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Abstracts of the

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Annual Meeting

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ORAL PRESENTATIONS

Student Competition: Immunology, Health and Disease: Immunology

1 Development and evaluation of a bovine adenovirus-based avian influenza vaccine for poultry

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Avian influenza virus (AIV) has been a threat to the poultry industry and public health. Current injectable AIV vaccines have limitations that necessitate the development of more effective alternative vaccines. This study aimed to develop and evaluate the immunogenicity and protective efficacy of a bovine adenovirus-vectored (BAds) AIV vaccine encoding the hemagglutinin (HA) gene of H5N1 and the nucleoprotein (NP) gene of H7N9 AIV subtypes. We constructed two live BAds-E1E3-deleted vectors: one expressing HA and NP (BAds-HA+NP) and the other as an empty vector without AIV inserts, and tested their efficacy in broiler chickens via three mucosal routes and doses. 85 broiler chickens were divided into 14 groups (n = 6birds/group) and vaccinated at post-hatch day 3 and 21 via oral (OR), intranasal (IN), and intraocular (IO) routes, using three doses (1×10^6, 1×10^7, and 1×10^8 PFU) in a prime-boost regimen. The empty vector was given at 1×10^8PFU via the same routes. Additional groups included a mock no-vaccine/no-challenge group and a mock-challenged group. All birds were housed in separate biosafety isolators.Two weeks post-booster, all birds (except the mock group) were challenged with 2×10^6 EID_{50} of a low pathogenic H5N2 AIV strain. Birds were monitored for clinical signs, body weight (BW), and feed conversion ratio (FCR). Samples (blood, cloacal, oropharyngeal and tracheal swabs) were collected on days post-vaccination/challenge (DPV/DPC) DPV 0, 21, and DPC 0, 2, 4, and 6. On necropsy day (DPC6), bile, small intestinal wash, cecal tonsil, and spleen were collected for mucosal IgA analysis, splenocyte isolation for T-cell profiling, and cytokine gene expression in the cecal tonsils. Humoral response was assessed using hemagglutination inhibition (HI) assay and ELISA (serum IgY and mucosal IgA). Cellular response was evaluated by flow cytometry ($CD4^+$, $CD8\alpha\beta^+$, and $TCR\gamma\delta^+$ T cells) from spleen of 1×10^8 dose, mock, and mock challenge groups. Viral loads in tracheal and cloacal samples were determined by RT-qPCR and hemagglutination assay (HA). Data were analyzed using two-way ANOVA with Bonferroni post-hoc tests ($p < 0.05$). Our results demonstrated that the BAds-HA+NP vaccine, 1×10^8 PFU dose inoculated IO, elicited the highest HI titers, serum IgY, and mucosal IgA levels ($p < 0.001$). This group also had increased splenic $CD4^+$, $CD8\alpha\beta^+$, and $TCR\gamma\delta^+$ T cells frequency, >2-log reduction in tracheal viral load ($p < 0.01$) compared to other groups. The 1×10^7 IO group showed moderate immune activation and protection, while OR and IN routes were less effective at lower doses. In conclusion, This study is the first to report BAds-HA+NP AIV vaccine efficacy in birds, and the 1×10^8 PFU IO route is the best.

Keywords: Avian Influenza, Adenovirus Vector, Vaccine Development, Mucosal Immunity, Broiler Chickens

2 Intradermal injection of peptidoglycan stimulates local and systemic inflammatory responses in turkeys: insights from a dual approach window

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The growing feather (GF)-pulp bioassay established in chickens was also recently validated as a dermal test-system in turkeys. Intradermal (i.d.) injections of test materials into the pulp of multiple GFs followed by concurrent sampling of injected GFs and blood (dual-window) at various times post-injection (p.i.) allow for assessment of local and systemic cellular responses to the test-material over time in the same bird. There is a lack of information regarding the immunomodulatory effects of Gram-positive bacterial components in turkeys. This longitudinal study examined local (GF-pulp) and systemic (blood) inflammatory responses in turkey poults to i.d. GF-pulp injections of peptidoglycan (PGN) from *Staphylococcus aureus*, a microbe-associated molecular-pattern (MAMP) of Gram-positive bacteria. To do this, 5-wk-old turkey poults were randomly assigned to two treatment groups consisting of i.d. GF-pulp injections of 16 GFs/poult (10µL/GF) with either PGN (1µg/GF; n=8) or endotoxin-free PBS (n=5). GF and heparinized blood were collected before (0h) and at 3, 6, 24, 48, and 72h p.i. to determine GF-pulp and blood leukocyte profiles by FACS. GF and blood data were analyzed by two-way ANOVA and two-way repeated measures RM-ANOVA, respectively. Student's t-tests multiple means comparison was applied when appropriate, with statistical significance set at $P\leq0.05$. I.d. GF-pulp injection of PGN triggered an early infiltration of heterophils and macrophages reaching highest levels (% pulp cells) by 3h and returned to near pre-injection levels by 72h p.i. ($P\leq0.05$). Infiltration of $CD28^+$, $CD4^+$, $CD8\alpha^+$, and B lymphocytes reached peak levels at 24h p.i. and remained above pre-injection levels thereafter ($P\leq0.05$). Blood leukocyte profiles were also impacted by i.d. GF-pulp injections of PGN. Blood heterophils reached highest ($P\leq0.05$) concentrations at 3h p.i. and returned to baseline by 24h p.i. A transient drop ($P\leq0.05$) in the concentrations of monocytes, $CD28^+$, $CD4^+$, $CD8\alpha^+$, and B lymphocytes was also observed at 6h p.i. The rapid GF-pulp infiltration of heterophils and macrophages followed by a later and sustained infiltration of T and B lymphocytes in PGN-injected GF-pulps, along with the accompanying changes in blood leukocyte profiles, indicate the capability of turkeys to mount an acute inflammatory response to this bacterial MAMP. The observed temporal changes in leukocyte recruitment in GF-pulps initiated by PGN are in line with those observed in chickens, although, lymphocyte infiltration was lower in turkeys than in chickens. The new knowledge gained from this study will find application in vaccine development against Gram-positive bacteria in turkeys.

Keywords: inflammatory response, peptidoglycan, turkey, leukocyte profiles, growing feather-pulp bioassay

3 Probiotic-induced microbiome modulation alters immune cell metabolism through the mevalonate pathway

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Optimizing immune function is essential for poultry health and productivity, and the microbiome plays a crucial role in immune system development. However, the mechanisms driving this are unknown. In previous studies, our laboratory observed increased ATP levels in circulating immune cells of chickens fed a probiotic consortium (*Lactobacillus acidophilus, Lacticaseibacillus casei, Bifidobacterium bifidum, Enterococcus faecium*). Further investigations indicated that avian lymphocyte cell lines treated with serum from probiotic-fed chickens had increased ATP levels compared to controls. This effect was eliminated by delipidation of the serum prior to treatment, suggesting that it was driven by a microbiome-derived serum lipid. To explore the metabolic basis of this effect, we performed differential abundance analysis (ANCOM-BC2) on 16S sequences and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2)-imputed functional pathways from ileum and cecum microbiota. Additionally, we conducted metabolomic profiling using Significance Analysis of Microarrays and Metabolites on serum, ileum, and cecum samples from probiotic- and control-fed chickens. Our results showed differential abundances of several lipid metabolites, including increased levels of mevalonate and mevalonolactone in the serum and ileum, and more abundant predicted mevalonate-related pathways in the ileum microbiome. These findings were further supported by transcriptomic data from Ingenuity Pathway Analysis of T-lymphoblastoid cell line treated with probiotic-fed chicken serum, revealing downregulation of mevalonate and cholesterol-related pathways, which can be regulated by mevalonate itself. Together, these results suggest that microbiome-derived mevalonate metabolites enter circulation and influence immune cell metabolism. Therefore, we hypothesize that mevalonate enhances ATP production in lymphocytes. To test this, a T-lymphoblastoid cell line was cultured with increasing concentrations of mevalonate 5-phosphate (0, 6.25, 12.5, 25, 50, and 100 µM). After a four-day incubation, ATP levels were measured, normalized to cell number, and analyzed using Kruskal-Wallis and Wilcoxon tests. Lymphocytes treated with 25 µM mevalonate exhibited significantly higher ATP production per cell ($p = 0.01$) compared to those cultured in control media. Together, these findings suggest that the probiotic-modulated microbiome induces enhanced lymphocyte ATP production through mevalonate pathway metabolites. Future research will investigate the broader immunometabolic effects of mevalonate in adaptive immunity and its potential applications for improving poultry health and performance.

Keywords: Probiotics, Microbiome, Mevalonate, Immunometabolism, Lymphocytes

4 Dietary supplementation of ginger root extract reduced intestinal *Salmonella* colonization in broiler chicks

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Due to the ban on antibiotic growth promoters, ginger root extract (GRE) is being explored as a natural alternative in poultry feed for its antimicrobial, anti-inflammatory, and antioxidant properties and ability to support immune function, gastrointestinal health, and nutrient absorption in poultry. The study investigated the effect of ginger root extract supplementation on intestinal colonization of *Salmonella* Typhimurium (ST) in broiler chicken. Day-old broilers (Ross 708 male, n = 600) were distributed into six treatments with five replicates of 20 birds each. All 6 treatment groups were fed a corn-soybean meal basal diet throughout the experiment. Control (CON) and CON-ST (positive control) were fed basal diet with no medication, BMD (basal diet + bacitracin methylene disalicylate 0.055 g/kg diet), GRE-A (basal diet + 0.75% GRE), GRE-B (basal diet + 1.5% GRE), and GRE-C (basal diet + 3% GRE). On d 2, all treatments were orally gavaged with 7.46 × 108 CFU ST /mL except control (CON). Body weight gain (BWG), Feed intake (FI), feed conversion ratio (FCR) and mortality (%) were recorded over a 6-week period. Data were subjected to One-way ANOVA. The highest ($P<0.05$) cecal ST concentrations reduction among the challenged groups was observed in GREC-ST at d 5 (3.62 CFU/g) and d 12 (1.50 CFU/g), indicating a dose-dependent manner. At 0-3 weeks, GREC-ST (0.70kg) had the poorest ($P<0.05$) BWG when compared to CON (0.84kg), while birds in CON-ST (0.88kg), BMD-ST (0.87kg), GREA-ST (0.84kg) and GREB-ST (0.84kg) had similar (P>0.05) BWG to CON. The FI followed similar trend as the BWG, while FCR was similar in CON and across all other treatment groups ($P=0.0545$). At 4-6 weeks, CON displayed higher (P<0.05) BWG similar to other challenged groups except GREC-ST, birds in GREA-ST (P<0.05) had improved BWG than GREB-ST and GREC-ST. The FI and FCR values were however similar across all treatments. The overall performance (0-6 weeks) revealed significant differences ($P<0.05$) only in BWG. While birds in CON had higher BWG similar to those obtained in CON-ST (3.35), BMD-ST (3.32), GREA-ST (3.26), and GREB-ST (3.07), birds in GREC-ST had a depressed BWG compared to other GRE groups and BMD-ST. Supplementing broiler diets with GRE demonstrated a dose-dependent reduction in cecal *Salmonella* colonization, however, moderate GRE levels (0.75% and 1.5%) maintained BWG comparable to control group. These findings indicate that while GRE exhibits antimicrobial properties against ST, optimizing its inclusion level is crucial to balance growth performance and health benefits in broiler chickens.

Keywords: Ginger root extract, Salmonella Typhimurium, bioactive compounds, broiler chicks

5 Macronutrient specific initiation of the metabolic switch in newly hatched chickens

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A characteristic feature of the embryonic-to-post-hatch transition in chickens is the metabolic shift from relying heavily on yolk lipid oxidation in the embryonic stage to a dramatic upregulation of *de novo* lipogenesis in the post-hatch liver. While this metabolic switch is generally attributed to the provision of diet immediately post-hatch, the role of specific dietary macronutrients towards this biochemical transition remains unclear. We hypothesized that carbohydrates by itself can initiate the metabolic switch, similar to a complete diet containing all three macronutrients. Newly hatched Ross 708 broiler chickens were randomly subjected to one of the following five treatments for 48 hours post-hatch (n=8/group). Sets of chickens were orally gavaged (three times/day) with isocaloric amounts of semi-purified diets, either complete with all the three macronutrients in the form of dextrin, casein hydrolysate, and corn oil (SP-Com), or 85% of the calories derived from dextrin (SP-Dex), or corn-oil (SP-Oil), or were given *ad libitum* access to starter diet (fully fed; FF), or were fasted for 48-hrs (DF). Additionally, one group of chicks were sampled on the day of hatch (DOH) for baseline measurements before metabolic switch. Plasma metabolites from these groups were measured using gas-chromatography mass spectrometry and hepatic gene expression profiles were determined using qPCR analysis. The results were analyzed in GraphPad Prism version 10.0.0 (Boston, Massachusetts USA) using one-way ANOVA followed by pairwise mean comparisons, with the statistical significance set at $P \leq 0.05$. While plasma levels of pyruvate, lactate and alanine all remained similar between the DOH, DF and SP-Oil groups, their levels were significantly higher in FF, SP-Com and SP-Dex plasma ($P \leq 0.05$), reflective of higher rates of carbohydrate oxidation. In addition, plasma β-hydroxybutryrate levels remained high in DOH, DF and SP-Oil groups, but decreased ($P \leq 0.05$) following FF, SP-Com and SP-Dex treatments, reflecting the downregulation of lipid oxidation. Furthermore, FF, SP-Com and SP-Dex treatments all resulted in the upregulation of hepatic lipogenic gene expression (e.g., *SCD1*, *ELOVL6*; $P \leq 0.05$) compared to their DOH, DF and SP-Oil counterparts. The ability of SP-Dex treatment to elicit responses similar to those of FF and SP-Com highlights how dietary carbohydrates by itself can initiate multiple aspects of the metabolic transition in chickens. Further understanding of macronutrient-specific modulation of the biochemical events involved in the metabolic switch will allow us to optimize nutritional strategies for the healthy growth and development of hatchlings.

Keywords: chicken, liver, macronutrients, metabolic switch, lipogenesis

6 Western Canadian faba beans (*Vicia faba*) as a feedstuff for laying hens: A tale of two varieties

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The high starch (~35%) and protein (~28%) content of Western Canadian faba beans (*Vicia faba*) suggests suitability as a feedstuff for laying hens, however little empirical evidence is available to support their use as such. A particular knowledge deficit pertains to the biological significance for laying hens of two related anti-nutritional factors that are naturally occurring in faba bean – vicine and convicine (**V/C**). The low input requirements to grow faba bean also results in a significantly lower greenhouse gas emission intensity (**GHGI**) than other feedstuffs, which might offer the potential to in turn reduce the GHGI of egg production. The purpose of the present study therefore was to compare the effect of increasing dietary inclusion of two varieties of faba bean differing in V/C content in laying hen diets on productivity, egg quality and feed associated-GHGI of egg production. Laying hens (n=140; 42 wk of age) were fed either a wheat-soybean meal-based control diet or one of 6 nutritionally balanced test diets consisting of 12%, 24% or 36% dietary inclusion of either low ('Nevado'; **LVC**) or high V/C ('219-16'; **HVC**) varieties of faba bean. Each diet was fed to 5 test cages of 4 hens in a randomized complete block design in a 12-wk experiment. Feed disappearance, egg production and objective egg quality were measured for each of three, 4-week periods. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS statistical software. Models included main effects of variety and inclusion level and the 2-way interaction. Significance level for all statistical comparisons was 5% (α=0.05). There was no significant effect of increasing dietary inclusion ($P > 0.64$) of either faba bean variety ($P > 0.10$) on feed intake. Increasing dietary inclusion of HVC however resulted in linear reductions in average egg weight ($P < 0.01$), egg production ($P < 0.01$), daily egg mass production ($P < 0.01$) and egg mass:feed ($P < 0.01$). There was no adverse effect of inclusion level ($P > 0.10$) or variety ($P > 0.10$) on proportional weight of egg components. Increasing dietary inclusion of either faba bean variety linearly reduced the calculated feed-associated GHGI of egg production (using egg production data and feedstuff GHGI values from the ECOALIM database), with a maximal reduction of 29% (36% LVC vs. control). In conclusion, our results suggest that up to 36% inclusion of LVC faba bean in laying hen diets can reduce the GHGI of egg production without any adverse impacts on productivity or egg quality. Further, our results demonstrate the biological significance of V/C as an anti-nutritional factor for modern laying hens.

Keywords: laying hen, faba beans, productivity, antinutritional factor, greenhouse gas emission intensity

7 Maximizing 27 to 35 d growth performance and processing metrics of broilers with and without a subclinical necrotic enteritis challenge through corn particle size and feed quality

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The current study was conducted to conclude a series of studies aimed at optimizing corn particle size (CPS) and feed quality (FQ) for maximized broiler performance and health. Within the first study, 0 to 17 d broiler growth was improved when feeding the combination of 900 µm (Medium; M) CPS (MCPS) and High FQ (HFQ) crumbles (3200 µm). The second, utilizing the optimal CPS and FQ combination from study 1 for a pretest, determined improved 17-28 d performance in birds with and without a

subclinical necrotic enteritis challenge (SubNEC; two experiments (EXP)), and reduced jejunum lesion scores with MCPS and HFQ (80% pellets). The SubNEC was induced via orally gavaging birds on d 14 with 10x live coccidiosis vaccine and on d 18 with *C. perfringens* (8.2 log cfu/mL). The current objective was to use previously established optimal CPS and FQ until 27 d, then determine the CPS and FQ combination to maximize 27 to 35 d male Ross YP x 708 broiler performance; growth and processing metrics within 1) an unchallenged EXP (EXP1) and 2) SubNEC EXP (EXP2; no processing). Each EXP was conducted simultaneously in separate facilities, following a 3 x 3 factorial arrangement with 3 CPS: LCPS (600 µm), MCPS (900 µm), and HCPS (1200 µm) and 3 FQ: low (LFQ; 40% pellets), medium (MFQ; 60% pellets), and high (HFQ; 80% pellets). The CPS and FQ variations were created by pelleting common basal diets, differing only in CPS. After hand-sieving batches by CPS, pellets and fines were blended to create FQ treatments. Birds were reared in floor pens with used litter (0.05 m^2/bird); EXP1 had 10 replications, 5 birds/pen were utilized for processing, and EXP2 had 5/trt replications without processing due to ABSL-2 facilities required. For EXP2, birds were orally gavaged on d 14 with 10x live coccidiosis vaccine and on d 18 with *C. perfringens* (8.2 log cfu/mL). Data were analyzed using a two-way ANOVA in SAS 9.4 ($\alpha \leq 0.05$); there were no significant CPSxFQ interactions ($P>0.05$), regardless of EXP. In EXP1, birds fed MCPS and HCPS had improved d 35 BW vs. LCPS ($P=0.0429$). Also, HFQ had the highest d 27-35 BWG ($P=0.0201$) and numerically improved FCR ($P=0.0759$). Processing at d 36, resulted in HFQ birds having a heavier carcass, breast, and total breast weight when compared to MFQ and LFQ ($P<0.05$). In EXP2, birds that consumed HFQ had a heavier d 35 BW when compared to MFQ and LFQ ($P=0.0084$). Birds fed HFQ had improved d 27-35 FCR ($P=0.0157$) by ~5pts vs LFQ, but similar to MFQ birds; there were no differences found for CPS ($P>0.05$). Overall, these data demonstrate a consistent benefit when feeding HFQ, regardless of disease status (EXP1&2) and response to CPS was more pronounced in EXP2.

Keywords: Corn particle size, feed quality, broiler performance, subclinical necrotic enteritis

8 Full-fat soybean nutrient and antinutrient characterization and variability from two origins

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A comprehensive understanding of nutrient and antinutrient variability of full-fat soybeans (**FFSB**) is essential for precision nutrition. This study aimed to evaluate and compare the composition and variability of FFSB sourced from Brazil (**BRA**, N = 6,181) and the United States (**USA**, N = 1,682). Commercial data (ABVista) containing user-submitted near-infrared spectroscopy (**NIRS**) readings from 2019 to 2024 were analyzed in JMP 18. A mixed-model regression was used to compare FFSB composition between origins, with the harvest year and NIRS sample submittal country included as random effects. Distributions were fitted to all composition data and best represented by mixtures of normal distributions. The Normal Mixtures platform was used to cluster FFSB into three groups (C1, C2, C3) within each origin, based on proximate composition (88% DM-basis). Clusters were analyzed using one-way ANOVA, and means were separated using Tukey's HSD. Differences ($P \leq 0.05$) were detected for all evaluated composition data. Compared with BRA, samples from USA had higher average total non-starch polysaccharide (**NSP**, 13.72 vs. 12.18%), insoluble NSP (11.08 vs. 9.30%), total arabinose + xylose (**A+X**, 3.77 vs. 3.32%), ash (5.46 vs. 5.03%), TDF (15.91 vs. 15.45%) and lignin (1.41 vs. 1.28%). The BRA FFSB samples had higher average CP (35.84 vs. 35.25%), crude fat (21.85 vs. 19.57%), CF (5.11 vs. 4.83%), phytic P (0.37 vs. 0.35%), ADF (7.83 vs. 7.40%), and NDF (10.43 vs. 9.62%). Within-origin cluster analysis revealed substantial internal variability, with differences ($P < 0.001$) in each variable evaluated. The USA clusters represented 30.5 (C1), 39.8 (C2), and 29.7% (C3) of samples, whereas the BRA clusters represented 30.8 (C1), 49.7 (C2), and 19.5% (C3) of samples. Notable variation was observed in the average cluster CP in BRA C1, C2, and C3 (34.87, 33.83, and 36.12%, respectively), with less variation in USA CP (35.31, 35.43, and 36.33%). Similar variability was observed for TDF in the USA clusters (17.12, 14.89, and 18.61%, respectively), with less variation in BRA TDF (16.45, 13.67, and 16.55%). While the range between average cluster total NSP was larger for BRA clusters (1.01 vs. 0.91%), the USA clusters had a larger range for insoluble NSP (1.40 vs. 0.79%). These results demonstrate substantial inter- and intra-origin variability in the nutrient and antinutrient composition of FFSB, providing a description of FFSB composition. In conclusion, leveraging commercial data on nutrient and antinutrient composition can enhance precision, enabling nutritionists to make more informed decisions about dietary constituents and optimize ingredient and enzyme inclusion for diet consistency, cost efficiency, and performance outcomes.

Keywords: full-fat soybean, nutrient, antinutrient, origin, variability

9 Not Presented

10 Effects of dietary fiber and feed form on production parameters of turkey toms

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Dietary fiber and feed form (i.e., pellets vs fines) modify gut health, behavior, and ultimately production efficiency in chickens, but less is known about the efficacy of these feeding strategies in turkeys. We hypothesized that turkey toms fed high-fiber, pelleted diets would display improved production performance compared to those on low-fiber, fines diets. A total of 740 male large white turkey poults were randomly assigned to pens with two fiber levels for the first 5 wks in a crumble feed form: 1) High fiber (3% CF) and 2) Low fiber (2% CF; 16 pens/treatment, 22 birds/pen). At 5 wks, two feed forms were applied to create four treatments for the remainder of the study (n=8 pens/treatment): 1) High fiber, pelleted (HFP; 4% CF), 2) High fiber, fines (HFF; 4% CF), 3) Low fiber, pelleted (LFP; 2% CF), and 4) Low fiber, fines (LFF; 2% CF). In both HF diets, CF increased by 1% every 3 wks to achieve 8% CF at 17 wks. All the formulations were based on breeder recommendations. Birds were weighed at 4, 9, 12, 15, and 18 wks. Data were analyzed in R software using linear models to assess the effects of fiber levels, feed form, and their interaction on body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) at each age. At 18 wks, there was a main effect of feed form on BW ($p=0.010$), where turkeys fed pelleted diets were heavier than turkeys fed fines diets (Pellet:21.33 [21.08,21.58], Fines:20.31 [20.06,20.56] kg, mean [95% CI]).

From 9 to 12 wks, LFP displayed the highest BWG, followed by HFP, HFF, and LFF (p=0.05; LFP:4.55 [4.44,4.67], HFP:4.38 [4.26,4.50], HFF:4.27 [4.15,4.39], LFF:4.21 [4.10,4.33] kg/bird). BW and BWG were not affected by feed form, fiber content, or their interaction at any other age (p>0.05). There was a main effect of feed form on feed intake from 4 to 9 wks (p<0.0001), 9 to 12 wks (p<0.0001), and 12 to 15 wks (p=0.0001), such that turkeys fed fines had a higher feed intake than turkeys fed pellets during each period. Feed form also had a main effect on FCR at 9 (p<0.0001), 12 (p<0.0001), and 15 wks (p=0.0001), where turkeys fed fines had a higher FCR than turkeys fed pellets at each age. At 18 wks, there was an interaction of feed form and fiber content on FCR (p=0.046), where LFP and HFP displayed similarly low FCRs, followed by HFF, and then LFF with the highest FCR (LFP:2.29 [2.14,2.46], HFP:2.39 [2.24,2.55], HFF:2.80 [2.64,2.95], LFF:3.02 [2.86,3.18]). Dietary fiber levels have minimal effects on turkey performance, but feed form primarily affects feed intake and consequently FCR. Turkeys fed fines appeared to waste or spill more feed while eating than turkeys fed pellets. Pelleted feed offers an advantage to optimize production efficiency.

Keywords: fiber, turkey, pellet, fines, feed

11 Impact of pellet diameter and particle size on the performance and processing yield of broiler chickens

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The objective of the study was to evaluate the performance and processing yield of broiler chickens fed with different pellet diameters and different geometric mean diameters (GMDs). A total of 960 one-day-old male Ross broilers were housed, and the experiment lasted from 18 to 42 days of age. The experiment followed a completely randomized design in a 3x2 factorial arrangement, with three different pelleting dies (3.5, 4.0 and 4.5 mm) and two different GMDs of the feed (900 and 1,200 µm), totaling 6 treatments with 8 replicates of 20 birds each. From 1 to 14d of age, the animals were fed a mash diet based on corn and soybean meal, and after a 4-day adaptation period, the experimental period began at 18d of age. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated from 18 to 25d, 18 to 32d and 18 to 39d of age. At 42d of age, two birds per replicate were weighed live, euthanized, and subsequently, the carcass, breast, thigh, drumstick and abdominal fat were weighed to calculate the yield. Data were subjected to the Shapiro-Wilk normality test, and when the assumption was satisfied, a two-way ANOVA was performed. When significant, the means were compared using Tukey's test at a 5% probability level. An interaction between die size and GMD was observed for BWG and FCR in the periods from 18 to 25 and 18 to 32 days of age (P<0.05), where the 4.5 mm die combined with a GMD of 1,200 µm resulted in the lowest values for these variables. In the total period (18 to 39d), an interaction was found for BWG (P<0.001), where the largest die size exhibited the worst results. When analyzed individually, the 4.5 mm die resulted in lower FI and BWG from 18 to 25 days (P<0.001) and lower BWG and worst FCR (P<0.001) from 18 to 32 days. In the same period, a GMD of 900 µm resulted in improved BWG and FCR values (P<0.05). Over the total experimental period, the lowest BWG was obtained with the 4.5 mm die (3.5 mm = 2183 g; 4.0 mm = 2154 g; 4.5 mm = 2023 g; P<0.001), while the 900 µm GMD resulted in a higher BWG than 1,200 µm (2154 g vs. 2086 g; P<0.001). Regarding processing yield data, no interaction was observed for the analyzed variables (P>0.05). However, when the factors were analyzed individually, the 4.5 mm die resulted in the highest percentage of abdominal fat compared to the other treatments (3.5 mm = 1.4%; 4.0 mm = 1.3%; 4.5 mm = 1.7%; P<0.05). It is concluded that a GMD of 1,200 µm and a pellet die size of 4.5 mm negatively affected the performance parameters of broiler chickens. Furthermore, the 4.5 mm die increased abdominal fat deposition, whereas processing yield was not affected.

Keywords: broiler nutrition, particle size, performance, pellet quality, poultry

12 Effects of black soldier fly larvae meal inclusion on digestibility, live performance and carcass without giblets yield at 40d in Cobb 500 broilers

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Feed costs represent around 75 to 80 percent of the total expenses in poultry production, corn and soybean are the primary ingredients providing energy and protein respectively, therefore, it is essential to evaluate alternative ingredients for poultry nutrition. This study aims to investigate the effects on digestibility, live performance and carcass without giblets (WOG) yield at 40d of Cobb 500 broilers of three levels (5%, 10%, and 15%) of black soldier fly larvae meal (BSFLM) compared to a control diet (basal diet). A total of 480 male-chicks were randomly located in 48 floor pens (10 chicks/pen). Data was analyzed using one-way ANOVA in a randomized complete design. Mean separation was performed using Tukey's test. Results obtained at 40d showed that BSFLM inclusion improved the protein crude digestibility (PCD) and metabolizable energy digestibility (MED) (p < 0,05). Chicks fed 10% were best in PD (92,55±0,63%) and MED (76,34±1,23%) followed by the 5% inclusion level showing values of PD (84,52±1,01%) and MED (76,55±0,62%) close to the best inclusion. At 40d the inclusion of 5% of BSFLM improved BW, BWG and FCR (p < 0,05) and when is compared to the basal diet, this inclusion could be provide around 200g up in BW (3249 *vs.* 3050 g), BWG (3207 *vs.* 3008 g) and improve FCR up to 7 points (1,37 *vs.* 1,44 g:g). The carcass WOG yield was similar across all treatments with no statistical differences. In conclusion, BSFLM enhances live performance of broilers and improves digestibility. About carcass WOG yield, the treatments and basal diet showed similar results and for that reason the inclusion of BSFLM can be considered a viable alternative to soybean meal. However, when up the inclusion in the diet formulation could decrease the broiler performance.

Keywords: Black soldier fly meal, Performance, Digestibility

Student Competition: Immunology, Health and Disease: Health and Disease I

13 Effects of early supplementation of postbiotic on performance, gut morphology and expression of intestinal genes in broilers during subclinical necrotic enteritis

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Necrotic enteritis (NE) is an enterotoxemic disease of poultry caused by the Gram-positive bacteria *Clostridium perfringens* and inflicts substantial economic losses to the poultry industry. Postbiotics have emerged as a promising solution for NE as they can improve birds' performance and nutrient absorption, and reduce NE-associated pathology. This study evaluated the effects of *in ovo* and post-hatch applications of a *Saccharomyces cerevisiae*-based postbiotic on growth performance, intestinal morphology, and mRNA abundance of structural and immune response genes in broilers during a subclinical NE challenge. At embryonic day (d) 18, fertile broiler eggs were injected with 0.2 mL of either sterile water or postbiotic directly into the amnion. Male hatchlings (n=288; 72 birds/group with 6 replicate cages each) were randomly assigned to: 1) NC (*in ovo* water, no challenge); 2) PIW (postbiotic *in ovo* and in drinking water, no challenge); 3) NC+ (NC with NE challenge); and 4) PIW+ (PIW with NE challenge). On d 14, all birds in the NE-challenged groups were orally gavaged with 3,000 *E. maxima* sporulated oocysts followed by two doses of ~1×10^8 CFU/mL/bird of *C. perfringens* on d 19 and d 20. Growth parameters, intestinal lesion scores, intestinal morphology, and mRNA abundance of gut health and immune response related genes in the jejunum, ileum, and cecal tonsils (CT) were assessed. Data were analyzed by student's *t*-test and two-way ANOVA with significance set at $P \leq 0.05$. A significant interaction effect of postbiotic and NE challenge was observed during d 21-28 with a greater average daily gain (ADG) in PIW compared to NC and PIW+. Lesion scores in the jejunum and ileum were significantly reduced in PIW+ compared to NC+. On d 14, PIW birds had a lower crypt depth (CD) and a higher villus height-to-crypt depth (VH:CD) ratio in the jejunum. In addition, mRNA abundance of mucin-2 and olfactomedin-4 was greater in PIW birds, while the levels of tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS), interferon-gamma, and interleukin-10 were lower compared to the NC group. During peak infection on d 21, postbiotic supplementation resulted in better villi and crypt structures as manifested by a higher VH:CD ratio. Furthermore, NE-challenged birds receiving the postbiotic had a greater mRNA abundance of zonula occludens-1 and TNF-α in the jejunum, and iNOS in the CT compared to NE-challenged control group. In conclusion, the supplementation of postbiotic *in ovo* and in drinking water presents a potential to improve intestinal health and regulate immune responses in broilers during a subclinical NE challenge.

