-ME diets. Economically, all experimental diets produced higher net returns than PC, with ED3 yielding the greatest profit per bird (USD 0.345), representing a 13.1% improvement over PC. In conclusion, phytase supplementation enhanced crude protein, ether extract, and gross energy digestibility, improving growth performance and production efficiency. Strategic reductions in ME to 2,970.8 kcal/kg during the starter phase and 3,134.6 kcal/kg during the grower-finisher phase optimized lipid digestibility and supported a linear improvement in protein digestibility. Diets ED1 and ED3 produced superior economic outcomes, with ED3 achieving the highest profitability without increasing production costs. These findings demonstrate that phytase inclusion, together with targeted ME reductions, is an effective and economically viable strategy for optimizing broiler productivity.

Keywords: Digestibility; protein; energy; metabolizability; phosphorus

368P Efficacy of a blend of a botanical species, citric acid and thymol (AMN) for the control of a necrotic enteritis infection model as a treatment in drinking water and as a preventative in feed

David Díez^{*1}, Claudia De Andrade¹, Anna Tesouro¹, Richard Churchill² ¹*Biovet S.A., Technical department, Constanti, Tarragona, Spain;* ²*Madras Veterinary College, Chennai, India*

480 Vencobb400Y broilers were distributed following a RBD into 4 groups each having 6 replicates of 20 chicks: non-infected (T1);

infected (T2); infected with AMN at 0.5 kg/t offered, continuously (T3); infected receiving AMN Liquid in water at 1ml/l as treatment. Birds in T2, T3 and T4 were challenged with toxigenic strains of *C. perfringens* (1.0×10^8 CFU/ml) inoculated on days 14, 15 and 16 of age. Results showed the birds fed with AMN in feed (T3) withstood bacterial challenge as evidenced by no reduction in body weight on 21 day and thereafter. Supplementation of AMN in water (T4) and feed (T3) evidenced significantly ($p < 0.05$) higher body weight in T3 (+8.03%) and T4 (+6.03%) compared to control. T3 had significantly ($p < 0.05$) better cumulative FCR (1.77) at 40 days compared to T1 and T2 with 1.96 and 1.99 respectively. T4 had intermediary value (1.85). Total proteins, cholesterol and triglycerides levels were restored to normal as that of control in T3. The mean lesion scoring was 0.00 ± 0.00 , 2.33 ± 0.24 and 1.02 ± 0.16 in control (T1), bacteria challenged group (T2) and AMN feed supplemented group (T4) on d17. Results showed that AMN as a treatment in infected birds (T4) was effective in controlling NE, as mortality was reduced by 38.5% in T3 (6.66%) and by 69.2% in T4 (3.33%) compared with

T2 (10.83%), indicating a statistically meaningful improvement in survival. In T2, histopathological examination of intestine revealed complete loss of the intestinal architecture with hemorrhages and total necrosis with ulceration at the tip in the villi and reduction or complete loss of GALT and goblet cells. Capillary engorgement and clubbing of the villi with desquamated intestinal epithelial cells in the lumen were noticed. Whereas, the villi appeared almost normal in birds challenged with *Clostridium perfringens* (T4) but supplemented with AMN in feed as preventative. Haemorrhages were not seen prominently as that of T2 and GALT tissue was seen normally and goblet cells were seen in adequate numbers. CFU of *C. perfringens*/g of caecal content on d17 was 0.012, 2.269 and 1.479 in T1, T2 and T4 respectively. In conclusion, AMN in feed can be used as a preventive for NE in poultry farms.

Keywords: Gut health; Necrotic enteritis; Natural antimicrobials; Pathology; Prevention and treatment

Metabolism and Nutrition: General Nutrition

369P Prosin, a lysine-rich single-cell protein as a sustainable soybean meal alternative in broiler nutrition

Kye J. Lee^{1,2}, Jea Hong Park^{1,2}, Chorong Park³, Eunyoung Park³, In Ho Kim^{*1,2} ¹Dankook University, Animal Biotechnology, Cheonan, Choongnam, Korea (the Republic of); ²Smart Animal Bio Institute, Dankook University, Cheonan, Choongnam, Korea (the Republic of); ³CJ Cheiljedang Bio, Seoul, Korea (the Republic of)

Soybean meal (SBM) has traditionally been the primary protein source in poultry diets due to its favorable amino acid profile and availability. However, the sustainability of SBM is increasingly challenged by fluctuating market prices and environmental concerns (Samtiya et al., 2020). These issues highlight the need for alternative protein sources that can partly replace SBM without affecting animal performance. Recently, single-cell proteins (SCPs) from microorganisms such as bacteria, yeasts, and algae have emerged as promising alternatives (Anupama & Ravindra, 2000) however the application of SCPs in broiler nutrition remains limited. Thus, we intend to evaluate the effect of Prosin as a protein source on growth performance, nutrient digestibility, excreta noxious gas emissions, and meat quality traits in broilers. A total of 630 one-day-old mixed-sex Ross 308 chicks (initial BW: 47.22 ± 0.50 g) were assigned to five dietary treatments 0, 1, 3, 5, and 7% Prosin, each with 7 replicates of 18 birds per pen. Birds had ad libitum access to feed and water and were fed in three phases: starter (d 1–7), grower (d 8–21), and finisher (d 22–35). Data were analyzed using one-way ANOVA (SAS 9.4), and treatment means were compared using Tukey's HSD test. $p < 0.05$ was set as statistically significant. Growth performance including body weight, daily gain, feed intake and feed conversion ratio were not significantly affected by Prosin inclusion ($p > 0.05$). Also, the apparent digestibility of dry matter, nitrogen, and energy remains unaffected by the dietary treatments ($p > 0.05$). Moreover, no significant differences were observed in the relative organ weight, fecal gas emissions, fecal consistency, or breast meat quality traits. These findings suggest that Prosin can effectively replace SBM in broiler diets without adverse effects on performance, nutrient utilization, or product quality. In conclusion, Prosin appears to be a sustainable and efficient alternative protein source for poultry, capable of maintaining animal performance and environmental standards, making it a viable substitute in broiler nutrition.

Keywords: broilers; growth performance; nutrient digestibility; Single cell Protein; alternative protein

370P Growth performance and economic benefits of finisher broilers fed sundried false yam (*Ipocina tricantha*) meal based diets

Stanley O. Omoikhoje^{*1}, Dick O. Obasoyo¹, Eromosele T. Obasoyo¹ ¹Ambrose Alli University, Ekpoma, Edo State, Nigeria, Animal Science, Ekpoma, Edo, Nigeria

The impact of varying concentrations of sun-dried false yam meal (SFYM) on the growth performance and economic benefits of finisher broilers were investigated in an eight weeks feeding trial. A total of 150 four weeks-old broiler chicks were randomly assigned to five dietary treatments with SFYM concentrations of 0, 4, 8, 12, and 16%. Each treatment was replicated three times in a completely randomized design (CRD) with 10 birds per replicate. Results indicated that the partial replacement of SFYM for maize significantly ($P < 0.050$) affected the growth performance of broiler chicken with respect to the average live weight, daily weight and feed intake, while the feed conversion ratio (FCR) and protein efficiency ratio (PER) were not ($P > 0.05$) affected. The overall result in this study indicated that sun-dried false yam meal can successfully be included in broiler chicken rations up to 4% level without any adverse effect on growth performance and economic benefit of the birds.

Keywords: Broilers; false yam; growth; processing; substitution

371P Different pelleting temperature with variable conditioning period effects on post peak production performance, egg quality parameters, nutrient digestibility and pellet durability index in commercial layer

Sohail Ashiq^{*1}, Ahsan Jamal¹, Muhammad Farhan Shamshad¹, Anjum Khalique¹, Ehsaan Ullah Khan¹ ¹University of Veterinary and Animal Sciences Lahore, Faculty of Animal Production and Technology, Lahore, Punjab, Pakistan

Variation in pelleting temperature and conditioning times may affect pellet quality, nutrient digestibility and animal performance. Current study designed to evaluate the effects of two different conditioning periods (30s, 60s) with two different pelleting temperatures (75 degrees centigrade, 90 degrees centigrade) on post peak production performance, egg quality parameters, nutrient digestibility and pellet durability index in commercial layer. There were 192 birds (LSL lite) were placed in four different treatments with 48 birds per treatment, each treatment was subdivided in three replicates with 16 birds per replicate. Treatments were arranged as a 2×2 factorial with 2 conditioning

period (30s, 60s) with two different pelleting temperatures (75 degrees centigrade, 90 degrees centigrade). Diets were formulated according to production stage of the birds containing 16.5% crude protein and 2800 kcal/kg metabolize energy. Conditioning period × pelleting temperature interactions were observed for production performance, egg quality parameters, nutrient digestibility and pellet durability index of commercial layer. There were non-significant effect of conditioning period and pelleting temperature on overall production performance of layers. Pelleting temperature and conditioning period had no effect on egg weight, yolk color, albumen height and Haugh unit. However, interaction of pelleting temperature and conditioning period affects the Haugh unit. Conditioning period and pelleting temperature had strong influence on pellet durability index. The birds fed diets manufactured at 90 degrees centigrade pelleting temperature had higher pellet durability index than 75 degrees centigrade irrespective of conditioning period. Birds fed diet manufactured at 60s conditioning time had higher pellet durability index than 30s. Conditioning period, pelleting temperature and its interaction did not influence on nutrient digestibility parameters crude protein, ether extract and ash contents. Conditioning period, pelleting temperature and its interaction did not exert any significant effect on production performance, egg quality parameters and nutrient digestibility, however, increase of conditioning period and pelleting temperature had positive effect on pellet durability index.

Keywords: Conditioning period; Pelleting temperature; Production performance; Egg quality

372P Utilization of near-infrared spectroscopy to predict mycotoxins in corn DDGS: a three years survey

Isadora Fabris Laber*^{1,2}, Cristina T. Simoes^{1,2}, Denize Tyska^{1,2}, Adriano Mallmann¹, Carlos A. Mallmann² ¹*Pegasus Science, Santa Maria, Rio Grande do Sul, Brazil;* ²*Federal University of Santa Maria, Department of Preventive Veterinary Medicine, Santa Maria, Brazil*

The search for alternative raw materials in poultry feed has led to increased use of corn by-products, such as dried distillers' grains with solubles (DDGS). This study used near-infrared spectroscopy (NIRS) calibration curves to predict mycotoxins in samples of corn DDGS from different countries during three consecutive years. A total of 1,363 DDGS samples were obtained through routine analyses performed in feed mills in Brazil (n=1294), Mexico (n=35), El Salvador (n=14), France (n=11), Paraguay (n=5), Portugal (n=3), and Colombia (n=1) during 2023, 2024, and 2025 (data up to 31st October). Aflatoxin B₁ (AFB₁), fumonisins (FBs - sum of FB₁ and FB₂), zearalenone (ZEN), and deoxynivalenol (DON) contamination levels were predicted using previously developed NIRS calibration curves based on partial least squares regression with cross-validation. HPLC-MS/MS served as the reference method. Correlation coefficients for AFB₁, FB₁, FB₂, DON, and ZEN were 0.85, 0.88, 0.88, 0.84, and 0.85, respectively, and limits of quantification (LQ) (µg/kg) were, respectively, 5, 200, 200, 250, and 30. Samples were milled and scanned using Foss and Bruker equipment. DON was only assessed in samples from 2024 and 2025. Descriptive statistics was performed using the software Statgraphics Centurion. FBs were the most prevalent mycotoxin, with 99% of the samples testing positive in 2023 (mean concentration 2,382±878 µg/kg), 97% in 2024 (mean concentration 1,980±753 µg/kg), and 99% in 2025 (mean concentration 2,527±838 µg/kg). DON was also frequently detected, with 60% positive samples (mean concentration 253±179 µg/kg) in 2024 and 61% (mean concentration 213±200 µg/kg) in 2025. The occurrence of AFB₁ and ZEN was low. AFB₁ occurrence was 1% in 2023 and 2024, and 0% in 2025, while ZEN occurred in 1% (2023), 0.9% (2024), and 0.4% (2025) of the

samples, both at very low mean concentrations (<LQ to 0.03 µg/kg for AFB₁ and 0.28 to 4.8 µg/kg for ZEN). The only relevant co-occurrence was observed between FBs and DON, found in 55.8% of the samples. The present results highlight the critical importance of monitoring mycotoxins, especially FBs and DON, in corn DDGS intended for poultry nutrition. These findings may contribute to defining a safe inclusion level of this ingredient in poultry diets.

Keywords: DDGS; fumonisins; mycotoxins; NIRS; poultry feed

373P Microbiome exploration to identify biomarkers related to crude protein level variation in broilers

Fernanda L. Castro*¹, Jean E. de Oliveira¹, Hsuan Chen¹, Anne I. Goderis¹, Henk Enting¹, Luisa A. Gene¹ ¹*Cargill, Wayzata, Minnesota, United States*

This study conducted a database exploration exercise of different nutrition research trials to identify potential microbiome biomarkers changes related to variations in the crude protein (CP) levels of the diets. The explored database included 2736 microbiome samples from 5 different Ross 308 research trials. The CP levels by age were distributed, averaged, and a cutoff level was determined. For 7, 14, 21, 28, and 35d, the min/max CP levels were: 21.2/22.4, 21.5/24, 20/21.5, 20/21.5, and 19/ 21.5%, respectively. Thus, CP1 and CP2 groups were established at being below and above the cutoff CP level of 21.5%, 22.8%, 20.6%, 20.6%, and 20.6% for each sampling d of age, respectively. Microbiome data was obtained from cloaca swab samples from one bird/pen at each timepoint (min 18 birds/treat). Microbiota analysis was done using a commercially available platform that assesses key bacteria biomarkers by a fluorescence customized microarray (Galleon™). Body weight (BW) was individually assessed in all sampled birds. Relative intensity for each bacteria DNA probe was submitted to ANOVA in a factorial arrangement with fixed effect of CP level, age and their interaction. Array was added as a random effect. Pairwise comparisons between standardized LS-means were made for each bacteria and variable combination adjusting for FDR (False Discovery Rate) test with P = 0.05. At 14d, BW of the CP2 group was 49g higher than CP1, and at 35d, the CP1 group was 130g heavier than CP2 (P<0.05). Throughout the study, CP2 had a higher relative prevalence of lactate producing bacteria (P<0.05) compared to CP1. In contrast, CP1 had a higher relative prevalence of proteolytic bacteria at 7d, and from 14d onwards, showed higher prevalence of proteolytic and short chain fatty acid producing bacteria (SCFA) than CP2 (P<0.05). The higher *Lactobacillus* population on CP2 at later ages (21 and 35 d) was not expected as it is usually a sign of delayed microbiome maturation linked to lower levels of SCFA-producing bacteria. Additionally, *Campylobacter jejuni*, one of the microbial biomarkers assessed, was highest in CP2 at 35d. Thus, based on the studies in this database, CP differences influenced the microbiota by modulating, mainly, lactate and SCFA-producing bacteria in broilers up to 35d of age.