Keywords: necrotic enteritis, postbiotic, in ovo, gut integrity, immune response

14 Effects of green tea extract on performance and immune response during experimentally induced necrotic enteritis in broiler chicks

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This study aimed to evaluate the efficacy of polyphenol-rich Green Tea Extract (**GTE**) in mitigating necrotic enteritis and regulating immune responses in broiler chickens. In a 28-day experiment, 600-day-old Ross 708 male broiler chicks were randomly allotted into 6 treatment groups, having 5 replicates of 20 birds each in a completely randomized design. All 6 treatment groups were fed a corn-soybean meal basal diet throughout the experiment. **T1 (control)** and **T2 (positive control)** were fed basal diet with no medication, **T3** had 55 mg/Kg Bacitracin Methylene Disalicylate supplemented in their diet, while **T4**, **T5**, and **T6** diets contained GTE (top-dressed in the diet) at **250, 500, and 1000 mg/Kg diet**. On d 13, all chicks except CON received 1 mL (10× dose) coccidia vaccine, followed by 3 mL *Clostridium perfringens* (**CP**, 3.2×10^8 Colony Forming Unit (**CFU**)/mL) on d 18 and 19. T1 chicks were mock challenged with sterile thioglycolate media on d 18 and 19. Performance data 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 ileo-jejunal content for the enumeration of CP on blood agar. Additionally, small intestine segments from these chicks were evaluated for lesion scores. Splenocytes were isolated from spleen samples and analyzed by flowcytometry on d 28. All data were analyzed using one-way ANOVA. At the end of the experiment (d 28), results showed that birds in T2 (3.91) had poorer FCR ($P<0.05$) compared to T1 (1.48), while birds in T3 (3.44), T4 (3.26), T5 (2.83) and T6 (3.62) showed FCR that were comparable to T1. While T1 (1.52 kg) showed higher ($P<0.05$) weight gain compared to all challenged treatments, T4 (1.29 kg) tended to improve weight gain compared to challenge treatments T2, T3, T5, and T6 (1.19 kg, 1.21 kg, 1.25 kg and 1.03 kg) Mortality was higher ($P<0.05$) in all challenged treatments compared to T1, howbeit, T4 showed the lowest mortality rate ($P>0.05$) among challenge treatments. No intestinal lesion was observed in T1 birds, while birds fed GTE supplementation showed lower ($P<0.05$) jejunal lesion scores compared to T2 on d 20 and 26. On d 20, T6 (3.47 Log10 CFU/g) had intestinal CP comparable to T1 (1.78 Log10 CFU/g), while T3 (2.31 Log10 CFU/g) was similar to T1 (2.11 Log10 CFU/g) on d 26. Splenocytes showed a higher ($P<0.05$) monocyte/macrophage frequency in all challenged treatments, with T5 exceeding ($P<0.05$) T2. Only T6 showed a higher ($P<0.05$) Bu-1a frequency compared to T1. It was concluded that GTE supplementation at 500 mg/Kg enhanced immune response while abating growth depression during necrotic enteritis in broiler chicks.

Keywords: Green Tea Extract, Necrotic enteritis, Clostridium perfringens, Immune response, Broiler chicks

15 Host immunological responses in Ross 308 broilers fed anti-IL-10 diet during *Eimeria maxima* and *Clostridium perfringens* challenge

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Understanding host response mechanisms to intestinal pathogen-induced tissue damage may lead to improved treatment methods for diseases such as necrotic enteritis, which reduces broiler

performance and causes mortality. Three experimental replicates (R1, R2, R3) of Ross 308 broilers were placed in 32 cages (20 birds/cage) and received diets±anti-IL-10 antibody, a dietary intervention to improve immune activation. R1 and R2 received $1x10^8$ CFU *Salmonella Typhimurium* (ST) on day (d) 0. In a 2×3 factorial design, birds were then assigned to unchallenged control, *Eimeria maxima* (EM) challenge on d14 (15,000 sporulated oocysts gavage), or EM d14 + *Clostridium perfringens* d18–19 ($1x10^8$ CFU) to induce necrotic enteritis (NE). Six birds per treatment were euthanized at baseline, peak infection, and post-infection. Intestinal tissue and digesta samples were collected to measure luminal IL-10 (ELISA) and visualize IL-10 and IFNγ production via immunohistochemistry (IHC; HALO Area Quantification Module). ANOVA was completed in SAS 9.4 for host IHC and luminal content data ($P<0.05$). RNA was purified from jejunal tissue (R3 only) at baseline and peak infection, sequenced on an Illumina NovaSeq 6000, and mapped to the chicken genome using STAR. DESeq2 identified differentially expressed genes ($|\log_2$ fold change$|>1$; FDR≤ 0.05). In R1 and R2, baseline IL-10 was significantly greater in the duodenum of unchallenged vs. *Salmonella*-challenged ($P<0.05$), and the anti-IL-10 diet had increased IL-10 vs. control ($P=0.006$). Duodenum luminal IL-10 was higher at peak infection in birds challenged with NE vs. *Eimeria* ($P=0.008$). Birds fed the control diet in R3 had more IL-10 vs. anti-IL-10 diet in the duodenum at baseline and ceca post-infection ($P<0.05$). NE increased IL-10 at peak infection in jejunum and ileum, while EM increased IL-10 in ceca post-infection ($P<0.05$). Ileal IFNγ production area trended towards an increase at peak necrotic enteritis challenge in R1 ($P=0.06$), significantly differed in R2 at baseline ($P=0.03$) and due to challenge ($P=0.02$), and significantly increased due to challenge in R3 ($P=0.04$). Jejunal transcriptomic contrasts for unchallenged vs. EM, unchallenged vs. NE, and EM vs. NE at peak infection showed 1,803, 1,353, and 0 significantly differentially expressed genes, respectively, with 56.6% shared between the first two contrasts. EM challenge upregulated genes involved in cell cycle, lipid metabolism, and B cell proliferation, while necrotic enteritis also increased defense response and catabolic metabolism genes. Overall, more differences in intestinal host responses, both cytokine production and transcriptional changes, were due to challenge type than diet.

Keywords: Eimeria, IFNy, interleukin-10, necrotic enteritis, transcriptome

16 Effects of different *Eimeria maxima* infection doses on growth performance, gut and bone health in dual-infection model of necrotic enteritis in layer pullets

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Necrotic enteritis (NE), on average, is causing billions of dollars in losses annually to the global poultry industry. While most NE studies focus on broilers, there is limited research in laying hens. Interestingly, many cases of NE reported in laying hens urging a need to understand their susceptibility to NE. *Eimeria maxima* (EM) is the major predisposing factor which increases vulnerability to NE. The objective of this study was to investigate the effects of various doses of EM on growth performance, gut and bone health in dual infection model of necrotic enteritis in layer pullets. A total of 300 twenty one days old layer pullets (Hy-line-36) were allocated into five treatments with six replicates, each containing 10 birds. The treatments included 1) Non-Challenge control (NC); 2) Challenged with 1×10^9 colony forming units (CFU) of *Clostridium perfringens* (CP) alone; 3) Challenged with 10,000 sporulated oocysts EM and 1×10^9 CFU of CP; 4) Challenged with 20,000 sporulated oocysts of EM and 1×10^9 CFU of CP; and 5) Challenged with 40,000 sporulated oocysts of EM and 1×10^9 CFU of *CP*. Birds were challenged with EM immediately after the allocation, *and* the initial inoculation timepoint was considered as 0 Days post inoculation (DPI). *C. perfringens* was inoculated on 4 and 5 DPI. Growth performance parameters were observed over a period of 14 DPI. All the data were analysed using one- way Anova in JMP Pro-18, with Tukey's test for comparing the means. Birds in the dual challenge groups showed a decrease in body weight on 7 DPI. ($P < 0.0001$), whereas the CP challenge alone did not show significant reduction compared to the NC group. The dual challenge groups had significantly increased feed conversion ratio ($P < 0.0001$) and significantly reduced feed intake ($P < 0.0001$) compared to the non-challenge and CP challenge alone groups during 0-7 DPI. The lesion scores for NE in the jejunum showed a significant increase ($P < 0.0001$) in the group 5 compared to the CP alone group. In addition, the jejunal and cecal colony counts of CP significantly increased ($P < 0.001$) in the groups 4 and 5 compared to the NC group. On further analysis, Dual-Energy X-ray Absorptiometry (DEXA) body composition on 7 DPI showed a significant increase ($P < 0.001$) in the tissue fat percentage of the CP alone or dual challenge groups. In addition, on 14 DPI, a decrease ($P =0.005$) in the Bone mineral density was observed in the groups 4 and 5 compared to the NC. In conclusion, challenging with both EM and CP potentiated NE, proving the susceptibility of laying hen pullets, and studies at various production stages are required to fully understand the disease dynamics which is necessary for finding a potential mitigation strategy.

Keywords: Necrotic enteritis, Layer Pullets, Bone, Growth performance

17 Growth kinetics assessment and NetB toxin quantification of necrotic enteritis-associated *Clostridium perfringens* strains

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The poultry sector is an integral component of the global food system, providing a significant protein source to a growing world population. However, the industry faces several hurdles, including diseases, that adversely affect sustainable growth. *Clostridium perfringens*-induced necrotic enteritis (NE) remains a significant economic and health concern, causing $6 billion in annual losses to global poultry production. Among a wide array of toxins *Clostridium perfringens* produces, NetB, a pore-forming cytotoxin, is considered a key contributor in NE. This study investigated the growth kinetics and quantified NetB toxin production in two CP strains. Two *C. perfringens* strains (strain 1: netB+/TpeL-; strain 2: netB+/TpeL+) from the stock at -80 °C were cultured anaerobically overnight in 10 mL fluid thioglycolate (FTG) broth at 37 °C. The netB is a pore-forming toxin, whereas TpeL is a glycosylating toxin. Growth kinetics of strains were assessed via microdilution assay at multiple time points (0, 6, 12, 18, and 24 hours). The strains were inoculated independently into 100µL FTG broth at a starting inoculum containing ~log 5 CFU/ml. A total of 6 wells per strain (3 wells X 2 experiments)

were loaded into a 96-well microtiter plate, and optical density (OD_{600}) was measured using the plate reader. The growth curve was generated by plotting the mean OD_{600} values against the corresponding time points. Quantification of NetB toxins at multiple time points (0, 6, 12, 18, 24 hours) for strains using both toxin-specific 96-well ELISA kits per the manufacturer's instructions. NetB toxin levels were calculated by generating a standard curve using a linear regression model with a correlation coefficient (R) above 0.9. Toxin quantification was performed independently for each strain with six biological replicates (n=6) and analyzed using ANOVA in R Studio. The results indicated that NetB toxin concentration varied across the 24-hour growth period, with strains differing in toxin production at all time points (P<0.05). Toxin levels were recorded as high as 73 pg/ml, with the highest production observed at 6 hours compared to other time points for both strains (P<0.05). A significant interaction was observed between time and strain on toxin production (P<0.05), indicating both strains differed in their toxin production at all time points except at the beginning (P<0.05). The study highlights the strain-specific differences in NetB toxin release to characterize potential toxin-mediated pathogenesis of NE-causing strains in chickens.

Keywords: Necrotic enteritis, Clostridium Perfringens, NetB toxin, Growth kinetics, ELISA

18 Evaluating the potential for vertical transmission of Enterococcus cecorum in broilers through microbiome analysis

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Enterococcus cecorum has evolved into a significant pathogen of poultry. Clinical outbreaks in broilers cause septicemia and locomotive disorders such as bacterial chondronecrosis with osteomyelitis and enterococcal spondylitis. While horizontal transmission is documented and *E. cecorum* has been identified in yolk sacs, and the timing of early disease suggests vertical transmission, definitive evidence is lacking. We conducted a study to analyze evidence of vertical transmission by using 16S rRNA sequencing analysis from intestinal and extraintestinal samples collected from ten 47-week-old female broiler breeders and their eggs (Experiment 1), including small intestine, ceca, reproductive tract, cloaca, and eggshell swabs. Additionally, whole intestines of day of hatch (DOH) chicks from a different flock, as well as small intestines at D3 and D10, were evaluated (Experiment 2). Experiment 1 samples were stored in saline at -80°C before DNA extraction, purification, and amplification (16S rRNA V4, F515/R806), followed by sequencing on the Illumina MiSeq platform at UNC Microbiome Core. In Experiment 2, DNA samples were sequenced using Illumina MiSeq with 2 × 300 paired-end reads. Experiment 1 revealed that the Enterococcus genus comprised 3.96% of total microbiome from internal hen samples. *Enterococcus_E* was the most prevalent genus, with *Enterococcus cecorum* as the dominant species. *Enterococcus* abundance varied by sample site, highest in the small intestine, followed by cloaca, eggs, then reproductive tissues. ANOVA (p>0.05) showed no significant differences, suggesting uniform distribution and that presence at one site does not promote vertical transmission over another. This demonstrates that presence of *E. cecorum* in a breeding system may increase the likelihood of pathogen transmission to broilers. In Experiment 2, the *Enterococcus* genus was present at 3.73% on Day of Hatch (DOH), which increased significantly in the upper intestine (UI) to 53.39% by D3, and remained relatively high at 37.68% on D10, indicating that low-level presence of *Enterococcus* at DOH can lead to significant presence as broilers age. Microbial richness and diversity did not differ between treatments (p > 0.05) at D3 and D10. The detection of *Enterococcus* at DOH, along with its presence in the cloaca and eggs, suggests potential vertical transmission. To improve hatchery biosecurity and early disease detection techniques, it is essential to understand the possibility of vertical transmission of *Enterococcus cecorum* in broilers. If maternal transfer contributes to early colonization, breeder-level interventions may reduce *E. cecorum* in offspring, minimizing production losses.

Keywords: Enterococcus, vertical transmission, microbiome, disease, broiler

19 Effect of oral and ontraperitoneal *Staphylococcus aureus* on bacterial chondronecrosis and osteomyelitis, broilers' performance, bone health and microbiome

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Bacterial chondronecrosis and osteomyelitis (BCO) is a bacterial infection of femur and tibia which is a poultry industry's global concern with an approximate annual loss of US$80-120 million. To understand this global issue, bacterial challenge (Oral and Intraperitoneal (IP)) models are sparse. The current study fills the above lacuna by unveiling effects of *Staphylococcus aureus* challenged orally and intraperitoneally on broilers' performance, bone health, and microbiome. A total of 300 forty-day-old broilers were allocated to 5 treatments and 4 replicates on floor pens. Treatment 1 (T1) was non-challenged control, T2 and T3 were orally challenged with 10^9 CFU *S.aureus* on day 42 and days 42, 43, and 44, respectively. Similarly, T4 and T5 were IP injected with 10^7 CFU on day 42 and days 42, 43, and 44, respectively. On day 49 and 56, growth performance, bone parameters using Dual Energy X-ray Absorptiometry (DEXA), gait, and BCO scoring were recorded. Additionally, femur bones for micro computed tomography, and femoral head and cecal microbiome and microbial counts. Data were analyzed with ANOVA, Kruskal wallis, and Wilcoxon each pair using JMP Pro 17 with a p-value less than 0.05. This study revealed a significant decrease in body weight gain for birds challenged intraperitoneally (T4 and T5) which are 400 grams lower compared to birds challenged orally (T2 and T3) (P < 0.05). Correspondingly, DEXA bone mineral density and bone mineral content were significantly lower for those treatments. micro-CT result of Day 49 femur revealed that total volume of pore space was significantly higher for the challenged treatments compared to the non-challenge control. Interestingly, bacterial counts in ceca and femoral head were significantly higher for all challenged treatments on day 49, but counts in bone were significantly higher for T3 and T5. Moreover, gait scores were significantly higher for T3 and T5 on day 49 and 56. In addition, significantly higher BCO scores were observed in T3, T4, and T5 (P < 0.05). Moreover, bone microbiome analyses revealed Faith's phylogenetic diversity which was significantly lower for T4 and T5 besides significant

phylogenetic distance (unifrac) among the treatments. Cecal microbiome also revealed significant unweighted and weighted unifrac distance between T2, T3, T4, and T5. The current study shows that *S.aureus* induces BCO in broilers through oral and IP routes which is supported by the increased gait and BCO scores, bacterial counts, DEXA, micro-CT, and microbiome data. In conclusion, this study establishes a BCO induction model besides orchestrating *S.aureus* effects on microbiome, and bone health which further helps in identifying therapeutics for BCO in broilers.

Keywords: Bacterial chondronecrosis and osteomyelitis, bone health, microbiome, Staphylococcus aureus

20 Effect of heat stress on egg production, blood biochemistry, immune response, and disease susceptibility in cage-free laying hens infected with *Salmonella Enteritidis*

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Heat stress (HS) has detrimental effects on commercial layers, such as decreased egg production, egg quality, and increased disease susceptibility. The shift from conventional cage to cage-free (CF) housing systems has further exacerbated these challenges, posing economic concerns to the CF system. This study aimed to assess the effects of HS on egg quality, blood biochemistry, immune response, and disease susceptibility in commercial layers raised in a CF system. A total of 144 Hy-Line W-36 layers (48 weeks old) were randomly assigned to four groups housed in separate rooms: thermoneutral control (TNC, 22°C), HS control (HSC, 35°C), thermoneutral + infected (TNI, 22°C), and HS + infected (HSI, 35°C), with 36 birds in each group (density of 0.139 m^2/bird). After a week, nine birds in the TNI and HSI groups were inoculated with *Salmonella Enteritidis* (10^8 CFU/mL). Cloacal swabs were collected at 5 and 7 days post-infection (dpi) to assess bacterial shedding, and eggs were tested for bacterial contamination. Egg production and quality, body weight, feed intake, and cloacal temperature were recorded throughout the study. At 14 dpi, cecal content, liver, and shell gland were collected from eight birds per group for *Salmonella* colonization. Moreover, the intraepithelial lymphocyte (IEL) populations were isolated from the ileum, stained with anti-chicken antibodies for flow cytometry analysis. Blood biochemical parameters were measured at pre- and post- infection using the iSTAT analyzer. *Salmonella* data were analyzed using binomial logistic regression, flow cytometry data with the Kruskal-Wallis test, and the remaining data were analyzed using one- or two-way ANOVA. Birds under HS conditions had significantly ($p < 0.05$) higher cloacal temperature but showed no difference in egg production rates compared to TN birds. HS hens consumed less feed and produced smaller eggs, yet had a better feed conversion ratio (based on egg weight) than the TN birds. Eggs from HS birds exhibited significantly ($p < 0.05$) lower shell thickness and vitelline membrane strength, while shell breaking strength and Haugh unit remained comparable between groups. HS also significantly ($p < 0.05$) reduced blood gases and electrolyte levels, including the HCO_3^- (mE q/L), PCO_2 (mmHg), TCO_2 (mE q/L), K (mE q/L), HCT (%PCV), and Hb (g/dL). HS did not impact *Salmonella* colonization and transmission at 5, 7 and 14 dpi. There was no difference between groups in the number of IEL populations. Under our experimental conditions, HS adversely affected egg production parameters and blood biochemistry but did not influence host immune response and *Salmonella* susceptibility.

Keywords: Heat stress, Layers, Cage-free, Salmonella, Intraepithelial lymphocytes

Student Competition: Physiology and Reproduction I

21 Effects of delayed feeding and semi-purified diets on anterior pituitary mRNA expression in broiler chicks

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Provision of feed after hatching induces a metabolic switch in chickens, where they undergo a shift from hepatic lipolytic metabolism during embryonic growth to lipogenic metabolism post-hatch, and delayed feeding is known to delay these metabolic changes. Hypothalamic gene expression is also affected by delayed feeding, as neuropeptide Y and thyrotropin-releasing hormone (*TRH*) mRNA levels are increased by 48 hours (hr) of fasting. The pituitary gland also controls growth and metabolism, but the effects of fasting and refeeding on the pituitary gland have never been studied. The objectives of this study were to determine the effects of fasting and delayed feeding on mRNA expression in the anterior pituitary, and to determine which macronutrient in feed elicits these effects. Ross 708 broiler chicks were divided into 14 treatment groups (n=8/group): embryonic day 18 (e18), day of hatch (DOH), 2 days after hatch fed with starter diet (fully fed; FF), fasted for 48 hr (delayed feeding; DF), and fed semi-purified (SP)-complete, SP-dextrin, SP-casein hydrolysate, and SP-corn oil diets for 48 hr. The macronutrient-specific SP diets consisted of 85% total caloric intake of their respective macronutrient on an isocaloric basis, and were orally gavaged 3 times/day for 48 hr. All remaining chicks were then provided starter diet until day 4. The following genes related to the regulation of metabolism and growth were measured in pituitary samples using reverse transcription-quantitative-PCR and normalized to phosphoglycerate kinase 1 (*PGK1*) before being statistically analyzed with the PROC MIXED model (SAS Version 9.4): thyroid stimulating hormone β-subunit (*TSHB*), TRH receptor (*TRHR*), pro-opiomelanocortin (*POMC*), corticotropin releasing hormone receptor 1 (*CRHR1*), growth hormone (*GH*), and GH-releasing hormone receptor (*GHRHR*). No significant changes were seen in the expression of *TSHB*, *CRHR1*, and *GHRHR* mRNA between the day 2 FF and DF groups. However, chicks fasted for 48 hours (DF) had significantly increased mRNA levels ($p<0.05$) of *TRHR*, *POMC*, and *GH*, all of which returned to fed levels after 48 hr on feed (day 4). All four of the SP diets had *TRHR*, *POMC*, and *GH* mRNA levels that were statistically similar to the fasted chicks on day 2 ($p<0.05$, relative to FF). Levels of *TRHR*, *POMC*, and *GH* mRNA in the SP-complete, SP-dextrin, and SP-corn oil diets returned to FF levels following the 48-hr refeeding period. We conclude that fasting newly hatched chicks for two days alters the thyrotropic, corticotropic, and somatotropic axes, as shown by the increased mRNA levels for *TRHR*, *POMC*, and *GH*. However, none of the SP diets mimicked the 48 hr-FF state.

Keywords: broiler, metabolic switch, pituitary, fasting, refeeding

22 *In vitro* transcriptomic response of pituitary cells from low and high turkey egg producers to thyroid hormone and gonadotropin releasing hormone

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Gonadal activity and ovulation rates in commercial turkey hens (Meleagris gallopavo) are regulated by the pituitary. Differential gene expression was investigated to characterize pituitary responsiveness of low egg producing turkey hens (LEPH) and high egg producing turkey hens (HEPH) to gonadotropin releasing hormone (GnRH) with and without thyroid hormone (TH) pretreatment. Thyroid hormones have been shown previously to be both essential for and inhibitory to egg production in turkey hens. Our research has shown that LEPH have decreased triiodothyronine (T3) levels during the preovulatory surge, when compared to HEPH and average egg producing hens (AEPH). We hypothesized that the pituitary's response to T3 likely diverges between LEPH and HEPH, affecting sensitivity to subsequent GnRH stimulation, hence ovulation. To explore this, pituitary cells from HEPH and LEPH were isolated, cultured, and treated with 1.5 ng/mL T3 for 12h, followed by 0, 10^{-9}, or 10^{-8} M GnRH treatment for 6h. This experimental design becomes a 2x2x3 factorial analysis with an n=4 for each group. RNA was extracted with RNAeasy kits, and quality was assessed via a bioanalyzer. RNA sequencing libraries were prepared using SMART-Seq v4 PLUS kit, paired-end sequenced on the Ilumina NovaSeq S4 platform and mapped to the latest turkey genome (Mgal_WU_HG_1.0). Differentially expressed genes (DEGs) (q-value < 0.05, fold change > | 1.5 |, FPKM > 5) were identified by Wald Test pairwise comparisons in CLC Genomics Workbench, with DEG functional annotation analysis performed in Ingenuity Pathways Analysis (IPA) and the Database for Annotation, Visualization, and Integrated Discovery (DAVID). DEGs counts comparisons revealed similarity between gene expression profiles at 10^{-8} M of GnRH in LEPH pituitary cells and at 10^{-9} M GnRH in HEPH pituitary cells, indicating that a higher concentration of GnRH was required for stimulation in LEPH pituitary cells. T3 downregulated genes in the TGF-β signaling pathway in both LEPH and HEPH, a pathway crucial for activin feedback on FSH-β release. Additionally, FoxO and PPAR signaling pathways were upregulated in LEPH in response to T3, while phosphatidylinositol signaling, a pathway used by the GnRH receptor, was downregulated by T3 in HEPH. The pathways and transcription factors identified are potential candidates underlying the effects of T3 on egg production in turkey hens.

Keywords: RNA-seq, thyroid, turkey, pituitary, reproduction

23 Evaluation of estradiol concentrations during sexual maturation in cage-free pullets synchronized with body weight and photostimulation

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Pullets reared in cage-free environments have higher activity levels than those in cages, resulting in altered body weight (BW) due to changes in body composition. Aside from photostimulation (PS), metabolic cues, such as BW, can play a role in the timing of sexual maturation. Estradiol (E_2) is a primary indicator of sexual maturation as circulating levels increase with follicular development. In this study, we aim to determine the timing of

sexual maturation based on different BW and timing of PS. At 12 weeks of age (woa), 600 Lohmann LSL-lite pullets were randomly assigned to four rooms, each containing six aviary-style pens (n=25 birds/pen). Two feeding regimens were imposed at 13 woa (n=3 pens/room): *ad libitum* (AL) and restriction (R) to achieve a 10% lower body weight (BW). PS was based on the age at which the average BW of each treatment reached a set target based on the breeders guideline (1.45 kg; AL at 18 woa or R at 20 woa). This resulted in 4 treatments (n=6 pens each; 150 hens): 1) AL + 18-PS (AL-18), 2) AL + 20-PS (AL-20), 3) R + 20-PS (R-20), and 4) R + 18-PS (R-18). From 12 to 26 woa, BW was recorded weekly. Repeated blood samples were collected from 4 birds per pen weekly from 13 woa to 26 woa. Plasma was recovered and extracted using cold ethanol, and E_2 concentrations were measured using a 17β-E_2 ELISA. Statistical analyses in SAS v9.4 (PROC MIXED) included feeding regimen, PS, and age as fixed effects. Pearson's correlations determined a relationship between BW and E_2. Birds fed AL and those PS at 18 woa reached the maximal level of E_2 faster than R birds and those PS at 20 woa, respectively (P<0.01). Overall, an interaction between feeding, age, and PS was observed on E_2 concentration (P<0.01). The peak in E_2 was observed one to two weeks post-PS in AL-18, R-18, and R-20 birds, demonstrating that PS can induce sexual maturation on its own. However, the peak in E_2 levels prior to PS in AL-20 birds also suggests that reaching a metabolic threshold is sufficient to induce sexual maturation regardless of PS. As a result, the rise in E_2 was quicker in AL than R birds (P<0.01), with a tendency (P=0.061) for birds PS at 18 woa to rise quicker than those at 20 woa. However, no interaction was observed. AL birds were heavier than R birds at the initiation of the E_2 rise (P<0.05). Interestingly, all birds initiated the E_2 rise within a 43-gram window of BW. Regardless of treatment, BW and E_2 concentration were positively correlated (r^2=0.68; P<0.01), suggesting a threshold of BW is present for sexual maturation. This study demonstrated that both PS and metabolic status can independently control sexual maturation. However, synchronizing the BW threshold with PS appears to be most effective.

Keywords: Reproduction, Estradiol, Cage-free, Photostimulation, Body weight

24 Sperm mobility phenotype impact on broiler breeder sperm cell and seminal plasma proteome

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Semen biomarkers indicative of sperm mobility in broiler breeders would provide the ability to screen for fertility potential, with a positive correlation previously established between mobility and fertilization potential in poultry. The objective of this study was to characterize the seminal plasma (SP) and sperm cell (SC) proteomes isolated from Cobb broiler breeder roosters with different sperm mobility phenotypes to identify possible fertility potential biomarkers. Whole semen samples were collected from six high sperm mobility roosters and six low sperm mobility roosters during the early, mid, and late phases of semen production (30 weeks, 42 weeks and 55 weeks, respectively). SC were separated from SP by centrifugation. SC and SP extracts were prepared using the EasyPep Mini MS Sample Prep Kit (Thermo Fisher Scientific), with protein and peptide quantification performed using BCA Protein Assay (Pierce) and Quantitative Colorimetric Peptide Assay (Pierce) kits, respectively. Mass spectrometry analysis was performed on an Orbitrap Fusion Lumos (Thermo Scientific), with protein identification and validation performed using the Mascot search engine (Matrix Science) and Scaffold 5 (Proteome Software). A t-test was used to identify differential protein abundance in low and high mobility samples. Functional annotation of differentially abundance proteins was performed using Database for Annotation, Visualization, and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA). SC proteins across the early, mid, and late timepoints were related to: cell cycle control, spermatogenesis, and acrosome formation, respectively. SP proteins across the early, mid, and late timepoints were related to: cellular stress functions, metabolic ATP generation, and sperm binding, respectively. Proteins exhibiting differential abundance between low and high sperm mobility phenotypes showed repetition across timepoints, with 35 and 22 proteins appearing across at least two timepoints in the SC and SP, respectively. These proteins in the SC regulate lipid metabolism, cilium movement, protein binding, and propanoate metabolism, while these proteins in seminal plasma regulate platelet activation, proteolysis, monocyte chemotaxis, and cysteine/methionine metabolism. Overall, the SP and SC proteome differed due to sperm mobility phenotype at each timepoint examined. The key proteins identified provide a further understanding of the physiological mechanisms that regulate sperm mobility and could potentially serve as putative biomarkers for male fertility. Future directions include validation of these proteins in larger numbers of males and determining the abundance of these proteins at more timepoints.

Keywords: Proteome, sperm cell, seminal plasma, sperm mobility, broiler breeder

25 Temporal dynamics of sperm morphology in broiler breeders

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Poultry production and consumption patterns have increased over the past few decades, and it is projected to increase over the next two decades. Despite this growth, there has been a steady decline in broiler breeder fertility over the last decade. We hypothesized that the decline is associated with changes in semen quality, particularly sperm morphology features over time. Our objective was to characterize the progression of sperm morphological defects in broiler breeder males and identify the trends that explain declining fertility. We assessed sperm morphology in high-efficiency commercial broiler breeders (Gallus gallus domesticus) from 19 weeks to 44 weeks of age. We collected semen samples weekly using the abdominal massage method. Samples were stained with Eosin-Nigrosin and evaluated under light microscopy, with approximately 100 sperm cells per slide classified into normal and defective categories. The morphological defects were categorized into head, midpiece, tail defects, and data were analyzed for trends over time. Once the data were collected, we performed a series of advanced statistical analyses to determine patterns of change over time, as well as the rate of change in defects over time. Finally, we used Bayesian generalized linear models with simulations to estimate the window of peak breeder

utility, which considered both normal morphology and defects. Overall, all semen morphology features showed a non-linear trend over time. Normal morphology was the most common form (~70%), which started at lower proportions in younger birds but stabilized by 30 weeks of age. However, several sperm morphological defects including cytoplasmic droplets, bent midpiece, and bent tail increased with age. Regression analysis showed that sperm parameters did not follow linear trends. Instead, quadratic models provided the best fit across most categories (9/14), confirmed using Kolmogrov-Smirnov tests (P>0.05). Next, our Bayesian analyses identified that defects increase in proportion with age, even as the rate of change in normal sperm remains stable. Tail defects showed the steepest increases in frequency with age, suggesting that increasing tail defects may be a key driver of fertility outcomes with age. Lastly, our Bayesian estimate of the time of peak breeding utility, estimated from semen morphology changes, indicates that 9.48 months (~38 weeks of age) represents the point of highest breeding utility, after which breeding performance is expected to decline. Our novel analyses underscore the need for an improved understanding of semen parameters to remedy ongoing fertility declines.

Keywords: spermatozoa, Sperm morphology, Breeder Age, Poultry, Fertility

26 Using neural networks to identify semen parameters impacting broiler breeder fertility outcomes

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Poultry is the top consumed animal protein product worldwide, but the sustainability of poultry is threatened due to declining fertility. We hypothesized that the declining fertility of broiler breeders is correlated with specific semen parameters. We focused on male parameters as fertility is somewhat restored when replacing males with younger males (spiking). Our objective in this study was to determine if and which sperm morphology and sperm kinetics parameters determine fertility outcomes. We investigated sperm parameters from male high-efficiency broiler breeders(Gallus gallus domesticus), which we acquired at 19 weeks of age and performed weekly assays until 44 weeks age. Motility was analyzed using the iSperm mCASA (mobile computer-assisted spermatozoa analysis) software (Aidmics Biotechnology Co. LTD), and we characterized sperm morphology using Eosin-Nigrosin staining and light microscopy. Eggs were collected to track fertility percentages during the production cycle. At the end of the trial, we arranged the data for statistical analyses. We examined our motility variables for linearity over time, and found that the Quadratic model was the best fit. Following this, we applied screening and regularization (LASSO regression) techniques to identify the most important features predicting the target variable (percentage fertility). We, then, implemented a deep neural network (DNN) using the Keras library in Python. We used a tuner to identify the best model architecture, by screening 20 model variations for the input layers, number of hidden layers, and number of neurons per layer. The best model was evaluated using Mean-Squared Error. We split the data into training and test sets, and the best model was used to generate the Shapley Additive Explanation (SHAP) importance scores for the parameters. Our screening and regularization methods showed (predictably) the important role that Age and Individual body weights played in determining fertility outcomes. Next, our best DNN model had one input layer and three hidden layers. The model confirmed the importance of Age, showing that younger birds predicted lower fertility, and fertility improved with bird age, to a point. Besides age, the motility parameters Beat Cross Frequency (BCF), Linearity, and Midpiece defect were the next most important factors. High values of BCF were associated with better fertility percentages. As the BCF is a measure of flagellar motion of the sperm, morphological defects that impact the sperm body, particularly midpiece or tail defects, were likely the underlying cause of decline in fertility. Overall, our study identified key motility and morphology relationships that determine fertility outcomes in broiler breeders.

Keywords: Spermatozoa, iSperm mCASA, Poultry, Fertility, Importance

27 Characterizing germ cell dynamics of the post-hatch ovary across three turkey lines

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Commercial turkeys have been intensively selected for carcass and growth efficiency traits, which has reduced their reproductive capacity. This, in turn, has compromised economic efficiency at the parent stock level due to reduced poult production. We hypothesize that low reproductive potential can be identified shortly after hatch by a reduction in the number of germ cells, which will limit the future follicular pool. While we predict ovary weight and cortex volume will increase with body weight (**BW**), cortex volume will be negatively correlated with the number of germ cells due to lower density. Three turkey lines with known differences in egg production rates were used for this experiment: a commercial line (**C**), a research line selected for high egg production (Ohio State University E-line; **E**), and a random-bred research line (random bred control 1; **R**). The BW and the left ovaries of three females per line (n=3) were collected at 1, 7, 14, 35, and 56 days post hatch (dph). Ovaries were fixed and embedded in paraffin, then sectioned (5 µm) and stained (H&E). Images from ovaries at 1 dph were analyzed in Volocity software to determine cortex volume and count prefollicular germ cells. Data were analyzed using the GLM procedure with Tukey's adjustment and Pearson correlations in SAS. As anticipated, BW was age ($P<0.0001$), line ($P<0.0001$) and age by line ($P<0.0001$) dependent, with C having the steepest growth trajectory from 1 to 56 dph and E the shallowest. At 1 dph, C were 64 g in BW, already 18 and 20 g heavier than R and E, respectively ($P<0.001$). Although not statistically significant ($P=0.2027$), cortex volume at 1 dph was nearly two-thirds greater in C ($1.0\pm0.29mm^3$) than E ($0.64\pm0.16mm^3$) with R being intermediate ($0.81\pm0.24mm^3$). The number of germ cells at this age also did not differ between strains ($P=0.5287$). However, contrary to our hypothesis, the number of germ cells was highly positively correlated with cortex volume ($r^2=0.8028$; $P<0.01$). Although no significant difference in reproductive capacity was evident at 1 dph, it is possible that loss of germ cells may occur following follicular organization past 7 dph (analysis currently underway).

Keywords: turkey, ovary, reproductive potential, germ cell dynamics

28 The culturable reproductive tract microbiota of chickens

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While the gut microbiota of chickens is well researched, the reproductive tract microbiota has only recently come into focus. In this study, we focused on three key sections of the reproductive tract, the infundibulum into which a mature ovum ovulates to and where fertilization takes place, the magnum which secretes egg white, and the uterus which secretes the calcium shell. We and others have previously utilized 16S rRNA sequencing to show that bacterial DNA is present throughout the chicken reproductive tract. However, as the reproductive tract secretes many antimicrobial peptides and proteins, it was possible that identified DNA represents dead bacteria. To determine if live bacteria are found in the reproductive tract of chickens, we cultured samples from the different parts of the reproductive tract of 37-week-old Cobb broiler breeders on rich YCFA plates in both aerobic and anaerobic conditions. We then used 16S rRNA sequencing to identify what grew. A diverse phylogeny of bacteria were cultured from the shell gland, magnum, and infundibulum. This included many Lactobacillales bacteria but also obligate anaerobes such as Bacteroidales, Lachnospirales, and Oscillospirales. Interestingly, fewer amplicon sequence variants were cultured from the magnum compared to the shell gland and infundibulum (Friedman test with Dunn's multiple comparisons test). Furthermore, bacteria of orders Oscillospirales, Lachnospirales, Erysipelotrichales and Enterobacterales were less frequently cultured from magnum samples compared to shell gland and infundibulum samples, whereas Lactobacillales were equally prevalent and culturable from all three sample types (Kruskal-Wallis test with Dunn's multiple comparisons test). Of note, culture-free DNA based analysis of these same samples incorrectly implied equal existence of these orders along the reproductive tract. This study highlights the fact that culturomics is an important supplement to DNA-based microbiota analyses. Furthermore, these results imply that live bacteria are in the right place to integrate into the forming egg and perhaps reach progeny, and that chicken physiology in the magnum section perhaps regulates this process. Finally, future work is required to understand if the bacteria found in the reproductive tract of hens affect sperm motility or survival, fertilization itself, or the secretion of egg elements.

Keywords: Chicken, reproductive tract microbiota, culturomics, selective environment, magnum

29 The effects of a novel precision biotic on modulating the gene expression of tight junctions, nutrient transporters, immune responses, and ceca protein levels in *Eimeria*-challenged broiler chickens fed wheat-based diets

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We previously reported positive effects of a precision biotic (PB) on growth performance and ileal amino acid digestibility. The current study explored additional modes of action of the PB precipitating the phenotypic response observed. The 42-day experiment investigated the effects of PB on tight junction proteins, nutrient transporters, immune response, and ceca soluble protein levels (μg/ml) in broiler chickens challenged with *Eimeria* (CH) fed corn-SBM (C) or wheat-corn-SBM (W) diets. 1,012 one-day-old chicks were allocated to 6 treatments with at least 7 replicates each in a randomized complete block design. The treatments were: C diet, no challenge (C-NCH); or with challenge, without PB (C-CH-0); W diet plus challenge, without PB (W-CH-0); W diet plus challenge, plus 300 (W-CH-300), 600 (W-CH-600) or 900 (W-CH-900) g/ton of PB. Cecal contents, and jejunal and spleen tissues were collected on d 20 and 42 to determine ceca protein levels and mRNA expression of nutrient transporters (GLUT2, SGLT4, b0+AT, CAT1, PEPT1, EAAT3), gut integrity (CLDN1, OCLDN, JAM2), and immune response (IL-6, IL-10, IL-1 β, IFN-γ, TGF-β1, iNOS) respectively. On d 12, birds from all treatments, except treatment 1, were challenged with a mixed inoculum of *E. acervulina*, *E. maxima*, and *E. tenella* through feed to induce enteric stress. Data were analyzed as one-way ANOVA using JMP Pro 18. Orthogonal polynomial contrasts were used to determine the linear and quadratic responses to increasing levels of PB in wheat-based diets. One-df orthogonal contrasts were used for pairwise comparisons. No significant treatment effects were observed for ceca soluble protein contents in d 20 and 42 samples. On d 20, PB linearly increased ($P < 0.05$) the expression of OCLDN gene whereas *Eimeria* reduced ($P < 0.05$) the gene expression of GLUT2 and amino acid transporter (EAAT3) in broilers fed C-CH-0 compared with W-CH-0 diets. On d 20, PB supplementation decreased ($P < 0.05$) the expression of IL-1β in a quadratic manner. On d 42, the treatments had no significant effects on the expression of gut integrity and nutrient transporter genes. However, IFN-γ expression was lower ($P < 0.05$) in the challenged birds. To conclude, precision biotic supplementation increased OCLDN expression, possibly positively affecting gut integrity in the *Eimeria*-challenged birds. This may partly account for the beneficial effect of PB on the growth performance of the *Eimeria*-challenged birds. The impact of PB on the nutrient transporters assayed is marginal and did not explain the observed positive effect of PB on amino acid digestibility.

Keywords: precision biotic, Eimeria, wheat, corn, broilers

30 Effects of dietary supplementation with organic zinc on the growth performance and tissue zinc concentration in broiler chickens

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The objective of the current study was to determine the optimal supplemental level of zinc (Zn) as proteinate Zn in broiler diet by observing growth performance and tissue Zn concentration. A total of 240 day-old male broiler chicks were assigned randomly to 5 dietary treatments with 8 replicate cages per treatment and 6 chicks per cage for 20d growing period. All diets were formulated to meet or exceed nutrient requirements for birds of this age except for Zn. The 5 treatments were: (1) A corn-soybean meal (C-SBM)-based diet supplemented with 40 ppm Zn as $ZnSO_4 7H_2O$; (2) A C-SBM-based diet without supplemental Zn; (3) Diet 2 + 5 ppm of Zn as proteinate Zn (Bioplex Zn®, Alltech, Nicholasville, Kentucky, USA); (4) Diet 2 + 10 ppm of Zn as Bioplex Zn; (5) Diet 2 + 20 ppm of Zn as Bioplex Zn. Weight gain, feed intake, and feed-to-gain ratio from d 0 to 20 were calculated after adjusting for mortality. On d 20, tibia from two birds per cage were sampled and Zn concentration in tibia ash was determined. A one-way ANOVA was performed using Statistix V9, and means were separated using the LSD test. Polynomial contrasts were used to assess the linearity of Zn concentration in tibia ash vs. dietary supplemental levels of Zn. The optimal dietary supplemental level of Zn as proteinate Zn for bone Zn concentration was estimated by using a one-slope broken-line model based on the NLIN procedure in SAS. Statistical significance was set at $P < 0.05$. Chickens fed diets supplemented with 40 ppm Zn as sulfate and 20 ppm Zn as Bioplex Zn had higher ($P < 0.05$) feed intake and weight gain compared with those fed diets supplemented with no Zn or 5 ppm Zn as Bioplex Zn. A linear relationship ($P < 0.001$, $R^2 = 0.959$) was observed between dietary Zn supplementation as Bioplex Zn and Zn concentration in tibia ash. The minimum dietary supplemental level of Zn as Bioplex Zn required to maximize Zn concentration in tibia ash was 16.5 ppm ($P < 0.001$). In summary, supplementation of Zn to a C-SBM-based diet improved growth performance and linearly increased tibia Zn concentration in broiler chickens. Supplemental levels of 10 or 20 ppm Zn as proteinate Zn resulted in weight gain and feed intake comparable to 40 ppm Zn as Zn sulfate. Maximized Zn concentration in tibia ash was determined to occur with a supplemental level of 16.5 ppm Zn as proteinate Zn in this trial.

Keywords: broiler, organic zinc, performance, tibia zinc

31 Effects of microencapsulated copper sulfate on the performance of 0 to 35 d old broiler chickens raised on used litter

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An experiment was conducted to determine the effects of microencapsulation of copper sulfate on broiler performance, intestinal health and copper excretion. A total of 1,656 d old male Ross 708 male chicks were housed in floor pens and randomly assigned to four dietary treatments with 18 replicate pens of 23 chicks. Treatments included all birds raised on used pine shaving litter seeded with coccidial oocysts and fed experimental diets including negative control (NC) diet (without supplemental Cu); the same diet with 150 g/MT of 100% copper sulfate (100CuSO4); the same diet with 233 g/MT of 60% microencapsulated copper sulfate (60CuSO4); and the same diet with 200 g/MT of 70% microencapsulated copper sulfate (70CuSO4). The encapsulated CuSO4 was provided by Maxx Performance Roanoke, VA. The used litter was generated by providing a 10x CocciVac® B52 dose

to 39 birds per pen via feed for three days and allowing these birds to shed oocysts into the litter for 21 d. All pens were then top dressed with equal amounts of this seeded litter at the start of the experiment. Body weight gain (BWG) and mortality corrected feed conversion ratio (FCRm) were calculated for the 0 to 13, 0 to 28, and 0 to 35d periods. On D13 and D28, 1 bird per pen was orally gavaged with FITC-dextran and one hour later, blood collected from the same bird to assess intestinal permeability via serum FITC-dextran. On D13, D28, and D35, 1 bird per pen was euthanized for gizzard scoring and litter samples were collected from each pen for dry matter determination. On D 11 to 13 and D 21 to 23, three composite fecal samples were collected per pen to determine oocyst shedding. Data were analyzed using ANOVA in JMP Pro 18.0, and means were separated using Student's T-test ($P \leq 0.05$). All CuSO4 treatments had no response over the 0 to 13d period, but increased BWG compared to the NC for 0 to 28d ($P < 0.05$) and 0 to 42d ($P = 0.06$). Copper sulfate treatment, 100CuSO4, 60CuSO4 and 70CuSO4 increased BWG over the NC by 54, 56 and 61 g over 0 to 28d and 84, 74 and 90 g over 0 to 35d, respectively. Feed intake and FCRm were not different over any time measured ($P > 0.05$). Although CuSO4 was able to increase BWG, gastro-intestinal health was unaffected as gizzard scores, FITC-dextran, and litter dry matter were not altered by CuSO4 supplementation ($P > 0.05$). Oocyst shedding was also unaltered by CuSO4 supplementation ($P = 0.60$). All CuSO4 sources were able to increase broiler BWG over the 35-day period and did so without altering gastro-intestinal health.

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

32 Dietary Spirulina supplementation in low-protein diets modulates cecal short-chain fatty acids and jejunal morphology in broiler chickens

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This study evaluated the impact of dietary Spirulina **(SP)** on the growth performance, nutrient utilization, cecal short-chain fatty acids, and the jejunal morphology of broiler chickens fed an adequate (20%) or low (16%) crude protein **(LCP)** diet. Three hundred birds were fed a commercial starter diet from d 1 to 8 post hatching. On d 8 post hatching, 256 birds were assigned to 4 dietary treatments in a 2 × 2 factorial arrangement with two crude protein **(CP)** levels (20 or 16% CP) and two added SP levels (0 or 50 g/kg). There were 8 replicate cages per treatment with 8 birds per cage from d 8 to 20 post hatching. Body weight was used as a blocking factor. On d 20 post hatching, birds were weighed, euthanized, and samples were collected. Data were analyzed using the MIXED procedure of SAS as ANOVA. The model incorporated CP level, SP inclusion, and their interaction as fixed effects, while replicate blocks were considered random effects. When a significant interaction between SP and CP level was detected, Tukey's test was used to separate the means. Body weight gain, feed intake and feed efficiency were reduced ($P < 0.05$) by feeding the LCP, but there was no SP × CP level interaction on the growth performance of broiler chickens. Dietary SP addition increased ($P < 0.01$) villus height to crypt depth ratio and ileal digestible energy in broilers, regardless of dietary CP level. Dietary SP increased ($P < 0.05$) the concentration of propionic acid in the cecal digesta of birds, irrespective of dietary CP level. Moreover, there was an SP × CP level interaction ($P < 0.05$) on the proportion of propionic acid in the cecal digesta short-chain fatty acids of birds. On the other hand, the LCP increased ($P < 0.05$) both the concentration and proportion of isovaleric acid in the cecal digesta of broilers, regardless of dietary SP addition. Also, dietary SP inclusion reduced ($P < 0.05$) the jejunal crypt depth of birds, irrespective of dietary CP level. There was a tendency ($P = 0.09$) for an SP × CP level interaction on the jejunal villus area of broiler chickens. In conclusion, this study highlights shifts in cecal short-chain fatty acid proportion and potential jejunal morphology modifications in broilers fed a low-crude protein diet with Spirulina supplementation.

Keywords: Spirulina, growth performance, intestinal morphology, low protein diet, short-chain fatty acids

33 Microalgae (*Spirulina Platensis*) improves broiler breast yield under heat stress conditions: role of protein synthesis pathway

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Heat Stress (HS) negatively affects many aspects of broiler production, including decreased performance and profitability. Nutritional modifications are one avenue for mitigating HS effects. Here, we examined the effect of algae (*Spirulina Platensis*) supplementation () on growth performance of Heat-stressed broilers. Day-old male Cobb 500 chicks were allotted randomly by body weight (BW)-matched groups to 12 controlled-environmental chambers (2 pens/chamber, 25 birds/pen), and assigned to 1 of 6 treatments: Thermoneutral-basal diet (C-TN), HS-basal diet (C-HS), TN-basal diet-pair fed (PF), TN-0.5% algae-supplemented diet (A-TN), HS-algae diet (A-HS), or TN-algae diet-pair fed (A-PF) in a 2x3 factorial design. On d29, 4 chambers were subjected to 8 h daily cyclic HS (36°C) until the end of the trial. Feed and water intake were recorded daily and BW weekly. On d40, breast muscle (n=8/diet/environment) was collected for gene expression, as determined by qPCR. At d42 birds were processed and carcass parts were weighed. Data was analyzed by Two-Way ANOVA and Tukey's multiple range test using Graph Pad Prism. Data are expressed as mean ± SEM and were considered significant at $P \leq 0.05$. Prior to HS there were no significant effects of diet on BW, yet, after 2 weeks, both HS and PF birds weighed less than their TN counterparts ($P<0.001$). There was no effect of supplemental algae on BW. Cumulative feed intake was decreased by HS and PF ($P<0.05$) but not with algae supplementation. Cumulative water intake increased with HS ($P<0.05$) yet was unaffected by diet ($P>0.05$). Feed conversion ratio (FCR) for the entire trial was increased in HS and PF ($P<0.01$). During the finisher phase, HS increased FCR but to a lesser degree with algae. Diet and environmental conditions had a significant effect on breast yield. The lowest yield was in the HS group compared to TN and PF (21.5 ± 0.02 *vs.* 22.3 ± 0.02 *vs.* 22.4 ± 0.02, $P<0.01$) and a higher yield was seen in algae compared to control diet (22.3 ± 2 *vs.* 21.8 ± 2, $P<0.05$). Molecular analysis showed that protein synthesis (PS) pathway (mTOR, P70S6K, 4EBP-1, eiF4B) was affected by both diet and environment. These results indicate that algae supplementation enhances breast yield under HS challenge potentially through, activation of PS pathway.

Keywords: Heat stress, Broiler, Spirulina Platensis, Breast Yeild, Protein synthesis

34 Assessment of black soldier fly larvae (BSFL) as a dietary supplement to mitigate necrotic enteritis and improve gut health in broiler chickens

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With restrictions on antibiotic use, sustainable alternatives are needed to mitigate necrotic enteritis (NE) while maintaining productivity. Black soldier fly larvae (BSFL) meal, a nutrient-rich protein source, offers promise as an antibiotic-alternative due to its antimicrobial properties. This study evaluated BSFL meal as a dietary intervention to mitigate NE and assessed its dose-dependent effects on growth performance and gut health in broilers. A total of 540 one-day-old Cobb 500 broiler chicks were randomly assigned to six dietary treatments with six replicates: a non-challenged control (**NC**), a challenged control (**CC**), and four challenged groups supplemented with 2.5, 5.0, 7.5 and 10.0 % BSFL meal. Birds in the challenged groups received 10,000 sporulated oocysts of *E. maxima* on day 14, followed by NetB-positive *Clostridium perfringens* (CP) (1 × 10^9 CFU/mL) at 4 days post-inoculation of *E. maxima (*dpi). Growth performance and different gut health parameters were evaluated. Data were analyzed using one-way ANOVA with orthogonal polynomial contrasts assessing linear and quadratic dose-response trends and the Kruskal-Wallis test for lesion scores. Statistical significance was set at $P < 0.05$. The results showed that increasing BSFL meal levels linearly increased body weight in the challenged group at 6 dpi (linear, $P = 0.09$) and 8 dpi (linear, $P = 0.05$), with the highest BW observed in 5.0 % BSFL compared to other groups.. Similarly, feed intake increased linearly during 0–6 dpi (linear, $P = 0.007$) and 0–8 dpi (linear, $P = 0.015$), also peaking at 5.0% BSFL, but no significant differences were observed among the challenged groups. The CC group had the highest gut permeability ($P < 0.05$), while BSFL groups showed numerical reductions ($P > 0.05$), with the lowest at 5.0% BSFL. Additionally, jejunal *C. perfringens* counts decreased linearly with increasing BSFL levels at 6 dpi (linear, $P = 0.023$), while a similar trend was found in cecal counts at 8 dpi (linear, $P = 0.09$). However, BSFL supplementation did not significantly affect NE and coccidiosis lesion scores at 6, 7, and 8 dpi ($P > 0.05$). In summary, dietary inclusion of BSFL meal showed a linear dose-response relationship. While 5.0 % BSFL showed optimal growth benefits, higher doses (up to 10.0 %) induced pathogen suppression without negatively affecting growth performance. These findings support the use of BSFL up to 10.0 % as a functional feed ingredient for reducing the negative effect of necrotic enteritis.

Keywords: Black soldier fly larvae, necrotic enteritis, Clostridium perfringens, broiler chickens, gut health

35 Not Presented

36 Supplementing corn-soybean meal diet with admixture of xylanase and glucanase increased the weight gain of broiler chickens during compensatory growth phase following an *Eimeria* challenge

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It is hypothesized that supplementing diets with exogenous carbohydrate-hydrolyzing enzymes will enhance growth performance of *Eimeria*-infected broilers, possibly by their positive effects on gut health metrics. A total of 960 male broiler chicks were allocated to 4 treatments in a randomized complete block design. Each treatment had 10 replicates with 24 chicks per replicate pen. The 4 treatments are: 1) corn-soybean meal (SBM) diet without challenge, NCH; 2) corn-SBM diet with *Eimeria* challenge, CH; 3) CH + 50g/ton xylanase + glucanase, CHXG; 4) CH + 100g/ton xylanase + glucanase, CH2XG. On d 15, birds in the challenged treatments were orally gavaged with 1 ml solution containing 45,000 sporulated oocysts of *E. acervulina,* 9,000 *E. maxima,* and 9,000 *E. tenella,* and birds in the NCH group received a placebo of 1 ml distilled water. Feed and birds were weighed on d 0, 14, 28, and 42 to compute feed intake, weight gain, and mortality-adjusted FCR. On d 20, gut permeability assay using FITC-d was done using one randomly selected bird per pen. On d 21, two birds per pen were randomly selected and euthanized for intestinal lesion scoring, and on d 21 and 42, one randomly selected bird per pen was euthanized to collect cecal content for short-chain fatty acid analysis. All data obtained were analyzed using one-way ANOVA, followed by post-hoc analysis via the Tukey HSD test. The lesion score data were analyzed using the Kruskal-Wallis nonparametric test. There were no treatment effects on growth performance on d 14. From d 14 – 28 (acute infection and recovery phases), weight gain (WG), and feed intake (FI) were lower, whereas FCR was higher for all challenged treatments compared to NCH ($P < 0.001$). During the compensatory growth phase (d 28 – 42), birds in CHXG and CH2XG had higher WG ($P < 0.001$) than NCH birds, and FCR tended to be lower in the CHXG treatment than the NCH treatment. Also, during the overall phase (d 0 – 42), WG was greater ($P < 0.001$) for CHXG than CH group, but there were no treatment effects on FCR. Gut permeability and lesion scores did not differ among challenged treatments. On d 21, birds in the CHXG and CH2XG treatments had lower acetate levels ($P = 0.003$), and all challenged treatments showed a reduced acetate:propionate ratio ($P = 0.007$). However, on d 42, no treatment effects were observed in the SCFA profile. In conclusion, given no differences in the measured gut health indices, the superior weight gain during the compensatory growth phase and overall phase in the xylanase + glucanase-supplemented treatments may be due to factors not currently evaluated such as improved nutrient utilization and positive modulation of the microbiota.

Keywords: xylanase, glucanase, eimeria, growth performance, short-chain fatty acid

37 Effects of cyclic heat stress and phosphorous and calcium reduction with phytase supplementation on growth performance and bone ash in 28-d-old broilers

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The objective was to investigate the effects of cyclic heat stress and diet type on growth performance and bone mineralization in 28-d-old broilers. A total of 480, d-old male by-product Cobb breeder chicks were randomly assigned to 6 treatments in a 2 (temperature [thermoneutral, TN vs. cyclic heat stress, HS]) x 3 (diets: PC diet– adequate level of P and Ca with no phytase; NC diet - 90% of P and Ca in the PC diet with no phytase; and NC+phytase diet - NC diet supplemented with 1,500 FTU/kg of phytase) factorial arrangement with 8 replicate cages per treatment and 5 birds per cage. Each temperature was replicated twice. All diets were formulated to meet or exceed energy and nutrient requirements of the birds except for the NC and NC+phytase diets where P and Ca were marginally deficient. The trial ran for 28 d with heat stress commencing on d7 (34.0 °C for 6 hr/d). Weight gain, feed intake, and feed-to-gain ratio from d0 to 7 and 7 to 28 were calculated after adjusting for mortality. At the end of the study, the left tibia and femur were collected from one bird/cage from 6 replicate cages/treatment/room for bone mineralization determination. Data were analyzed using the MIXED 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. From d0 to 7, average daily gain (ADG) decreased ($p<0.05$) in birds on the NC diet, but no difference was seen between birds raised on the PC and NC+phytase diets. From d7 to 28, ADG and average daily feed intake (ADFI) were higher ($p < 0.05$) in TN group compared to birds in the HS group. From d7-28, ADG, ADFI, and percent femur ash were lower ($p<0.05$) in birds fed the NC diet compared to the other diets. Significant interaction between temperature and diet type was found for percent tibia ash with birds fed the PC diet under the TN condition having higher ($p<0.05$) value than the other treatment groups. Data from this study shows that within the first 7 d, phytase significantly improved birds' ADG. Furthermore, heat stress had a negative effect on ADG, ADFI, and tibia ash.

Keywords: broiler, diet, heat stress, temperature, thermoneutral

38 Impact of protease supplementation on performance and pancreas weight in broilers fed diets containing soybean meal with different levels of trypsin inhibitor activity

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Soybean meal (**SBM**) quality has been continuously scrutinized by industry nutritionists, with several challenges surrounding the variation between SBM batches, particularly regarding anti-nutritional factors such as trypsin inhibitor activity (**TIA**). One potential solution is the use of exogenous protease enzymes to overcome the negative effects of TIA on nutrient digestibility and performance. This study aimed to evaluate the effect of protease supplementation on broiler performance when fed SBM diets with varying TIA levels, and to assess the impact of TIA and protease on pancreas size. This study used a 3x3 factorial design with three TIA levels (low: 0.87, 0.81, 0.76, 0.61 mg/g; medium: 2.52, 2.33,

2.15, 1.76 mg/g and high: 4.17, 3.85, 3.54, 2.91 mg/g for pre-starter, starter, grower, and finisher, respectively) and three protease doses (0, 4000, 8000 U/kg diet), resulting in 9 dietary (**TRT**) with at least 10 replications each. A total of 5376 Ross 308 male hatchlings were assigned to 96 floor pens (56 birds per pen). The TRT diets were fed using a 4-phase feeding program (d 0-8, d 9-16, d 17-24 and d 25-31) with BW and FCR corrected for mortality (mFCR) and determined at the end of each phase. Data were analyzed using JMP 16.1 using the fit model platform where the main effects and interactions were assessed. Block was considered as a random effect with means separated using a protected Tukey HSD test ($P < 0.05$). When no interactions were present, main effects were reported. A main effect of TIA was observed, where broilers fed high levels of TIA exhibited a lower ($P < 0.05$) BW compared with broilers fed low TIA diets on d 16, 24 and 31d (669.26 g vs 684.12 g, 1256.61 g vs 1282.14 g and 1864.80 g vs 1900.00 g). Broilers fed 4000 and 8000 U protease/kg diet, showed higher ($P < 0.05$) BW compared to broilers fed no supplemental protease on d 24 (1277.25 g and 1277.73 g vs. 1253.13 g) and d 31 (1898.40 g and 1897.10 g vs 1853.8 g). Similarly, broilers fed protease at 4000 and 8000 U protease/kg diet exhibited lower ($P < 0.05$) mFCR in comparison to broilers fed no supplemental protease on d 24 (1.148 and 1.148 vs 1.157) and d 31 (1.303 and 1.304 vs 1.319, on d 31). The pancreas weight as a percentage of broiler BW was heavier ($P < 0.05$) for broilers fed high TIA diets compared to low and medium TIA fed broilers (0.283 % vs 0.255 % and 0.263 %, respectively). Protease supplementation enhanced performance regardless of TIA level, and the increased pancreas weight indicates it was insufficient to overcome TIA's negative effects.

Keywords: Soybean meal, Trypsin inhibitor activity, Protease, Broiler, Pancreas

39 Enzyme addition improves performance and the caecal microbial community of broilers fed high rye diet

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The objective of the study was to investigate the effects of enzyme addition on performance, histology, short chain fatty acids (SCFA) and caecal microbiome, when birds were fed high rye diets. A total of 168 birds were distributed equally into two groups with 7 replicate pens each. The groups were allocated into 2 treatments, control (No Enz), and test (Plus Enz) with xylanase (0.1g/kg, 16000 BXU/kg feed) and phytase (1g/kg, 5000 FTU/kg feed) addition. Starter (D0-21) and finisher (D21-35) diets contained 45.0% and 63.3% of rye respectively and were fed as mash. Bird weight and feed intake were recorded weekly and two birds per pen were euthanized by cervical dislocation on D7, 14, 21, 28 and 35. Caeca samples were collected and pooled per pen. Histology samples were taken from the ileum at Mekkel's diverticulum from D14 and onwards. Bird performance, SCFA and histology were analysed by ANOVA and means separated by T test. Caecal samples were analysed for SCFAs on D21 and D35 and 16s rRNA sequencing was performed for all the days by using Schloss MiSeq WetLab SOP and data processed by using Mothur v.1.39. Enzyme addition significantly improved cumulative body weight gain for all the sampling periods and feed conversion ratio from D0-14 and onwards at ($P \leq 0.05$). Enz supplemented birds had significantly different beta diversity. Alpha Diversity was significantly higher on D21, D28 & D35 for Plus Enz. There were no significant differences between treatments in histology, but the values were higher for villus to crypt ratio in the Plus Enz birds on D14,21 and 35. The Plus Enz treatment resulted in higher concentrations of acetic acid, butyric acid, propanoic acid, iso butyric and valeric acid on D21 while propanoic acid and butyric acid concentrations were greater on D35. Plus Enz promoted an increased number of linear discriminant features, notably probiotic and/or SCFA producing bacteria such as Clostridium IV, Blautia, Oscillibacter & Faecalibacterium. No Enz had increased linear discriminant features for Subdoligranulum, a bacteria associated with lower chicken body weight. In summary, enzyme supplementation of rye-based diets resulted in better performance which was likely driven in part by the subsequent improved villus to crypt ratio, key higher SCFAs production, significantly higher microbiome beta diversity and from D21 increased alpha diversity.