Keywords: biomarkers; broilers; crude protein; microbiome; microarray

374P Effects of dietary black soldier fly (*Hermetia illucens*) larvae meal on growth performance and gut health of broilers under intestinal challenge

Yuri K. Dalmoro¹, Guilherme L. Godoy¹, Jessica C. Agilar¹, Valeria Biselo¹, Jessica A. Alencar¹, Catarina Stefanello*¹ ¹*Federal University of Santa Maria, Santa Maria, RS, Brazil*

The inclusion of black soldier fly (BSF) larvae meal in poultry diets has gained attention as an alternative protein source due to its

high nutritional value, efficient bioconversion of organic substrates, and potential functional contributions to gut health. This study evaluated the effects of including 5% BSF larvae meal in broiler diets on growth performance, nutrient digestibility, energy utilization, intestinal integrity, gene expression, lipid profile, and short-chain fatty acid (SCFA) production under an induced intestinal challenge. Eight hundred one-day-old male broilers were assigned to four dietary treatments with eight replicates (25 birds/pen) and reared in floor pens. Birds received either a basal corn–soybean meal diet or a diet containing 5% BSF larvae meal, which partially replaced conventional protein and energy sources. Each diet was provided to nonchallenged birds or to birds exposed to an intestinal challenge consisting of oral inoculation with *Eimeria* spp. on day 1, followed by *Clostridium perfringens* on days 11 and 14 (1×10^8 CFU/mL; 3 mL/bird per day). Growth performance was monitored until day 40, while nutrient digestibility, breast muscle lipid profile, intestinal histomorphology, and gene expression were assessed at day 21. The concentration of SCFA was measured at both 21 and 40 days. A two-way analysis of variance was performed using the MIXED procedure of SAS, with diet and challenge as the main effects. Mean comparisons were conducted using Fisher's LSD test ($P \leq 0.05$). No interactions between the diet and challenge were observed in this study ($P > 0.05$). The intestinal challenge induced dysbiosis and impaired performance, whereas the inclusion of BSF meal partially alleviated these detrimental effects. In the overall period, broilers fed the BSF diet had an improved feed conversion ratio and exhibited higher cecal SCFA concentrations and increased lauric and myristic acid levels in breast muscle ($P \leq 0.05$). Reduced interleukin-6 gene expression was observed with the BSF meal. In conclusion, using 5% BSF larvae meal supported broiler performance without compromising nutrient digestibility or intestinal morphology, while enhancing cecal butyrate and acetate levels and promoting a more favorable lipid profile.

Keywords: broiler; digestibility; gene expression; muscle lipid profile; insect meal

375P Trends in mycotoxin contamination in 2025 United States corn

Chasity Pender*¹, Paige Gott¹, Lan Zheng¹ ¹*dsm-firmenich, Animal Nutrition & Health, Pendergrass, Georgia, United States*

Mycotoxins—secondary fungal metabolites—are common contaminants in feed ingredients and pose significant risks to animal health. While classic signs such as reduced feed intake and gastrointestinal lesions are well recognized, they often underestimate broader impacts, including heightened disease susceptibility, inflammation, and alterations to gut ecology. This study aims to assess mycotoxin prevalence and contamination levels in U.S. corn from the 2025 harvest and compare findings with prior years. Samples were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A (OTA). Statistical analysis was performed using GLIMMIX procedure of SAS with harvest year as a fixed effect and sample as the experimental unit. Means were separated using Tukey-Kramer with significance reported at $P \leq 0.05$. A limited number of samples are available thus far ($n = 45$) as analysis of this harvest is still ongoing, therefore the risk profile of this crop year is likely to change as the sample pool expands. Preliminary results indicate that 98% of samples evaluated contained at least one mycotoxin, which is similar to the prevalence observed in 2024. Co-occurrence in corn thus far is significantly less than seen in 2024 (45 vs. 77%). Currently, FUM is the most prevalent group (84 vs. 65% in 2024), followed by ZEN (40 vs. 73% in 2024), and B-Trich (38 vs. 70%

in 2024). The occurrence of A-Trich has numerically decreased in 2025 vs. 2024 (11 vs. 16%), while occurrence of Alfa has numerically increased (16 vs. 5%). As the mycotoxin risk of this harvest season is still coming into focus, these trends may shift; however, preliminary results of the 2025 survey indicate a continued risk of mycotoxin contamination. Ongoing monitoring is essential to characterize risk and guide mitigation strategies as new crop corn is fed out over the coming months.

Keywords: Corn; Mycotoxins; LC-MS/MS; Prevalence; United States

376P Efficacy of a natural calcium & phosphorus bioavailability enhancer (MagaCal) as a replacement for synthetic vitamin D3 on performance, egg quality and yolk vitamin D content in layers

Paramartha Roy*¹, Shivi Maini¹ ¹*Indian Herbs Specialties, Chandigarh, Chandigarh, India*

A 20-week controlled study was conducted to evaluate the efficacy of a natural calcium and phosphorus bioavailability enhancer (MagaCal, M/S Indian Herbs Specialities) as a replacement for synthetic vitamin D3 on performance, egg quality and yolk vitamin D3 content in BV300 layers. One hundred birds were allotted to two dietary groups (50 birds/group; 5 replicates of 10). Group T1 received a standard basal ration containing synthetic vitamin D3, whereas Group T2 received a basal ration devoid of synthetic vitamin D3 & supplemented with MagaCal, formulated to completely replace synthetic vitamin D3. MagaCal is a unique synergistic combination of phytochemicals & a feed supplement product. The trial was conducted from 16–35 weeks at an accredited CRO in Bengaluru. Weekly performance traits (egg production, HHE, feed intake and feed efficiency) and egg quality parameters at weeks 27 and 35 were assessed. Yolk vitamin D3 content was analysed at both time points. Data were statistically interpreted following Snedecor and Cochran procedures ($p < 0.05$). MagaCal supplementation resulted in a clear performance advantage, with mean egg production (weeks 25–35) significantly higher in T2 (95.84%) than T1 (90.93%). Shell weight was also significantly greater in T2 (7.14 g) compared to T1 (6.66 g), alongside improvements in egg weight and yolk index. Yolk vitamin D3 levels were significantly higher in T2 at both 27 weeks (0.62 $\mu\text{g/g}$ vs. 0.55 $\mu\text{g/g}$) and 35 weeks (0.73 $\mu\text{g/g}$ vs. 0.56 $\mu\text{g/g}$). The consistently higher yolk vitamin D3 deposition demonstrates functional effectiveness of medicinal plant-based bioactives that mimic vitamin-D3-like activity, supporting superior mineral absorption, better eggshell formation, maintenance of peak lay & enrichment of eggs with enhanced nutritional value. Overall, This study highlights the potential of phytochemical vitamin-D3 mimetics to deliver reliable mineral nutrition and consistent production performance without relying on synthetic vitamin D3. The findings reinforce MagaCal as a robust, nature-derived strategy for improving eggshell quality & yolk nutrient enrichment while supporting sustainable layer nutrition programs. MagaCal proved to be a sustainable & efficient natural alternative to synthetic vitamin D3 in commercial layer nutrition.

Keywords: natural calcium–phosphorus enhancer; synthetic vitamin D₃; egg vitamin content; shell quality; layer performance

377P Fatty acid profile of soybean oil extracted from soybeans differing in maturity groups

Md Raihanul Hoque¹, Alexis McDade², Virginia R. Sykes³, Ondulla Toomer⁴, Lisa Dean⁴, Pramir Maharjan*¹ ¹*Texas A&M University, Department of Poultry Science, College Station, Texas, United States;* ²*Medvet, Fairfax, Ohio, United States;* ³*University of Tennessee, Department of Plant Sciences, Knoxville, Tennessee, United States;* ⁴*United States Department of Agriculture, Food*

Science & Market Quality and Handling Research Unit, Raleigh, North Carolina, United States

Soybean meals are primarily used as a protein source in poultry diet, while full-fat soybean meals and soybean oil serve as energy sources. The nutritional composition of soybeans can vary depending on growing location, variety, season, and maturity group (MG). This study aimed to evaluate the variation in fatty acid profile of expeller-extracted soybean oil while producing soybean meals from different soybean varieties belonging to four different maturity groups. Soybeans from 12 different soybean varieties belonging to four maturity groups MG 3, MG 4, MG 4.5, and MG 5 were analyzed for oil content and fatty acid composition. Three representative oil samples from three soybean varieties belonging to each MG were analyzed for fatty acid profile as percent of total fat (w/w) with a GC-FID system. Mean values were separated by one-way ANOVA Tukey's HSD test at $P < 0.05$. MG 4 and MG 4.5 soybeans exhibited significantly higher ($P < 0.05$) levels of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), vaccenic acid (11c-18:1), and linolenic acid (18:3n3) compared to MG 3. In contrast, MG 3 soybeans had higher ($P < 0.05$) concentrations of stearic acid (C18:0) and linoleic acid (18:2n6) than MG 4 and MG 4.5. MG 5 soybeans contained the highest ($P < 0.05$) levels of stearic acid (C18:0), arachidic acid (C20:0), and behenic acid (C22:0). Overall, MG 3 soybeans were enriched in polyunsaturated fatty acids (PUFAs) and had the lowest saturated fatty acid (SFA) content, whereas MG 4 soybeans had the highest SFA and lowest PUFA content. Though MG 5 had highest levels of some SFA, in terms of total SFA, MUFA or PUFA, MG 5 was not different from any other groups. These findings suggest that MG 3 soybean oil may be preferable for health-beneficial PUFA content, while MG 4 soybean oil may be more suitable when better oxidative stability is desired. This information could influence soybean agronomic and production management practices for a more targeted nutritional profile of soybeans and extracted oil. Further studies are warranted to determine the dietary effects of PUFA components of MG 3 soybean oil as we supplement poultry diets with soybean oil.

Keywords: fatty acid; poultry feed; PUFA; SFA; Soybean maturity groups

378P Sorghum as a complete replacement for corn in layer diets with and without pigment supplementation

Maria Alvarenga^{*1}, Ahmed Ali¹, Mireille Arguelles-Ramos¹ ¹*Clemson University, Animal and Veterinary Sciences, Clemson, South Carolina, United States*

Global fluctuations in corn availability and cost have increased interest in alternative grains for poultry nutrition. Sorghum offers comparable metabolizable energy and protein to corn but differs in pigment content, which may influence egg quality traits such as yolk color. Evaluating its suitability as a full corn replacement is therefore relevant to both economic and production efficiency. The objective of this study was to determine whether sorghum, with or without added pigment, affects feed intake, body weight, and egg quality in laying hens. A total of 480 Hy-Line Brown hens were assigned to four dietary treatments from 20 to 30 weeks of age: corn without pigment (C), corn with pigment (C+P), sorghum with pigment (S+P), and sorghum without pigment (S). Each treatment included six replicate pens of 20 hens/pen. Diets were formulated to be isocaloric and isonitrogenous, and data were analyzed using one-way ANOVA with Tukey's multiple comparison test. Feed intake did not differ among treatments at either 20 or 30 weeks ($p = 0.025$ and 0.041), and body weight remained within guideline ranges ($p < 0.05$). Egg weight, albumen height, shell thickness, and shell weight were not affected by diet ($p > 0.05$). Yolk color showed a strong treatment effect ($p < 0.001$), with sorghum-only

hens producing the palest yolks. Breaking strength ($p = 0.032$) and yolk weight ($p = 0.019$) were higher in pigment-supplemented groups. These findings demonstrate that complete corn replacement with sorghum does not negatively affect feed intake, growth, or external egg quality but results in reduced yolk pigmentation unless dietary pigments are included. In conclusion, sorghum is a suitable and nutritionally viable substitute for corn in layer diets when formulated to guideline specifications, but pigment supplementation remains essential to achieve commercially acceptable yolk color. Further work should evaluate responses to different sorghum varieties and pigment inclusion levels to optimize egg quality outcomes.

Keywords: sorghum; laying hen; egg quality; pigment supplementation; feed efficiency

379P Changes in broiler breeder feeding phases alters protein and lipid turnover leading to production differences

Cole Umberson^{*12}, Diego Martinez³, Joshua Wilkes³, Craig N. Coon³ ¹*University of Arkansas, Poultry Science, Lincoln, Arkansas, United States;* ²*Plaza Feed and Crop Nutrition, Lincoln, Arkansas, United States;* ³*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States*

A study was conducted to compare industry feeding strategies including: feed allocation, body weight gain (BWG), and metabolizable energy on breeder performance, protein, and lipid turnover. A completely randomized design with a factorial arrangement 2×5 (2 studies and 5 feeding phases) was used. Data were analyzed using one-way ANOVA and means were separated using Student-t test. Lipid turnover was measured via intraperitoneal infusion of deuterium oxide (99%) H₂O achieving 2.5% atom percent excess (APE), 5% deuterium water was supplemented for drinking water to maintain 2.5% APE. Breeders 4/age/treatment (trt) were euthanized by CO₂ asphyxiation, fat pad was sampled 24 hours post-infusion for synthesis and 1 bird was sampled 7 days post infusion for triglyceride degradation. Protein turnover was measured by infusion of isotopic ¹⁵N phenylalanine in the brachial vein, 4 breeders/age/trt were infused and euthanized by CO₂ asphyxiation. Synthesis was determined by ¹⁵N PHE incorporation in mixed skeletal protein; protein degradation was determined by loss of 3-methylhistidine. Body composition was measured using dual energy x-ray absorptiometry (DEXA); body crude protein, ash, and fat content/trt was determined by scanning 10 birds/trt/age. Breeders fed less grams/bird did not utilize lipid degradation from the fat pad compared to the other treatments ($p < 0.05$). Less grams/bird required breeders to rely on skeletal protein degradation and feed allocation causing early high egg production ($p < 0.05$). Average grams/bird breeders utilized lipid degradation to reduce expensive skeletal protein degradation ($p < 0.05$). Average grams/bird breeders gained 39% body weight from 20-25 weeks providing necessary amount of gain to buffer protein turnover with lipid turnover. The average and high grams/bird breeders are expensive to produce due to feed costs but have egg production longevity ($p < 0.05$). In conclusion, the average grams/bird breeders stored and mobilized lipids efficiently buffering protein turnover to reduce the loss of costly amino acids.