Keywords: short chain fatty acids, rye, enzyme, performance, alpha and beta diversity

40 Modeling of the thermal Inactivation of *Salmonella* Surrogate, *Enterococcus faecium,* in mash broiler feed pelleted in a university pilot feed mill

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The current feed manufacturers have not established effective standard processes and preventive control methods to control foodborne pathogens throughout the pelleting process. This study evaluated the thermal inactivation kinetic parameters of a *Salmonella* surrogate *Enterococcus faecium* during feed manufacture in a university pilot feed mill setting. A batch of 227 kg mash broiler feed was pelleted after being inoculated with 1,000 mL of nalidixic acid resistant *E. faecium* (5.4 $\log_{10}$CFU/g) at 70º, 75°, 80°, and 85°C for 0 to 115 s. Bacterial survival cell counts were analyzed by spread plating onto bile esculin agar plus 200 ppm of NaL.This pilot plant experiment was conducted by a 4 × 11 factorial design with 4 tested pelleting temperatures at 70, 75, 80 and 85ºC and 11 time periods including 15, 20, 25, 30, 40, 50, 65, 79, 94, and 115 s. The experiment was repeated three times with three samples per treatment for each replicate. To characterize the relationship between time and thermal inactivation of *E. faecium, m*icrobial data and thermal kinetic parameters [n=6, Global-Fit and United States Department of Agriculture (USDA)-Integrated-Predictive-Modeling-Program software] were analyzed by R-software (orthogonal polynomial model). Pelleting mash broiler feed at 70º, 75°, 80°, and 85°C decreased ($P < 0.05$) *E. faecium* cell counts by 0.81, 1.18, 1.69, and 1.94 $\log_{10}$ CFU/g after 115 s, respectively. D-values of orthogonal polynomial, Linear with Tail, Weibull models for *E. faecium* at 70º, 75°, 80°, and 85°C were 47.1 to 135.4, 42.1 to 135.2, and 51.4 to 118.8 s, respectively. These results suggest that pelleting at 80 or 85ºC reduces *E. faecium* populations the fastest, and it takes at least 50 s to reduce populations by 1 $\log_{10}$ CFU/g at these temperatures. Thermal inactivation for *E. faecium* took longer and required higher temperatures in the feed mill than lab estimates, highlighting the importance of testing thermal inactivation temperatures in the field to ensure proper feed hygiene. Results from this study can be used to develop a standard commercial feed mill operation procedure to reduce foodborne pathogens in feed manufacture.

Keywords: Enterococcus faecium, Thermal Inactivation, Surrogate Bacteria, Pilot Feed Mill, Mash feeds

41 Evaluation of O3 gas as a potential antimicrobial fumigant for commercial poultry feed trucks

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There are concerns regarding potential contamination of poultry feed trucks with salmonellae, especially among primary breeders. Presently, we evaluated the efficacy of ozone (O^3) generation (electrical arcing in air) as a mechanism to disinfect intentionally contaminated moistened cotton gauze pads during variable fumigation times. For these studies, we used an apathogenic isolate of *Escherichia coli* as a proxy for salmonellae as structural composition is expected to be similar. Moreover, in one study where a *Salmonella enterica* serovar Infantis was compared, the effects of O^3 exposure were similar with regard to colony forming units (CFU) recovery. For these experiments a minimum of 20ppm O^3 generation and air movement speeds of approximately 800ft/min were being measured out of an exhaust located in the front of the feed truck. We performed these experiments in two poultry feed trucks over 4 different time periods of exposure (3.5h, 8h, 10h, 24h). This evaluation was completed over 3 different experiments, including two treatments each time. Treatments were a 1) positive control truck and an 2) O^3 treated truck. Before fumigation, two 4in x 4in cotton gauze pads were placed into 4.5in x 8in sterile sample bags and allowed to pasteurize for 18h at 60°C. After pasteurization, 6mL of sterile tryptic soy broth (TSB) and 1mL of TSB enriched with *E. coli* grown for 12h at 37C to approximately $1x10^8$ $\log_{10}$ CFU/mL was added to each bag containing two cotton gauze pads layered together. The bags containing cotton gauze pads and *E. coli* were kept on wet ice in an insulated container during transport. Following fumigation, the cotton gauze pads were returned to the laboratory and promptly suspended in 25mL sterile saline and agitated by hand to disperse any bacteria present. Sample suspensions were serially diluted and drop plated onto MacConkey agar to enumerate CFU/mL bacteria recovered. CFU recovery was analyzed using a one-way ANOVA in JMP Pro 17 software. Exposure to O^3 fumigation for either 10 or 24h caused significantly ($P<0.05$) lower recovery as compared to 3.5 or 8h exposure times. The 24h exposure had no bacterial recovery, and the 10h exposure had a mean of 1.6 ± 0.34 $\log_{10}$ CFU/mL recovered. The 3.5 and 8h exposure times not being significantly different showed a mean recovery of 4.0 ± 0.83 log10 CFU/mL for 8h of exposure time. Whilst the 3.5h exposure had the highest mean recovery at 4.8 ± 0.75 log10 CFU/mL. These findings suggest that O^3 at a minimum of 20ppm with an air speed of approximately 800ft/min for the duration of at least 10h can be used to significantly reduce Gram-negative *Enterobacteriaceae* in poultry feed trucks.

Keywords: ozone , O3, disinfectant , feed truck , Escherichia coli

42 Effect of electron beam irradiation on the immunosuppressive properties of deoxynivalenol in HD11 chicken macrophage cells following lipopolysaccharide challenge

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Deoxynivalenol (DON), a prevalent mycotoxin in poultry feed, adversely impacts chicken health due to its immunosuppressive effect and results in production losses. Electron beam irradiation (EBI) is widely used for the decontamination of microorganisms in feedstuff. Hence, our objective was to assess the efficacy of EBI to denature DON. *In vitro* testing was used to determine the optimal dose in kilogray (kGy) that can effectively denature DON using a chicken HD11 macrophage cell line. We hypothesized that increasing exposure to EBI would denature the DON, which would

result in reduced immunosuppressive effects and restore nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated HD11 chicken macrophage cells comparable to the control + LPS group. HD11 cells at $1x10^5$ cells/mL concentration were plated with 0 or 1mg/mL of DON and EB irradiated DON (2.5, 5, 10, 15, 20, and 30 kGy), followed by stimulation with 10 mg/mL LPS. After 48h of incubation, the supernatant was used to measure NO production, and real-time qPCR was performed to analyze the Toll-like receptor (TLR)-2, TLR-4, and inducible nitric oxide synthase gene expression (iNOS) (n = 3). The statistical analysis was done using one-way ANOVA, and means were separated using the Tukey HSD test. The non-irradiated DON with the LPS challenge significantly reduced the nitrite concentration by 54% ($p < 0.05$). Similarly, the EBI treated DON with LPS challenge at doses of 2.5-5 kGy, 10-15 kGy, and 20-30 kGy significantly reduced nitrite concentration by 53-58%, 83-86%, and 76%, respectively, when compared to the control + LPS group ($p < 0.05$). Gene expression analysis results showed that there was no significant difference in TLR-4 expression ($p > 0.05$), whereas TLR-2 expression was significantly downregulated in both nonirradiated and irradiated treatment groups ($p < 0.05$) when compared to the control group, indicating immunosuppressive effects of the toxin even after irradiation. There was a significant reduction in iNOS expression by 0.35-fold in the non-irradiated DON group with the LPS challenge ($p < 0.05$). Similarly, there was a significant reduction in iNOS expression in irradiated treatment groups when compared to control+LPS ($p < 0.05$). These results indicate that EBI up to 30 kGy did not mitigate the negative impact of DON on HD11 cells, likely due to the formation of additional toxic metabolites resulting from EBI. In order to verify this, HPLC can be used to quantify DON metabolites in the cell culture to better understand their toxicity in future experiments. In conclusion, EBI up to 30 kGy is ineffective for pre-formed DON but may be useful for decontaminating fungi in feedstuffs.

Keywords: Electron beam irradiation, Deoxynivalenol, Toxicity, Lipopolysaccharide

43 Sweet cherry (*Prunus avium*) pomace extract on cell defences against *Salmonella enterica* serovar Enteritidis

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Salmonella enterica serovar Enteritidis (SE) is a major foodborne pathogen often associated with colonization, and persistence in host tissues. Cherries contain phenolic compounds, including polyphenols, flavonoids, and anthocyanins and may have antimicrobial and antioxidant properties. Thus, the objective of this study was to investigates the ability of a polyphenol-rich extract of sweet cherries pomaces (SCPE) to protect host cells from SE infection. Our preliminary results indicated a MIC of 64 mg/ml for the cherry pomace extract, indicating that using lower concentrations is not detrimental to SE. *In vitro* CCK-8 assay was conducted to assess the safe doses of SCPE on chicken liver (LMH) and human intestinal epithelial (Caco-2) cells. The effect of SCPE at concentrations of 0.5, 1 and 2 mg/ml on SE adhesion, invasion, and host defense responses were evaluated. The expressions of genes related to intestinal barrier integrity, inflammation, and antioxidative responses were analyzed using qPCR. Statistical analysis was conducted using one-way ANOVA with a significance threshold of $P < 0.05$. The results demonstrated that SCPE, at concentrations up to 2 mg/mL, was safe and effectively protected host epithelial cells from SE-induced cytotoxicity while significantly reducing SE colonization. SCPE upregulated tight junction genes (*claudin*, *ZO-1*), with strong effects being observed at 1 and 2 mg/mL ($P < 0.0001$). Additionally, SCPE activated antioxidant response genes (*Nrf2*, *HO-1*, and *Gclc*), with Nrf2, a central regulator of antioxidant gene expression, showing a 6- to 8-fold increase compared to untreated SE-infected cells. SCPE also suppressed the expression of inflammation-related genes (*TNF-α*, *IL-1β*, *IL-6*, *NF-κB*), with the strongest suppression observed with 2 mg/mL ($P < 0.0001$). This study demonstrates the potential of SCPE in protecting host cells from SE infection, offering a promising strategy to enhance food safety and public health in poultry production.

Keywords: Sweet cherry, S. Enteritidis, poultry cell colonization and defence, antioxidant

44 Not Presented

45 Modulating the poultry gut resistome: anti-horizontal gene transfer potential of in-feed cuminaldehyde supplementation

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Bacterial conjugation-based horizontal gene transfer (HGT) facilitates the dissemination of antimicrobial resistance genes (ARGs) within the poultry gut, posing a significant challenge to both public health and poultry production. This study evaluated cuminaldehyde (CA), a Generally Recognized as Safe (GRAS) phytochemical, for its potential to inhibit plasmid-mediated HGT of ARGs. A combination of *in vitro* and *in vivo* studies was conducted using multidrug-resistant *Salmonella* and commensal *Escherichia coli*. *In vitro* transconjugation assays utilized ceftriaxone-resistant *Salmonella Heidelberg* strains (S18 and S26) as donors and rifampicin-resistant *E. coli* J53R as the recipient. A completely randomized design (n = 9) tested CA efficacy at sub-inhibitory concentration of 3.8 mM (0.025%). Transconjugants were enumerated by selective plating, confirmed via colony PCR, and conjugation gene expression was assessed by RT-qPCR. Additionally, conjugative pili reduction was visualized using transmission electron microscopy. Two broiler trials (N = 60 birds/trial) evaluated in-feed supplementation of CA at 0.25% (T1) and 0.5% (T2). *Ex vivo* conjugation assays using cecal contents from first trial were used to assess post-treatment HGT. Whereas, cecal samples from the second trial from birds challenged with S18 and *E. coli* J53R, were subjected to metagenomic and Hi-C sequencing. Statistical analyses for *in vitro* and *ex vivo* data were conducted using two-way ANOVA ($p \leq 0.05$), while preliminary Hi-C data were analyzed using Student *t*- test. *In-vitro* CA treatment resulted in a 90% reduction in transconjugant frequency ($p \leq 0.05$), with significant downregulation of critical conjugative genes, and a visible reduction in conjugative pili structures. *Ex*

vivo analysis showed a significant decrease in conjugation frequency at 0.5% CA ($p \leq 0.05$). Although S18 and *E. coli* J53R did not reach sufficient abundance to be resolved via Hi-C analysis, a gross-level assessment of plasmid linkage was performed to gain an overall view of plasmid dynamics. This preliminary analysis revealed a decreasing trend in transfer of plasmids harboring ARGs, with mean ± SD values of 16.0 ± 4.58 (control), 11.25 ± 2.63 (T1); $p = 0.139$, and 13.5 ± 1.73 (T2); $p = 0.396$. Additionally, CA-treated groups exhibited increased relative abundance of beneficial gut commensals such as *Blautia*, *Mediterraneibacter*, *Flavonifractor*, *Lawsonibacter*, and *Allocoprobacillus*. These findings demonstrated that CA can effectively inhibit conjugation-mediated ARG transfer and modulate the gut microbiota, supporting its application as a non-antibiotic strategy to reduce ARG dissemination in poultry production.

Keywords: antimicrobial resistance, horizontal gene transfer, cuminaldehyde, microbiome, phytochemical

46 Impact of litter treatment and organic acid supplementation via drinking water on *Salmonella* prevalence and growth performance in *Salmonella*-challenged broilers

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This study assessed the effects of litter treatment (**LT**) and organic acid (**OA**) supplementation in drinking water on *Salmonella* prevalence and growth performance across two consecutive broiler grow-outs. Following a *Salmonella*-challenged grow-out, litter underwent windrow composting (**WC**) for 7 d, aluminum sulfate application (**AS**), or no litter treatment, with 24 pens per treatment. A total of 1,872 d-old male broilers were randomly assigned to the treated pens (26 chicks/pen) and provided one of three drinking water treatments: citric acid (**CA**); a mixture of lactic, acetic, and propionic acids (**OA blend**); or untreated water, with 24 replicate pens per treatment. Litter boot swabs and chick papers were analyzed at placement to assess initial *Salmonella* status. At the end of each grow out, cecal content and carcass rinse samples were analyzed for *Salmonella*. Body weight (**BW**), feed intake (**FI**), feed conversion ratio (**FCR**), and water intake (**WI**) were evaluated on d 14, 28, and 40. Data were analyzed using a 3×3 factorial arrangement, with the GLIMMIX procedure of SAS, applying a Poisson distribution for microbiology data. Statistical significance was set at $P \leq 0.05$. Chick papers tested 46.5% positive for *Salmonella* at placement. Interactions between LT and OA were not observed ($P > 0.05$). *Salmonella* prevalence after LT did not differ among treatments across grow-outs ($P > 0.05$). Ceca content showed no *Salmonella* detection, except for one sample (7 x 10^2 CFU). *Salmonella* prevalence in carcasses remained below 6% with no differences among treatments ($P > 0.05$). On d 14, broilers on untreated litter had lower BW ($P < 0.05$; 480 vs. 493 g) than broilers in the WC group. No differences were observed in other growth performance parameters ($P > 0.05$). On d 40, WI was higher ($P < 0.05$) in the CA group (8.3 vs. 8.0 L/bird) compared to the untreated water group. In the second grow out, *Salmonella* was undetected in cecal content, and carcass rinse prevalence was unaffected by treatments. Broilers in the WC group had higher ($P < 0.05$) BW (509 vs. 494 g), FI (458 vs. 441 g) and lower FCR (1.09 vs. 1.11 g: g) on d 14 and higher BW (1,704 vs. 1,652 g) and FI (2,197 vs. 2,139 g) on d 28 compared to AS group. Water-to-feed ratio was higher ($P < 0.05$) in the CA treatment (1.96 vs 1.84) compared to untreated water on d 28. Under these experimental conditions, LT did not reduce *Salmonella* prevalence but did impact broiler performance. Similarly, OA supplementation did not significantly reduce *Salmonella* prevalence, though it increased WI. Despite these findings, *Salmonella* prevalence declined over time, possibly due to natural environmental factors or the development of flock resistance.

Keywords: litter treatment, organic acids, Salmonella prevalence, growth performance, water intake

47 In-feed therapeutic supplementation of linalool reduces *Salmonella Enteritidis* and *Campylobacter jejuni* colonization in market-age broiler chickens

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S. Enteritidis (SE) and *C. jejuni* (CJ) are major foodborne pathogens transmitted through poultry products, together contributing to over 70% of foodborne infections and hospitalizations and 55% of the deaths related to foodborne illnesses in the United States. Chickens are the major reservoir host of SE and CJ, with their intestinal colonization leading to fecal shedding and meat contamination. Therefore, reducing SE and CJ populations in chickens could potentially reduce the contamination of poultry meat and products. This study investigated the efficacy of a GRAS (generally recognized as safe)-status phytochemical, linalool to decrease SE and CJ carriage in poultry. Day-old commercial broiler chicks (N = 60) were randomly assigned to 6 groups of 10 birds each (n = 10/group) consisting of a control group (challenged with SE/CJ, no linalool), SE treatment group (challenged with SE and feed supplemented with 1.5 or 1.8% [vol/wt] linalool) and CJ treatment group (challenged with CJ and feed supplemented with 1.5 or 1.8% linalool). On day 25, birds were challenged with a 4-strain mixture of SE or CJ (8 $\log_{10}$ CFU/bird). Two birds from each group were euthanized after 48 hours to check for SE and CJ colonization in the cecum. Birds were given feed supplemented with linalool (1.5 or 1.8%) for only the last 5 days before being euthanized on day 42 (n = 8 birds/group). The experiment was replicated twice and included two replications. Linalool supplementation at 1.5 and 1.8% significantly reduced SE counts in the cecum by 2 $\log_{10}$ CFU/g cecum and 4 $\log_{10}$ CFU/g cecum, respectively ($P < 0.05$). Linalool supplementation at 1.8% significantly decreased CJ counts in the cecum by 5 $\log_{10}$ CFU/g cecum ($P < 0.05$). There were no significant differences in the body weight gain, feed intake and feed conversion ratio of the linalool supplemented groups and control birds ($P > 0.05$). The results suggest that linalool could be used as a pre-slaughter dietary supplement for reducing SE and CJ counts in broiler chickens without adversely affecting production parameters.

Keywords: Salmonella Enteritidis, Campylobacter jejuni, Linalool, Broiler chickens, Therapeutic

48 In-water supplementation of Trans-Cinnamaldehyde Nanoemulsion reduces *Salmonella* enteritidis colonization in ceca and oviduct of layer chickens

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Salmonella Enteritidis (SE) is a leading foodborne pathogen responsible for human gastroenteritis, with contaminated eggs being a primary source of infection. In layer hens, SE can persist in the ceca, serving as a reservoir for intestinal shedding, and can also colonize the oviduct, leading to vertical transmission of the pathogen to eggs. This dual colonization increases the risk of egg-borne transmission and subsequent public health concerns. To reduce *Salmonella* colonization in hens thus reducing SE dissemination on eggs, pre-harvest strategies are employed with varied degrees of efficacy. Trans-cinnamaldehyde (TC) is a Generally Recognized as Safe status phytochemical obtain from cinnamon bark that exerts significant anti-*Salmonella* efficacy in chickens. However, the challenge working with TC oil is its low solubility in water. To overcome this challenge and enhance dispersion of hydrophobic TC oil in water, TC nanoemulsions (TCNE) were prepared using high energy sonication with food grade Gum Arabic and lecithin emulsifiers. Thereafter, the efficacy of in-water supplementation of trans-cinnamaldehyde nanoemulsion (TCNE) was investigated to reduce SE colonization in the ceca and oviduct of layer chickens. In two trials, 48 laying hens (White leghorns; seventy-week-old;12 birds/treatment/trial) were randomly allocated to 4 groups: control, emulsifier control, TC-0.005 and TCNE-0.005. Birds in the TC and TCNE groups were supplemented with TC oil or TCNE at 0.005% in drinking water for 9 weeks. One week after starting treatments, birds were orally challenged with a four-strain cocktail of SE (~9 log CFU/bird). Eggs were collected daily, and cloacal swabs weekly to test for the presence of SE. After 9 weeks, all the birds were sacrificed and SE colonization in the ceca and oviduct was determined. In addition, body weight gain, feed and water intake, and egg production were measured. Data from the two trials were pooled and analyzed using Fisher's exact test with statistical significance at $P < 0.05$. TCNE had a particle size of ~ 100 nm, polydispersity index of <0.3 and Zeta potential of approximately -30 mV. Supplementing birds with 0.005% TCNE in drinking water significantly reduced SE on eggshells and in cloacal swabs by 15% ($P < 0.05$). In addition, TCNE treatment decreased SE colonization in the cecum and oviduct by 66% and 34%, respectively ($P < 0.05$). No effect in body weight gain, feed and water consumption, or egg production were observed by treatments as compared to controls ($P > 0.05$). The impact of treatments on egg quality and consumer acceptability is currently under investigation. TCNE supplementation shows potential as an effective strategy for controlling SE transmission through eggs.

Keywords: Salmonella Enteritidis, Layers, Colonization, Trans-cinnamaldehyde, Egg quality

49 Optimizing eBeam strategies against *Campylobacter* colonization in broiler chickens

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Campylobacter jejuni (Cj) is a leading cause of foodborne illness globally, often linked to consumption of poultry. Unfortunately, current strategies to control Cj in poultry have had limited success, and the development of an anti-Cj vaccine could be an attractive alternative strategy. Electron Beam (eBeam) technology can be used for producing safe and effective strategies against poultry pathogens, including *Campylobacter*. Using electricity as its source, eBeam is eco-friendly, cost-effective, and free from health hazards. It inactivates pathogens by shredding the DNA, preventing reproduction, while preserving membrane integrity and antigenic epitopes, ensuring strong immunogenicity. This unique mechanism makes eBeam vaccines as safe as killed vaccines and as effective as live vaccines. The objective of this study is to determine the optimal dose of eBeam to develop a vaccine against *Campylobacter* for chicken. To achieve this, we treated two different *Campylobacter jejuni* wild strains, K1 and S1, with varying eBeam doses ranging from 0 kGy (negative control) to 5 kGy to determine the D10 value and total inactivation dose. Additionally, both strains were inactivated with formalin, one of the most common inactivation methods utilized in the development of vaccines, as a control. All samples were plated on *Campylobacter* enrichment agar for microbiology analysis. *Campylobacter* colonies on the eBeam-treated samples were enumerated and data were used to determine the D10 value (the dose required to reduce the bacterial population by 1 log) and the lethal dose, which is the dose required to completely inactivate the bacteria. Also, treated bacterial cultures were evaluated for membrane integrity (Bac Light staining) and cell viability (Bac Titer-Glo assay). D10 values were calculated using a dose-response analysis in RStudio. The microbiology and *in vitro* analysis data were analyzed by one-way ANOVA followed by Dunnett's *post hoc* comparison using GraphPad Prism 10 Software, and a P-value<0.05 was considered significant. The D10 values were calculated as 0.300 kGy for K1 and 0.226 kGy for S1 strains. eBeam-treated samples had a similar percentage of cells with intact cell membranes compared to the negative control, as opposed to the formalin control that presented a high percentage of damaged cells. Viability assay revealed that eBeam-treated samples exhibited increased metabolic activity compared to negative control and formalin-treated cells. In conclusion, eBeam treated cells are unable to multiply while maintaining membrane integrity and metabolic activity. Based on these positive results, we will evaluate effectiveness of this eBeam strategy on immune response in birds.

Keywords: Campylobacter jejuni, Electron beam, Broiler chickens, D10 value, Vaccines

50 Alterations to the phenotypic expression of *Salmonella enterica* due to environmental stressors

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Salmonella enterica has continued to be a significant concern in poultry and poultry products. Research indicates that environmental conditions can influence survival and persistence, posing food safety risks. Understanding these factors is crucial for developing control strategies to mitigate contamination and improve food safety. This study evaluated the phenotypic changes to *Salmonella enterica* serotypes Infantis (SI), Kentucky (SK), Schwarzengrund (SS), and Typhimurium (ST) in response to antimicrobial and temperature stresses. A pre-harvest isolated strain of each serotype was exposed to gradually increasing

concentrations of peracetic acid (PAA) and quaternary ammonium compounds (QAC), starting at 40 ppm and 1 ppm, respectively until growth ceased, and the minimum inhibitory and bactericidal concentrations (MIC and MBC) were established. The stressed cells underwent two shock treatments at 4°C (60 min) and 55°C (6 min) simulating the chiller and scalder, respectively. This resulted in three "conditioned" variants for each antimicrobial of each serotype. These variants, PAA/QAC-stressed, cold-stressed, heat-stressed, and unexposed control were evaluated for phenotypes including tolerance to PAA and QAC, biofilm formation, biofilm recovery post-PAA or QAC treatments for 5 min, and antibiotic susceptibility. The data was analyzed using one-way ANOVA in JMP Pro 18 at $p \leq 0.05$. The serotypes differed in their tolerance to PAA and QAC after daily exposure: SI (120 & 22 ppm), SK (100 & 25 ppm), SS (110 & 10 ppm), and ST (150 & 20 ppm). After temperature stress, the tolerance of the variants to PAA increased by 10-20 ppm, whereas QAC was up to 1.5-fold increment compared to the controls. The variants of ST significantly ($p=0.0002$) formed more biofilms compared the control, while there was no difference for the variants of the other serotypes and their controls. Furthermore, biofilm recovery was significantly higher for SI ($p=0.0001$) and SS ($p<0.0001$) variants following PAA exposure and lower for SK ($p=0.003$) and SS ($p=0.015$) variants after QAC exposure. All the variants and controls were exposed to 13 antibiotics and the QAC-stressed variants showed more phenotypic changes compared to their PAA-stressed counterpart. Interestingly, SI variants exhibited reduced MIC to 10 of the 13 antibiotics. The variants of the other serotypes showed an increased MIC to 5-8 antibiotics. Notably, SK and ST variants exhibited 2 to 4-fold increase in MIC to azithromycin, an antibiotic commonly used to treat bacterial/viral infection, including salmonellosis. This study suggests that stresses encountered in food processing environment can significantly enhance the survival, persistence, and virulence of *Salmonella enterica*.

Keywords: Salmonella enterica, PAA, QAC, Stress-adaptation, Biofilm

51 Impact of *Salmonella* Infantis inoculation on poultry litter microbiome

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Consumption and handling of contaminated poultry products contribute to 23% of human *Salmonella* infections. While *Salmonella* Infantis causes only 2% of cases, this serovar has a higher potential to carry antimicrobial resistance compared to other serovars. Thus, in this study, we aimed to assess the potential impact on the litter microbiome resulting from inoculation of *Salmonella* Infantis. *Salmonella*-free litter samples were allocated into 4 plastic boxes (300 g/box), and a prepared inoculum of *Salmonella* Infantis was sprayed with frequent agitation by hand for a final inoculation concentration of 3.33×10^4 CFU/g of litter. Litter samples were collected before inoculation and post-inoculation at 24 h, 72 h, and 7 d. Collected litter samples were homogenized using 1:10 w/v PBS, and litter pellets were obtained, then washed using Inhibitex buffer and subjected to DNA extraction. PCR amplification of the hypervariable regions (V1-V9) of 16S rRNA was conducted, followed by sequencing using MinIon (Oxford Nanopore). Raw reads were base-called and demultiplexed using MinKnow and analyzed using EPI2ME for taxonomic identification using the NCBI database. Raw reads with a quality score >10 and read lengths between 800–2000 bp were obtained, resulting in a sequencing depth of approximately 49702 reads per sample for downstream analysis. Microbial diversity was analyzed in R Studio using the Phyloseq package to evaluate community shifts across time points and treatments. The alpha diversity indexes indicated that there was no difference ($p > 0.05$) in richness and pielous evenness in the litter microbiome between treatments. Additionally, there was no significant difference between treatments for beta-diversity Bray-Curtis (Adonis $p > 0.05$). However, while comparing the differentially abundant species using DESeq2, there were significant changes in bacterial diversity over time due to *Salmonella* inoculation. Compared to 0 h control and 24 h inoculated samples, there was a significant increase in the abundance of bacteria such as *Escherichia fergusonii, Esherichia marmotae, Shigella dysenteriae, Shigella flexneri* in 72 h and 7 d samples ($p < 0.05$). There was a significant decrease in the abundance of bacteria such as *Enterococcus faecium* and *Enterococcus hirae* between 0 h control compared to 72 h and 7 d samples ($p < 0.05$). In addition, the abundance of environmental bacteria (*Anaerosalibacter bizertensis*, *Sphingobacterium cibi*, *Amphibacillus indicireducens*, *Cerasibacillus terrae*) was impacted by the inoculation of the *Salmonella* Infantis. In conclusion, *Salmonella* Infantis inoculation alters microbial community structure over time, leading to the increased presence of opportunistic pathogens.

Keywords: Salmonella Infantis, litter microbiome, full-length 16S rRNA, species-level identification

52 Impact of storage temperature on microbial composition in poultry litter over time

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Transporting poultry litter for microbial assessment poses challenges due to variable shipping durations and temperature conditions, ranging from overnight transit on ice to extended delays at room temperature. This study evaluated microbial compositional changes in poultry litter stored at three different temperatures (-20°C, 4°C, and 25°C) over five days, compared to freshly collected control samples (N = 140) from a laying hen facility. Litter samples (20 g) were stored in Whirl-Pak® bags at their respective temperatures, with 1 g subsampled daily, diluted in 9 mL PBS, vortexed for 30 seconds, and serially diluted. A 10-µL aliquot of each dilution was plated on selective agars to assess microbial populations: Tryptic Soy Agar (TSA) for total aerobes, MacConkey Agar for Gram-negative bacteria, and De Man, Rogosa, and Sharpe (MRS) Agar for lactic acid bacteria (LAB). Plates were incubated at 37°C for 24 hours, and colony-forming units (CFUs) were log-transformed for statistical analysis. A linear mixed-effects (LME) model was used to evaluate the effect of storage temperature over time, with significance set at $P < 0.05$. Microbial counts were significantly affected by storage duration across four days ($P < 0.05$). Aerobic counts were significantly higher in the -20°C group than in the control on day 3 ($P < 0.05$). LAB counts were significantly higher in the -20°C and 25°C groups compared to the control and 4°C groups. By day 3, all treatment groups exhibited higher LAB counts than the control ($P < 0.05$). By day 4, LAB counts in the -20°C and 4°C groups remained significantly elevated compared to the control, while the 25°C group showed similar counts to the control. However, Gram-negative bacterial populations did not significantly differ between treatments across time ($P > 0.05$), indicating that storage temperature had minimal impact on this group. These findings

indicate that microbial counts in poultry litter change significantly over time, regardless of storage temperature. The lack of a significant interaction between temperature and day suggests that bacterial growth patterns were primarily driven by storage duration rather than temperature effects. This was particularly evident for LABs, where bacterial counts increased significantly over time across all conditions. However, the stability of Gram-negative populations suggests that certain bacterial groups may be less influenced by storage conditions. These results highlight the importance of processing poultry litter samples promptly, as microbial shifts occur with extended storage, potentially impacting data interpretation in microbial assessments.

Keywords: Temperature, Poultry litter, Shipping conditions, Microbial characterization

53 Assessing transcriptomic responses of *Salmonella Infantis* in litter

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In recent years, even though overall *Salmonella* contamination has declined, illnesses caused by *Salmonella* Infantis infections have increased, along with a rising trend in carcass contamination. Therefore, the objective of this study was to elucidate the persistence mechanism of *S.* Infantis by analyzing transcriptomic changes under different environmental conditions. A 2 × 2 factorial design was utilized, with two different culture media BHI and PBS with or without poultry litter. For litter treatments, litter was mixed with either BHI or PBS at a 1:5 w/v ratio, and bacteria at 1.2×10^6 CFU/ml were inoculated and incubated at 37°C for 24 h before collecting samples. RNA was extracted and quality, quantity, and purity were assessed and subjected to Illumina Novaseq sequencing after rRNA depletion. Demultiplexing, quality control, and adapter trimming were done using bcl-convert. Reads were aligned to reference genome using HISAT2, and read counts were performed using HTSeq. Differential gene expression was analyzed using DESeq with a log2FC cutoff set at ≥2 or ≤-2. The compared treatments were BHI vs BHI-litter (BL), PBS vs PBS-litter (PL), and PBS vs BHI. Among 4611 genes, a total of 472 and 732 were significantly expressed in litter compared to their respective culture media BHI and PBS. Wherease408 genes were upregulated among 722 significant genes between PBS vs BHI. Starvation stress was pronounced in bacteria with upregulated expression of *rpoS* and *dksA* genes in nutrient-poor media like PBS in comparison to BHI and litter treatments. Additionally, genes like *fad, eut, and pdu* associated with alternative carbon utilization were upregulated in PBS compared to PL and BHI, indicating a lack of nutrient sources for bacteria in PBS in comparison to PL and BHI. Compared to non-litter media, alternative respiration system genes *dmsABC*, *ttrBC*, and *fdnHG* upregulation in litter highlights *S.* Infantis ability for metabolic adaptation to oxygen-limited environments. Oxidative stress genes *soxR* and *soxS* were upregulated in BHI compared to BL and PBS suggesting oxidative stress increases in nutrient-rich BHI at 24 h. The study found upregulation of *marA a*nd *marR* genes in litter compared to samples without litter which provides resistance towards environmental and antimicrobial stressors in litter. Furthermore, virulence genes such as *sseC*, *invA*, *sopD* and *sifA* were found to be upregulated in BHI in comparison to BL. In contrast, genes associated with amino acid-dependent acid resistance mechanisms like *adiA*, *cadA*, and *cadB* were downregulated in BHI in comparison to BL. In conclusion, *S.* Infantis upregulated stress response and metabolic genes to survive in challenging environments.

Keywords: transcriptomics, Salmonella Infantis, litter, survival

54 Enhancing the free-choice learning experience at CBATs through interactive poultry programs

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Agritourism farms are free-choice learning environments that are curated to be unique and immersive based on several dimensions and qualities that enhance the experiences. These dimensions include *authenticity, learning, and interaction*, vital aspects for the sustainability and operation of such attractions. interactive based on several dimensions and qualities that enhance the experiences. The design and intent of agritourism is to entice visitors to explore and experience "temporary escapism" through the rural landscape and simplistic agricultural lifestyle, while simultaneously promoting the local community's economy and other businesses. The Farm at Prophetstown is a community-based agritourism [CBAT] farm located in the Prophetstown State Park emulating American 1920s homesteading, their most notable feature are the heritage breeds owned on the property. To better understand the extrinsic and intrinsic characteristics impacting visitor retention, Purdue Poultry Extension developed a survey which was deployed during calendar season for The Farm at Prophetstown. 157 visitors participated in the survey through online links, QR codes, and a stand placed along the tour path on the property. The researcher identified and extracted themes from the data collected to assess what aspects the visitors find desirable. Most respondents (83%) were not first-time visitors, noting that they are aware of the establishment within the state park; however, when asked about the underlying reason for their visit "touring the state park" and "camping" were the biggest factors. The results showed that 63% of visitors had children or grandchildren, influential factors in their attendance at The Farm. When asked about their favorite aspects of the attraction results showed that "animals" and "history or antiques" were provided in the answer box most frequently. Similar findings were shown when the adults were asked about their favorite part of the visit with livestock (30%) being one reason, followed by the appreciation of history (20%). Demonstrations (28%) and seasonal events (37%) garnered more interest in attending, teaching the public homesteading skills and about animals they own. Poultry was the most common livestock owned by residents, presenting a niche opportunity in program development for the prospective future. Previous studies have found that overall experience impacts the promotion of the attraction from visitor-to-visitor, word-of-mouth (40%) communication spreading the entity's name and offerings they provide to others within the community. These findings were used to develop an interactive poultry program for youth and adults, which The Farm can modify in future iterations.

Keywords: free-choice learning, CBAT, experience, poultry, program

55 Assessing the development, implementation, and evaluation of an online poultry dual credit course for high school students across the state of Mississippi

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Poultry is Mississippi's top agricultural commodity, supporting approximately 99,000 jobs across the state. However, previous studies have indicated that there is a lack of poultry career opportunity exposure for students in MS high schools. To address this, previous research from our lab surveyed high school agriculture teachers across the state of MS (n=35) to determine if they would be willing to implement an online dual-credit poultry course. Data illustrated a high percentage of teachers indicating potential student interest in a dual-credit poultry course (86%) and a high percentage of teacher willingness to implement a dual-credit poultry course (88%), suggesting a need for a course. Therefore, the objective of this study was to develop, implement, and evaluate an online poultry dual-credit course utilizing pre/post-tests and surveys. High school students from five different schools across the state of MS (n=27) were enrolled in an online dual-credit poultry course facilitated by onsite teachers. Utilizing a paired t-test, data demonstrated student knowledge gained ($P<0.0001$) with a 23.55% improvement in test scores. A chi-square of independence indicated an increase in students' awareness of post-secondary ($P=0.0405$) and career opportunities in poultry ($P=0.0044$). Prior to completing this course, 26.1% of students stated they were likely to major in poultry science, with this number increasing to 79.2% post-course completion ($P=0.0003$). Furthermore, 92% of students noted their perception of the poultry industry changed post-course. Also, 58.3% of students noted that they were likely to pursue a career in the poultry industry, while not significant ($P=0.1058$), this is a 23.5% increase compared to pre-course responses (34.8%). Data also indicated that 96% of respondents would recommend this course to other students. Additionally, facilitating teachers were surveyed to determine their satisfaction with the course and ease of implementation. With a 75% return rate (n=3), survey data indicated that 100% of facilitators were satisfied with the course, would recommend it to future students, and would implement the course again. These data suggest that an online dual-credit poultry course has the potential to expose high school students to post-secondary and career opportunities in poultry and serve as a recruitment tool for poultry departments and industry. No course modifications were noted by facilitators, and minor course modifications were provided from students (e.g., virtual meetings, more interactive videos, and extending the course). These data, including suggested changes, provide an important roadmap to further develop student interest in poultry across the state of Mississippi and beyond.

Keywords: online dual-credit course, poultry, high school students

56 Development of interactive training modules for handling, herding, loading, and transporting of market-ready turkeys during live haul

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Formal training programs for crew members on proper handling, herding, loading, and transporting market-ready turkeys are scarce. The absence of structured training increases the risks for bird

injuries and mortality, compromising animal welfare and resulting in financial losses for processors. Additionally, negative media attention can jeopardize market access and result in economic consequences if legal action is taken. To address these challenges, six focus groups with 38 crew members across the U.S. were conducted to identify risk factors during live haul practices. Using insight from the focus groups and live haul resources from academia and industry, a comprehensive and interactive computer-based training program was developed. The virtual training program is structured into three modules to provide practical, science-based guidance to equip crew members with essential skills for correctly handling, herding, loading, and transporting birds. The modules cover critical aspects of turkey behavior and health as they relate to movement, including birds' sensory perceptions, effective herding techniques, environmental factors affecting movement, proper handling of non-ambulatory birds, and appropriate equipment use. The training showcases a structured step-by-step process, supported with real-world media from commercial farms, for crew members to follow during live haul, minimizing the chances of stress and injury for the birds and crew members. Incorporating commercially relevant videos ensures that trainees experience a realistic and immersive learning experience to improve the likelihood of effectively applying proper handling techniques. To reinforce learning and ensure comprehension, crew members complete knowledge-check questions designed to assess their understanding and ability to apply the material in real-world barn settings. This training tool can be implemented across the turkey industry for onboarding and annual training programs while also ensuring worker compliance with company policies and animal welfare auditing programs. Beyond training crew members, it is also a valuable resource for industry professionals and educational programs in poultry science and animal welfare to uphold industry standards and improve overall handling efficacy. Future research will assess the effectiveness of the training by evaluating crew performance in handling, herding, loading, and transporting turkeys.

Keywords: turkey, training, herding, loading, welfare

Student Competition: Immunology, Health and Disease: Health and Disease II

57 Cold stress in cage-free laying hens: effect on performance, mucosal Immunity, blood biomarkers and *Salmonella* susceptibility

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Non-typhoidal *Salmonella* is a major zoonotic concern in poultry, as chickens can asymptomatically harbor the bacteria and transmit it to humans through contaminated products. Environmental stressors, such as cold temperatures, can place undue stress on birds, detrimentally impacting their performances and increasing their susceptibility to infection. This study aimed to investigate the impact of cold stress in cage-free layer chickens on performance, blood physiological parameters, disease transmission, and immune response during *Salmonella* infection. A total of 144 Hyline white layers (48 weeks old) were housed in 4 rooms (36 birds/room). Two rooms were maintained under thermoneutral (TN) conditions (22°C), while the remaining rooms were exposed to cold stress (CS) at (5°C). After a week of acclimatization, 25% of birds in one TN and one CS room were orally infected with 1 x 10^8 CFU/ml of *Salmonella* Enteriditis, while 25% of birds in other rooms received PBS, resulting in four treatment groups, TN control (TNC), TN infected (TNI), CS control (CSC), and CS infected (CSI). Body weights, feed intake, cloaca temperature, and egg production and quality were recorded at multiple time points before and after infection. Blood parameters were measured using the i-STAT analyzer pre-infection and at 14 days post-infection (dpi). On 14 dpi, eight birds per treatment were euthanized for sample collection, including cecal content, liver, and shell glands for *Salmonell*a load assessment. Intraepithelial lymphocytes (IELs) were isolated from the ileum through mechanical disruption followed by percoll density gradient and stained for flow cytometry analysis. Flow data were processed using FlowJo and analyzed by Kruskal Wallis test. Data were analyzed using one-way and two-way ANOVA, or logarithmic regression, as appropriate, with significance set at P-value < 0.05. The results indicated that CS did not affect the weekly egg production rate, regardless of infection. However, CS hens produced eggs that were 3g lighter and consumed 8g more feed compared to the TNC birds. Notably, CS birds had significantly increased shell thickness before and after infection, without change in egg breaking strength. Blood parameter analysis showed that HCO_3 levels were significantly higher in CSC and TNC than CSI and TNI ($P<0.01$). No significant differences were observed between the groups in *Salmonella* colonization and transmission, resulting in comparable ileal IELs across groups. Under these experimental conditions, cage-free chickens raised in low ambient temperatures consumed more feed and produced lighter eggs without affecting immune responses and *Salmonella* susceptibility.

Keywords: Salmonella, Layer, Cold stress, cage free, Immune response

58 Impact of different doses of Intratracheal *Escherichia coli* challenge on growth performance, immune organs, and lesion severity in broilers

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Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is an infectious disease causing respiratory and systemic infection leading to significant economic losses in the poultry industry due to morbidity, mortality, carcass condemnation, and reduced productivity. The aim of the experiment was to investigate the effect of different challenge levels of APEC on growth performance, Bacterial loads, lesions severity, and body composition in broilers and to determine the optimal dose that can be used as an intratracheal APEC challenge model. A total of 240 eighteen-day-old Cobb 500 male broilers were randomly assigned to four treatments and six replicates in battery cages with adlibitum feed and water. The treatments consisted of non-challenged control (NC) and the three challenge treatments (T1, T2, and T3) which were intratracheally inoculated with 0.1ml of 10^5, 10^7, and 10^9 CFU/1ml Nalidixic acid resistance APEC *O78,* respectively, on day 18. On the 7th day post inoculation(dpi), growth performance was measured, and 2 birds per replicate per treatment are sampled to evaluate bacterial loads on the lung and liver. Furthermore, Lesions on the lung, pericardium, and liver were scored, and relative weights of immune organs were measured. Body composition of birds was measured using Dual-energy X-ray absorptiometry (DEXA). The data were analyzed with one-way ANOVA, Wilcoxon/Kruskal-Wallis tests, and Tukey HSD, using JMP Pro 17 statistical software. Body Weight and body weight gain were significantly decreased in birds inoculated with 10^9 CFU APEC *O78* (T3) compared to the NC group. Average daily feed intake gradually decreased from 1 dpi and significantly on 6, and 7 dpi in T3. Lesions were scored based on the severity of pericarditis, and fibrosis on the pericardium, liver, and lung, hemorrhages on the lungs. The severity of scores was observed to increase as the dose increased ($p<0.05$). Relative weights of the spleen and bursa were significantly higher in the T3 compared to the NC group. DEXA analysis revealed an increase in fat percentage corresponding to the increasing dose of APEC *O78*. In conclusion, the results showed that 10^9 CFU APEC would be the optimum in understanding the effects of APEC inducing production losses besides serving as an intratracheal challenge model.

Keywords: avian pathogenic Escherichia coli, Intratracheal inoculation, Avian colibacillosis, Systemic infection

59 The impacts of multi-species *Eimeria* vaccination on cecal microbiota diversity and composition of turkey poults

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The gastrointestinal tract experiences a significant state of disease that disrupts the microbiome; therefore, it is essential to evaluate the changes that occur and to determine the types of probiotics or

other gut-enhancing feed additives to provide to birds infected with *Eimeria*. As a result, this study aimed to characterize the effects of a multi-species turkey *Eimeria* vaccine (*E. adenoeides, E. meleagrimitis,* and *E. gallopavonis*) on the cecal microbiota of turkey poults. The experiment comprised two treatments: Control (CON) and Vaccinated (VAC), with 20 poults per treatment. Poults in the VAC group received 1,000 oocysts/mL of the vaccine on the day of hatch, and cecal samples were collected at 4, 9, and 14 days of age. DNA was extracted using Qiagen standard kits, and 16S rRNA amplicon sequencing was performed using the Illumina NextSeq 2000 PE250 platform. Data were processed in QIIME 2 with DADA2 for quality filtering and chimera detection. Alpha diversity indices such as Shannon, Chao1, and Observed Operational Taxonomic Units, OTUs, and beta diversity metrics Bray-Curtis, Jaccard, Weighted UniFrac were analyzed using Mann-Whitney/Kruskal-Wallis statistical methods and Microbiome Analyst Software and with a significance threshold of $p < 0.05$. Significant differences in alpha diversity were observed, including the Shannon index ($p = 0.007$), observed OTUs ($p = 0.0038$), and Chao1 ($p = 0.0034$), indicating shifts in microbiota diversity and richness over time. At d4, changes in diversity and richness were seen in the VAC group with Chao1 index of 45 vs. 52 in CON, indicating an early sign of gut dysbiosis. At d9, the VAC group exhibited a substantial decline in bacterial diversity with Shannon index of 1.65 vs. 2.6 in CON; observed OTUs of 35 vs. 54 in CON and richness shown in Chao1 index of 35 vs. 54 in CON), suggesting transient dysbiosis. Beta diversity indexes, including Bray Curtis ($p = 0.001$), Jaccard ($p = 0.001$), and Unweighted UniFrac distance ($p = 0.002$), showed a significant distance of the microbiome samples on d9 VAC group from the CON while the d4 and d14 showed a clustered overlapping among each other. Also, a higher abundance of potentially pathogenic bacteria *Enterobacteriaceae*, *Enterococcaceae*, *Clostridiaceae*, and *Peptostreptococcaceae*, was seen in VAC on d4 and d9 compared to CON. However, this was reduced at d14, which showed a recovery by the presence of increased levels of beneficial bacteria such as *Lachnospiraceae* and *Butyricicoccaceae*. Early disruptions in microbial diversity may have implications for gut health and performance. Future strategies should focus on mitigating transient dysbiosis to optimize the benefits of vaccination in commercial poultry production.

Keywords: Eimeria, coccidiosis, Turkey poults, microbiome, Vaccination

60 Evaluation of probiotic interventions against turkey reoviral arthritis in specific pathogen free (SPF) turkeys

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Turkey reoviral arthritis (TRA) is disease caused by turkey arthritis reovirus (TARV) that leads to lameness and hock joint swelling in turkeys. The disease increases culling, decreases market body weight, and reduces carcass quality, resulting in a $33.7 million loss in 2019. TARV has remained top 10 turkey industry concern for the last decade, yet no effective preventive strategies currently exist against this disease. The virus initially replicates in intestines and induces gut dysbiosis before translocating to tendons and other organs. To reduce virus replication in intestines and reverse gut dysbiosis during reoviral infection, probiotics administration could be a potential approach. This study investigated the effect of administration of probiotics on the pathogenesis and severity of reoviral arthritis. We hypothesized that probiotics administration will reverse gut dysbiosis and reduce the severity of TRA in turkeys. Specific pathogen free (SPF) turkey poults, which were free from reovirus, were used. Poults were randomly divided into ten groups (n=10/group). The probiotic groups were orally inoculated with 200 µl (10^8 CFU per bird) of *Lactobacillus aviarius* subsp. aviarius (LAV), *Lactobacillus aviarius* subsp. araffinosus (LAR), *Lactobacillus johnsonii* (LJ), and *Clostridium bartlettii* (CB) from one day of age to 2 weeks of age (WOA). The probiotics and mock infected groups were orally inoculated with 200 µl of 10^7 TCID50 TARV O'Neil per bird at 1 WOA, while mock group with 200 µl of phosphate buffered saline. Blood, cloacal swabs and body weights were taken on a weekly basis. The poults were euthanized at 4 weeks post-infection (WPI) and intestinal contents, hock joints, and tendons were collected. Statistical analysis among groups was performed by one-way ANOVA followed by post-hoc Tukey's test. At 4 WPI, the body weight between infected and non-infected poults was significantly different for the CB ($p< 0.05$) probiotic treated group but not for LAV, LAR, and LJ treated groups. We observed that antibody (reovirus specific and isotype nonspecific) titers for LAR ($p<0.0001$) and LJ ($p<0.05$) treated and infected groups were significantly lower than mock infected group at 3 and 4 WPI, while titers for LAV and CB treated and infected groups were numerically lower compared to mock infected groups. We observed a positive effect of probiotics on body weight for LAV, LAR and LJ treated groups. The lower antibody response in LAR and LJ groups might be correlated to a reduced dissemination of virus after initial replication in the intestines. Future studies will be conducted with a combination of probiotics in turkeys to study the effect on gut microbiome modulation and severity of reoviral arthritis.

Keywords: reovirus, arthritis, turkeys, probiotics, gut dysbiosis

61 Not Presented

62 Characterization of LysM protein in *Histomonas meleagridis*, a protozoan pathogen in turkeys

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Histomonas meleagridis is the infectious agent for histomonosis, also known as blackhead disease. This parasitic protozoan infects gallinaceous birds, primarily affecting turkeys, causing strong inflammation and severe necrosis of the ceca and liver. It is an economically deleterious disease as it can cause mortality rates of 80% to 100% in commercial flocks. Due to the limited understanding of the molecular mechanisms by which *H.*

meleagridis infects host cells, no prophylactics or treatments are currently available to control histomonosis, despite its significant agricultural impact. Previous studies on fungal pathogens indicate that LysM is a key protein within the cell wall that prevents recognition by host cell chitinase. We speculate that LysM protein is embedded in the chitin wall of *Histomonas* parasites to protect them against attack from turkey intestinal acidic chitinase. To test it, in this study, the recombinant *H. meleagridis* LysM (HmLysM) gene was PCR-amplified, cloned into a prokaryotic pQE30 expression vector, induced by IPTG, and purified by nickel column chromatography using fast protein liquid chromatography (FPLC). Rabbits were immunized with recombinant HmLysM for polyclonal antibody generation. Immunoblotting analysis revealed that the produced anti-HmLysM recognizes both the recombinant and native HmLysM. Purified *Histomonas* parasites were stained with anti-HmLysM and rhodamine-conjugated wheat-germ agglutinin (WGA) to observe the location of LysM in the parasites. WGA binds to chitin, serving as a marker for the cell's surface. HmLysM displayed puncta pattern and partial surface staining, suggesting it is located in vesicles within the parasite and traffics to the parasite surface. I will conduct another indirect immunofluorescent assay (IFA) without permeabilization to confirm the location of LysM on the surface of *H. meleagridis*. In conclusion, we successfully generated recombinant LysM protein in *E. coli* and determined its subcellular locations in the parasites. Future work will focus on cloning and purifying turkey chitinase to assess its efficacy in damaging putative *Histomonas* cysts *in vivo* and examining turkey chitinase expression in vivo following *H. meleagridis* infection.

Keywords: Histomonas meleagridis, Turkeys, LysM, Molecular Parasitology, Chitinase

63 Gene Expression of Immune Mediators in the Ceca, Ceca Tonsil, and Spleen Tissues of Poults Post Infection with *Eimeria*

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Infection with *Eimeria* spp. compromises the intestinal integrity of turkey poults, affecting their growth, immunological homeostasis, and ability to absorb nutrients. Persistent intestinal injury puts birds at risk of secondary infections and financial losses, even while the infection produces inflammatory and adaptive immune responses. Developing efficient vaccinations, immunomodulatory techniques, and focused disease control approaches requires understanding the immune response. The study investigated the cellular immune responses in the ceca, ceca tonsil, and spleen of young turkeys after a primary infection with mixed *Eimeria* species by specifically analyzing changes in the gene expression of key immune mediators such as IL-8, Il-10, IL-18, IFN-γ, IL-1β (IL1RN), LITAF, and HSP70. A total of 104 turkey poults were randomly assigned to two treatments with four replicate pens per treatment: Uninfected Control (UC) and Challenged (CC) group. Poults in the challenged group were inoculated with 25,000 sporulated oo/ml of *E. adenoiedes, E. gallopavonis* & *E. meleagrimitis* on d4. Birds were individually weighed on d1 and 6dpi (days post infection) to show infection was achieved. The tissues collected for gene expression analysis were ceca and spleen on 4dpi, 7dpi and 9dpi while cecal tonsils were collected on 4dpi, 7dpi, 9dpi, 11dpi, 13 dpi and 15dpi. Oocyst per gram (OPG) data was analyzed as a non-parametric test with Wilcoxon Rank Sum test. Gene expression data were analyzed as a t-test, with significance at $P < 0.05$. There were significant differences ($P<0.05$) in BW and Mortality on 6DPI, which confirms the presence of disease. The poults in the challenge group exhibited elevated fecal oocyst counts of *E. adenoiodes*, *E. gallopavonis*, and *E. meleagrimitis* at 6dpi ($P < 0.05$). Significant differences were also observed in the ceca for IL-8 at 7dpi, IFN-γ at both 7dpi and 9dpi, and IL-1RN at 9dpi ($P < 0.05$). Additionally, at 9dpi, HSP70 levels in the spleen showed significant variation among treatment groups ($P < 0.05$). In the cecal tonsils, significant changes were noted in IFN-γ at 4dpi, HSP70 at 7dpi, and IL-1RN at 9dpi ($P < 0.05$). In summary, this study offers insights into the cellular immunological responses of mixed *Eimeria* species-infected turkey poults. The results underscore the need to understand these pathways to create efficacious vaccines, immunomodulatory methods, and the dynamic immune response during infection. Overall, the study advances our knowledge of the host immune response to *Eimeria* spp. and establishes the foundation for better disease management and control in the production of turkeys.

Keywords: Eimeria infection, Turkey poults, Intestinal health, Immune response, Oocysts counts

64 Comparative proteomics of *Salmonella* Enteritidis reveal the differing adaptative mechanisms between egg contamination and cell invasion

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Salmonella Enteritidis (SE) is a leading foodborne pathogen, with contaminated eggs responsible for ~18% of salmonellosis cases in the U.S. Understanding SE's proteomic adaptations during infection (from egg contamination to cell invasion) provides key insights into its survival strategies and virulence mechanisms. This can help design effective control measures to reduce transmission and infection risk. SE modulates its proteome to support its survival and establishment in different systems such on the egg surface and during infection. SE was used to inoculate the egg surface (~10 log CFU) and Caco2 cells (~7 log CFU; n=6/group). After 6 h of incubation, SE was recovered from both the egg surface and the intracellular environment of Caco2 cells for protein extraction. Trypsin digested and desalted peptides were identified using a 2 h data-dependent UPLC-MS/MS method on an Orbitrap Eclipse mass spectrometer. Raw data files were processed in MaxQuant and searched against the Uniprot proteome database for peptide identification and label-free quantification of protein abundance. Abundance data output from Scaffold was analyzed in R studio. Using *limma* package, differentially abundant proteins were identified using Benjamini-Hochberg multiple testing adjustment to control the false discovery rate and control for multiple testing at $p \leq 0.05$. Comparative analysis revealed that on the egg surface SE proteins primarily associated with resistance to desiccation, harsh conditions, and oxidative stress (osmY, trxA, ftnA), membrane integrity and nutrient uptake (lolA, metQ), and alternate metabolic pathways (pduJ, eda, citF) were significantly more abundant. However, upon host cell invasion, SE shifted its proteome profile to enhance intracellular survival, with increased abundance of proteins involved in host adaptation and immune evasion (sopB, flgD, ydfZ), intracellular metabolism (leuD, acnA, trkA), host interaction regulation (csrA), and stress response (grcA). Despite differences between food contamination and infection, proteins essential for SE survival, adaptability, virulence, and metabolism remained consistent, suggesting their potential role across different systems ($p \leq 0.05$). The distinct shift in protein abundance between the two systems highlights SE's ability to adapt, enhance virulence, and optimize metabolic flexibility for intracellular replication. These findings provide valuable insights into system-specific bacterial adaptations, which could aid in developing targeted interventions to control SE contamination and infection.

Keywords: Salmonella Enteritidis, Comparative proteomics, differential abundance, egg contamination, host infection

65 Development of a CFU-calibrated qPCR assay for detection of a conserved pathogenicity factor in *Enterococcus cecorum*

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Enterococcus cecorum (EC) is an emerging poultry pathogen associated with septicemia, osteomyelitis, and enterococcal spondylitis. Accurate quantification of EC is essential for disease surveillance and pathogenicity studies, but selective agars for the genus *Enterococcus* provide limited information. This study aimed to develop a SYBR Green-based qPCR assay targeting the epaM-like gene (ECS3_2296), a conserved marker in pathogenic EC strains, and to correlate qPCR results with traditional CFU counts to establish a molecular quantification method. Four EC field-isolated strains were cultured on tryptic soy agar (24 h) for colony selection and in tryptic soy broth (10 h) for propagation at 37 °C under microaerophilic conditions. Serial ten-fold dilutions (10^0 to 10^7) were prepared in PBS. For CFU enumeration, 10 µL of each dilution was plated on TSA and incubated at 37 °C for 24 h. Concurrently, DNA was extracted from 10 µL of each dilution using the Invitrogen PureLink™ Genomic DNA kit and quantified and qualified using a Nanodrop spectrophotometer. Primers were designed using Primer3Plus (forward: 5'-CGGGATGGTACTTGGATTTACC-3'; reverse: 5'-ATCGCCACTGCAAAAGCTAG-3') and validated via NCBI BLAST. qPCR reactions were performed in triplicate using SYBR Green Master Mix at cycling conditions: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, with melt curve analysis. Standard curves for each isolate demonstrated strong linearity ($R^2 = 0.93–0.996$) over a range of 1 to 8 $\log_{10}$ CFU/mL. Cq values from original cultures were used to estimate bacterial load using regression equations. Predicted $\log_{10}$ CFU/mL values were 7.91 (Sample 2), 9.00 (Sample 7), 8.19 (Sample 9), and 8.15 (Sample 13), compared to plate count values of 8.02, 7.72, 7.20, and 7.82, respectively. Differences between predicted and observed values ranged from –0.11 to +1.28 $\log_{10}$ units, with most samples exhibiting qPCR-based overestimation within ±1.0 $\log_{10}$, an acceptable margin for semi-quantitative assays. These findings support the use of the developed SYBR Green qPCR assay as a rapid and reliable alternative to traditional culture-based methods for estimating EC load in poultry samples. Additionally, the ECS3_2296 gene remains a strong choice as a molecular target for the detection and quantification of pathogenic EC strains. Further studies with expanded isolate sampling are recommended to improve assay validation and strengthen the correlation between CFU counts and Cq values.

Keywords: Enterococcus cecorum, qPCR, pathogenicity factors, CFU, quantification

66 Transcriptomic analysis reveals myogenic effects of *Lactobacillus paracasei* and *Lactobacillus rhamnosus* on broiler embryo muscle development

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Probiotic supplementation has emerged as a promising alternative to promote growth performance and muscle development in broilers. Towards this our previous research demonstrated that early probiotic supplementation improved muscle development in broiler embryos. As a next step, in this study we aim to elucidate the transcriptomic changes associated with in-ovo probiotic supplementation and its role in muscle growth and development in broilers. In-ovo supplementation of *Lactobacillus paracasei* DUP-

13076 (LP) and *L. rhamnosus* NRRL-B-442 (LR) promotes embryonic muscle development in broilers by modulating key myogenic factors. Hatching eggs were randomly assigned to three groups (Ross 308; 24 eggs/group). Probiotic groups received in-ovo supplementation of either LP or LR (9 log CFU/egg) while the control received PBS. Embryos were sacrificed on day 10, 14, 18 and 21 (hatch) of incubation and pectoralis major muscle sections (PMM; n = 6/group) were collected for RNA extraction. Extracted RNA was subject to transcriptome sequencing. Following quality check and trimming, the filtered RNA-seq reads were mapped to the reference genome (Chicken Genome - GCF_000002315.5_GRCg6a) using HISAT2 (v 2.2.1). Then, expression levels of all genes were quantified using HTSeq-count (v 0.11.1) and differential gene expression was analyzed using normalized read counts with DESeq2 via log fold change (log2FC). Additionally, the QIAGEN Ingenuity Pathway Analysis (IPA) tool was used for the gene network and functional pathway analysis. Transcriptomic analysis identified key upregulated genes involved in growth, muscle hypertrophy, and myogenesis. Throughout incubation, IGF-1 and calcium signaling pathways were activated in the control group. Moreover, across all groups during incubation, genes involved in myogenic genes and regulatory factors were upregulated. Specifically with LP, at D14, the IGF-1 signaling pathway (IGF1, IGF1R) and myogenesis pathway (MYOD1, MYOG, MTOR) were significantly upregulated compared to the control group ($p<0.05$). Also, on D14 and D18, significant activation of the Wnt/β-Catenin Signaling Pathway (WNTs) and mTOR pathway (MTOR) was observed in the LP group when compared to LR supplementation, indicating a strain-specific effect ($p<0.05$). These findings align with our previous observation of improved muscle development in broiler embryos following in-ovo probiotic supplementation. Specifically, these data demonstrate that probiotics modulate myogenic genes to promote muscle development and may serve as a viable alternative to antibiotics in broiler production.