Keywords: Lipid turnover; Protein turnover; Broiler breeder; Isotopes; Feed allocation

380P Three evaluated approaches to refine the classical net energy system fail to match the predictive strength of productive energy in broilers

Diego Martinez^{*1}, Carol Ponce-de-Leon², Oscar H. Aguilar¹, Craig N. Coon¹ ¹*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States;* ²*Independent Researcher, Fayetteville, Arkansas, United States*

Classic net energy (CNE), calculated as apparent metabolizable energy (AME) minus the heat increment (HI), has shown limited ability to predict protein accretion (PAC) while performing better for fat gain (FG). This study evaluated three modifications intended to improve the predictive capacity of CNE for PAC and FG and compared them with productive energy (PE), defined for broilers as net energy for gain (NEg) + net energy for maintenance (NEm), both directly measured. Five energy systems were assessed: (1) NE-1, the classical NE estimate using HI calculated from calorimetry total heat production minus a reference fasting heat production (FHP); (2) NE-2, using the measured fasting heat production (FHPm); (3) NE-3, using directly determined NEg but still FHP; (4) NE-4, derived from AME efficiency to produce BW gain; and (5) PE. In this study, PE was determined as Arkansas Net Energy (NEg measured with dual-energy X-ray absorptiometry, DEXA; NEm measured with calorimetry chambers using FHPm). Eight experiments were conducted, each starting with 240 day-old chicks allocated to 12 pens and fed one of 12 diets differing in digestible amino acids (or dCP), digestible starch, digestible fat, and non-starch polysaccharides. All experiments began

simultaneously, and each 7-day feeding period was initiated one week apart. A digestibility study determined AME and AMEn. Recorded variables included BW gain (BWG), PAC (g/bird per day), FG (g/bird per day), NEg (kcal/kg; DEXA), FHPm, AME/BWG efficiency, NE-1 through NE-4, and PE. Data were analyzed using a mixed model with 12 treatments, 8 replications, and experiment as a random effect. PE strongly predicted PAC ($P = 0.006$; adj. $R^2 = 0.98$) and FG ($P < 0.001$). NE-1 ($P > 0.05$) and NE-2 ($P = 0.031$) predicted FG. NE-1 ($P > 0.20$) and NE-2 ($P = 0.15$) did not predict PAC. NE-3 improved the P-values for PAC prediction relative to NE-1 and NE-2, but it still failed to significantly predict PAC ($P > 0.05$). NE-4 likewise did not predict PAC ($P > 0.05$). In conclusion, none of the refined NE systems based on metabolizable energy matched the predictive strength of PE for protein accretion in broilers. Energy systems derived from metabolizable energy fail to predict PAC efficiently.

Keywords: Productive energy; Net energy; Protein accretion; Meat production; Fat gain

Metabolism and Nutrition: Vitamins and Minerals

381P Zinc, calcium, and phosphorus: Effects of supplementing broilers with a more bioavailable source of zinc on calcium and phosphorus absorption

Javier López-Paredes¹, Monica Puyalto¹, Karan Singh¹, D.S. Suryawanshi², Juan Jose Mallo Revilla*¹ ¹Norel SA, Madrid, Madrid, Spain; ²Omega Laboratories, Lonand, India

Zinc plays a crucial role in calcium and phosphorus metabolism by acting as a cofactor in various enzymatic processes, such as vitamin D activation, which is key for the efficient absorption of calcium (Ca) and phosphorus (P). Zinc also regulates the expression of calcium-binding proteins that facilitate Ca transport. Additionally, Zn enhances the activity of parathyroid hormone (PTH), influencing the balance of both Ca and P. Based on these functions, we hypothesized that supplementing with more bioavailable sources of Zn, such as zinc glycinate, compared to inorganic sources (ZnO and ZnSO₄), would improve the utilization of dietary Ca and P. This is particularly important for mitigating potential imbalances caused by high P levels in livestock. The objective of this study was to evaluate the impact of different Zn sources on Ca and P absorption in broilers. In this trial, 500 birds were divided into five treatment groups: T₁, received the control basal diet; T₂, supplemented with 100 ppm of Zn from ZnSO₄; T₃, supplemented with 100 ppm of Zn from ZnO; T₄, supplemented with 100 ppm of Zn from Zinc glycinate (Glymet Zinc 22%); and T₅, supplemented with 100 ppm of Zn from Zinc glycinate (Glymet Zinc 40%). Ten animals per group were randomly sampled, and blood samples were collected to measure serum levels of Zn, Ca, and P. Statistical analysis was performed using the Kruskal-Wallis test to evaluate treatment significance, followed by Wilcoxon test and bootstrap analyses. Results showed a significant increase in serum Zn content with organic zinc sources. In T₄, Zn levels increased from 33.7% to 34% ($p < 0.05$), and in T₅, levels rose from 34.4% to 35.6% ($p < 0.05$), compared to T₂ and T₃, respectively. Phosphorus was similar for organic sources, with an increase of 6.5% compared to T₀ and an increase of 5.0–5.3% compared to inorganic sources ($p < 0.05$). For Ca, serum content increased by 2.8% to 3.8% ($p < 0.05$) in T₄ and T₅. Those results were validated with bootstrap, with higher values of P (2.32 and 2.31), and Ca (3.31 and 3.27), for T₄ and T₅, respectively. In conclusion, supplementation with highly bioavailable Zn sources enhances the absorption of Ca and P, potentially reducing the need for higher dietary levels while positively impacting animal productivity.

Keywords: Organic Trace Minerals; Phosphorus; Zinc; Bioavailability; Calcium

382P Efficacy of a step-down tribasic copper chloride regimen on growth performance and mortality of broilers under necrotic enteritis challenge

Corey Johnson¹, Anthony Quant¹, Opeadura T. Osunbami¹, Tanner Wise¹, Elizabeth Miller¹, Yulin Ma², Kelly Chen*² ¹Kalmbach Nutritional Services, Upper Sandusky, Ohio, United States; ²Hanley International LLC, Belmont, Massachusetts, United States

Two experiments were conducted to evaluate the effects of a step-down regimen of tribasic copper chloride (TBCC; Hanley International, LLC) on growth performance, mortality, and gut health of broilers challenged with necrotic enteritis (NE). A total of 2340 Ross 708 (Exp.1) and 3600 Ross 308 (Exp.2) day-old chicks were allocated to floor pens (60 birds/pen) in a randomized complete block design. Birds received diets in a 39-d, 3-phase program (Ph1: d 1-14; Ph2: d 14-28; Ph3: d 28-39) formulated according to Aviagen recommendations. NE was induced by administering 10⁷ CFU of *Clostridium perfringens* per bird through feed on d 20 in Exp.1 or d 18 in Exp.2. In Exp.1, treatments included a commercial corn-SBM basal diet without NE challenge (PC), the basal diet with NE challenge (NC), NC plus a saponin-based feed additive (NC+SP), and NC plus a step-down TBCC at 200, 150, and 135 mg/kg in Ph1, 2, and 3, respectively, in addition to SP (NC+SP+TBCC). In Exp.2, treatments were PC, NC, NC plus 55 mg/kg bacitracin methylene disalicylate (NC+BMD), NC plus 135 mg/kg TBCC (NC+L-TBCC), and NC plus the step-down TBCC regimen used in Exp.1 (NC+S-TBCC). On d 22, 3 birds per pen were euthanized for intestinal NE lesion scoring. Data were analyzed using PROC MIXED of SAS with pen as experimental unit. In Exp.1, during d 14-28 post-challenge, NE reduced ($P < 0.01$) BW gain (BWG) and feed efficiency (FE) and increased mortality 6.94-fold (5.83% vs. 0.84%; $P < 0.01$) in NC compared with PC. Both SP and SP+TBCC improved BWG and FE, with SP+TBCC showing greater efficacy than SP alone. Although not significant ($P > 0.05$), mortality was lowered by 22.30% with SP and by 32.25% with SP+TBCC relative to NC. In Exp.2 (d 1-39), NE markedly depressed ($P < 0.01$) BWG and FE and increased ($P < 0.01$) lesion scores and mortality in NC compared with PC. Within challenged birds, BMD and S-TBCC reduced lesion scores compared with NC, with BMD being

the most effective ($P < 0.01$). BWG was increased ($P < 0.01$) by BMD and both TBCC regimens; however, only BMD improved ($P < 0.01$) FE. Notably, S-TBCC markedly reduced mortality by 35.77% (6.94% vs. 10.81%; $P < 0.01$), reaching a level similar to PC. In conclusion, a step-down TBCC regimen could improve growth performance and reduce mortality in broilers under NE challenge.

Keywords: tribasic copper chloride; broiler; growth performance; mortality; necrotic enteritis

383P Cost and compositional patterns of commercial vitamin–mineral premixes for broilers: Principal component analysis and cost comparison of combined vs. separate premixes

Ahmet Y. Pekel*^{1,2} ¹*Istanbul University-Cerrahpasa, Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul, Turkey;* ²*Gastrovet AS, Istanbul, Turkey*

This study evaluated the nutrient composition and cost relationships in 100 commercial broiler premixes, including both combined vitamin–mineral and separate formulations. Data included 21 variables (cost per ton and 20 nutrient components) analyzed using principal component analysis (PCA) and hierarchical clustering. All variables were standardized to z-scores, and PCA was performed on the correlation matrix using Excel and SPSS software. PCA explained 42.5% of total variance within the first three components. Correlation analysis revealed strong interdependencies between key nutrients, including a significant correlation between manganese (Mn) and zinc (Zn) (0.72), as well as moderate correlations between vitamins A, D₃, and E. PC₁ (25.3%) represented a fat-soluble vitamin and trace mineral axis (vitamins A, D₃, E, Zn, Mn, Fe), reflecting antioxidant and mineral density. PC₂ (10.8%) described a B-vitamin and metabolic cofactor axis (riboflavin, niacin, pantothenic acid, biotin, and choline chloride). PC₃ (6.3%) captured cost and formulation variation (cost_per_ton loading \approx 0.60), independent from nutrient axes. Hierarchical clustering grouped nutrients into three major functional clusters (B-vitamin/metabolic, antioxidant/trace mineral, and fat-soluble vitamins); the cost variable formed a distinct branch. These patterns demonstrate strong functional organization among nutrients and confirm that cost factors are largely independent from nutrient composition. A two-sample t-test showed that separate vitamin and mineral premixes were significantly cheaper ($t = 2.04$; $p = 0.022$) than combined formulations. Together, PCA, clustering, and cost comparison indicate that nutrient structuring in commercial premixes follows biologically meaningful patterns, while using separate premixes provides a measurable cost advantage without compromising nutrient diversity.

Keywords: broiler premixes; vitamin-mineral interactions; principal component analysis; cost efficiency; hierarchical clustering

384P Phytogetic E for peak performance: A comparative efficacy evaluation with synthetic vitamin E on productivity, egg quality and antioxidant status in commercial laying hens

Paramartha Roy*¹, Shivi Maini¹, G.V. Purushotam², S. Yogisha² ¹*Indian Herbs Specialties, Chandigarh, Chandigarh, India;* ²*Skanda Life Science, Bangalore, Karnataka, India*

In modern intensive poultry systems, birds are frequently exposed to environmental stressors, particularly heat, humidity, leading to oxidative stress, impaired immunity & reduced feed efficiency (Sahin et al., 2002). This study evaluated efficacy of a next-generation herbal antioxidant supplement (E Sel Power m/s Indian Herbs Specialties, India), formulated with natural vitamin E & organically complexed selenium, in broilers under high

Temperature-Humidity Index conditions. A total of 630 Vencobb 430Y chicks were assigned to three treatments: basal ration deficient in vitamin E (control), synthetic vitamin E (100 g/ton) & E Sel Power (100 g/ton). Birds were reared for 42 days under naturally occurring heat stress, with temperatures exceeding 35°C & RH >65%. Performance, antioxidant profile, stress indicators & immune responses were evaluated. Data were analyzed statistically following Snedecor and Cochran procedures, with significance at $p < 0.05$. Supplementation with E Sel Power significantly ($p < 0.05$) improved growth & feed efficiency, increasing final body weight by 80 g over control and 70 g over synthetic vitamin E, while improving FCR to 1.57 versus 1.61 in T1. Mortality decreased to 1.95% versus 4.29% in control, indicating enhanced resilience. Stress biomarkers were significantly ($p < 0.05$) reduced, with plasma cortisol lowering to 95.96 ng/mL compared with 198.06 ng/mL in control & MDA decreasing by 19.77%, reflecting reduced lipid peroxidation. Birds receiving E Sel Power showed enhanced antioxidant defense, consistent with elevated catalase, SOD & GPx activities reported for its precursor product Herbal E. Immunomodulation was evident from significantly higher IL-10 (66.43 vs. 44.62 pg/mL) and IgA & IgG increases of 20.3% and 34.9%, respectively, demonstrating stronger anti-inflammatory & humoral responses. The superior performance of E Sel Power over synthetic vit. E is attributed to synergistic action of mixed natural tocopherols, tocotrienols promoting cascading antioxidant effects & better selenium bioavailability. E Sel Power effectively mitigated oxidative stress, enhanced redox balance, improved immunity & supported overall productivity, offering a robust phytogetic alternative for sustainable poultry production under climatic stress.

Keywords: oxidative stress; vitamin E; selenium; antioxidant; phytochemicals

385P Natural vitamin C redefining layer performance: Stronger eggs, higher antioxidants and better productivity

Shivi Maini*¹, S Yogisha² ¹*Indian Herbs Specialties, Chandigarh, Chandigarh, India;* ²*Skanda Life Science, Bangalore, Karnataka, India*

Antioxidant supplementation in layer diets is essential to counter oxidative stress, sustain peak productivity, and maintain egg quality throughout the laying cycle. A 20-week controlled study was conducted to evaluate the efficacy of a phytogetic/herbal universal antioxidant & Vitamin C (C Power, Indian Herbs)—on performance, egg quality, and antioxidant status in BV300 layers. A total of 150 birds were allotted to three dietary groups (50 birds/group; 5 replicates of 10). Group T1 (negative control) received a basal ration without synthetic Vitamin C. Group T2 (positive control) received basal ration with synthetic coated Vitamin C. Group T3 received basal ration supplemented with Natural antioxidant & Vitamin C (C Power) @ 200 g/ton. The trial was conducted from 16 to 35 weeks of age at an accredited CRO in Bengaluru, India. Weekly performance parameters (egg production, HHE, feed efficiency) were recorded, while egg qualitative and quantitative traits were assessed at weeks 27 and 35. Antioxidant status was evaluated by FRAP assay for albumen and yolk, and IgG levels were analysed in selected groups. All data were subjected to statistical analysis following Snedecor and Cochran procedures, with significance determined at $p < 0.05$. Mean egg production (weeks 25–35) was significantly higher in natural Vit C group -T3 (94.96%) compared with synthetic vit. C-T2 (90.93%) and non-supplemental (T1) (83.81%). Egg weight and yolk index also improved significantly in T3. Ferric Reducing Antioxidant Power (FRAP) of egg albumen was markedly elevated in T3 (17.87 μ g/g) versus T2 (8.72 μ g/g) & T1 (4.26 μ g/g). Similarly, yolk FRAP values were highest in T3 (704.62 μ g/g)

compared with T2 (461.32 µg/g) & T1 (296.90 µg/g). Overall, Natural Vitamin C (C Power) substantially improved laying performance, enhanced egg quality & significantly increased antioxidant capacity in commercial layers. The results confirm superiority of C Power over synthetic Vitamin C & establish it as a potent, natural and safe phytochemical antioxidant for layer nutrition. Given the increasing global shift toward residue-

free, sustainable poultry production, phytochemical antioxidants offer a scientifically validated, practical strategy to mitigate climatic stress without reliance on synthetic additives.