Keywords: Broilers, In-ovo probiotic supplementation, Muscle development, RNA-seq reanscriptome, Myogenic factors

67 A landscape genomics approach to detect adaptive loci in local chicken breeds

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Gallus gallus domesticus has differentiated into numerous local breeds across the globe, specific to regions, culture, and climate. While, local chicken breeds exhibit a wide array of morphological phenotypes resulting from human driven isolation and selection, it has been hypothesized that these breeds have adapted to their environment in terms of climate and breeding practices. In the modern context of rapid climate shifts, these local breeds could contain valuable genetic resources essential for advancing the agroecological transition. However, low interest and limited funding for local breeds have resulted in poor breed characterization and genotyping, which makes the detection of genes underlying traits through traditional methods like GWAS (Genome Wide Association Studies) impossible. Consequently, few evaluations of the real adaptive potential of these breeds have been performed. Therefore, this study aims to highlight the value of multidimensional analysis in identifying adaptative loci when working with a limited number of SNPs. The samples included 1484 individuals from 32 breeds spanning over a large climatic area encompassing eight countries (Egypt, France, Germany, Iceland, Ivory Coast, Spain, Norway, Russia) and genotyped on either the 57K or 10K SNP Chip. A total of 4274 SNPS were identified to overlap between the two SNP Chips and used for the final analysis. Twelve climate variables were extracted from the WorldClim database for each breeds origin and were reduced into four principal components. Redundancy analysis (RDA) was used to evaluate Genomic-Environment Association (GEA). To account for the genetic structure, we computed admixture proportions and used them as a covariate in both analyses. The RDA analysis identified a total of 37 SNPs significantly associated with RDA reduced environmental variables (p-value < 0.05). Then, gene content and subsequent gene ontology-based enrichment tests were performed on those SNPs. A total of 25 genes were identified and were related to anatomical structure size and cell differentiation. Two genes were previously found to be associated with heat tolerance. The gene KIRREL3 was found to regulate the immune response under heat stress, and DBH, involved in homoeostatic processes to maintain internal body temperature constant. These results highlight the value of local breeds and landscape genomics for identifying genomic regions associated with climate adaptation even with a limited dataset (few SNPs and no individual performances) by taking into account numerous populations. Once validated, these adaptive loci can help to guide breeding practices towards a more climate resilient chicken. Funding: GEroNIMO (H2020 GA No 101000236)

Keywords: Landscape Genomics, Adaptation, Local Breeds, Climate Change, Europe

68 Epigenetic programming of the broiler hypothalamus by in-ovo green monochromatic illumination: Effects on growth and metabolism

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Early stages of brain development are highly susceptible to environmental stimuli, leading to lasting phenotypic changes. Recent studies have shown that in-ovo monochromatic green illumination (GMI), improves broiler's growth at 35 days of age, through elevation of the somatotrophic axis activity. This study examines whether GMI during broiler embryogenesis induces epigenetic programming in the hypothalamus, its stability, and phenotypic impact post-hatch. Ross 308 fertile broiler eggs (n=300) were exposed to GMI continuously throughout incubation (Green) or only during the last three days (G3D) with two control groups – incubation under dark conditions or under white illumination. To assess hypothalamic activity and molecular memory, half of each treatment group underwent an acute GMI exposure at day of hatch. Genome-wide transcriptional profiling, epigenetic analysis at gene promoters, retinal cell changes, immunofluorescent staining of cFOS-positive neurons were conducted at DOH, along with evaluation of growth and FCR levels until 35 days of age. Results reveal genome-wide transcriptional changes in the hypothalamus, with G3D exposure significantly altering gene expression linked to growth, appetite, immunity, and metabolism. This exposure resulted in a marked increase in both phosphorylated CREB1 (pCREB1) binding and Histone H3 Lysine 27 acetylation (H3K27ac) levels at the promoter sites of specific genes, reinforcing the epigenetic impact of GMI during these crucial developmental periods. Notably, these epigenetic modulations were exclusive to the G3D group, aligning

with the transcriptional upregulation observed in these pathways. Moreover, in response to a single pulse of GMI there was a significant elevation in the number of cFOS positive cells in comparison to those who are not exposed to the green pulse, only in the G3D group. Mechanistic insights suggest that retinal green photoreceptors mediate GMI effects. Pre-exposing embryos to blue light before GMI, which disrupts green photoreceptor activity, resulted in a significant reduction in retinal green opsin levels. This blue light intervention effectively nullified the typical epigenetic modifications observed in the G3D group. Respectively, the G3D group exhibited increased post-hatch body weight abd reduced FCR levels at several time points, indicating that fetal response to GMI enhances phenotypic plasticity, though these effects appear to wane with age. These findings demonstrate that late-stage in-ovo GMI induces stable epigenetic modifications, enhancing gene expression and growth. As a safe, non-invasive, and cost-effective method, GMI presents a promising strategy to optimize broiler performance without compromising animal welfare.

Keywords: epigenetics, green photostimulation, in ovo, broilers, hypothalamus

69 Alternative pathway for vitamin B_{12} absorption in chickens possessing mutated tumor virus A receptors

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The tumor virus A receptor (TVA) serves a dual function in chickens: as an entry receptor for avian leukosis virus (ALV) subgroups A and K, and as a receptor for vitamin B_{12} bound to transcobalamin. Mutations in *TVA* that confer resistance to ALV-A/-K typically disrupt the low-density lipoprotein A (LDL-A) domain, which is critical for both virus entry and vitamin B_{12} uptake. While genome-edited *TVA* knockout chickens exhibit impaired vitamin B_{12} metabolism, naturally occurring *TVA* mutant chickens have thrived for generations without apparent metabolic disorders. We investigated vitamin B_{12} metabolism in chickens with wild-type TVA (TVA^{WT},n=12), a single nucleotide polymorphism variant (TVA^{SNP},n=12), and deletion mutants (TVA^{R4}/TVA^{R5}, collectively TVA^{R},n=12) using protein interaction modeling, serum metabolite measurements through ELISA, and gene expression analysis (n=4 per group). Statistical analysis was performed using Student's t-test and two-way ANOVA with Bonferroni's multiple comparison test. Contrary to our predictions from protein structure modeling using AlphaFold 2, TVA^{R} chickens showed significantly higher serum vitamin B_{12} levels than TVA^{WT} chickens, with no significant differences in methylmalonic acid (MMA) levels. Gene expression analysis revealed progressive upregulation of vitamin B_{12} carrier-related genes (*AMN*, *GIF*, *TCN2*) across genotypes (TVA^{WT}<TVA^{SNP}<TVA^{R}) in the liver, kidney, and especially small intestine. Our findings demonstrate that naturally occurring TVA mutant chickens maintain normal vitamin B_{12} metabolism through compensatory upregulation of alternative vitamin B_{12} uptake pathways, illustrating an evolutionary adaptation that balances viral resistance with essential nutrient absorption. These results provide new insights into the complex interplay between viral resistance mechanisms and nutrient metabolism in poultry.

Keywords: Avian leukosis virus -A/-K, Chicken, TVA, Transcobalamin 2, Vitamin B12

70 Exploring the transcriptomic regulation of muscle development in Chickens

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Muscle development is regulated by several genes whose expression levels vary throughout growth, ultimately influencing muscle tissue formation. In this study, we investigated the gene expression profiles of muscles from 5-week-old and 10-week-old Cornish and Korean Ogye chickens to identify the stage-specific messenger RNAs, and elucidate the transcriptional regulation of these mRNAs. Through RNA-seq analysis, we identified 190 up-regulated and 127 down-regulated differentially expressed genes (DEGs). DEGs used in transcriptome analysis have log2FC<1, log2FC>1 values, and *p*-values less than 0.05. Functional enrichment analysis of the DEGs revealed several key KEGG pathways, including "Cell adhesion molecules", "Focal adhesion", "Cytokine-cytokine receptor interaction", "ECM-receptor interaction", "Regulation of actin cytoskeleton", and "Mitogen-activated protein kinase (MAPK) signaling pathway." It is of note that DEGs involved in energy metabolism were significantly down-regulated in both 10-week-old Cornish and Ogye muscles. To investigate the role of the MAPK signaling pathway in the transcriptional regulation of DEGs, we conducted *in vitro* assays using chicken primary myoblast (pCM) cells treated with specific MAPK inhibitors, SB203580 (p38 MAPK inhibitor), SP600125 (JNK inhibitor), and PD98059 (MEK inhibitor). Our results revealed that *COL6A3* and *CCDC80* mRNA expression were down-regulated in a dose-dependent manner in the JNK and MEK-inhibited myoblasts. The significance of the DEG expression levels were calculated using one-way ANOVA test. These findings suggest that *COL6A3* and *CCDC80* may play a significant role in muscle tissue development and present a potential role of the MEK/ERK signaling pathway in regulating muscle development-related genes, in contrast to previous studies that primarily focused on the p38 signaling pathway. This study contributes to the fundamental knowledge of *COL6A3* and *CCDC80* transcriptional regulation and expands the investigation about their bio-functional aspect.

Keywords: Muscle development, Differentially expressed gene, MAPK, COL6A3, CCDC80

71 Supporting the poultry industry with Extension, instruction, and applied research

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The Morrill Act of 1862 helped establish land grant institutions and the Smith-Lever Act of 1914 created the cooperative extension system associated with these land-grant institutions. As the agricultural landscape has changed throughout our history, Extension specialists and agents have been on the frontlines delivering science-based education, helping to translate research into actionable items. This presentation will discuss how Extension resources can be leveraged during a foreign animal disease (FAD) outbreak, such as highly pathogenic avian influenza (HPAI). The objective is to discuss how applied research programs can be used to generate research findings pertinent to your poultry clientele during typical times and the best avenues for dissemination. However, these same resources, within the cooperative Extension system, can be leveraged to help overcome significant threats to the poultry industry. The presentation will discuss 1) local, regional, and national funding mechanisms to support applied research; 2) approaches to providing science-based education; 3) importance of industry relations; and 4) leveraging resources to support the poultry industry during FAD. These topics will reflect the efforts of our lab during times of both FAD freedom and presence. In conclusion, "Extension" has a different meaning for everyone. Extension efforts are scalable to the needs of your industry but a healthy, functional relationship with your industry is a critical component of success.

Keywords: Extension, instruction, applied research

72 Science-based instruction and student demographics influenced undergraduate student opinions of the poultry industry in an introductory poultry science course

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Effective teaching methods are essential to shaping informed perspectives on the poultry industry and undergraduate comprehension of poultry-related topics. To evaluate the impact of instruction and demographic factors on student perceptions and opinions of the poultry industry, fifty total undergraduate students enrolled in a poultry science course (ANS 2230) across two consecutive years at Iowa State University were provided identical surveys during weeks 1 and 16 (2023 n=27, 2024 n=23). The survey included three demographic questions regarding gender identity, previous livestock experience, and career goals. Thirteen statements covered nutrition, health, welfare, behavior, products, and industry practices. Students drew a single vertical line on a 130mm horizontal line to indicate their agreement or disagreement with each statement. Self-assigned codes were used to anonymously identify and compare each survey within year. Responses were categorized using a Likert scale of strongly disagree, disagree, neutral, agree, and strongly agree for statistical analysis. Data were analyzed using the PROC MIXED procedure with Tukey–Kramer adjustment to account for multiple comparisons (SAS 9.4). Fixed effects included gender, livestock experience, career goals, and pre- versus post-instruction. Demographics and instruction significantly influenced student perceptions ($P \leq 0.04$). Males were more likely to agree that broilers and layers have similar nutritional requirements and that all eggs sold for human consumption are fertile, while females showed 18.2-23.0% greater agreement on adequate downtime between bird exposures and mandatory backyard poultry vaccinations ($P \leq 0.04$). Students with farming backgrounds were 16.1-19.8% more likely to support culling and current industry practices ($P \leq 0.003$). Students interested in livestock careers were more likely to choose animal products based on welfare standards over pricing than those interested in poultry or veterinary careers ($P = 0.02$). Those with non-animal-related career goals had 38.7–53.6% higher agreement that all eggs sold for human consumption are fertile ($P = 0.02$). Post-instruction surveys had decreased agreement on misconceptions about nutrition and antibiotic use by 25.0–29.8%, while support for mandatory vaccinations in backyard flocks increased by 11.8% ($P \leq 0.04$). Post-instruction, awareness of product safety improved by 11.9-25.0%, and 9.9% more students viewed current industry management as humane ($P = 0.04$). These findings highlight demographic and instruction-driven differences in poultry industry perceptions and emphasize the need for teaching methods that support diverse student backgrounds in higher education.

Keywords: demographics, education, poultry science, teaching methods, undergraduate instruction

73 Echoes of Experience: The role of previous poultry experience in student interest in the poultry industry

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Online modules offer flexible lesson plans for classrooms, allowing teachers to integrate poultry science content into their classes. Poultry exposure through elementary-agriculture exploration may result in increased student interactions with poultry compared with previous generations. However, increased exposure has not translated into careers in poultry science. The overarching goal of our project was to design an online poultry and animal health program to convey One Health and STEM lessons. Specific objectives included identifying the relationship between poultry exposure and interest in the poultry industry in our population. Program participants included 5th grade students enrolled in Indiana (n=3) and Michigan (n=1) schools. Students participated in an online classroom unit focused on the poultry industry and animal health. The program consisted of five online modules, an interactive notebook, simulation games, and an in-class activity. At the start of the program, students (n=116) self-reported their poultry experience and poultry production knowledge as no experience, some experience, or poultry ownership. Additionally, the individual interest questionnaire (IIQ) was administered to determine participants' interest in the poultry industry. Similarly, students self-reported their poultry production knowledge as No, Yes, or A little. After completing the online module and an in-class activity, 49 students completed the IIQ again. Individual interest was calculated based on five individual interest questions that had been previously validated for elementary students. Statistical analysis consisted of one-way proportion tests and ANOVA. The majority (54%) of students had no poultry experience and reported having a little poultry production knowledge (61%). The overall IIQ score pre-program was 2.93 ± 1.1, just below neutral 3. The IIQ score for students with no poultry experience was greater than students with some experience or ownership ($P < 0.0001$). Students who reported A

little or Yes poultry production knowledge had lower IIQ scores than No knowledge scores (P = 0.002). After completing the program, the overall IIQ score was 2.78 ± 1.2, similar the pre-program score (P = 0.335). Previous experience with poultry negatively impacted participants' interest in the poultry industry, though none of the IIQ scores were inherently negative. We can't tease apart whether the low interest was due to students who had poultry experience not wanting to engage with poultry or if the low interest was due to the online format of the program.

Keywords: Interest, Elementary Education, Previous Experience, Survey, Online Education

74 The impact of a poultry-focused CURE on scientific self-efficacy and persistence in STEM

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Course-based undergraduate research experiences (CUREs) provide hands-on opportunities for students to engage in relevant research, which has been found to increase students' intent to persist in science. Poultry-focused CUREs not only provide general research experiences but expose students to the critical role that poultry science plays in the global agricultural industries. Five undergraduate second-year students in animal science were chosen to participate in this Fall 2024 CURE based on their involvement in a campus program designed to support students of diverse backgrounds. The objectives of this mixed-methods study were to examine the impact of the CURE on the following: scientific self-efficacy, scientific identity, challenge, frustration, meaning making and persistence intentions. Additionally, individual and situational interests were evaluated. Participants completed Likert-type scales and reflections during five different time points throughout the Fall 2024 semester. A descriptive analysis of the quantitative results show that self-efficacy and scientific identity increased over time, which aligns with participants' growing confidence and skill development. However, challenge and frustration peaked mid-semester as the students dealt with more complex research tasks. Qualitative reflections highlight how overcoming challenges contributed to sense of accomplishment and reinforced persistence in scientific research. While individual interest and perceived value in the scientific community remained stable, reflections suggest that peer support and strong mentorship played key roles in maintaining engagement. Situational interest remained consistently high throughout the CURE. These findings demonstrate that while challenges can affect confidence in the moment, productive struggle contributes to motivation and long-term persistence. Our results support the concept that CUREs can improve self-efficacy and foster a sense of belonging in science, particularly for students from underrepresented backgrounds. Future CUREs should balance challenging tasks with structured support to enhance student retention and self-efficacy in STEM fields.

Keywords: CURE, STEM Persistence, Poultry Science, Scientific Self-Efficacy, Challenge and Frustration

75 Not Presented

76 Machine-learning enabled real-time ammonia monitoring in poultry house

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Poultry meat and eggs are a source of affordable protein, but ammonia from their manure has a negative impact on bird health and productivity. High ammonia levels (> 25 ppm) can lead to respiratory issues, reduce weight gain, increase mortality, poor feed conversion and lower egg production. This project aims to develop an economical intelligent sensor to maintain poultry health, production and welfare through effective ammonia monitoring. This sensor is a nanomaterial based gas sensor array, fabricated using aerosol jet printing. This sensor continuously monitors and analyzes ammonia data in the dynamic poultry house environment (0 – 80 ppm) without the need of waiting for sensor equilibrium. During Phase 1 of the project, a low-fidelity prototype was fabricated, and field-tested. The prototype sensor integrated with a custom control electronics board that managed data acquisition and processing. Multiple units connected to a laptop via USB, allowing easy deployment and integration. A custom Python program handled data logging and real-time visualization, enabling simultaneous monitoring of up to 3 sensor arrays (9 sensors total) at a rate of 4Hz. A machine-learning model was developed and implemented for signal processing on device. This provides a robust platform for evaluating the system efficacy in poultry house environments. Two field tests were carried out on commercial broiler farms in Northeast Georgia. The first field test showed excessive signal noise, promoting sensor updates for the second field test. The second prototype included three sensor array units: Green, Yellow, and White. The White unit, using the original fabrication and served as a benchmark, while the Green and Yellow units were updated versions. In the second field test, NH_3 was measured by Dräger X-act 7000 equiped with ammonia microtubes, DOL 53, and three of our sensor array units for half-day period inside the broiler farm at the height of 50 cm. The Green and Yellow units showed significantly better performance, with reduced noise and stable response signals while the White unit continued to have the higher variability observed in the first field test. The readings from the Green and Yellow units closely tracked the ammonia levels, recorded by the DOL 53 sensor and Dräger X-act 7000. The Root-mean-square deviation (RMSE) of our model is about 7 ppm for data ranging from 0-100 ppm. In our field test data, we took the measurements of Dräger X-act 7000 as ground truth of ammonia level, and used it to fit our model. The R-squared value of our model is 0.912, as compared to a baseline sensor DOL 53 whose R-squared value is 0.880. Additional tests are planned to evaluate the sensor performance in longer deployments.

Keywords: Machine Learning, Real-Time, Ammonia Monitoring, Poultry House, Ammonia Gas Sensor Array

77 Field Report: Study on practices for utilizing nitrogen foam euthanasia in Pennsylvania swine and poultry operations

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The use of high expansion firefighting foam as a method of euthanasia for poultry flocks has been established and used primarily in floor-based housing. Developments in foam generator technology have been made to insert nitrogen gas instead of atmospheric air to create a nitrogen rich foam that is lighter than water-based foams alone. A test to assess the physical properties of the foam was made by building a wrapped 30 ft. high scaffold (9.1 meter) and filling from above the tower and from ground level. Time to fill the tower was less than 90 seconds. Latency (dwell time) of the foam was over 20 minutes duration. Encasement of nitrogen in bubble may prove helpful in containment of gas near the birds. In another test 26 swine cadavers were covered in nitrogen foam for over 2 hours. The cadavers were then placed in a composting windrow for over six months. The use of nitrogen foam did not affect the composting process as temperatures did reach composting levels and allowed for full breakdown of tissues. Further testing in poultry field structures is planned.

Keywords: ethanasia, nitrogen, foam, compost, swine

78 Not Presented

79 Examining the cecal microbiota of conventional and slow growing broilers in floor pen and silvopasture rearing systems

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As consumer concern for animal welfare grows, limited information exists on how alternative rearing systems affect poultry physiology and composition. This study evaluated the outcomes of raising conventional broilers (Ross 708) and slow-growing broilers (Sasso) in conventional floor-pen and silvopasture systems on cecal microbiota. We hypothesized that differences in cecal microbiota would arise between the strains and rearing environments. Chicks were acquired from commercial hatcheries and placed in their respective environments in a 2x2 factorial design. The Ross 708s grew for 42 days and the Sassos grew for 56 days. All birds were fed a standard commercial diet. Cecal contents were aseptically collected at processing (after picking) from 10 birds per treatment (40 birds total) and placed in liquid nitrogen for microbiota analysis using 16S ribosomal RNA (rRNA) amplicon sequencing. Sequencing of the V3-V4 hypervariable region of the 16S rRNA was performed using Illumina's MiSeq platform. Raw sequences were processed with Quantitative Insight into Microbial Ecology 2 software (Qiime2). Non-parametric tests were used for data analysis, with significance at $q \leq 0.05$ and trends identified when $0.05 < q < 0.1$. The number of amplicon sequence variants (ASVs), richness, and Shannon index did not significantly differ between experimental groups. Floor-pen microbiota trended toward higher evenness compared to silvopastured birds (q=0.0559). Sasso broilers exhibited higher overall evenness regardless of environment (q=0.0061). Furthermore, floor-pen-reared Sassos were more even than silvopastured Ross 708s (q=0.0271). PERMANOVA analysis based on the weighted UniFrac revealed statistically significant differences in the cecal microbiota of the broiler strains, irrespective of rearing system (q=0.0003), and between rearing systems, regardless of broiler strain (q=0.0010). Significant differences were also observed between all experimental combinations ($q < 0.05$). Principle coordinate analysis based on Bray-Curtis showed distinct groupings based on rearing environment and all four experimental combinations. Altogether, these results indicate that rearing in outdoor environments may lead to lower evenness from factors like a more variable diet by foraging outside the feeders and developing more dominant taxa in the gut from exposure to various microbes. Furthermore, greater beta diversity distances demonstrate differences in phylogenetic composition between broiler strains and environments, highlighting the impact of rearing system and genetics on microbiota composition. Ultimately, this study contributes to the growing body of knowledge of effects from poultry rearing systems and shows the need for further research.

Keywords: Ceca, Microbiota, Conventional, Slow-growing, Silvopasture

80 *Campylobacter hepaticus* Used as an Indicator Organism to Evaluate Biosecurity at a Commercial Egg Farm

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Spotty liver disease (SLD) has become an increasing problem in cage-free poultry production in brown egg layers, leading to increased mortality and decreased egg production. *Campylobacter hepaticus* (CH) has been reported as a common etiology. Limited research exists on monitoring the presence of CH in aviary style systems in the United States. The objective of this study was to investigate sources of CH in aviary style systems as an indicator organism for biosecurity audits. A layer farm with two pullet and seven hen houses with a history of SLD was experiencing symptoms of an SLD outbreak in one flock of 25-week-old single combed white Leghorns (SCWL). Samples were collected from environmental surfaces including fans (n=7), fan shrouds (n=8), cool cells (n=9), exterior doors (n=9), water lines (n=9), entry door knobs (n=9), manure belts (n=9), feed troughs (n=9), rodent stations (n=18), vehicle floor mats (n=5), fly specks (n=9), egg belts (n=7), locker and breakroom floors (n=20), as well as insects (n=17) and livers from pullets (n=21) and hens (n=48), for a total of 214 unique samples. Samples were enriched for 48 h and struck onto brucella agar with 5% lysed horse blood and incubated in microaerophilic conditions for 72 h at 37 °C. Colonies from presumptive positive plates underwent DNA isolation and PCR analysis of CH using two primer pairs targeting the glycerol kinase gene and the 16S rRNA gene. Livers from pullets (SCWL in both houses) were 90% positive for CH. Birds from all hen houses were 78% positive for CH, regardless of age or feather color. Birds in the one barn exhibiting clinical symptoms and gross liver lesions were 80% positive for CH. All insects collected throughout the complex were positive for CH. Rodent stations were 75% and 93% positive in pullet and hen houses, respectively. We found that environmental samples from each barn were 77-100% positive for CH. Floor samples in restrooms, locker and break rooms were 100% positive at pullet facilities, but only 50% at hen housing. Vehicle floor mats were 80% positive. These results suggest widespread contamination with CH, therefore this bacterium may not be a viable biosecurity indicator organism. This is the first reported evidence of SLD beginning during pullet stage. Next steps for this research should include evaluating genotypic differences between CH isolated from the environment versus the bird so that epidemiology and host-pathogen interactions can be further elucidated.

Keywords: Campylobacter hepaticus, Laying hens, Cage free, Biosecurity, Egg production

81 Unraveling aerotolerancy of *Campylobacter jejuni* and *Campylobacter coli*: A transcriptomic approach

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Campylobacter spp. are major causes of foodborne illness worldwide. Despite a sensitivity to atmospheric oxygen levels, *Campylobacter* spp. often survive exposure to oxygen during meat processing. The genetic mechanisms behind *Campylobacter*'s aerotolerance are not well understood. The objective of this study was to conduct transcriptomic analysis using RNA-Seq Illumina technology for survival under

atmospheric oxygen aerobic conditions of two species, *Campylobacter jejuni* (S2-20) and *Campylobacter coli* (WA333). Strains were exposed to aerobic conditions in Mueller Hinton Broth media and triplicate samples were taken at various time points from 15 min through 15 h for RNA isolation. Ribosomal RNA depletion and RNA Seq libraries for Illumina were prepared with NEBNext rRNA Depletion kit (Bacteria) and NEBNext Ultra II Directional RNA Library Preparation Kit for Illumina and sequenced on a NovaSeq 6000 platform. Annotation was performed using Bakta v1.11.0. Sequence reads were trimmed and aligned to the reference genome using Python packages (Trimmomatic, HISAT2 2.2.1, SAMtools) and total counts were generated using featureCounts from Subread 2.0.8. A differential gene expression analysis was performed with RLE normalization using a visual interface for DESeq2, DEBrowser v1.26.3. Differential gene expression under aerobic conditions at timepoints 15 min through 15h was compared to microaerobic incubation at Time 0. Both species showed significant changes in gene regulation under aerobic conditions. However, the *C. coli* strain differentially regulated more genes overall in response to aerobic conditions than *C. jejuni*. Upregulation of several genes involved in the uptake or synthesis of nutrients was noted in both species. *C. coli* appeared to be upregulating more genes associated with iron transport and acquisition than *C. jejuni*. Downregulation of a high number of genes involved in ribosome or flagella formation was observed in both species. Both species exhibited downregulation of several genes involved in anaerobic respiration and protein folding. Downregulation of genes associated with energy production and conversion was observed in both species during the first hour. The *C. coli* strain downregulated genes associated with amino acid metabolism and transport during the first 30 minutes, but this response was not noticeably observed in *C. jejuni*. In conclusion, these findings suggest that *C. jejuni* and *C. coli* utilize different strategies to cope with survival under aerobic conditions. While flagella formation was suppressed in both species, regulation of iron acquisition was enhanced in *C. coli*. To our knowledge, this is the first study to use RNA-Seq to investigate *C. coli* aerotolerancy.

Keywords: Campylobacter, microbiology, aerotolerance, genes, bacteria

82 Microbial mapping of pathogen load and indicators in a small pastured-poultry processing plant

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There is a growing consumer demand for pasture-raised poultry, and so is the increase in small-scale processing operations. However, most of these small plants have limited resources to routinely assess their microbial status, and there is limited information on pastured processed poultry. Periodic bio-mapping can help small processors understand the microbial ecology of their operations, enabling them to establish an effective monitoring plan. This study evaluated the prevalence and microbial load of *Salmonella* and *Campylobacter* in poultry carcass rinse and processing water at different stages of production to identify potential points of contamination and assess microbial trends over time. A small processing plant was visited twice monthly for nine months with each visit representing different flocks sourced from separate farms (V1 and V2). During each visit, a total of ten samples (five each of carcass and water rinses) were collected at key points along the processing line, from pre-slaughter to post-chill (N=180). All samples were analyzed for *Salmonella* and *Campylobacter* using standard culture methods and quantified with the BAX® system. All confirmed isolates and strains were further identified while aerobic count (AC) and Enterobacteriaceae (EB) were determined using Petrifilms™. The data was analyzed using the Fit Least Square model in JMP ($P \leq 0.05$). Overall, the prevalence of *Salmonella* and *Campylobacter* in the samples were 36% and 48%, respectively. Assessing the prevalence of both pathogens over time, there was no significant difference between the visits ($P > 0.05$). For carcass rinse, there was a reduction ($P = 0.003$) of *Salmonella* from pre-slaughter (78%) to post-chill (56%) while *Campylobacter* remained the same (78% to 72%). For processing water, contamination ranged from 0% to 6% in the scalder to 0% in the chiller for both pathogens, except picker water at 39% and 56%, respectively. *Salmonella* concentration in the samples averagely ranged from <1 to 4.37 Log CFU/mL while *Campylobacter* was generally high with levels ranging from <10 to 6.26 Log CFU/mL. *Salmonella* serogroup C2-C3 predominantly dominated (86%) the samples while a mixed species of *Campylobacter coli* and *jejuni* were detected in 53% of the samples. Additionally, the AC and EB counts had a significant reduction ($P < 0.001$) of 3 to 4 Log CFU/mL from pre-slaughter with an average count of 7.41 and 8.12 Log CFU/mL, respectively to post-chill with 4.34 and 4.50 Log CFU/mL, respectively. This study provides insight into the microbial status and monitoring plan of a pastured processing environment, which will be useful for developing effective control measures in reducing contamination risks to ensure food safety.

Keywords: Salmonella, Campylobacter, pasture poultry, bio-mapping, microbial contamination

83 Assess West Virginia very small poultry producers' knowledge of applying mobile poultry processing unit and evaluate vinegar water against *Salmonella* Typhimurium, *Campylobactor jejuni*, and surrogate bacteria *Enterococcus faecium* during chilling of chicken carcasses

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As the demand for locally grown products increases, pastured poultry production and applying on-farm mobile poultry processing unit (MPPU) becomes more prevalent. 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 1) assess the knowledge of WV very small poultry producers' knowledge of applying MPPU and 2) evaluate efficacy of applying vinegar water against pathogens and surrogate bacteria during chilling of chicken carcasses. In Study I, an anonymous survey was conducted to assess the practices of applying MPPU from 38 very small poultry producers in WV. In Study II, fresh organic broiler carcasses were surface inoculated with *Salmonella* Typhimurium, *Campylobacter jejuni*, and surrogate bacteria *Enterococcus faecium* followed by dip-chilling in refrigerated 0, 25%, and 75% vinegar water for 1 or 24 h. The broiler carcasses were then rinsed with D/E neutralizing solution in a standard poultry sampling bag for 30 seconds followed by 10-fold serial dilution and spread plating onto XLT-4, Brucella, and

Bile-esculin agars (BEA) for *S.* Typhimurium, *C. jejuni*, and *E. faecium*, respectively. XLT-4 and BEA were incubated at 35ºC for 48 h and the Brucella were incubated at a microaerophilic jar at 42ºC for 48 h. Chi^2 test and mixed model procedure in R-program were used for data analysis of survey and the 3 × 3 × 3 factorial design vinegar water studies with significant level at P=0.05. Survey results showed that only 34% of survey participants measured temperatures of scalding water, 22% added sanitizer into the chilling water and among them 25% use vinegar water, and only 19% applying antimicrobials during poultry processing other than chilling. Results of study II showed that chilling in 50 and 75% of vinegar water for 1h achieved 0.8-1.9 and 1.5-2 log CFU/g reductions (P < 0.05) of *S.* Typhimurium, *C. jejuni*, and when the chilling time extended to 24h, the reductions increased to 2.2-2.5 and 1.6-2.3 log CFU/g, respectively. *E. faecium* indicated less reductions (P < 0.05) of 0.2-0.7 log CFU/g vs 0.8-1.9 log CFU/g of *Salmonella*, which suggested that *E. faecium* is resistant to chilling in vinegar water regardless of chilling times (1h and 24h). These results suggested that applying 75% of commercial vinegar water for 24h during chilling process is effective to control foodborne pathogens on broiler carcasses and *E. faecium* is a promising *Salmonella* surrogate during post-harvest chilling process, which will be beneficial for developing MPPU outreach material.