Keywords: oxidative stress; vitamin C; natural; C Power; FRAP of egg

Pathology

386P Transcriptomic insights into the immune modulatory signatures of Pullorum disease in red-feathered native chickens

Wen-Yuan Yang*^{1,2} ¹National Taiwan University, Veterinary medicine, Taipei, Taiwan; ²Zoonoses Research Center, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Salmonella Pullorum (SP) causes systemic infection in young chicks. Birds that recover often become asymptomatic carriers, developing ovarian infection at sexual maturity and transmitting the pathogen vertically to offspring. Pullorum disease (PD), therefore, remains a persistent problem, particularly in native chicken production systems across Asia. However, the immunological basis underlying SP persistence remains unclear. In this study, cecal tissues were obtained from 19-day-old red-feathered chicks naturally infected with SP for 2–3 days, showing clinical white diarrhea and acute mortality, along with age-matched uninfected controls from the same farm. RNA sequencing was conducted on six infected and six control samples to characterize host immune responses. A total of 420 differentially expressed genes were identified, including 326 upregulated and 94 downregulated genes. Gene Ontology enrichment analysis revealed that the upregulated genes were primarily associated with cytokine activity, immune system processes, and defense responses. KEGG pathway analysis revealed activation of acute proinflammatory and cell-mediated immune signaling, with notable upregulation of IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-18, IL-22, INHBA, and multiple chemokines. These genes were enriched in Toll-like receptor, cytosolic DNA-sensing, C-type lectin receptor, and cytokine–cytokine receptor interaction pathways, which collectively promote pyroptosis, Th1 and Th17 differentiation, and strong inflammatory responses. Meanwhile, the concurrent increase in IL-10 expression suggests the presence of immunoregulatory mechanisms that may dampen excessive inflammation and facilitate bacterial persistence. These transcriptomic findings indicate that early SP infection triggers both robust proinflammatory signaling and compensatory immune suppression, forming a balanced immune state that may enable chronic carriage. Targeting this immune equilibrium through immunomodulatory strategies, such as probiotics, prebiotics, or immune-enhancing interventions, may provide effective approaches for preventing and controlling PD in native chickens.

Keywords: Pullorum disease; Immune modulation; Native chickens; RNA sequencing

387P Dietary supplementation with *Lactobacilli* and chickpea-derived fibers mitigates necrotic enteritis in broiler chickens

Ari Bragg*¹, Shreeya Sharma¹, Sukhman Kaur¹, Hosni Hassan², Khaled Abdelaziz¹ ¹Clemson University, Animal and Veterinary Sciences, Central, South Carolina, United States; ²North Carolina State University, Prestage, Raleigh, North Carolina, United States

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is a significant poultry disease resulting in global economic losses of approximately \$6 billion annually. This study investigated the protective effects of prebiotics, probiotics, and their combination

(synbiotics) against NE in broiler chickens. Ninety-five Ross 708 broiler chicks were randomly assigned to eight treatment groups, including chickpea-derived prebiotics (low or high dose), probiotics (*Lactobacillus animalis*), synbiotics (low or high dose of prebiotic combined with *L. animalis*), antibiotics (bacitracin), and control groups (positive and negative). Birds were fed a high-crude-protein diet containing the respective treatments, orally challenged with *C. perfringens* from days 21 to 24, and necropsied on day 25. Intestinal lesion scores were assessed, jejunal samples were collected for cytokine gene expression analysis, and cecal contents were collected for microbiome profiling using 16S rRNA. Data was analyzed using a One-Way ANOVA with a significance level set at $p < 0.05$ and Tukey's HSD post-hoc test was applied to significant results. The lesion scores were lower in the probiotic-only, low and high prebiotic, and the low prebiotic+ probiotic groups compared to the positive control group. In contrast, the high prebiotic + probiotic group exhibited high intestinal lesion scores, comparable to those of the positive control. No lesions were observed in the antibiotic or negative control groups. Interestingly, the groups with high lesion scores—including the positive control and the high prebiotic + probiotic groups—also showed higher expression of the proinflammatory cytokines IL-1β, IL-6, and IL-17 compared to the other treatment groups. Microbiome analysis is still ongoing. These data suggest that dietary supplementation with *L. animalis*, low doses of chickpea-derived fibers, or their combination can mitigate NE in broiler chickens. However, further optimization of the inclusion rate is warranted to achieve better protection.

Keywords: Necrotic Enteritis; Chicken; Prebiotic; Probiotic; Lactobacillus

388P Genotype-Phenotype associations reveal distinct pathogenic mechanisms in avian pathogenic *Escherichia coli*

Huanling Xing*², Ulunna Rita Ugoh², Xin Ye², Jeffrey D. Evans¹, Kelsy Robinson¹, Pratima Adhikari², Linan Jia², Li Zhang² ¹USDA, Agriculture Research Service, Poultry Research Unit, Mississippi state, Mississippi, United States; ²Mississippi State University, Department of Poultry Science, Mississippi state, Mississippi, United States

Avian pathogenic *Escherichia coli* (APEC) causes significant economic losses in poultry production worldwide. Traditional serological classification provides limited insights into virulence diversity, highlighting the need for genomic approaches to elucidate pathogenic mechanisms. This study integrated whole-genome sequencing with in vivo pathogenicity testing to identify genetic determinants of APEC virulence. We evaluated eight APEC isolates with varying embryo lethality and one nonpathogenic strain in 7-day-old unvaccinated Ross R7xYP broiler chicks via subcutaneous challenge (0.1 mL of 1×10^8 CFU/mL). A randomized complete block design (RCBD) was used. A total of 10 treatments, consisting of 9 isolates and one negative control injected with sterile saline, were evaluated. Rooms were considered as blocks, and each treatment included a total of 36 chicks, 6 cages, 6 chicks per cage (NC and PC with 72 chicks, respectively). Survival and lesion scores were analyzed using Kaplan-Meier and Mann-Whitney *U* test with Holm's

correction for multiple comparisons ($P \leq 0.05$). Two strains (MS1657 and MS1679) exhibited high pathogenicity with mortality rates of 61.11% and 41.67%, respectively ($P < 0.001$), and severe pericarditis and perihepatitis. Despite lacking canonical high-virulence markers (*chuA*, *ibeA*, *kpsM/kpsT*), MS1679 exhibited comparable virulence to MS1657, suggesting an alternative pathogenic mechanism. Genomic screening with ABRicate (v1.0.1) against VFDB identified 27 virulence-associated genes uniquely shared by both highly pathogenic strains. Notably, MS1679 harbored 13 additional unique virulence genes, including *espLL*, extended pap operon members (*papH-K*), and yfc-encoded fimbrial genes associated with adhesion and toxin production. These factors likely compensate for the absence of classical markers and drive an alternative adhesion-and toxin-based pathogenic strategy. These findings demonstrate that APEC pathogenicity is not defined by a static set of classical markers but by diverse and adaptable virulence repertoires. This understanding is critical for developing next-generation diagnostics and vaccines capable of targeting the multiple pathogenic strategies employed by APEC, ultimately leading to more effective disease control in poultry production.

Keywords: Avian colibacillosis; mortality; pathogenicity lesion score; virulence factors; genotype-phenotype association

389P Characterizing H9Nx after serial blind passage in avian cells

Mariana Andrioli Pinheiro*¹, James Krehling¹, Miria Ferreira Criado¹ ¹Auburn University, Pathobiology, Auburn, Alabama, United States

Avian influenza virus (AIV) poses a significant threat to wildlife, poultry production, and global food security. Among low pathogenicity avian influenza viruses (LPAIV), the H9Nx subtype circulates widely across various bird hosts, raising concerns about its potential for cross-species transmission and adaptation. To investigate host-specific replication patterns and adaptability, we conducted *in vitro* sequential passages of AIV H9Nx strains using continuous chicken and duck cell lines to . AIVs from H9 subtypes strains isolated from turkey (H9TK), wood duck (H9WD), and ruddy turnstone (H9RT) were inoculated into chicken (DF-1), duck, and MDCK cell lines at a multiplicity of infection (MOI) of 1. At 72 hours post-infection (hpi), cell monolayers were scraped, supernatants collected, and the supernatants used to infect a new cell. This procedure was repeated for five sequential passages under the same conditions. Infectious titers were measured using TCID₅₀/ml, and Real-time RT-PCR quantified viral RNA loads. Statistical analyses were performed with GraphPad Prism software. All H9Nx viruses showed detectable infectious titers during the first passage in all cell lines. Most of the H9 strains had viral titers decreased by the third passage (H9TK: 4.11 to 2.92 Log₁₀ TCID₅₀/ml; H9WD: 3.8 to 0.6 Log₁₀ TCID₅₀/ml; H9RT: 1.8 to 0 Log₁₀ TCID₅₀/ml), but a significant increase was observed at the fifth passage for H9TK (6.04 Log₁₀ TCID₅₀/ml) in duck cell lines. In contrast, all H9Nx strains exhibited a decrease in infectious viral titers across passages in DF1 cells. The Real-Time RT-PCR followed similar results. Overall, H9Nx viruses displayed distinct replication patterns after passage in ducks, with some advantages in adaptation. Additional analyses are essential to elucidate the underlying mechanisms driving these differences. These findings emphasize the role of host-virus interactions in influencing viral replication efficiency and potential adaptation across avian species.

Keywords: Viral replication; Cell tropism; H9 subtype; Influenza virus; Cross-species transmission

390P Tissue tropism of H9Nx influenza viruses in intravenously inoculated chicken embryos

Diego E. Ventura Urbina*¹, Rocio Gerez¹, Mariana Andrioli Pinheiro¹, James Krehling¹, Miria Ferreira Criado¹ ¹Auburn University, Department of Pathobiology, Auburn, Alabama, United States

The H9 subtype of avian influenza virus (AIV) poses a threat to both poultry and human health. To better understand AIVs and host interaction, the objective of this study was to evaluate tissue tropism of H9Nx AIVs isolated from turkey (H9TK), ruddy turnstone (H9RT), and wood duck (H9WD) in embryonated chicken eggs (ECE) systemically inoculated by intravenous injection. Specific pathogen free ECE (n=40) were incubated for 16 days and inoculated with one of the three viruses (titer of 10⁶ EID₅₀/ml). The opening in the shell used for injection was sealed with surgical tape containing antimycotic and antibiotic agents, and the ECE were returned to the incubator and candled to confirm viability prior to sampling. Tissue samples (kidney, liver, brain, duodenum, lungs, and trachea) and allantoic fluid were collected at 1 and 2 days post-inoculation (dpi) from five eggs per treatment and stored at -80°C until further analysis. Hemagglutination test was performed in allantoic fluid samples. Tissues were homogenized utilizing pre-filled tubes with ceramic beads, and RNA was extracted by Trizol methodology and quantified with spectrophotometer. Viral titer was determined by Real Time RTPCR methodology targeting the AIV matrix gene. Statistical analysis was performed using the two-way ANOVA with GraphPad Prism 10. Virus strains and dpi served as fixed effects, and means were considered statistically different when $p < 0.05$. No differences were detected in lung. In kidney at 2 dpi, H9WD had a lower titer compared with H9RT ($p = 0.031$). In tracheal samples at 1 dpi, H9TK exhibited a higher viral load compared to H9RT ($p = 0.023$). In duodenal samples at 2 dpi, H9WD had a lower viral titer compared to H9TK ($p = 0.019$) and H9RT ($p = 0.034$). At 1 dpi, H9RT presented a lower titer compared to H9TK ($p = 0.013$) and H9WD ($p = 0.038$). In brain samples at 1 dpi, H9TK treatment showed a higher titer compared to H9RT ($p = 0.213$), and at 2 dpi, H9TK had a higher titer compared to H9WD ($p = 0.024$). Tissues exhibited viral replication, with viral loads varying by strain. Detection of certain strains in tissues occurred independently of whether the ECE was viable or whether allantoic fluid tested positive by HA. Further tests are underway to better understand which factors can influence the AIV tropism.

Keywords: Avian Influenza; Tissue tropism; Virus; Low Pathogenic; Intravenous

391P Saponin-rich additive Is as efficient as conventional program in coccidia risk management: field trial report

Hoa Bui¹, Sekhou Cisse¹, Mohammed El Amine Benarbia*¹ ¹Nor-Feed, R&D, Beaucaouzé, France

Several studies have demonstrated that saponin-rich plants are efficient in reducing coccidial risk and improving resilience of infected broilers. However, most investigation had been conducted *in vitro* or experimental conditions while really few data from large commercial-scale settings. The present study aims to reevaluate the effectiveness of a standardized saponin-rich feed additive (SP, Norponin® XO2, Nor-Feed) under field conditions. A total of 573668 broiler (Ross 308) from 22 houses located in Centre-Val de Loire regions (France) implemented from 22nd November to 27th December 2024 were included. 9 buildings (TC, n= 265284) had the basal diet supplemented with the conventional anticoccidial program (monensin 40ppm + nicarbazin 40ppm); while 13 buildings (SP, n= 308384) received the same basal diet supplemented with the saponin-rich additive at

300g per ton of complete feed. In each house, 5 broiler chickens per building in both groups were randomly selected from 9 to 24 days of age to score cocci-related intestinal lesion (ILS). Growth performances were also recorded. Fisher's exact test was used to analyze differences in coccidial occurrence, while Student's t-test (parametric) or the Mann-Whitney test (non-parametric) was applied to compare overall ILS and performance parameters between groups. No difference was observed in coccidial incidence ($P > 0.999$) and global ILS ($P = 0.43$) between groups. The average slaughter age was 33 in TC Day and 34 days in SP buildings. Consequently, SP birds were slightly heavier (1,800 g) than TC birds (1,757 g), although not significantly ($P = 0.317$). Feed conversion ratio was comparable between 2 groups (1.54 vs.

1.56 in TC and SP group respectively, $P = 0.186$). Mortality was recorded at 3.3% (TC) and 3.7% (SP) with no significant difference. These findings revealed that SP supplementation was as efficient as monensin-nicarbazin combination in preventing coccidial from spreading and maintaining animal performances under commercial field condition. These results consolidate previous results about standardized saponin premixture supplementation in managing coccidial risk in broiler chicken farms.

Keywords: Saponins; coccidiosis; broiler chicken; commercial conditions; ILS

Physiology, Endocrinology and Reproduction

392P How does late chick placement affect performance parameters of broilers raised to 28 days?

Oscar Tejada*¹, Jordan Smith¹ ¹*Southern Arkansas University, Magnolia, Arkansas, United States*

An experiment was conducted to evaluate the effects of late chick placement on the growth performance, relative organ weights, and breast muscle of broiler chickens. A total of 100 chicks were randomly allocated into 2 experimental treatments ($n = 5$ pens per treatment; 10 birds per pen). Treatment 1 consisted of chicks placed in the farm 24 hours after hatching and treatment 2 consisted of chicks placed in the farm the same day of hatching. All birds were fed a common corn and soybean meal diet formulated to meet or exceed nutritional requirements. Growth performance, relative organ weights, and breast muscle weights were evaluated on days 0, 7, 14, 21, and 28. No statistical differences were observed in the percentage body weight of organs during the rearing period. However, treatment two had a non-significant better feed conversion ratio compared to treatment 1 during the whole rearing period ($P > 0.05$). Relative body weight gain showed that birds placed on the farm the same day of hatching (T2) had heavier relative weights on days 7, 14, and 21 ($P < 0.05$). In conclusion, shorter periods of time between hatching and placement can improve growth performance and increase body weight gain of broiler chickens.

Keywords: Growth performance; Relative organ weights; Breast muscle; Chicken placement

393P The effect of heat stress on egg production of broiler breeder hens selected for water conversion ratio

Allison Weaver*¹, Karen M. Rivera¹, Rosie Whittle¹, Maricela A. Maqueda¹, Sara K. Orłowski-Workman¹, Shawna Weimer¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Heat stress is a significant challenge for the poultry industry, resulting in substantial losses not only through increased mortality

but also decreased production. Broiler breeder females produce billions of eggs each year, and reduced breeder reproductive capacities could have a dramatic impact on the supply of chicken meat. We aimed to determine the effect of heat stress on productivity in broiler breeder hens from three genetic lines: high-water conversion ratio (HWCR), low-water conversion ratio (LWCR), and modern-random bred (MRB). A total of 108 hens were evenly distributed across 12 environmentally controlled chambers within individual cages (3/line/chamber). At 31 wks, cyclic heat stress (HS; $32 \pm 2^\circ\text{C}$ for 8 h per day for 3 wks) was applied to 6 chambers. The other 6 chambers remained thermoneutral (TN; $22 \pm 2^\circ\text{C}$), creating a 3x2 split-plot design. Eggs were collected twice daily, and hen-day-egg production (HDEP, %) was calculated. Eggs collected on the last two days of HS were analyzed for egg quality metrics, including weight (g), albumin height (mm), Haugh unit (HU), peak force (kg), and shell thickness (mm). Follicle counts were recorded for 36 hens (6/line/treatment) at the end of HS and again after a one-week post-HS recovery (R) period. Data were analyzed using linear regression models for line, HS, and period (follicle count only). For HDEP, HWCR TN had the highest egg production ($87.6 \pm 1.9\%$) while LWCR HS had the lowest ($69.9 \pm 1.9\%$; $P = 0.0003$). For egg quality, albumen height and HU were lower ($P = 0.02$) for LWCR ($4.41 \pm 2.0\text{mm}$ and 60.5 ± 2.0) compared with MRB ($5.15 \pm 0.19\text{mm}$ and 68.2 ± 1.9) and HWCR ($4.90 \pm 0.19\text{mm}$ and 65.4 ± 1.9), with no differences in other metrics. Similar to egg quality, LWCR had fewer ($P = 0.03$) follicles (5.38 ± 0.16) than MRB (5.96 ± 0.16), but not HWCR (5.62 ± 0.16). Hens in the HS group had the fewest follicles during HS (5.11 ± 0.19 ; $P = 0.04$) compared to TN during the HS period and both groups in the post-HS recovery period (5.83 ± 0.185). These results suggest that HS reduces broiler breeder hen follicle development, and genetic selection for LWCR may reduce egg production and quality that could be exacerbated by HS.

Keywords: Broiler breeders; Heat stress; Production; Water conversion

Processing and Products

394P Impact of *Pediococcus acidilactici* CNCM I-4622 supplementation on egg and eggshell quality across the laying cycle: a multi-trial approach

Marion Schiavone¹, Anais Nozeran*¹, Audrey Sacy², Louise Vigier¹ ¹*Lallemand SAS, Animal Nutrition, Blagnac (31), France, France;* ²*Lallemand SAS, Animal Nutrition, Blagnac (31), Midi Pyrénées, France*

The continuous growth in global egg demand, combined with increasing concerns about animal welfare, is driving the layer industry to extend laying cycles. Producers aim to maintain high

laying persistence while preserving eggshell quality and minimizing negative impacts on hen welfare and bone health. However, extending the laying cycle presents several challenges, including increased egg size and reduced calcium metabolism efficiency, resulting in more fragile eggshells. These factors contribute to a higher rate of downgraded eggs and economic losses. This study aimed to evaluate the effects of dietary supplementation with the probiotic *Pediococcus acidilactici* (PA) on egg and eggshell quality at different stages of the laying cycle. A total of 990 eggs were collected from four independent trials. Analyses focused on key parameters: egg weight and size, eggshell

weight and thickness, specific gravity, eggshell strength, and the proportion of downgraded eggs. The impact of PA was assessed at three critical stages: early lay (≤ 26 weeks), mid-lay (26–47 weeks), and late lay (≥ 47 weeks). Continuous variables were analyzed using mixed models (treatment as fixed effect; trial as random factor), while categorical data were compared using Chi-square tests. Statistical significance was set at $p < 0.05$. Results showed that PA supplementation significantly improved egg and eggshell quality across all parameters. Overall, PA increased egg weight (+2.5%), eggshell thickness (+13.9%), and eggshell strength, while reducing downgraded eggs. The proportion of L and XL eggs rose by 9.8 points, and eggs with optimal specific gravity (>1.08) reached 90.1% versus 69.8% in controls. Age-specific effects were also observed: mid-lay hens exhibited the greatest improvements in egg weight (+6.1%) and eggshell strength (+63.9% workload at sharp pole), while older hens had thicker shells (+6.2%). Young hens also benefited, though to a lesser extent. In conclusion, *Pediococcus acidilactici* effectively enhances egg and eggshell quality throughout the laying cycle, helping counteract age-related declines. These improvements may support the extension of laying cycles while maintaining product quality and economic performance.

Keywords: egg quality; probiotic; meta-analysis; Bactocell; eggshell quality

395P Effect of dietary inclusion of roasted and unroasted peanut skin on growth performance and carcass characteristics of broiler finishers

Johnson A. Olupona^{*1}, Olubukola Lawrence-Azua¹ ¹*Federal College of Animal Health and Production Technology, Ibadan, Animal Production, Ibadan, Oyo, Nigeria*

This study investigated the effects of graded levels of roasted and unroasted peanut (*Arachis hypogaea*) skin on growth performance and carcass traits of broiler finishers. A total number of One hundred and fifty (150) broiler chickens were randomly allocated to five (5) dietary treatments in the Completely Randomized Design (CRD). Each treatment had three replicates and ten (10) birds per replicate. The broilers were fed diets containing varying levels of roasted and unroasted peanut skin which contain 0.0g/kg, 2.5g/kg, 5.0g/kg, 2.5g/kg, 5.0g/kg of Roasted and Unroasted peanut skin designated as T1, T2, T3, T4, and T5 respectively. Birds were fed the experimental diets for eight weeks. Growth parameters including body weight gain, feed intake, and feed conversion ratio (FCR) were recorded. Carcass characteristics were evaluated at the end of the feeding trial. Results on the nutritional characteristics revealed that the peanut skin has a protein level of 13.95% for unroasted and 13.11% for roasted. The tocopherol, pyridoxine, thiamine and niacin for unroasted peanut skin are 0.93; 0.22; 0.19; and 0.08 respectively. Results showed the broilers on diets T4 (2.5% unroasted peanut skin) and T1 (control diet) had significantly higher body weight gain ($P < 0.05$) and average daily weight gain compared to those of T2, T3 and T5. Average daily feed intake was higher in T3 and T4 suggesting improved palatability and nutrient utilization. Feed conversion ratio (FCR) was lowest in T5 followed by T1 with significantly higher FCR observed in T2, T3 and T4 reflecting poorer feed efficiency. Carcass characteristics revealed that higher dressed weights was obtained in birds fed diets with 2.5% unroasted peanut skin (T4) and 2.5% roasted peanut skin (T2) diets which indicated that birds on these two diets produced more edible meat and was significantly better ($P < 0.05$) than other treatments. Higher levels (5%) of peanut skin, particularly unroasted, reduced growth performance and feed efficiency, possibly due to elevated fiber and anti-nutritional factors. The study concludes that peanut skin can

be included at 2.5% in broiler diets without adverse effects on performance, offering a sustainable feed ingredient alternative.

Keywords: Peanut skin; Broiler; Carcass characteristics; Growth performance; Feed conversion ratio

396P Effects of corn type and coccidiosis challenge on broiler meat quality

Jean C. Caceres^{*1}, Camila Hammel Sobreira¹, Sawyer W. Smith¹, Jennifer Rainbow¹, Christina S. Sigmon¹, Yan Campbell¹, Samuel Mwangi¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Coccidiosis is a common enteric disease in broiler production, often compromising growth performance, gut integrity, and meat quality. Implementing nutritional strategies that provide carotenoids through corn inclusion may enhance broiler resilience to intestinal stress, thereby maintaining meat quality. This study evaluated the effect of feed corn type (yellow [YC] vs. orange [OC]) on broiler meat quality, both with (C+) and without (C-) a coccidiosis challenge. Meat quality traits evaluated included yield (live, chilled, drip loss at 2 and 24h), pH, color, and woody breast (WB) incidence/severity. A 2x2 factorial design was evaluated with a two-way ANOVA, including fixed effects of corn type, coccidiosis challenge, and their interaction. The pen was assessed as the experimental unit (8 reps/treatment with 15 birds/pen). For yields, meat quality, and WB score, one bird/pen was processed. Significance was determined using Duncan's multiple range test ($P < 0.05$). The corn type x coccidiosis interaction influenced the broiler meat yields. Birds fed with YC/C+ exhibited the lowest live and breast meat weights ($P \leq 0.05$). In contrast, those fed with OC showed a stable yield performance regardless of C status. C+ alone resulted in a reduction of chilled weight, indicating an adverse effect ($P \leq 0.05$). Meat quality traits (pH & color) were mainly not affected by any of the main effects. Only meat from birds fed with OC/C+ showed a greater yellowness ($P \leq 0.05$). For WB incidence, OC/C+ exhibited greater WB severity, while YC/C+ had the lowest, with no severe cases ($P = 0.032$). The C-group, regardless of corn type, exhibited intermediate severity levels. Overall, corn type and coccidiosis had an impact on broiler growth, meat quality, and the incidence of WB. Corn type OC helped maintain meat yields under coccidiosis, likely due to a diet rich in carotenoids, showing enhanced resilience, albeit with an increased WB incidence. In contrast, YC broilers had a lower yield during the coccidiosis challenge, with fewer WB incidences, which may be attributed to their slower growth. Overall, these results suggest a trade-off between growth performance, yield, and WB incidence.

Keywords: Meat quality; Corn; Coccidiosis; Woody Breast

397P Correlation study using dynamic and isothermal rheological analysis, and texture profile analysis in chicken thigh patties formulated with citrus fiber and phosphates

Thiago Belem^{*1}, Rudro Deb Sardar¹, Harleen Kaur¹, Chinelo S. Onah-Kachiside¹, Elavarasan Krishnamoorthy¹, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Phosphates are used in meat products formulation to enhance meat functionality and processing yield. Replacing phosphates with equally functional alternatives is important to meet customer demand for more natural ingredient label. The objectives of this study were to evaluate the relationship between rheological and texture profile analysis (TPA) of raw and cooked chicken thigh patties, incorporated with citrus fiber and phosphate. Four treatments were prepared: control, 2% citrus fiber, 0.3% trisodium phosphate, and fiber+phosphate. Raw batters were analyzed for pH and instrumental color, and patties (n=3 per treatment; weight 90g,

diameter 86 mm, high 14 mm) were cooked to a core temperature of 75°C and cooled to room temperature before measuring TPA parameters. Rheological properties (G' , G'' , and $\tan \delta$) were measured under dynamic and isothermal heating at 55°C. Data were analyzed using one-way ANOVA with Tukey test ($\alpha=0.05$), Pearson correlation and principal component analysis (PCA). Treatment significantly affected batter pH ($p < 0.05$), with FI (6.21) < CON (6.35) < FI+phosphate (6.46) < phosphate (6.56). Pearson correlation showed that hardness was moderate associated with G' and G'' during time sweep at 55°C ($r=0.66$ and $r=0.668$, respectively), suggesting that at early-stage gelation behavior (demonstrated in the temperature ramp by $\tan \delta$) may be used to predict the final product firmness. PCA showed that PC1 was defined primarily by springiness, cohesiveness and resilience, suggesting gel elasticity. PC2 was represented by gumminess,

chewiness, G' and G'' of time sweep at 55°C, and hardness, suggesting firmness or gel strength. Overall, PCA showed similar relationship in hardness and viscoelastic behavior at 55°C as Pearson correlation. In conclusion, citrus fiber and phosphate had a similar TPA and rheology properties. Rheological behavior at 55°C had a reliable indicator for predicting final textural quality. It suggests that early stage of thermal gelation can provide useful predictive insights into the final texture of cooked chicken thigh patties, allowing rheological measurements to be used as a fast analytic tool instead of full cooking and TPA testing. This study may support the development of phosphate free chicken products using plant-based fiber.