Keywords: Enterococcus faecium, Salmonella, Campylobacter, Chilling, Vinegar Water

84 Evaluating free chlorine decay kinetics, efficacy in pathogen reduction, and interactions with organic matter during poultry processing

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Ensuring food safety and minimizing foodborne illness risk linked to poultry consumption remain challenges in the industry. This study investigates the kinetics and efficacy of free chlorine (FC), a commonly used disinfectant, during the chilling stage of poultry processing. Experimental data and mathematical modeling were integrated to improve decision making towards optimal, effective sanitization approaches, recognizing that disinfectant concentration, efficacy, and decay trends can serve as predictive indicators from lab-scale to commercial applications. This study also elucidated pathogen inactivation and transfer dynamics during chilling and post-chill stages. Whole chicken carcasses (~4 lbs) sourced from retail and processing plants were exposed to 300mg/L FC in a lab-scale immersion chill tank at 4°C, with pH ~6.5. Plant-sourced samples are not always readily available or feasible for repeated access, and therefore we included store-bought samples as well. Chicken thighs (100±20 g) were inoculated with a five pathogenic strain *Salmonella* cocktail,and their shedding and inactivation were measured. Chiller tank samples were analyzed for pH, FC, total dissolved solids (TDS), and chemical oxygen demand (COD). High (10^7 CFU/10µL) or low (10^5 CFU/10µL) *Salmonella* cocktail loads were inoculated onto chicken thighs and evaluated across FC levels (0.1–50 ppm) and times (0.5–10min). Conditions were tested in the absence of organic load and/or pH adjustment using neutralized rinse samples. Rinses were analyzed by plate counts (CFU/rinse) on brilliant green agar with sulfapyridine (BGS) plates. Water chemistry and inactivation data were integrated into a spatial model to predict post-chill carcass levels under commercial conditions. Statistical significance was evaluated using Student's t-test to compare FC decay rates, bacterial reduction, and the influence of organic matter. All experiments were replicated at least three times with sample size of n=12. Organic matter and suspended solids significantly influenced FC decay. Store-bought carcasses showed faster FC decay (286mg/L to 6mg/L in 1 h) compared to plant-sourced carcasses (314mg/L to 84.5mg/L in 1 h). TDS spiked after FC addition, indicating chemical interactions. Residual (0.1–5 ppm) and 10–50 ppm FC reduced bacterial loads under pH-adjusted conditions. Model results, partially validated against commercial data, highlighted the roles of pH and COD in determining FC levels and *Salmonella* reduction. This study provides a detailed analysis of FC efficacy, decay kinetics, and interactions with organic matter under poultry chilling conditions, offering insights to optimize FC dosages and exposure regimens for improved poultry processing safety.

Keywords: poultry chiller water, salmonella, total dissolved solids, decay kinetics, Free Chlorine

85 Identifying chemical methods to optimize the lifetime of peracetic acid

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Maintaining efficient and effective sanitation practices is an ongoing battle faced by all portions of the poultry processing industry. Peracetic acid (PAA) has been utilized during the immersion chilling process as an antimicrobial agent to ensure food safety, but is limited by its short lifespan. This study explored varying the concentration of the following chemicals for lengthening the lifetime of PAA: ethylenediaminetetraacetic acid (EDTA), aspartic acid diethoxy succinate (AES), and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP). These were tested individually and in varying combinations in simulated and pre-chiller water. Simulated chiller water contained sodium, potassium, and magnesium cation total mixture at 1000ppm and further experimentation was conducted by adding 1800ppm BSA (bovine serum albumin). All tested solutions were buffered to pH 9, a common pH in industry, using 4M NaOH. Once adjusted, a stopwatch was started and initial PAA concentrations were obtained using a MP-9700E digital photometer. Concentration values were recorded at specific time intervals, and PAA's half-life was calculated using the half-life exponential decay formula. A 2-Way ANOVA was used to analyze the impact of the various chemicals and concentrations. In only pH-adjusted water, the baseline for all experiments, PAA's half-life was 593 minutes. All concentrations of AES examined in simulated chiller water reduced PAA half-life. EDTA concentrations of 0.5% and 0.1% (in simulated chiller water) increased the half-life of PAA by 78% and 185%, respectively. While low levels of HEDP (15 and 25 ppm) decreased PAA half-life, concentrations of 50, 75, and 100 ppm resulted in gradual increases in PAA half-life from 76% up to 410%. The robustness of HEDP was further challenged by the addition of BSA to better simulate chiller water; the greatest increase in half-life was observed at 50ppm HEDP, resulting in a 49% increase. Finally, the combined effect of BSA, 100ppm HEDP, and 0.1% EDTA resulted in a 22% increase in half-life. This study shows that the addition of various stabilizers, specifically HEDP, can significantly increase PAA's half-life, even when presented with the harshest of chiller environments.

Keywords: Peracetic acid, Decay Kinetics, Half-life, Lifetime, Immersion Chillers

86 Differences in susceptibility of peracetic acid (PAA) across multiple *Salmonella* serovars

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Over the past several decades, the application of peracetic acid (PAA) has become the main antimicrobial intervention to ensure safe poultry meat. Poultry processing plants have favored this antimicrobial for its broad-spectrum activity and its ability to break down into non-toxic byproducts. However, recent evidence of the persistence and coexistence of multiple *Salmonella* serovars in poultry environments and products has raised new concerns, particularly regarding the unknown potential of high-risk serovars to selectively withstand PAA interventions. Therefore, the goal of this study was to assess the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of PAA across multiple *Salmonella* serovars. Using a 96-well broth microdilution method, seven bacterial suspensions (1-7 Log_{10} CFU/mL) were challenged with eleven concentrations of PAA (50-1300 ppm). This study included six strains representing four serovars: Enteritidis, Heidelberg, Infantis, and Typhimurium. The MIC was defined as the lowest PAA concentration that inhibited visible bacterial growth after incubation, while the MBC was the lowest concentration that resulted in a loss of bacterial viability, as confirmed by plating. Data were analyzed using one-way ANOVA, and differences were established using Least Squared Means; effects were considered significant at $p<0.05$. For all serovars, bacterial inhibition and loss of viability coincided at the same PAA concentration, ranging from 50 to 120 ppm across strains. No growth or viability was observed above 120 ppm in any of the serovars, demonstrating the strong bactericidal effect of PAA against *Salmonella*. However, differences in susceptibility were noted between serovars and were influenced by their initial inoculum ($p<0.05$). Serovar Typhimurium showed lower susceptibility than other serovars, requiring 120 ppm to inhibit and render non-viable an initial inoculation of 4–5 Log_{10} CFU/mL. Intermediate susceptibility was observed for serovar Infantis and Heidelberg; both required higher initial bacterial concentrations (>6 Log_{10} CFU/mL) to withstand PAA but became susceptible at 80 ppm. Lastly, serovar Enteritidis was the most susceptible, losing its ability to grow and its viability at 50 ppm regardless of the initial inoculum. This study highlights the inherent differences among serovars in their ability to withstand PAA interventions, presenting a new challenge for poultry processors. While the high efficacy of PAA has been vastly documented, it is important to further understand its dynamics against different serovars, as this could help tailor interventions to improve the safety of poultry products.

Keywords: MIC, MBC, serovar, susceptibility, peroxyacetic acid

87 Pre-harvest heat stress and its effect on the post-harvest shelf-life of chicken carcasses

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Although extensive heat stress (HS) research on broiler performance, health and carcass characteristics, and their underlying molecular mechanisms exists, there is a paucity of information on how HS affects post-harvest poultry shelf-life. The objective was to determine pre-harvest HS effect on post-harvest shelf-life. Male Cobb 500 chickens (N=600) were randomly assigned to 12 environmentally controlled chambers (2 pens/chamber, 25 birds/pen) at University of Arkansas Division of Agriculture Broiler Research Farm (Fayetteville, AR) and provided commercial corn-soybean meal diet and water ad libitum until d29. On d29, the following treatments were applied: Control (CTL): thermoneutral conditions (23°C); Cyclic HS (HS): exposure to cyclic high ambient temperature (35°C) for 8h/d; Pair-fed (PF): thermoneutral conditions (23°C), but pair-fed to the CHS group (1.05× HS intake). On d42, birds (N=180) were processed and deboned at University of Arkansas Pilot Processing Plant (Fayetteville, AR). Boneless-skinless breasts were split, packaged (overwrapped foam trays with absorbent packs), transported on ice to University of Wyoming Meat Laboratory (Laramie, WY), and placed under simulated retail display (4°C). On d1, 5, 7, and 13 (n=10), objective color (CIE L*, a*, b*), intramuscular pH, and microbial quality (total mesophiles (APC), *Enterobacteriaceae* (EB), *Pseudomonas*, lactic acid bacteria (LAB) and *phychrotrophs*) were determined. Color, pH, and microbial count data (Log CFU/g) were analyzed as GLM in R Studio with pairwise differences determined using Tukey's ($P\leq0.05$). There was treatment×time interaction on intramuscular pH and L*. On d1, HS breast (5.66 pH) pH was lower than those from TN (5.85 pH; $P<0.05$). On d13, HS (47.7 L*) had lighter breasts than PF (45.4 L*; $P<0.05$). There was a treatment effect on breast a* and b* values where TN (3.82 a*, 10.19 b*) were more red and yellow than HS breasts (2.67 a*, 8.79 b*; $P<0.05$). There was a treatment×time interaction on APC, EB, and *Pseudomonas* ($P<0.05$). There were no APC and EB differences until d 13 of shelf-life, where PF had higher APC and EB (6.13, 5.66 Log CFU/g) than TN (5.24, 4.79 Log CFU/g) and HS (4.78, 4.36 Log CFU/g; $P<0.05$). On d9, breasts from PF had higher levels of *Pseudomonas* than TN or HS (6.27, 5.03, and 4.94 Log CFU/g; $P<0.05$). There was a treatment effect on LAB and psychrotrophs ($P<0.05$). HS (3.30 and 4.03 Log CFU/g) had lower levels of LAB and Psychrotrophs than PF (4.15 and 4.93 Log CFU/g). Results indicate feed restriction (PF) may potentially have a greater role in reduced microbial shelf-life than HS. Microbiome elucidation will be necessary to determine HS and PF's exact impact on microbial shelf-life.

Keywords: heat stress, shelf-life, microbial quality

88 Evaluate the efficacy of commercial marinades with/without microwave heating against *Salmonella* Typhimurium on chicken breasts

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Salmonella adulteration of chicken products, primarily seen in uncooked non-ready-to-eat (NRTE) products, can also occur in precooked ready-to-eat (RTE) products due to post-processing contamination. This study aims to analyze the effect of commercial marinades with and without microwave heating against *Salmonella* Typhimurium on prepared chicken breasts. Commercial fresh chicken breasts were trimmed into 4 × 5 cm and surface inoculated with *S.* Typhimurium (*Salmonella* was manufactured to be resistant to 100 ppm of nalidixic acid [NaL] by "spot-mutation" method). Marinades including A1, barbecue, and Italian dressings were applied to chicken sample surfaces either before or after microwave heating (1,100 W) for 15 or 30 s prior

to storage at 10ºC in vacuum-sealed packaging bags to simulate NRTE chicken products for up to 10 days. Microbial analyzed on days 0, 1, 3, 7 and 10 by spread-plating onto tryptic soy agar plus 100 ppm of NaL. The experiment design is a 3 × 2 × 5 factory design and analyzed by a mixed model procedure of R-program with *p*-value at 0.05 with a total of 540 samples with 3 replicates (180 samples per replicate). *Salmonella* counts on non-marinated control samples increased from 4.74 ± 0.12 $\log_{10}$CFU/g to 8.47 ± 0.32 during the 10 days storage. Microwave reheating for 15 s only slightly (P>0.05) reduced the pathogen counts by 0.20 ± 0.09 $\log_{10}$CFU/g, while reheating for 30 s increased (P<0.05) the reductions to 1.64 ± 0.27 $\log_{10}$CFU/g. The addition of a marinade, regardless of type, showed an increase (P<0.05) in bacterial cell reduction as time endured. Samples marinated before cooking and inoculation showed bacterial reduction progress from no significant difference (P>0.05) at day 0 to 3.18 ± 0.36 $\log_{10}$ CFU/g at day 10. Marinading after cooking and inoculation produced a similar, but exaggerated reduction of no difference at day 0 to 4.46 ± 0.19 $\log_{10}$ CFU/g at day 10. Combined effects of marination and 30 second microwave reheating achieved a 5.16 ± 0.49 $\log_{10}$ CFU/g reduction at day 10 when chicken was marinated prior to microwave heating, compared to 6.23 ±0.57 $\log_{10}$ CFU/g when marinade was applied after microwave heating. Therefore, this study indicates that adding marinades after microwave heating is the most effective approach for inactivating *Salmonella* on NRTE chicken breasts.

Keywords: Salmonella, Microwave, Chicken Breast, Marinades

89 Evaluating the efficacy of photonic decontamination treatment on *Salmonella* reduction on packaged poultry parts

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This study sought to assess the efficacy of pulsed light (PL) on reducing aerobic plate counts (APC), *Enterobacteriaceae*(EB), and *Salmonella* on packaged chicken wings and tenders. The machinery used to apply the PL treatments was developed by PulseForge Inc. (Texas, USA). Control (no PL application) or 200 V PL treatments were conducted on both packaged and un-packaged whole chicken wings or tenders. To evaluate *Salmonella* reduction, four packages of four wings or tenders along with four sets of four unpackaged wings or tenders were used (128 samples). An additional set of 64 uninoculated parts were used to evaluate APC and EB reductions (128 additional samples). Parts assessed for *Salmonella* were inoculated with 0.1 mL of 10^6 CFU/mL *Salmonella* and allowed to sit for 30 min for bacterial attachment. Samples were packaged manually using a manual over wrapper. The entire package for the packaged parts was placed in the PL machine for treatment application, and the unpackaged parts were placed four at a time in the machine. Each wing or tender was sampled individually. Bacterial counts were log transformed and are reported as $\log_{10}$ CFU/mL. Data were analyzed using the general linear models procedure and means were separated by Tukey's HSD with significance at P≤0.05. For whole wings, untreated, non-packed wings had the highest APC (3.30) and EB (2.60) counts while the remaining treatment combinations were not different from each other (P≤0.0047). Packed wings had higher levels of *Salmonella* (1.74) compared to non-packed wings (1.27, P=0.0026). PL treatment did not influence *Salmonella* levels on wings (P=0.4364). Packed tender APC and EB (2.74, 1.98) were lower than non-packed tenders (3.06, 2.37, P<0.0001). However, PL did not influence APC or EB counts. For *Salmonella* on tenders, untreated, packed tenders had the highest counts (1.75) while untreated, non-packed (1.44) and PL treated, non-packed tenders (1.51) had the lowest counts, with PL treated, packed tenders intermediate (1.58, P=0.0044). Overall, PL treatment was not effective in reducing bacteria on wings or tenders regardless of whether or not they had been packaged.

Keywords: pulsed light, salmonella, wings, tenders

90 Biofilm formation and antimicrobial resistance profile of *Salmonella* Newport isolated from rubber plucking fingers

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Salmonella spp. are responsible for foodborne disease and represent a control challenge for the poultry industry. Certain steps in poultry slaughterhouses, such as defeathering using rubber plucking fingers, can act as sources of *Salmonella* spp. cross-contamination. This study aimed to assess the biofilm formation and antimicrobial resistance profile of *Salmonella* spp. isolated from rubber plucking fingers in a poultry slaughterhouse. Fourteen pools, each containing three used rubber fingers, were washed and incubated in 1:10 peptone water for *Salmonella* isolation. Thirteen (n=13) isolates were recovered and serotyped using the Check & Trace Salmonella® assay. For biofilm analysis, isolates were incubated in TSB at 35 °C for 24 h, adjusted to 0.5 McFarland, aliquoted into ELISA plates, and incubated again in TSB at 35 °C for 24 h. Plates were washed with PBS, biofilms fixed with methanol (99%), stained with crystal violet (1%), and resuspended in glacial acetic acid (33%). Absorbance was measured at 630 nm. Tests were performed in triplicate with *Escherichia coli* EAEC ATCC 042 as control. Antimicrobial resistance was assessed by disk diffusion using the following antimicrobials: ceftiofur (CTF - 30 µg), ciprofloxacin (CIP - 5 µg), fosfomycin (FOS - 200 µg), sulfamethoxazole + trimethoprim (SUT - 25 µg), cefoxitin (CFO - 30 µg), tetracycline (TET - 30 µg), florfenicol (FLF - 30 µg), imipenem (IPM - 10 µg), ceftazidime (CAZ - 30 µg), aztreonam (ATM - 30 µg), cefepime (CPM - 30 µg), ceftriaxone (CRO - 30 µg), and amoxicillin + clavulanic acid (AMC - 30 µg). Spearman's correlation test was used to assess the association between biofilm formation and antimicrobial resistance. For all analyses, *p*-values < 0.05 were considered statistically significant. All isolates (100%, 13/13) were identified as *Salmonella* Newport. Regarding biofilm formation, isolates were classified as weak (7.69%, 1/13), moderate (69.23%, 9/13), or strong (23.07%, 3/13) producers. For antimicrobial resistance, 7.69% (1/13) were resistant to CTF, CIP, and CRO, and 15.38% (2/13) to FOS. Intermediate resistance was observed in 30.76% (4/13) for CIP and 15.38% (2/13) for AMC. One isolate (7.69%) was resistant to three classes and classified as multidrug-resistant (MDR). There was no statistically significant correlation (p > 0.05) between antimicrobial resistance and biofilm formation. In conclusion, all *Salmonella* Newport isolates exhibited biofilm formation, with the majority classified as moderate producers, indicating a potential risk of cross-contamination. Most isolates were susceptible to the tested antimicrobials, however, a resistance profile to ciprofloxacin and fosfomycin was observed, highlighting a public health concern.

Keywords: Poultry slaughterhouse, Bacterial resistance, Food safety, Cross-contamination, Defeathering

91 Influence of the origin of the soybean meal on the metabolizable energy content and ileal digestibility of crude protein in broilers

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We studied the total tract apparent retention of N (TTAR of N), the AMEn content, and the standardized ileal digestibility of protein (SIDP) of 27 soybean meals (SBM) from Argentina (ARG), Brazil (BRA), and USA in broilers. The samples (n = 9 per origin), were randomly collected from European feed mills in 2024 and analyzed for chemical composition and protein quality indicators. From hatch to 14 d of age, broilers were fed a common commercial crumble diet based on corn, wheat, and SBM. From 15 to 20 d of age, they received a first set of experimental diets consisting of 65% of a basal diet based on corn and soy oil, and 35% of the SBM tested. From 21 to 26 d of age, all the birds received a second set of experimental diets that consisted of 53% of a N-free diet (based on dextrose and soy oil) and 47% of each of the same tested SBM as unique source of N. The TTAR of N of the diets were determined at both period. Additionally, the AMEn and SIDP of the SBM was determined at 20 and 26 d of age, respectively. Each treatment was replicated 6 times and the experimental unit was a cage with 6 broilers. Data were analyzed as a completely randomized design by ANOVA, with SBM origin as main effect. On 88% DM basis, the content of CP and ether extract of the SBM were, as an average, 46.2 and 1.93% for the ARG, 46.2 and 1.76% for the BRA, and 45.8 and 1.22% for the USA meals. From 15 to 20 d of age, broilers fed the USA SBM consumed more feed (82.9 vs. 80.3 and 80.7 g/d; $P < 0.01$) and grew faster (57.0 vs. 55.6 and 55.1 g/d; $P < 0.05$) than broilers fed the ARG or the BRA meals. Also, from 21 to 26 d of age, ADFI, ADG and feed conversion ratio were better for broilers fed the USA meals than for broilers fed the South American meals ($P < 0.05$). On 88% DM, the AMEn content was not affected by country of the origin of the SBM (2,410, 2,394, and 2,378 kcal/kg, for ARG, USA, and BRA meals, respectively). The SIDP of the SBM were 87.0, 86.2, and 85.5% for the USA, BRA and ARG meals, respectively, but the differences were not significant. At 20 d (62.8 vs. 60.1 and 60.8%; $P < 0.01$) and at 26 d (53.6 vs. 49.4 and 50.7%; $P < 0.001$) of age the TTAR of N was higher for the USA meals than for the ARG and BRA meals. In conclusion, despite the greater CP and ether extract content of the South American SBM, broilers fed these meals showed poorer growth performance and TTAR of N than the USA SBM, but no significant differences among soybean origins were observed for the AMEn content or SIDP. Consequently, data from proximal analysis may not be sufficient to determine the nutritional value of the SBM. It is advisable to control also the origin of the soybeans to optimize broiler performance and feed formulation cost.

Keywords: apparent metabolizable energy, broiler, ileal protein digestibility, nitrogen retention, soybean meal origin

92 Impact of increasing dietary nutrient density with high levels of canola product in broiler diets

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A key challenge in broiler diets with high canola product inclusion is reduced feed intake. Increased dietary nutrient densities could compensate for the resulting nutrient losses. Therefore, this study aimed to evaluate the impact of increasing amino acid and energy densities in diets with higher inclusion of canola products. A total of 1080 Ross 308 mixed-sex broiler chickens were used in four dietary treatments which included wheat-soybean meal-based control diet (T1), a diet with high inclusions of canola products (T2), and two diets (T3 and T4) with increased nutrient densities compared to T2. In T3 nutrient densities were increased 1.5%, 2.0% and 2.5% than T2 in starter, grower and finisher phases, respectively. Likewise, in T4 nutrient densities increasing by 3.0%, 4% and 5.0% in respective feeding phases. Each dietary treatment was offered to six replicates of 45 birds per pen from 0 to 35 days post-hatch. Data was subjected to one-way ANOVA using the General Linear Model of the JMP pro 17 software package. Body weight gain ($P < 0.001$), feed intake ($P < 0.001$) and FCR ($P = 0.010$) were influenced by the dietary treatment from 0 to 35 days post-hatch. Birds fed T2 reduced feed intakes by 8.4% (3223 versus 3520 g/bird), and weight gain by 9.8% (2325 versus 2581 g/bird) than those fed T1. There was no significant difference between T2 and T3 on growth performance. However, birds fed T4 reduced weight gain by 5.4% (2203 versus 2328 g/bird), reduced weight gain by 7.4% (2986 versus 3223 g/bird), but improved FCR by 2.1% (1.356 versus 1.385). Canola meal inclusions in all the feeding phases were negatively correlated with PDI of diets ($r = -0.792$, $P = 0.019$). Moreover, pellet durability indices (PDI) which were positively correlated with feed intake; in the grower ($r = 0.700$, $P<0.001$) and finisher ($r = 0.700$, $P<0.001$) phases. Therefore, reduced feed intake in high canola product diets could be resulted by the adverse impact of canola meal to PDI. In conclusion, increasing nutrient densities in high canola product diets did not ameliorate weight gain and feed intake, but improved FCR in broiler chickens. Acknowledgment: The study was supported by the AgriFutures Chicken Meat Consortium (PRO-016111).

Keywords: Canola meal, Amino acid, Energy, Growth, Pellet quality

93 Early post-hatch delayed feeding stress alters gastrointestinal development, enteroendocrine hormone expression, and long-term gut function in broiler chickens

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Enteroendocrine (EEC) functions of the gastrointestinal (GI) tract are critical for regulating feed intake, nutrient absorption, and metabolism, yet remain understudied in agricultural animals. Early-life events are known to significantly influence GI development and have lasting effects on animal health. This study investigated whether a 72-hour post-hatch delayed feeding (DF) disrupts EEC development and predisposes animals to altered responses under subsequent nutritional stress. A total of 120 Cobb-500 broilers were assigned to either DF or normal feeding (NF) groups post-hatch and raised under industry-standard conditions.

At d 42, both groups were subjected to either a 24-hour fasting stress or left unstressed. Tissue samples from the proventriculus, duodenum, jejunum, and ileum were collected on days 3, 15, 21, 28, and 43. Gut length and yolk sac weight were recorded on d 3. Expression of EEC gene markers—chromogranin A (*CHGA*) and neurogenin 3 (*NEUROG3*)—along with key peptide hormones—ghrelin (*GHRL*), cholecystokinin (*CCK*), peptide YY (*PYY*), and glucagon-like peptide 1 (*GLP-1*)—was analyzed by RT-qPCR. Protein expression of selected EEC targets was measured by Western Blotting or ELISA. CCK-positive cells in the ileum were quantified by immunofluorescence. Data were analyzed using two-way ANOVA and Student's *t*-test. Results showed that the DF stress significantly impaired intestinal development at d 3, evidenced by shorter gut length and larger yolk sac residuals ($P < 0.05$). At day 3, expression of all EEC-related genes was downregulated in response to DF stress, except for the upregulation of *GHRL* (appetite-stimulating hormone) and *NEUROG3* (EEC differentiation marker) ($P < 0.05$), indicating adaptive response to early life stress. Persistent downregulation of *CCK* (appetite-suppressing hormone) in the duodenum and *PYY* in the ileum was observed at d 15 ($P < 0.05$). By d 21, expression of *CHGA* (EEC specific marker) was significantly upregulated across most gut segments ($P < 0.05$), suggesting compensatory EEC maturation. In DF birds, *GHRL* was consistently elevated in upper gut segments ($P < 0.05$) at d 43, though this difference disappeared under fasting. Notably, *CCK* expression in the duodenum and ileum was significantly reduced only after the secondary fasting stress ($P < 0.05$), consistent with decreased CCK-positive cell counts in the ileum. These findings demonstrate that early post-hatch DF stress causes lasting changes in EEC populations and hormone expression, impairing the gut's ability to regulate endocrine responses during fluctuating nutritional conditions. This disruption may ultimately affect appetite regulation, nutrient absorption, metabolism, and overall health in broiler chickens.

Keywords: Delayed feeding stress, Appetite, Gastrointestinal function, Broilers

94 Synergistic effects of soluble and insoluble fiber combinations on the jejunal and cecal microbiota of broilers with a subclinical enteric challenge

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Dietary fiber plays a crucial role in poultry gut health, with insoluble fibers improving digestive function and soluble fibers enhancing microbial fermentation. This study investigated the effect of various fiber types and combinations on jejunal and cecal microbiota in broilers experiencing subclinical enteric challenge. Eight dietary treatments were tested from hatch: six fiber-supplemented diets and two controls (challenged and unchallenged, both without supplemental fiber). Fiber treatments included 3% oat hulls (OH), 3% soy hulls (SH), and combinations of 1.5% OH or SH with either 1.5% wheat middlings (WM) or sugar beet pulp (SBP). Challenged birds received a tenfold coccidia vaccine dose on d14 and 10^8 CFU *C. perfringens* on d18. Jejunal and cecal contents from 9 birds per treatment were collected on d21 for microbiota analysis using shotgun whole genome sequencing. Microbiota data were analyzed using R software (version 4.3.2) with phyloseq, vegan, and DESeq2 packages to assess alpha diversity, beta diversity, and differential abundance. Alpha diversity analyses by calculating Shannon and Simpson indices revealed challenge-induced reductions in cecal diversity (Kruskal-Wallis, $P < 0.05$), with fiber treatments showing trends toward diversity restoration (Kruskal-Wallis, $P = 0.099$). The challenge significantly disrupted the cecal community structure (PERMANOVA, $P = 0.001$) but had a limited impact on the jejunal microbiota. Beta diversity indicated distinct community structures between fiber treatments and challenged control in the cecum but not jejunum. OH+SBP treatment most effectively enhanced *Lactobacillus* species (*L. pontis*, $\log_2$FC +5.96; *L. fermentum*, $\log_2$FC +2.59), while SH+SBP promoted butyrate-producing bacteria including *Agathobaculum massiliense* ($\log_2$FC +3.25) and *Lachnoclostridium sp.* ($\log_2$FC +23.52). All fiber treatments significantly reduced potential pathogens, with OH+SBP treatment reducing *Escherichia* species ($\log_2$FC -25.19), and SH+WM reducing pathogens like *Pseudomonas hunanensis* ($\log_2$FC -24.11) and *Streptomyces* species. The pronounced effects in cecum versus minimal effects in jejunum suggest that fiber combinations primarily exert benefits through hindgut fermentation. These findings demonstrate that strategic combinations of soluble and insoluble fibers effectively modulate the cecal microbiota, promoting beneficial bacteria while suppressing potential pathogens and offering a dietary approach to enhance resilience against enteric challenges in broiler production.

Keywords: dietary fiber, microbiota, broilers, enteric challenge, gut health

95 Interactions of deoxynivalenol, *Eimeria* challenge, and cyclic heat stress on productivity, egg quality, body composition, and gut health in laying hens

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This study investigated the three-way interaction of deoxynivalenol (**DON**), *Eimeria* challenge, and cyclic heat stress on the productivity, egg quality, body composition, and gut health of laying hens. A total of 384 (26-wk-old) Hy-Line W36 laying hens were arranged in 2×2×2 factorial design, with 8 treatment groups (n = 6 replicates, 8 birds each) exposed to dietary DON (control: 2.6 mg/kg and contaminated: 15.6 mg/kg) from day 0 to 35, cyclic heat stress (31°C at 60% relative humidity for 8 h/day from day 21 to 35), and a mixed *Eimeria spp.* challenge (125,000 oocysts/mL *E. acervulina*, 25,000 oocysts/mL *E. maxima*, and 25,000 oocysts/mL *E. tenella* on day 21) over a 35-day experimental period. Statistical analysis was performed using three-way ANOVA, with Student's t-test for main effect comparisons and Tukey HSD for interaction effects ($P < 0.05$). Results showed that *Eimeria* challenge significantly reduced body weight, feed intake, and hen-day egg production (**HDEP**) compared to non-challenged hens ($P < 0.001$). A significant interaction between *Eimeria* challenge and heat stress was observed for feed intake ($P < 0.05$), where heat stress significantly reduced feed intake in non-challenged birds but only a numerical decrease in *Eimeria*-challenged birds. Heat stress independently reduced body weight ($P < 0.05$) but partially mitigated the negative effects of *Eimeria* challenge on HDEP during 1–14 days post-inoculation (**DPI**; $P = 0.015$). *Eimeria* challenge, heat stress, and DON negatively affected the intestinal morphology, with interactions observed between *Eimeria*-challenge and heat stress and between *Eimeria* challenge and DON ($P < 0.05$). However, no

interaction was observed between heat stress and DON ($P > 0.05$). DON exposure alone did not significantly affect productivity, egg quality, or body composition ($P > 0.05$) but increased coccidial lesion severity at 5 DPI in the mid-intestine ($P = 0.036$) and ceca ($P = 0.025$). A body composition analysis showed that heat stress reduced bone mineral density ($P = 0.002$) and body fat percentage ($P = 0.001$), whereas DON decreased fat weight ($P = 0.002$). Egg quality was compromised by *Eimeria* challenge, with reductions in egg weight, albumin height, and Haugh units ($P < 0.001$). Heat stress independently reduced egg weight, eggshell weight and thickness ($P < 0.05$). No significant three-way interactions were observed ($P > 0.05$). In conclusion, *Eimeria* challenge and heat stress were the primary factors reducing productivity in laying hens, however heat stress partially mitigated HDEP losses in *Eimeria* challenged birds. DON played a secondary role, exacerbating intestinal lesions and altering fat metabolism without affecting productivity or egg quality.