Keywords: chicken thigh; rheology; texture; physicochemical; correlation

SCAD

398P Comparing FTA cards and fresh spleen tissue for measuring HVT vaccine takes

Benjamin N. Alig^{*1}, Po-Hsin Yu¹, Ellie Wollet¹, Jennifer Schlenker¹, Kalen Cookson¹ ¹Zoetis, Durham, North Carolina, United States

Over the last several years, various molecular diagnostic techniques have become more accessible to the poultry industry, allowing producers to better understand their vaccine applications and field challenges. Many diagnostic labs in the United States accept fresh tissue samples domestically for diagnostic analysis; however, some labs have an interest in accepting samples from outside countries to better support products used in international markets. One challenge includes the USDA policy which forbids the importation of fresh tissue samples which may contain infectious organisms. While more expensive than tissue samples, FTA cards have become a popular tool to inactivate biological agents while preserving genetic material for diagnostic analysis without cold chain. The goal of this study was to compare HVT PCR takes to a commercial recombinant HVT vaccine using spleen tissue vs. spleen impressions preserved on FTA cards. 120 Ross broiler chicks were incubated under standard conditions and given Poulvac® Procerta® HVT-IBD via in-ovo at E18. Birds were hatched, placed, and reared under standard conditions. 20 birds were euthanized at days 3, 7, 10, 14, 21, and 28 to collect spleen samples. Each spleen was split in half and one half was collected in tubes containing PBS and frozen until extraction, while the other half was smeared on the FTA card and dried at room temperature. DNA was then extracted, and qPCR was performed in duplicate to determine the load of HVT in each sample. A CT-40 was considered negative. Results were analyzed as percent positives and mean CT values. Statistics was performed using a generalized linear mixed model. At day 3, FTA cards showed 20% higher percent positives with similar CT values. For days 7 through 28, however, there was no statistical difference between FTA and spleen tissue with each age point showing 95%+ positives and CT values around 32.5. This research revealed that using FTA cards for analyzing HVT vaccine takes yields similar results to directly testing spleen tissue. FTA cards are a viable option for international HVT sampling and transporting for diagnostic purpose.

Keywords: HVT; Diagnostics; FTA Cards; Broiler; Marek's Disease Virus

399P Evaluating broiler response to *Eimeria* challenge after commercial Coccidia vaccine administration when birds are fed Ecodiar, an oregano essential oil

Matthew K. Jones^{*1}, Charles L. Hofacre¹, Rafael Cabrera², Dolores Cabrera², Gabe Cabrera², Santiago Sasia² ¹Southern

Poultry Research Group, Watkinsville, Georgia, United States; ²Nutrinae LLC, Palo Alto, California, United States

Eimeria species are one of the most important intestinal diseases in the poultry industry, causing primary disease and secondary conditions. Many producers utilize live coccidia vaccination or a combined strategy using vaccine and a feed additive. In the current trial, Ecodiar (Nutrinae LLC), an oregano essential oil, was evaluated in broilers vaccinated with a commercial coccidia vaccine and subsequently challenged. The goal was to understand whether this product would impact the vaccine's ability to cycle and protect broilers. Treatments included an unchallenged control, a challenged control, challenge + Ecodiar (150 ppm), and challenge + Ecodiar (300 ppm). Each treatment was represented by six replicate floor pens containing 25 male Ross broiler chickens. All groups received a 1x dose of commercial coccidia vaccine via coarse spray prior to placement onto fresh pine shavings. On day 22, challenged groups received ~100,000 *E. acervulina*, ~35,000 *E. maxima*, and ~75,000 *E. tenella* oocysts/bird. On day 28, gastrointestinal sections from three birds in each replicate were scored for coccidia lesions. Excreta samples were collected on days 14 and 22 to assess the cycling of the coccidia within the pen. Broilers and feed were weighed on days 0, 14, 28, and 42 to calculate performance metrics. Data were subjected to an ANOVA and LSD procedure at a p-value of 0.05. The higher Ecodiar inclusion had higher feed conversion early which was sustained for the duration of the trial. There were no body weight or feed conversion differences between the vaccinated and lower Ecodiar treatments. Challenged groups had greater lesion scores than the unchallenged control ($P < 0.05$). Coccidia lesion scores were similar across vaccinated treatments for each intestinal section evaluated and OPG outcomes were not different among vaccinated groups. The reduction in feed conversion in the highest Ecodiar inclusion is a phenomenon observed routinely in additives that stimulate immune function. In the current evaluation, Ecodiar did not have a negative influence on the coccidia vaccine's efficacy. Based on the perceived immunostimulatory involvement, it may complement in a shuttle program or be used strategically for other health solutions.

Keywords: Coccidia; Eimeria; Vaccination; Essential Oil; Intestinal Health

400P Optimizing Sequencing of Variable VP2 Region of IBDV from Bursa Field Surveillance Samples

Jennifer Schlenker^{*1}, Po-Hsin Yu¹, Kalen Cookson¹ ¹Zoetis, Durham, North Carolina, United States

Infectious Bursal Disease Virus (IBDV) is a highly contagious RNA virus that causes immunosuppression in chickens. This virus

is most effectively controlled through proper vaccination programs. Due to the high levels of antigenic drift seen in IBDV, IBDV vaccination programs require constant monitoring and surveillance to ensure expected protection. The two key goals of IBDV surveillance are to establish the window of field infection and then characterize the IBDVs in positive bursal samples. A crucial tool in monitoring IBDV vaccination programs is through sequencing the variable region of the VP2 gene, which allows the identification and differentiation of both vaccine and wild type IBDVs. In 2024, our diagnostic lab had a 63% sequencing success rate from 288 qPCR positive field samples using Sanger Sequencing. In 2025, our goal was to optimize sample processing and sequencing workflows to improve VP2 sequence recovery from IBDV-positive samples. A subset of bursal samples from trial samples, commercial broiler, and layer flocks of varying ages submitted to our diagnostic laboratory for surveillance testing were selected. qPCR was performed to identify positive samples ($qCt < 40.0$), followed by endpoint RT-PCR amplification of the VP2 gene and submission for sequencing. Bursa samples were evaluated under various extraction methods, storage conditions, primer configurations, and sequencing methods. By using a shorter homogenization method, storing samples frozen in PBS prior to extraction, amplifying a shorter VP2 fragment, and employing third generation Nanopore Sequencing, we were able to increase sequencing success rate to 98.0% for the 371 qPCR positive samples evaluated under the new protocol so far in 2025. In conclusion, optimizing sample processing methods and utilizing Nanopore sequencing allowed us to sequence positive samples at a much higher rate. This allows us to provide a more accurate and detailed snapshot of the IBD challenge and impact of the vaccination program.

Keywords: IBDV; VP2; Nanopore Sequencing; Endpoint PCR; Viral Surveillance

401P A study comparing two live IBD vaccines in commercial broilers challenged before two weeks of age with AL2 variant IBDV

Kalen Cookson^{*1}, Benjamin N. Alig¹, Brian Jordan¹, Po-Hsin Yu¹ ¹*Zoetis, Durham, North Carolina, United States*

The injection of live IBD vaccines in the hatchery either by themselves or in combination with recombinant HVT-IBDV vaccines has become a popular practice in the US and Canada. Live vaccines have the potential to protect the most susceptible chicks possessing low maternal antibodies and, thus, delay the time when field virus starts to infect a critical mass of birds and spread through the entire flock. We've conducted similar challenge studies in broilers against Del-E and Group-6 variants and, more recently, in leghorns against the predominant AL2 virus. The goal of this study was to apply the broiler model using an early AL2 challenge. Study Design: Commercial broiler chicks were vaccinated *in ovo* on E-18.5 with either a live Del-E type or a Lukert type or no vaccine (positive and negative controls). At hatch, 30 chicks were tested for IBD ELISA serology. 54 chicks from each treatment were divided into 3 isolators. At 11 days of age, all vaccinated birds and half of controls were challenged with 3.5 EID50 AL2. At 18 days of age, all birds and bursas were weighed, bursas were scored and then each was split for real time PCR and histopathology scoring. Results: The day of age flock GMT was 4,784 on Idexx-XR. There were no significant differences in mean bursameter scores, bursa to body weights (B:BW) or even histopathology scores between any of the treatments. However, there were differences in mean Ct values of the bursas with the negative controls and Lukert vaccinates (40.0 and 39.9, respectively) being significantly higher than the Del-E vaccinates (38.8), followed by the significantly lower challenge

controls (34.1). The incidence of IBD PCR positives (Ct<40) post challenge was, in ascending order, Lukert (8%), Del-E (37%) and Controls (92%). Conclusion: The higher-than-expected maternal antibody levels in this flock (breeder flock source was 60+ weeks) helps explain why there wasn't much lymphoid depletion or atrophy a week after the 11-day challenge. However, the sensitivity of the PCR assay was able to detect the eventual AL2 replication by the tail end of the study as well as show subtle but significant differences between the two live vaccines in delaying the infection.

Keywords: IBD; AL2; PCR; in ovo; vaccines

402P Development and optimization of an APV ELISA for disease monitoring in SPF chickens

Roxanne E. Lopardo^{*1}, James Murray², Bianca Heinrich¹, Kimberly Rankin¹, Dawn Alderman¹ ¹*AVS Bio, New Product Development, Groton, Connecticut, United States*; ²*Benchmark Antibodies, Eugene, Oregon, United States*

Avian Pneumovirus (APV) remains a significant respiratory pathogen in poultry, contributing to production losses and complicating the differential diagnosis of respiratory diseases. Historically, subtype C was the only strain detected in the United States, with sporadic outbreaks across limited regions. However, in recent years, subtypes A and B have been introduced and are now widely prevalent. Beyond commercial poultry operations, the European Pharmacopoeia (section 5.2.2) mandates that specific-pathogen-free (SPF) flocks be free of APV infection including subtypes A, B and C. This highlights the critical need for a highly specific diagnostic assay that minimizes false positives and repeat testing while maintaining reliable and consistent detection of true positives. This project aimed to develop an enzyme-linked immunosorbent assay (ELISA) specifically optimized for disease monitoring in SPF birds, with a focus on minimizing background signal in the SPF serum matrix. The indirect antibody-capture ELISA was systematically optimized for antigen coating type and concentration, blocking buffer formulation, sample diluent composition, and substrate incubation time to achieve the highest signal-to-background ratio. The goal was to produce an assay with equal or greater sensitivity and limit of detection, coupled with improved specificity, compared to currently available commercial kits, particularly for use with SPF samples. The developed ELISA demonstrated high sensitivity and specificity for the detection of Avian Pneumovirus, particularly within the SPF sample matrix. Plate coating was performed with defined ratios of recombinant proteins that can detect subtypes A, B and C, and the finalized ELISA protocol was optimized for blocking conditions and substrate kinetics. Elevated antibody titers in serum samples from APV infected chickens were detectable as early as 14 days post infection (dpi). Studies showed no cross-reactivity against various poultry pathogens such as Chicken Anemia Virus, Newcastle disease virus, infectious bronchitis virus, or avian influenza virus. Further work is ongoing to fine-tune the assay's detection capabilities, ensuring comprehensive surveillance across circulating strains.

Keywords: Avian Pneumovirus; ELISA; sensitivity/specificity; SPF layer chickens; disease monitoring

403P Reduction in *E. coli* airsacculitis in broiler chickens

Charles L. Hofacre^{*1}, Matthew K. Jones², Ramesh Selvaraj⁴, Bertrand Medina³, Ivan Girard³, Graziela Alves da Cunha Valini⁵, Glycon L. Santos⁵, Melina L. Bonato⁵ ¹*Southern Poultry Research Center, Watkinsville, Georgia, United States*; ²*Southern Poultry Research Group, Watkinsville, Georgia, United States*; ³*Probiotech International, Saint-Hyacinthe, Quebec, Canada*; ⁴*University*

Canada; ⁴University of Georgia, Athens, Georgia, United States; ⁵ICC Animal Nutrition, Jundiaí, São Paulo, Brazil

Escherichia coli remains the primary bacterium associated with airsacculitis in broilers. *E. coli* airsacculitis most often results from elevated ammonia in chicken houses that damages the trachea. Respiratory vaccine viruses (NDV/IBV) further affect the trachea, allowing an avian pathogenic *E. coli* (APEC) to infect deeper into the respiratory system. Sugarcane ethanol fermentation yields a yeast cell wall particularly rich in β -glucans and mannan-oligosaccharides (MOS). β -glucans are recognized for immunomodulatory properties, while MOS can bind gram-negative bacteria, promoting gut pathogen clearance. Limited research has evaluated sugarcane-derived MOS and β -glucans, with or without phytochemical feed additive (PFA) based on thyme, clove, and cinnamon oils, during APEC challenge. This study followed the model of Glisson et al. (2004): birds were reared on reused litter, coarse-sprayed at 24 days of age with NDV (LaSota), IBV (Mass) vaccines, then on day 29, coarse-sprayed with APEC (X-7122 Curtiss). Samples for intestinal integrity, FITC-d, and cytokine response were collected on day 35. Treatments included T1 (No Treatment); T2 (IMW50 – 0.5 kg/mT); and T3 (IMW50 – 460 g/mT + PFA – 140 g/mT). Fifty broiler chicks were placed in each of 24 pens with 8 replicates/treatment. Data was evaluated using ANOVA with a comparison of means using a T-test at $P < 0.05$. Airsacculitis related mortality was 6.25%^A T1; 5.00%^{AB} T2; and 3.50%^B T3. Airsacculitis lesion scores on day 42 (10 birds/pen) were not significantly different at 0.500^A (T1); 0.462^A (T2), and 0.475^A (T3). Performance results throughout the study were not significantly different; however, at the peak of the challenge (day 35), the numerically heaviest weight was observed in T2. At the peak of the challenge, there were no differences in FITC-d permeability. On d14, there were no significant treatment effects on liver IL-1, LITAF, IFN γ , and IL-21. The T2 and T3 had significantly higher IL-10 mRNA levels than T1. On d35, the T2 had significantly higher IL-1, LITAF, and IFN γ mRNA levels. The challenge model successfully induced *E. coli* airsacculitis and mortality in the birds. The combination of IMW50 and the PFA had a significant impact on airsacculitis-related mortality and numerically benefited performance.

Keywords: Airsacculitis; *E. coli*; B-glucan; MOS; phytochemical blend

404P Functional metabolic pathway dynamics of gut microbiota during early response to *Eimeria* vaccination in turkey poults

Philip Yeboah*¹, Demilade Ibiwoye¹, Natalie Roberts¹, Lauren Anderson¹, Thaciane L. Amaral¹, Muhammad Wajeeh Ul Hassan¹, Renata Gomes¹, Tracy Omasanuwa¹, Lisa Bielke¹ ¹North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United States

The use of live *Eimeria* vaccination is a primary non-anticoccidial approach for controlling *Eimeria*. However, infection can cause changes to the enteric microbiome, leading to decreased growth performance. This study focused on assessing the effects of live *Eimeria* vaccination on key microbiota-associated metabolic pathways in the ileum and ceca of poults. The experiment consisted of Control (CON) and Vaccinated (VAC) groups, each comprising 20 poults. Poults in the VAC group were administered 1x dose of commercial live vaccine on the day of hatch, with samples collected at 4, 9, and 14 days of age. 16S rRNA amplicon sequencing was performed, targeting the V4 region, and predicted metabolic functional analysis was performed using the PICRUSt 2 pipeline and MetaCyc databases, with a p-value and false discovery rate (FDR) threshold of 0.05. Cumulative analysis of cecal microbiota showed the upregulation of lysine biosynthesis II

($p = 0.014$, log₂ FC, 0.79) in the VAC group, while energy metabolism pathways, including fucose degradation ($p = 0.04$, log₂ FC, -1.15), and amino acid pathways such as L tyrosine ($p = 0.04$, log₂ FC, -0.68), were downregulated compared to the CON. However, in the ileum, the VAC group exhibited upregulation of only cyanophycin metabolism ($p = 0.001$, log₂ FC, 3.9) and oxobutanoate degradation pathways ($p = 0.006$, log₂ FC, 3.9). On d4, both cecal and ileal microbiota exhibited a significant upregulation of the pyruvate fermentation to propanoate I ($p = 0.002$, log₂ FC, 3.08) and cyanophycin metabolism pathways ($p = 0.01$, log₂ FC, 5.08) in the VAC group. On d9, cecal microbiota showed downregulation of pyruvate fermentation to propanoate I ($p = 0.001$, log₂ FC, -6.19), while ileal microbiota recorded a downregulation of propane 1,2 diol degradation ($p = 0.002$, log₂ FC, -2.81) in the VAC group compared to CON. On d14, while the cecal microbiota showed a downregulation of formaldehyde oxidation ($p = 0.004$, log₂ FC, -2.19) in the VAC group, those on the ileal microbiota recorded downregulations in L-rhamnose degradation ($p = 0.042$, log₂ FC, -1.25). *Eimeria* vaccination triggers an initial downregulation of critical energy and amino acid metabolic pathways; thus, nutritional strategies can be adapted to offset the resulting stress.

Keywords: *Eimeria*; metabolic pathways; turkey poults; microbiome; Vaccination

405P Epidemiological analysis of avian influenza viruses H5N2 and H7N3 in Mexico: a phylogeographic inference study

Inkar Castellanos-Huerta¹, Alejandro Aranda³, Sankari Castellanos², Aaron Forga¹, Danielle Graham*¹ ¹University of Arkansas, Fayetteville, Arkansas, United States; ²Northwest Arkansas Community College, Bentonville, Arkansas, United States; ³Universidad Autónoma de Querétaro, Santiago de Querétaro, Mexico

Influenza A virus is considered a significant agent due to its substantial socio-economic and eco-epidemiological impact, stemming from its ability to circulate in diverse species, where it can cross interspecies barriers and infect other hosts, demonstrating its high zoonotic potential. In Mexico, the virus first appeared in 1994 with the H5N2 subtype, and later in the 20th century, the H7N3 subtype emerged. It was established that migratory waterfowl facilitate the transmission and persistence of the virus in the national poultry population. Therefore, this research aims to evaluate the evolutionary dynamics over time and space of highly pathogenic avian influenza (HPAI) strains in relation to strains circulating in wild bird populations in the Americas, comparing them with recent isolates from wild birds to establish the evolutionary relationships between viruses of wild and domestic origin circulating in the country. Fifteen H5N2 strains, thirteen H7N3 strains, and one Mexican wild-origin strain, which differ from each other based on their spatiotemporal dynamics, were evaluated to determine evolutionary relationships using phylogenetic inference analysis in BEAST. Phylogenetic trees were constructed by applying discrete trait variables to develop inference networks and perform discrete spatiotemporal diffusion analysis using geospatial software. Phylogenetic inference established that the Mexican lineage originated primarily from intraspecific diffusion of wild origin; however, it has not developed an evolutionary network with wild viruses circulating in the country. Domestic populations have been the focus of regional geographic persistence, continental genomic diffusion, genomic variability, and interspecies transmission, especially to mammals. Based on the results, it is established that poultry production in Mexico plays a significant ecological role in the circulation of the avian influenza virus, and that wild birds play a crucial ecological role in the geographic dispersal of the virus.

Still, domestic populations promote regional and continental genomic persistence and dynamism.

Keywords: Avian influenza; phylogeographic inference study; Mexico; H5N2; H7N3

406P Leukocyte responses of broiler chickens against BCO lameness, elicited by electron beam or formalin-killed multi-bacterial vaccines

Layla Al-Mitib*¹, Ruvindu Perera², Palmy Jesudhasan¹, Jossie M. Santamaria¹, Gisela Erf¹, Adnan Alrubaye¹, Douglas Rhoads¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ²University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States

We evaluated the immune responses in broiler chickens vaccinated with either electron beam (eBeam) or formalin-killed mixed-species bacterial vaccines targeting Bacterial Chondronecrosis with Osteomyelitis (BCO). This condition leads to significant welfare issues and economic losses in the poultry industry. BCO occurs when bacteria from the gut or respiratory tract enter the bloodstream and infect bone growth plates, resulting in necrosis and lameness. Our eBeam-killed vaccine included ten strains from *Staphylococcus*, *Escherichia coli*, and *Enterococcus* species, which are commonly found in BCO lesions. Using fluorescent staining and flow cytometry, we measured the concentrations of total and subset lymphocytes in the blood at six time points post-vaccination (days 3, 7, 10, 15, 22, and 29). The groups included sham, eBeam, formalin, and wire-floor. Two-way ANOVA revealed significant time effects for all leukocyte populations; however, only the counts of B lymphocytes and $\gamma\delta$ T lymphocytes differed significantly across treatment groups. Chickens that received the eBeam vaccine showed higher responses in B and $\gamma\delta$ T lymphocytes compared to those in the formalin vaccine group. The results suggest that eBeam-killed bacterial vaccines may produce a stronger cellular immune response than those killed with formalin, which could lead to better protection against BCO. We are actively continuing our research to compare antibody responses and to better understand the humoral immunity generated by each vaccine.

Keywords: eBeam; BCO; vaccines; antibody responses; bacteria

407P *Campylobacter hepaticus* challenge study using two methods of challenge administration

Ashley Gatewood*¹, Charles L. Hofacre², Matthew K. Jones³, Jennie Baxter² ¹Southern Poultry Research Group, QA, Athens, Georgia, United States; ²Southern Poultry Research Center, Watkinsville, Georgia, United States; ³Southern Poultry Research Group, Watkinsville, Georgia, United States

Campylobacter hepaticus is the primary bacteria that causes Spotty Liver Disease (SLD) in poultry. SLD can cause birds to experience lethargy, a drop in egg production and possibly death. Devising a way to induce disease in order to combat this bacterium is imperative to the egg laying poultry industry. A repeatable method of creating SLD is critical for product validation. In this study, birds were challenged with *Campylobacter hepaticus* (NCTC) using two different methods of administration. Each pen consisted of 30 layer hens in egg production (30+ weeks) (10 direct challenged birds and 20 indirectly exposed horizontal birds). On DOT 0, one group was challenged with *C. hepaticus* via intravenous injection and the other group via oral gavage. Birds were given environmental stressors of elevated heat and feed withdrawal as part of the model approved by SPRG IACUC. Bile and liver samples were collected 7 days post challenge from 1 direct challenged bird and 2 horizontal birds in each challenge model prior to environmental stressors being introduced. Bile was

collected 16 days post challenge (3 challenged and 6 unchallenged per pen). No lesions were observed at the first collection. All remaining birds at 35 days post challenge were evaluated for liver lesions and bile was taken for culture. No orally gavaged birds were positive for *C. hepaticus* while 1 horizontal and 1 direct challenged bird in the IV group were culture positive. At termination, DOT 35, hens in the IV challenge group had a numerically higher incidence of lesions present in the horizontal birds (6/11 remaining birds, 54.5%) compared to the oral gavage method (3/10 remaining birds, 30%). Lesion scores in horizontal hens were relatively low in both intravenous and orally challenged groups, 0.6% and 0.3% respectively. The current study represents reproducible model of a *C. hepaticus* (SLD) challenge.

Keywords: Spotty Liver Disease; *Campylobacter hepaticus*; Egg Production

408P Development of an Infectious laryngotracheitis virus (ILTV) whole genome sequencing strategy to genotype clinical samples and gain understanding of current genotype VI ILTV evolution

Rachel Jude*¹, Brittany Henry¹, Roshan Paudel¹, Lok Raj Joshi¹, Maricarmen Garcia¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

Infectious laryngotracheitis (ILT) is an acute respiratory disease of chickens caused by Infectious laryngotracheitis virus (ILTV), an *alphaherpesvirus*. ILTV strains are phylogenetically distinguished into nine genotypes (GT), five of which circulate in the United States (U.S.). Genotype VI represents non-vaccine related virulent viruses first identified in 2004 which are highly prevalent across poultry operations in the U.S. and are associated with clinical cases in broiler flocks and subclinical cases in broiler breeder flocks vaccinated with the TCO vaccine. Recently, there has been an escalation of CEO vaccine administration in broiler operations located in regions with high prevalence of GT VI infections. Meanwhile the status of GT VI prevalence in layer flocks vaccinated with either TCO or CEO within these regions remains unknown. Taken together, the above scenarios create a breeding ground for the emergence of more transmissible and virulent recombinant viruses. Although genotyping assays of partial genome regions have been very helpful in identifying circulating strains not related to vaccines, these assays are not sufficiently informative to uncover the origins of geographically distinct GT VI sub-lineages and to ascertain whether the use of CEO and TCO vaccines has contributed to their evolution. To address this issue, we are currently designing and validating an amplicon-enriched long-read sequencing assay to facilitate ILTV whole genome sequencing (WGS) to be used supplementally with SISPA-Seq (sequence-independent, single-primer amplification sequencing). Using long range PCR facilitated by high-fidelity polymerases, we have been successful in amplifying 10+kbp overlapping fragments of the ILTV genome which can be subsequently sequenced using nanopore sequencing. Use of long range AmpSeq enables rapid sequencing of full ILTV genomes from clinical samples without the need for expansion in cell culture and therefore allows us to build a more comprehensive database of complete ILTV genomes of currently circulating GT VI strains to gain better understanding of how they are evolving under vaccination pressures.

Keywords: Infectious laryngotracheitis; genotype VI; whole genome sequencing; AmpSeq

409P *Campylobacter hepaticus* persistence and intervention in a challenge trial

Catherine M. Logue*¹, Roel Becerra¹, Charles L. Hofacre², Matthew K. Jones² ¹University of Georgia, Population Health,

Athens, Georgia, United States;²Southern Poultry Research Center, Watkinsville, Georgia, United States

Spotty liver disease (SLD) has reemerged as an important cause of disease in table egg layers. *Campylobacter hepaticus* and *Campylobacter bilis* have been reported as the etiologic agents causing multifocal lesions in infected birds' livers, resulting in reduced egg production, and increased mortality. The objective of this study was to evaluate persistence of gross liver lesions and the potential impact of an antimicrobial intervention following challenge with *C. hepaticus*. One hundred and fifteen commercial, 24 weeks of age, layer hens were divided into 2 groups and 80 birds were challenged with *C. hepaticus*, (10^9 cfu/mL/hen) for three days by oral gavage; the negative control group (n=10) received a placebo (PBS) using the same regimen. At 16 days post challenge (dpch), the hens were subdivided into two groups, and one group was treated via feed with chlortetracycline (CTC)/Aureomycin® for 5 days (n=40) while the second group remained untreated (n=40). Also, at 16 dpch, 11-layer hens were added as sentinels to each group to evaluate potential horizontal transmission. At 16, 23, and 32 dpch, a subpopulation of hens was euthanized and necropsied for sample collection including liver for histopathology, bile for bacteriology, and to record gross spotty liver lesions. Results found that gross liver lesions were persistent in birds that did not receive CTC; however, for birds that received CTC, gross liver lesion prevalence was lower and significantly different compared to untreated birds especially at 32 dpch. The CTC could not clear the infection as over 60% of the hens in each group remained positive on bacteriological analysis. The CTC did not prevent transmission of *C. hepaticus* to sentinel hens. Overall, this study confirms persistence of liver lesions in flocks where antibiotics are not used. Further research is needed to understand the pathophysiology of how *C. hepaticus* causes liver lesions leading to mortality and a drop in egg production in layer hens.

Keywords: *Campylobacter hepaticus*; challenge; Layers; spotty liver disease; antibiotic

410P VG/GA Newcastle Vaccine administered with precocious coccidia vaccine benefits performance in broilers compared to non-precocious coccidia vaccine during a necrotic enteritis challenge

Christa K. Goodell*¹, John McCarty¹, Matthew K. Jones², Ramesh Selvaraj⁴, Charles L. Hofacre³, Richard Phillips¹ ¹Boehringer Ingelheim, Duluth, Georgia, United States; ²Southern Poultry Research Group, Watkinsville, Georgia, United States; ³Southern Poultry Research Center, Watkinsville, Georgia, United States; ⁴University of Georgia, Athens, Georgia, United States

The Villegas–Glisson/University of Georgia (VG/GA) vaccine strain of Newcastle disease virus (NDV) is considered primarily enterotropic, though it also replicates in the respiratory tract of chickens. Enterotropic VG/GA vaccine strain induces strong interferon γ (IFN γ), Th1, and Th2 immune responses in the gut, which can be expected to co-protect against other intestinal infections like coccidiosis and *Clostridium perfringens* (CP). The objective of this study was to evaluate the effect of the VG/GA vaccine strain of NDV when administered with a precocious coccidia vaccine on mortality, feed conversion (FC), and gain (BWG) during a necrotic enteritis (NE) challenge, compared to a non-precocious coccidia vaccine. A total of 1500 birds were randomly allotted to 1. Negative control; 2. Non-precocious coccidia vaccine+CP challenge [NCVCP]; 3. Precocious coccidia vaccine +CP challenge [CVCP]; and 4. VG/GA+CVCP in 12 replications. VG/GA vaccine (AVINEW®, Boehringer Ingelheim, Duluth, GA), precocious CV (VAXXILIVE® COCCI 3; Boehringer Ingelheim) and non-precocious coccidia vaccine were

administered according to label dose on D0. On D12, 13, 14, and 15, 1×10^8 CFU/bird CP was mixed in chicken feed. Data were analyzed using ANOVA and means were separated using Tukey's HSD ($p < 0.05$). On D21, D28 and D42, birds in VG/GA+CVCP had comparable BWG and FC to negative control and CVCP groups, and significantly better ($p < 0.05$) BWG and FC than the NCVCP group. Additionally, the VG/GA+CVCP group had the lowest mortality. On D12, birds in the VG/GA+CVCP had a significant increase ($p < 0.05$) in the cecal tonsil IFN γ , IL-17 and IL-21 mRNA compared to the NCVCP and CVCP groups, which were also significantly greater ($p < 0.05$) than the negative controls. On D16, birds in the VG/GA+CVCP had significantly higher ($p < 0.05$) cecal tonsil IFN γ , IL-1 and IL-17 mRNA and spleen IFN γ mRNA compared to all other groups. It can be concluded that the VG/GA vaccine stimulated local and systemic proinflammatory cytokine mRNA levels and improved production performances during a NE challenge.

Keywords: VG/GA; vaccine; coccidiosis; Necrotic enteritis; broiler performance

411P Rooster testis as a reservoir for *Mycoplasma synoviae* strains: Implications for vertical transmission

Eniope Oluwayinka*¹, Naole Ferguson-Noel¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

Mycoplasma synoviae (MS) is a significant avian pathogen associated with respiratory disease, airsacculitis, and reproductive problems, including decreased egg production, eggshell abnormalities, and infertility in hens, yet its role in male reproductive tract colonization is under-documented. This study aimed to determine the capacity of virulent field strains of MS to colonize the testes of roosters. Roosters were inoculated with five distinct MS strains isolated from chickens in the United States and observed for two weeks post-challenge. Testicular tissues were collected for both MS culture and strain-specific PCR, air sac and footpad lesions were scored, while the trachea was simultaneously collected for histopathology and PCR. Necropsy revealed significant air sacculitis and footpad lesions, along with tracheal thickening. Two of the five challenged MS strains, both originating from North Carolina, were isolated from testicular samples, achieving isolation rates of 29.2% and 42.9% in the respective groups. The testicular samples were also confirmed positive via MS-specific PCR. Sequence typing of the *vhhA* gene indicated that the two testicular isolates represented a distinct genotype. The testicular isolates showed 99.11% genomic identity with the ATCC reference strain (WVU). Crucially, proteomic comparison with the three other non-testicular isolates revealed non-synonymous mutations in proteins located downstream of the siderophore-mediated iron transport protein, a pathway vital for iron acquisition that influences biofilm formation, motility, and survival. These findings furnish direct evidence that virulent MS strains can colonize rooster testicular tissue. This colonization suggests the male reproductive tract may serve as an additional reservoir for MS persistence. These results have substantial implications for understanding venereal and potential vertical transmission in poultry breeder flocks and underscore the necessity for further research into the mechanisms of MS dissemination within the male reproductive system.

Keywords: *Mycoplasma synoviae*; Testis; Reservoir; Poultry; United States of America

412P Booster immunization with *Histomonas meleagridis* cathepsin protease recombinant protein vaccine elicited strong immunogenic responses in turkeys

Jossie M. Santamaria*¹, Chrysta N. Beck², Aaron Forga¹, Kasey A. Matusik¹, Diego Cortes¹, Melissa Martinez¹, James Higuaita¹,

Karen Echevarria¹, Zhicheng Dou³, Gisela Erf¹, Danielle Graham¹ ¹University of Arkansas System Division of Agriculture, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States; ²Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States; ³Clemson University, Department of Biological Science, Clemson, South Carolina, United States

There is a critical need to develop effective vaccines against *Histomonas meleagridis* (HM) in turkeys, as no treatments are available to prevent disease. This study monitored the local tissue leukocyte profiles and systemic plasma-IgG responses to a booster immunization with an HM cathepsin-protease vaccine using the growing feather (GF)-pulp bioassay in sensitized female turkey poults. Poults received a primary and a secondary s.c. vaccination (250 µL/poult) at 1d and at 14d of age, respectively, with either HM-protein in emulsion (HM-Emul), in PBS (HM-PBS), or vehicles alone (3 poults/treatment). At 5 weeks, poults were immunized by intradermal (i.d.) GF-pulp injections (10µL/GF; 250µL/poult) with respective treatments. GF-pulps sampled before (0d) and at 3h, 1d, 2d, 3d, and 5d post-GF-pulp injection (p.i.) were subjected to immunofluorescent staining and FACS analysis. Heparinized blood was collected before (0d) and at 3 and 5d p.i. to measure plasma HM-specific IgG using ELISA.

Data were analyzed using a 2-way ANOVA for GF and a 2-way repeated-measures ANOVA for blood, with significance placed at $P \leq 0.05$. Booster immunization with HM-PBS and HM-Emul into GF-pulps stimulated the recruitment of heterophils, reaching peak levels (% pulp cells) at 3h, and returning to pre-injection levels at 2d p.i. ($P < 0.001$). Independent of treatment, MHCII⁺ levels remained at baseline up to 1d, increased to highest 2d, and returned to baseline on 5d p.i. ($P = 0.015$). The main effects of time revealed maximal recruitment of CD4⁺ T cells at 1d, with a return to baseline at 3d p.i. ($P < 0.001$), while CD8⁺ cells reached highest levels at 3h that were sustained up to 5d p.i. ($P < 0.001$). B cell levels increased at 1d and remained elevated up to 5d p.i. ($P = 0.006$). Independent of time, recruitment of CD28⁺ T cells ($P < 0.001$), CD4⁺ T cells ($P = 0.010$), and B cells ($P < 0.001$) was higher with vaccines than with vehicles. Independent of vaccine, HM-specific IgG levels in plasma were elevated at all time points ($P < 0.001$) p.i. Although the two prior immunizations elicited high HM-specific plasma IgG, the GF-pulp booster vaccinations with HM-PBS and HM-Emul stimulated rapid and sustained lymphocyte responses, suggesting potent immunogenicity that may confer protective immunity against HM in turkeys.

Keywords: *Histomonas meleagridis*; Vaccine; Cathepsin protease; Growing feather-pulp bioassay; Immune responses

Teaching, Pedagogy, Extension

413P Building teacher confidence through experiential poultry processing instruction

Jesse Bower*¹, Laura Huisinga², Katy Tarrant¹ ¹Fresno State, Animal Science and Agricultural Education, Fresno, California, United States; ²California State University, Fresno, Department of Art and Design, Fresno, California, United States

The purpose of this project was to strengthen agricultural educators' confidence and teaching skills in poultry processing by combining online curriculum training with hands-on learning. A professional development workshop titled Poultry Processing 101: Best Practices for Safety and Compliance was presented to 22 high school agriculture teachers during the 2025 California Agricultural Teaching Association AgriSkills Conference at California Polytechnic State University, San Luis Obispo (Cal Poly). The workshop was designed in two parts. Day 1 focused on classroom instruction using the Broiler Key Welfare Indicators (KWI) curriculum developed by Fresno State's Center for the Optimization of Poultry (COOP). Participants explored poultry production systems, welfare assessment, and food safety practices through digital certification modules and guided discussion. Day 2 centered on experiential learning, where participants processed

meat birds at the Cal Poly Meat Processing Unit following humane handling, welfare, and HACCP-aligned procedures from shackling through chilling and packaging. Post-workshop evaluations collected by Cal Poly demonstrated consistently high satisfaction amongst participants. On a 5-point scale, teachers rated the usefulness of content at an average score of 4.95 (SE=0.05), hands-on activities at an average score of 4.68 (SE=0.18), and materials and resources at an average score of 4.91 (SE=0.06). All participants rated the pace "Just Right," and one respondent noted the content was "Above" their current skill level. Comments reflected excitement and classroom relevance, such as "Super awesome hands-on!", "So much useful information," and "The best class I've ever participated in." Results show that pairing digital certification materials with live poultry processing improved both confidence and instructional readiness among educators. This blended approach demonstrates an effective model for bridging classroom instruction with industry standards in agricultural education.

Keywords: agricultural education; poultry science; experiential learning; professional development; certification

Welfare and Behavior

414P Strain and aviary design shape daytime aviary use in cage-free laying hens

Gretchen Peckler¹, Huaijun Zhou¹, Maja Makagon¹, Richard Blatchford*¹ ¹University of California, Davis, Animal Science, Sacramento, California, United States

Cage-free aviaries offer a more complex environment than conventional cage systems, with the goal of allowing hens to express a broader range of natural behavior. However, the welfare benefits of these systems are contingent on hens actively using them. We investigated how aviary design and genetic strain affect daytime usage of aviaries. We randomly assigned 320 Hy-Line Brown and 320 Hy-Line White laying hens to standard (3-tier, metal round perches) or enhanced (3-tier, plastic mushroom shaped perches, ramps) aviaries, with four pens (40 hens/pen) representing each strain by treatment combination. Using

instantaneous scan sampling with 15-minute intervals, we assessed the proportion of hens using the aviaries during a morning (9:00am-11:00am) and an afternoon period (1:00pm-3:00pm) at 23, 28, 33, and 37 weeks of age. Data were analyzed by fitting a generalized linear mixed model with a binomial distribution with the fixed effects of age, housing treatment, period, hen strain and two-way interactions between housing and hen strain, housing and age, as well as hen strain and age, with pen as a random effect. Overall, daytime aviary use was low. However, hens used aviaries more in the morning (proportion of hens \pm SE: 0.20 ± 0.005) than in the afternoon (0.18 ± 0.005 ; $P < 0.001$) and Hy-Line White hens (0.21 ± 0.007) used aviaries more than Hy-Line Brown hens (0.17 ± 0.006 ; $P < 0.001$). A greater proportion of hens used the enhanced (0.198 ± 0.006) compared to the standard aviary (0.183 ± 0.006 ; $P = 0.004$). Aviary use was affected by hen age with the greatest proportion of hens using the aviaries at 23 weeks of age ($0.234 \pm$

0.006) and least at 28 weeks of age (0.149 ± 0.005 ; $P < 0.001$). There were no interaction effects. While overall low daytime usage of aviaries was limited for both strains, enhancing aviaries to facilitate movement between tiers did increase usage. This study highlights the importance of aviary design in promoting system use, particularly for Brown hens, which have previously been reported to prefer lower surfaces.

Keywords: laying hen; aviary design; behavior; strain

415P Genetic impact on laying hen keel bone fractures

Cloude Shao¹, Maja Makagon¹, Anna Wolc², Hao Cheng¹, Huaijun Zhou¹, Richard Blatchford¹¹*University of California, Davis, Animal Science, Sacramento, California, United States;* ²*Iowa State University, Ames, Iowa, United States*

A major welfare concern for laying hens are keel bone fractures (KBF), which have been shown to impact production and are likely painful to the birds. The prevalence of KBF may vary across genetic strains, yet the existent data is limited. The goal of this study was to investigate the prevalence of KBF in three different genetic lines of breeder laying hens housed in identical individual cage systems via radiographic images. We hypothesized that certain genetic lines would be more likely to develop fractures due to underlying genetic differences. Breeder hens were x-rayed at both 30 and 60 weeks of age (woa). Three genetic lines were examined: one brown (30 woa: $n = 985$, 60 woa: $n = 500$), and two white (whiteA, 30 woa: $n = 200$, 60 woa: $n = 484$; whiteB, 30 woa: $n = 1000$; 60 woa: $n = 537$). The data was analyzed using chi-square to compare fracture presence across the three genetic lines. At 30 woa, all three lines (brown = 1.1%, whiteA = 27%, whiteB = 4.4%) differed in the percentage of fracture presence ($X^2 = 236.18$, $df = 2$, $p < 0.05$). At 60 woa, whiteB had a lower fracture prevalence (3.2%; $X^2 = 387.64$, $df = 2$, $p < 0.05$) compared to brown and whiteA, while brown (55.2%) and white A (53.1%) no longer differed from each other ($p = 1.00$). As all hens in the study were housed in identical single-cage systems with limited behavioral opportunities, environmental variation was likely not a contributor to the presence of KBF. However, differences in body weight or size between the lines, as well as differences in temperament or reactivity may have contributed to the results, although these were not measured in this study. The findings from this study provide insight into genetic predisposition to KBF, contributing to breed selection and management strategies aimed at mitigating KBF. Future studies should focus on how heritable KBF are, and how those genes flow into subsequent generations, which are frequently crosses of genetic lines.

Keywords: laying hen; keel bone fractures; genetic strain

416P Automated tracking of individual broilers in group settings

Oluwadamilola M. Oso^{*1}, Guoming Li¹²³, Anderson A. Alves²⁴, Samuel E. Aggrey¹, Khaled Rasheed⁵¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States;* ²*University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States;* ³*University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States;* ⁴*University of Georgia, Department of Animal and Dairy Science, Athens, Georgia, United States;* ⁵*University of Georgia, School of Computing, Athens, Georgia, United States*

Tracking individual broilers is essential to account for within-group variability during behavioral evaluation, disease detection, or digital phenotyping. However, the previously developed algorithm has challenges of identity (ID) switches, tracking errors and occlusion due to complex poultry environment and similarity in bird appearance. This study aimed to (1) develop hybrid algorithms of YOLO v12n and ByteTrack to track individual broilers in group settings with high detection rates, good tracking accuracy and ID consistency; and (2) evaluate the effect of high-, medium, and low-level activity on tracking performance. 1,776 Cobb-500 broilers were randomly allotted to 8 treatments with 6 replicates containing 37 broilers each. The broilers were fed diet supplemented with 5, 125, 250, or 500 mg/kg of CuSO₄·5H₂O and raised either on fresh or reused litter. To track and assess individual responses to diet, each group was video recorded for 24 hours using one-hour time lapse schedule at 5 frames per second. Ten of the 37 broilers were color marked for continuous tracking. Result shows that YOLO v12n and ByteTrack had multi-object tracking accuracy (MOTA) of 98.2%, multi-object tracking precision (MOTP) of 94.3%, identification F1 score (IDF1) of 97.0%, identification precision of 97.9%, and identification recall of 97.3%. Longest tracking duration was 55 min and 22 secs with an average tracking duration of 17 mins and 53 secs. Average rate of ID switches was 3.80. Activity level affected tracking duration. For low activity level, seven birds were tracked 100% of the observation period of 1.67 mins, while three birds were tracked between 90.13 and 99.67% of the observation time. For medium activity level, eight birds were tracked 100 % of the time, while two birds were tracked 54.5% and 92.21% of the time. For high activity, five birds were tracked 100 % of the time, four birds were tracked between 70.20 and 98.93 % of the time, while one bird was tracked for 1.40% of the time. The developed algorithm is suitable for tracking individual birds with high accuracy, and the tracking duration per bird was inversely related to the activity level. High activity birds exhibited a decreased tracking duration per bird, and the opposite was true for low activity birds.

Keywords: poultry; tracking; object detection; animal welfare; artificial intelligence

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