Keywords: deoxynivalenol, Eimeria challenge, cyclic heat stress, laying hens, productivity

96 Chemical composition, amino acid profile, and protein quality indicators of soybean meals from different origins collected from 2006 to 2024

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Numerous factors affect the chemical composition and protein quality of soybean meals (SBM), including latitude, area of production, type of soil, agronomic practices, and weather conditions, which result in significant annual fluctuations of the nutritional value of SBM. The objective of this research was to evaluate the influence of crop year period and country of origin of the beans on the chemical composition, amino acid (AA) profile, and protein quality indicators (PQI) of commercial SBM. Samples originated from Argentina (ARG; n = 291), Brazil (BRA; n = 308), and United States of America (USA; n = 315) were randomly collected from EU crushing plants and feed mills between 2006 and 2024. The meals were classified in two time periods: old (from 2006 to 2014; n = 477) and recent (from 2015 to 2024; n = 437). The abundance of AA of the SBM were determined by NIRS whereas all the other variables were analyzed by wet chemistry. All the analysis were conducted in the same official laboratory. The data were analyzed as a completely randomized design with 6 treatments arranged as a 3 x 2 factorial with country of origin of the beans and crop year period as main effects, using the MIXED procedure of SAS. Both country of origin of the soybeans and crop year period affected most of the variables studied. On 88% DM bases, CP (47.0 vs. 46.5 vs. 45.8%), CF (4.99 vs. 4.09 vs. 4.31%), NDF (10.2 vs. 8.63 vs. 9.04%), and raffinose (1.35 vs. 1.05 vs. 1.22%) were higher ($P < 0.001$) but sucrose (5.75 vs. 7.23 vs. 6.84%) and stachyose (4.38 vs. 5.31 vs. 4.79%), were lower ($P < 0.001$) for the BRA meals than for the USA and ARG meals. Also, in % CP, Lys (6.07 vs. 6.15 vs. 6.10%), Thr (3.88 vs. 3.90 vs. 3.92%), Arg (7.27 vs. 7.30 vs. 7.23%), and TSAA (2.77 vs. 2.83 vs. 2.82%) were lower ($P < 0.001$) for the BRA meals than for the USA and ARG meals. In addition, Ca, P, and K were lower but Mg and Fe contents were higher ($P < 0.001$) for the BRA than for USA and ARG meals. Most of the PQI studied (PDI, KOH, TIA, and Reactive Lys presented as a percentage of Lys) were higher for the USA meals than for the South American meals ($P < 0.001$). Crop year period affected most of the variables studied, with oligosaccharides content, TSAA profile (%CP), and all PQI values (UA, PDI, KOH, and TIA) being lower ($P < 0.001$) and CF, NDF, Ca, and P, higher ($P < 0.05$) for the SBM collected from 2015 to 2024 than for meals collected from 2006 to 2014. It is concluded that both, crop year period and country of origin of the beans affect significantly the chemical composition, AA profile, and the protein quality of the SBM.

Keywords: amino acid profile, chemical composition, collection period, protein quality indicators, soybean meal origin

97 Production performance and nitrogen balance in laying hens in response to dietary protein levels

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This study investigated the effects of different dietary protein levels on laying performance, egg quality, and nitrogen balance in laying hens from 18 to 62 weeks of age. A total of 84 Hy-Line Brown hens at 18 weeks old were randomly assigned to two dietary groups, each with six replicates. The control group (CON) received diets containing 17.5%, 16.5%, 15.5%, and 14.5% protein from weeks 18-38, 39-46, 47-54, and 55-62, respectively. The lower-protein group (LP) was fed diets with 16.0%, 15.0%, 14.0%, and 13.0% protein during the same periods, representing a 1.5% reduction. Lysine, methionine, and threonine were supplemented using synthetic amino acids to achieve an ideal amino acid balance in the diet. All data were analyzed using an independent t-test with SPSS software (version 29, Armonk, NY). Compared to the CON group, hen-day egg production in the LP group was lower ($p < 0.05$) during weeks 18-38 and 54-62. Egg weight was also reduced ($p < 0.01$) in the LP group from weeks 54-62, and feed efficiency was lower ($p < 0.05$) in weeks 18-38, 38-46, and 54-62. Egg quality was negatively affected, with lower ($p < 0.05$) Haugh units at week 38 and lighter ($p < 0.01$) yolk color at week 62. Nitrogen intake decreased ($p < 0.01$) in the LP group at weeks 46, 54, and 62, and nitrogen excretion was consistently lower ($p < 0.05$) throughout the experiment. Additionally, nitrogen retained in eggs was lower ($p < 0.05$) in the LP group at weeks 26, 38, and 62. In conclusion, reducing dietary protein effectively reduces nitrogen excretion, highlighting potential environmental benefits. However, it negatively impacted laying performance at weeks 18-38 and both laying performance and egg quality at weeks 54-62, posing challenges to productivity.

Keywords: Dietary protein, egg quality, laying hen, laying performance, nitrogen balance

98 Exploring laying hen responses to graded levels of dietary crude protein reduction

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The capacity of graded levels of dietary protein reduction to maintain laying hen performance and egg quality; modulate tibia characteristics; reduce feed costs; and lower environmental stress due to nutrient excretion was investigated until 44 weeks of age. A total of 252 26-week-old Hy-Line Brown hens were housed in enriched cages (7 birds/cage) and randomly allocated to 1 of 4 diets with 9 replicates per treatment. The diets were iso-energetic, and fed over two phases from 26 to 34, and 36 to 44 weeks of age.

Experimental diets contained 4 varied levels of balanced dietary protein ranging from 18.0, 17.5, 17.0, and 16.5% for Phase 1; and 17.0, 16.5, 16.0, and 15.5% for Phase 2. Limiting amino acids including lysine, methionine, threonine, and tryptophan were supplemented to maintain constant amino acid concentrations across diets. The diets are respectively denoted as T1, T2, T3, and T4 series. Routine data collection and sampling was done; and collected data were analyzed for statistical significance at $P < 0.05$ and tendencies were measured at $0.05 < P < 0.10$ using the General Linear Model (GLM) procedure for one-way ANOVA technique of IBM SPSS Statistics software. Several parameters of growth and productive performance including body weight, egg production in hen-day-egg production (HDEP), feed intake, egg mass, and egg loss percentages were unaffected ($P > 0.10$). Balanced dietary protein level reduction was a limiting factor for overall egg weight and feed conversion ratio from weeks 26 to 44. Feeding the T4 diets (15.5 and 16.5% CP) led to reduced ($P < 0.05$) egg weights and feed conversion efficiency. For egg quality indices, the shell color, egg specific gravity, and percent shell, albumen, and yolk were unaffected ($P > 0.10$) by balanced dietary crude protein reduction. Increasing dietary crude protein in the T1 and T2 series led to improved Haugh units ($P < 0.05$). Marginal improvements for the yolk color at week 44 ($P = 0.093$), and egg-breaking strength at week 26-44 ($P = 0.092$) were also observed with balanced dietary crude protein reduction at the T3 and T4 levels. Balanced dietary crude protein reduction reduced nitrogen consumption and excretion ($P < 0.05$). Furthermore, some tibia traits including length, volume, density, and breaking strength were unaffected ($P > 0.10$) by dietary treatments. As to economic performance, while reducing dietary protein levels led to lower feed costs at weeks 34 and 44 ($P < 0.10$), the profit margins were unaffected ($P > 0.10$) with balanced dietary protein levels. Conclusively, feeding reduced CP/AA diets could decrease nitrogen excretion and feed costs without adversely affecting productive performance, egg quality, and some tibia traits of laying hens.

Keywords: egg quality, laying hen, nitrogen excretion, performance, protein

99 *In ovo* supplementation of branched-chain amino acids and hepatic mitochondrial function in developing chicken embryos

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The embryonic chicken liver sustains high rates of yolk lipid oxidation, while the neonatal chicken liver dramatically upregulates *de novo* lipogenesis following feeding. Emerging literature from multiple species also demonstrates mechanistic links between branched-chain amino acids (BCAAs; Valine, Leucine, Isoleucine) and facets of lipid metabolism and mitochondrial function. We hypothesized that *in ovo* BCAAs will promote lipid oxidation and upregulate mitochondrial function in the livers of developing chicken embryos. Single Comb White Leghorn eggs (60.3g±0.7 SEM) were incubated at 37.5°C and 55% relative humidity. Starting embryonic day 9, a once daily *in ovo* injection (100µL) of either saline, 1X BCAA mix (58 mM Valine, 69 mM Leucine, 44 mM Isoleucine), or 2X BCAA mix (114 mM Valine, 138 mM Leucine, 83 mM Isoleucine) was given for a period of 5 days. On embryonic day 14, plasma and livers (n=8-12/group) were collected for targeted metabolomics of amino acids, β-hydroxybutyrate, and mitochondrial intermediates using gas-chromatography mass-spectrometry (GC-MS). Hepatic gene expression profiles were determined using qPCR. The results were analyzed using one-way ANOVA and pairwise mean comparisons, with the statistical significance set at $P \leq 0.05$. Plasma BCAAs (mM±SEM) progressively increased with 1X and 2X *in ovo* BCAA treatments (e.g., Valine; Saline, 369±20; 1X, 455±26; 2X, 647±31; $P \leq 0.05$). A similar trend was evident with the corresponding keto acids of BCAAs (mM±SEM, e.g., a-ketoisovaleric acid of valine; Saline, 33.1±1.9; 1X, 37.1±1.6; 2X, 44.3±2.5; $P \leq 0.05$) indicating higher rates of whole body BCAA degradation. Interestingly, plasma β-hydroxybutyrate (mM±SEM), a byproduct of lipid oxidation, trended higher in the 2X BCAA group (Saline, 2881±127; 2X, 3307±207; $P = 0.09$). In addition, levels of glycolytic intermediates (mM±SEM) were higher in plasma following supplementation of BCAAs (e.g., Pyruvate; Saline, 118±8; 2X, 145±9; Lactate; Saline, 905±52; 2X, 1092±60; $P \leq 0.05$), while hepatic tricarboxylic acid (TCA) cycle intermediates (mM±SEM) were higher following BCAA supplementation (e.g., α-ketoglutarate; Saline, 13.6±1.3; 2X, 20.9±2.9; $P \leq 0.05$; Malate; Saline, 256±19; 2X, 302±8; $P \leq 0.05$), indicating changes in mitochondrial function. Furthermore, hepatic gene expression of Carnitine palmitoyltransferase IA (*CPT1A*) was higher following 2X BCAAs ($P \leq 0.05$). In summary, *in ovo* BCAAs, while resulting in an increase in BCAA degradation, also altered lipid oxidation and mitochondrial TCA cycle metabolism in embryonic chicken. Identifying the mechanisms by which BCAAs impact lipid metabolism and mitochondrial function will allow us to improve the overall metabolic health of the chicken embryos and hatchlings.

Keywords: branched-chain amino acids, liver, mitochondria, lipid oxidation, embryonic chicken

100 The effect of low-protein soybean meal with graded inclusion levels of soyhull on immune parameters, jejunal tissue histomorphology in broiler chickens challenged with mixed *Eimeria* spp.

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Water-soluble fiber can act as a substrate for intestinal microbiota to synthesize short-chain fatty acids (SCFA), providing energy to intestinal cells and modulating immune responses by regulating cytokine production. This 42-d study investigated the effects of standard soybean meal (SSBM, 464 g/kg crude protein) or low-protein SBM (LPSBM) on inflammatory cytokine gene expression in spleen and cecal tonsils (CT), CD8+:CD4+ cell ratio, jejunal tissue histomorphology in broiler chickens challenged with mixed *Eimeria spp*. Three LPSBM were produced by adding soyhull (SH) to SSBM at 27, 55, or 86 g/kg rates to produce LPSBM1, 2, and 3, with 449, 437, and 417 g/kg crude protein, respectively. 1,056 male broilers were allocated to eight treatments in a 4 x 2 factorial arrangement, consisting of four corn-based diets with challenge (CH) or without challenge (NCH). All diets were balanced for metabolizable energy, crude protein, and standardized digestible amino acids. Challenge with mixed *Eimeria* spp was done on d 14. Spleen and cecal tonsils were collected on d21 and d28 to assess immunological responses, and jejunal tissue was collected on d21 to assess villus height

(VH), crypt depth (CD), and VH:CD ratio. The data were analyzed by two-way ANOVA in a 4×2 factorial arrangement using JMP Pro 17.There were significant *Eimeria* × diet (P<0.05) interactions where birds in the NCH group fed LPSBM2 had greater VH:CD ratio than the birds in the CH groups. Significant *Eimeria* × diet (P<0.001) interactions were observed for CT IFN-γ mRNA on d21 and d28 and for IL-1 on d21. Birds in the CH group fed LPSBM diets had decreased expression of these genes compared to birds in SPSBM diets. A diet effect was observed on d21 in CT where birds fed LPSBM2 had lower (P<0.05) CD8+:CD4+ cells than LPSBM1 diet. Bile IgA was significantly higher (P<0.05) in CH birds fed SPSBM than LPSBM3. There were no significant interaction effects on inflammatory cytokines in spleen on d21 and d28. The LPSBM3 decreased (P<0.05) IFN-γ concentration in spleen on d21 compared to LPSBM1 and on d28 there was a tendency (P= 0.064) where SPSBM had higher concentrations than LPSBM1. In conclusion, the inclusion of fermentable fiber in the diet during infection period decreased the proinflammatory cytokines concentration, likely due to the fiber anti- inflammatory properties. These findings suggest that LPSBM diets may help mitigate excessive immune activation during *Eimeria* challenge while modulating gut health and immune response.

Keywords: Eimeria, low protein soybean meal, soyhull, inflammatory cytokines, histomorphology

101 Effect of increasing dietary amino acid levels and starch-to-lipid ratios on broiler performance exposed to cyclic heat stress

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Improving broiler growth performance during heat stress (HS) can be achieved by reducing dietary protein while supplementing with amino acids, resulting in improved feed intake, body weight gain (BWG), and feed-to-gain ratios (F:G). In contrast, increasing dietary protein can result in higher dietary starch-to-lipid ratios (S:L) due to the increased inclusion of grains, which could adversely affect amino acid utilization and overall performance. Thus, the objective of this study was to evaluate the effect of increasing dietary amino acid levels (AAL) and S:L ratios on performance of broiler exposed to cyclic heat stress. Isocaloric diets (3,089 kcal/kg) were formulated with 6 AAL at 80, 90, 100, 110, 120, and 130% of the digestible lysine requirement (1.01% dLys) with all other amino acids formulated to a fixed ratio with dLys and two S:L ratios (4:1 and 10:1) for a total of 12 dietary treatments arranged in a 6 x 2 factorial design. The reference values for S:L ratio, energy, and dLys were derived from previous trials with broilers exposed to cyclic heat stress of 31°C and 21°C during the finisher phase. Mixed sex 0 d-old Ross 308 broilers were housed in 8 individual rooms with 12 pens/room containing 60 birds/pen (final stocking density of 31 kg/m^2). Birds were fed common starter and grower diets before being switched on d21to the experimental finisher diets. Room temperatures of 32°C on d0, were reduced to 21°C by d21. From d28-35, birds were exposed to cyclical HS with 12h of 31°C and a cool temperature of 21°C (50% relative humidity). Body weight and feed weights were measured on d21, 28, and 35. Data were analyzed as a two-way ANOVA using Proc Mixed and Proc NLIN to fit a broken line model using SAS (SAS 9.4, Cary, NC). The model used was Y = L + U * (R-AAL), where (R-AAL) is 0 when AAL > R, Y is the dependent variable (BWG or F:G), L is the plateau, U is the slope and R is the breaking point. An interaction in BWG from d21-28 was observed, where birds fed 120%, and 130% AAL at S:L of 10 exhibited significantly higher BWG than birds fed diets with a S:L of 4 (P<0.05). No interactions between S:L and AAL were detected for BWG and F:G ratios in the other periods evaluated (P>0.05). The estimated AAL requirement for the overall finisher period (d21-35) was 108% for a BWG of 1.472 kg (P<0.05; R^2=0.487). For F:G ratios estimated AAL was also 108% for a F:G of 1.536 (P<0.05; R^2=0.884) during the finisher period (d21-35). In conclusion, estimated AAL requirements of 108% maximized growth performance of heat stressed Ross 308 broilers during the finisher phase, however, reducing dietary S:L ratio to 4:1 from 10:1 did not affect growth performance under these environmental conditions.

Keywords: Amino acid, Body weight, Feed efficiency, Nutrition, Energy

102 Effects of dietary reduction of metabolizable energy and amino acid density on the performance, carcass traits, and nutrient digestibility of broilers from 1 to 49 days

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This experiment evaluated the effects of apparent metabolizable energy (**AME**) and amino acid (**AA**) density on performance, carcass traits, and nutrient digestibility of broilers from 1 to 49 d of age. A total of 1,200-d-old male broilers were sorted in 48 floor pens and assigned to 4 treatments. Diets were formulated by the combination of standard (**SE**) and reduced (**RE**) AME (-100 kcal/kg) levels with 100 and 90% AA densities. Feed intake (**FI**), body weight (**BW**), and feed conversion ratio (**FCR**) were determined at 14, 28, 42, and 49 d of age. On d 49, ileal digesta was collected from 5 birds per pen for nutrient digestibility analysis. On d 50, 10 birds per pen were processed for carcass traits analysis. Data were analyzed as a 2 × 2 factorial (AME × AA density) arrangement in a randomized complete block design to evaluate main effects and interactions using JMP PRO 15. Means were separated by Tukey's HSD test with statistical significance considered at P ≤ 0.05. Broilers fed SE diets had a higher (P ≤ 0.05) BW and lower (P ≤ 0.05) FI on d 28, 42, and 49 compared to those fed RE diets. Furthermore, broilers fed diets with 100% AA had a higher (P ≤ 0.05) BW and FI than those fed diets with 90% AA on d 28, 42, and 49. An interaction between AME and AA density was observed for cumulative FCR on d 28 and 42. Reducing AA density increased (P < 0.05) FCR in both SE and RE diets; but the effect was greater in RE diets. In SE diets, FCR increased by 5 points on d 28 (1.38 vs. 1.43 g:g) and 42 (1.52 vs. 1.57 g:g). In contrast, in RE diets, FCR increased by 8 points on d 28 (1.40 vs. 1.48 g:g) and 42 (1.55 vs. 1.63 g:g) when the AA density was reduced from 100 to 90%. Broilers fed SE had lower (P < 0.05) FCR at 49 d of age compared to those fed RE diets (1.63 vs. 1.67 g:g). Similarly, broilers fed diets with 100% AA had a lower (P < 0.05) FCR than those fed 90% AA (1.62 vs. 1.68 g:g) on d 49. Broilers fed SE diets had a higher (P ≤ 0.05) wing and leg weights compared to those fed RE diets. Likewise, broilers fed 100% AA had higher (P ≤ 0.05) chilled carcass, breast, tender, and wing weights, along with greater breast yield compared to those fed a 90% AA. Apparent ileal digestibility (**AID**) of crude protein, fat, potassium, and phosphorus was unaffected (P > 0.05) by AME. Broilers fed 90% AA had higher (P ≤ 0.05) calcium digestibility

(56.56 vs. 41.49%) but lower phosphorus digestibility (83.49 vs. 85.62%) compared to those fed 100% AA. Overall, reductions in AME and AA density negatively impacted BW and carcass traits of broilers throughout the grow-out period.

Keywords: broiler performance, carcass traits, nutrient digestibility, AME, amino acid density

103 Determination of apparent metabolizable energy and digestibility of energy and lipids in palm oil blends and other feed-grade lipid sources in Ross broilers

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The volatile cost of traditional energy sources in diet formulation has driven the poultry industry to explore alternative lipid sources. The objective of this study was to determine the apparent metabolizable energy (AME) and energy utilization of palm oil and its blends compared with traditional lipid sources in broilers from 1 to 21 d of age. A total of 720 Ross® Yield Plus × 708 broilers was randomly distributed in 80 battery cages (9 birds/cage) and assigned to 8 dietary treatments (10 replicates/treatment) including 7 test diets and a basal diet containing dextrose. Lipid sources evaluated included palm oil (PO), soybean oil (SO), poultry fat (PF), acidulated soapstock (AS), and 3 blends of PO and SO (75:25, 50:50, and 25:75). Body weight, body weight gain, feed intake, feed conversion ratio, and mortality were evaluated. Energy utilization was determined by measuring the gross energy (GE), nitrogen-corrected AME (AME_n), the ratios of AME_n to GE (AME_n:GE), and apparent total tract energy (ATTED) and lipid digestibility (ATTLD) of each lipid source. Data were analyzed using a one-way ANOVA and treatments were separated using Tukey's multiple comparison test. Additionally, pre-planned orthogonal contrasts were performed to determine differences between PO and all other treatments. Significance was declared at $P < 0.05$. No performance differences ($P > 0.05$) were observed between treatments, indicating a general acceptance of the alternative energy sources. The GE of the oils ranged from 9,233 (AS) to 9,401 (SO) kcal/kg, with an average of 9,366 kcal/kg. The AME of the oils varied ($P < 0.05$) by more than 2,000 kcal/kg, with the lowest values determined for PO (8,307) and the highest value for PO50:SO50 (10,339). This corresponded to AME:GE ratios ranging from ~88.5% for PO and PO75:SO25 to 110% for PO50:SO50 ($P < 0.05$). Differences were observed in ATTED ($P < 0.05$) of the oils, with PO and AS having the lowest values (~90.55%) and SO having the highest value (97.38%). The ATTLD of the oils ($P < 0.05$) ranged between 80.55% and 91.98% with PO and PO75:SO25 treatments having the lowest values and PO25:SO75 and SO having the highest. Values of AME:GE for PO50:SO50 may indicate an extra caloric effect of this blend and an overestimation of the energy value derived from the lipid source per se. Nonetheless, the data demonstrate that PO can be blended with SO to create alternative lipid sources with desirable digestibility and energy utilization in broilers.

Keywords: Palm Oil, AMEn, lipid digestibility, broiler nutrition, lipid sources

104 Relative metabolizable energy of soybean gums and soap stocks in precision-fed roosters and relative bioavailable energy in restrict-fed broiler chickens

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The objective of these experiments was to determine the available energy content in soybean gums and soap stocks using precision-fed roosters and restrict-fed broiler chickens. Soybean gums and soap stocks are lipid-based impurities from crude soybean oil refinement. In Experiment 1, relative metabolizable energy of gums and soap stocks was determined in precision-fed roosters. Leghorn roosters with intact ceca were fasted for 26 h, prior to being precision-fed (crop intubated) 25 g of 1 of 7 dietary treatments. Excreta were quantitatively collected for 48 h. Diet 1 was 100% corn. Diets 2 through 7 were the same as diet 1, except that 2 increasing levels of soybean oil, gums, and soap stocks (7.5 and 15.0%) were mixed with corn. In Experiment 2, relative bioavailable energy of gums and soap stocks was determined in broiler chickens. Birds were provided a nutrient-adequate starter diet from 0 to 10 d-of-age. Broilers were provided experimental diets from 11 to 22 d-of-age. Diet 1 was an energy-deficient corn-soybean meal-based diet (2,485 kcal/kg). Diets 2 through 7 were the same as diet 1, except that 2 increasing levels of soybean oil, gums, and soap stocks (5.0% and 10.0%) were added at the expense of Solka Floc. Birds were feed restricted to 70% of the expected ad libitum feed intake per breeder guidelines. Data were analyzed using a 1-way ANOVA for a completely randomized design. Relative availability of energy was determined by regressing either nitrogen-corrected true metabolizable energy (**TME_n**; Experiment 1) or BW gain (Experiment 2) against test ingredient inclusion level. The relative TME_n of test ingredients in Experiment 1 was also calculated by difference using the basal substitution method. Soybean oil was used as the standard reference source for RBV calculations in both experiments. The significance value for all analyses was $P < 0.05$. In Experiment 1 with precision-fed roosters, TME_n increased linearly with increased lipid inclusion ($P < 0.05$; $R^2 = 0.92$). The TME_n of soybean oil was 8,954 kcal/kg (as-fed), compared with 4,695 and 4,414 kcal/kg in gums and soap stocks, respectively. The ME of gums and soap stocks relative to soybean oil ranged from 20 to 23% using multiple linear regression and 49 to 52% using the basal substitution method. In Experiment 2 with restrict-fed broilers, BW increased linearly with increased lipid inclusion ($P < 0.05$; $R^2 = 0.88$). The bioavailable energy content of gums and soap stocks were 51 and 40%, respectively, compared with the soybean oil. In conclusion, the relative energy values of gums and soap stocks compared with soybean oil were approximately 40 to 50%, indicating that these products have potential to be used as an energy source in poultry diets.

Keywords: gums, soapstocks, metabolizable energy, soybean oil, broiler

Student Competition: Physiology and Reproduction II

105 FGF23 receptor expression in the kidney and shell gland of laying hens indicates it is a potential regulator of phosphorus homeostasis for eggshell formation

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Renal phosphorus (P) homeostasis is key to maintaining an adequate mineral balance for eggshell formation and bone mineralization in laying hens. Fibroblast growth factor 23 (FGF23) has been shown to mediate renal P homeostasis in avian species by promoting excess P excretion. Additionally, P is an important component of the crystalline hydroxyapatite layer beneath the cuticle of the eggshell that may be involved in the termination of eggshell calcification. Interestingly, our previous work suggests the shell gland is a novel FGF23 target. To better understand the role of FGF23 in mediating P homeostasis for eggshell mineralization, this study evaluated expression of FGF23 receptors across the daily egg formation cycle from early through extended production. To this end, shell gland and kidney tissue was collected from Nick Chick hens (H&N International) at 25, 43, 80, and 95w of age during times of the oviposition cycle representing bone mineralization (1.5 hours post-oviposition [HPOP]), eggshell mineralization (15 HPOP), and transitions between them (6 and 21 HPOP) (n=8/age/HPOP). Levels of mRNA were determined for FGF receptors (FGFR*)* 1-4 and coreceptor Klotho (*KL*) by RT-qPCR. Data were analyzed by ANOVA and Fisher's LSD test. Main effects of age and HPOP were observed for *FGFR2* and *3* in the shell gland, with significantly greater expression at 95w and variation across the daily lay cycle ($P \leq 0.05$). Levels of each receptor decreased between 1.5 and 6 HPOP and remained low through 15 HPOP before increasing to their highest levels at 21 HPOP ($P \leq 0.05$). An age-by-HPOP interaction was observed for *KL*, where HPOP expression patterns were similar to that of *FGFR2* and *3* at all ages but with 80w exhibiting the lowest levels ($P \leq 0.05$). In the kidney, main effects of age and HPOP were observed. Expression of *FGFR2* increased between 25 and 43w and maintained high levels through 95w ($P \leq 0.05$), while *FGFR3* linearly increased through 80w then decreased at 95w ($P \leq 0.05$). Expression of *KL* also increased between 25 and 43w followed by a steady decrease thereafter ($P \leq 0.05$). Levels of all genes were lowest at 21 HPOP in this tissue, consistent with the need to retain P for eggshell formation at this time ($P<0.05$). The shell gland is a potential target for FGF23 action, and its sensitivity may increase with age. Elevated receptor expression at 21 HPOP in the shell gland indicates FGF23 could regulate P utilization for the crystalline hydroxyapatite layer and cuticle formation occurring during late stages of eggshell mineralization. Moreover, changes observed across the oviposition cycle indicate decreased renal excretion of P at 21 HPOP, likely to ensure P availability in the shell gland for late eggshell mineralization.

Keywords: Crystalline layer, Hydroxyapatite, Eggshell cuticle, Phosphorus homeostasis, Extended lay

106 Probing the validity of reference point indentation for detecting bone quality differences between caged and cage-free laying hens

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Reference point indentation (RPI) has recently been explored as a more robust and less invasive method of assessing bone biomechanical properties in poultry, but data interpretation challenges remain. Mammal literature commonly reports first indentation distance (FID), total indentation distance (TID), and indentation distance increase (IDI) as these measurements appear to have correlations with whole bone strength and toughness. This has not been the case in poultry. Other RPI outputs, such as average creep indentation distance (AvgCID), average energy dissipated (AvgED), and average loading or unloading slope (AvgLS and AvgUS) may be better suited for assessing poultry bone. The objective was to evaluate the capability of RPI to distinguish between two populations of laying hens with known bone strength differences: caged versus cage-free layers. Cage-free tibias were expected to have reduced RPI distance and energy parameters, and increased slope parameters. Tibias were obtained from caged hens at a commercial facility at 5 ages from 22 to 70 weeks of age (woa; N = 70 bones) and from 41 woa cage-free hens at a research facility (N = 10 bones), resulting in 6 age-groups. Soft tissue was removed with a scalpel and marks drawn at 25, 50, and 75% of the bone length to denote proximal, middle, and distal locations, respectively. Bones were subjected to RPI testing with three measurements taken at each location. Measurements consisted of 10 loading cycles at 5 N. Data were analyzed as a repeated measures model with main effects of age-group and location, and random effect of bone nested within location. Contrasts comparing the cage-free group to each of the 5 cage groups were performed. Whole bone breaking strength was conducted and analyzed to confirm the assumption that cage-free tibias were stronger than cage tibias. Cage-free hens did have a higher breaking strength, but had an increased FID, TID, and IDI than the 40, 52, and 70 woa caged hens ($P < 0.04$), contradicting the expected pattern. The AvgCID was lower in cage-free hens compared to all cage groups ($P < 0.0001$), indicating cage-free tibias were better able to resist deformation under constant force. Less energy was absorbed by cage-free tibias as evidenced by reduced AvgED ($P < 0.007$) compared to all cage groups, relating to reduced microcrack propagation. Cage-free hens had a higher AvgLS than all cage groups ($P < 0.0001$) and a higher AvgUS than 32 and 52 woa cage hens ($P < 0.03$), implying greater resistance to plastic deformation. The commonly reported parameters of FID, TID, and IDI are not sufficient when assessing poultry bone quality with RPI; creep, energy, and slope parameters may be more appropriate.

Keywords: reference point indentation, bone quality, biomechanics, breaking strength, laying hens

107 Yolk sac tissue gene expression in the context of embryonic development

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The incubation period accounts for roughly one-third of a modern broiler's lifetime, placing emphasis on understanding the factors that control embryonic development. The yolk sac tissue (YST)

has been identified as a potential target for novel strategies during incubation aiming to increase hatchability and improve post-hatch growth performance, as prior work in our lab suggests it could serve as a source of hormonal factors to regulate embryo growth prior to the development and maturation of other endocrine organs. The objective of this study was to evaluate yolk sac tissue gene expression in comparison to select somatic tissues during embryogenesis. Broiler eggs were incubated at standard conditions and sampled on embryonic days (E) 3, 6, 9, 12, 15, 18, and day of hatch (DOH). The entire YST was collected at each age, embryo body was collected on E3 and E6, and liver and breast muscle were collected for the remaining ages. Male embryos were used for mRNA analysis by RT-qPCR (n=8/age) to determine relative expression of genes within the corticotropic and somatotropic axes. Data were analyzed using a one-way ANOVA and Fisher's LSD test. The somatotropic axis regulates growth and development through the actions of insulin-like growth factors (IGFs) and their associated binding proteins (IGFBPs). In YST and liver, *IGF2* increased from E15 to DOH and levels in YST were greater than liver on DOH ($P \leq 0.05$). For *IGFBP2* and *7* and IGF acid labile subunit (*IGFALS*), expression increased in YST from E18 to DOH, and YST had the greatest expression in comparison to other tissues ($P \leq 0.05$). *IGFBP1* increased in the YST from E3 to E9 and from E12 to E15 before decreasing at E18; however, YST expression levels were lower than liver at all ages but higher than embryo body and muscle ($P \leq 0.05$). Embryo body had the greatest expression of *IGF1R*, with expression in YST decreasing at E9 and E12 before increasing at DOH ($P \leq 0.05$). Corticosterone binding globulin (*CBG*) in the YST steadily increased from E6 to E15 before decreasing at E18 ($P \leq 0.05$). Liver *CBG* expression at E12 and E15 was comparable to the YST and exhibited a decrease from E15 to E18 ($P \leq 0.05$). High expression of *IGF2*, *IGFALS*, and *IGFBP2* and *7* in the YST suggests they could mediate YST and embryo growth. Circulating corticosterone in the embryo increases during mid-embryonic development, indicating YST CBG might facilitate that. Dynamic gene expression in the YST when compared to other somatic tissues implies its broader role in embryonic development and highlights the need for further studies to elucidate the extent in which it mediates its own growth versus that of the embryo.

Keywords: incubation, embryogenesis , CBG, corticotropic axis, somatotropic axis

108 The effects of supplemental choline chloride on egg quality parameters in Ross 708 broiler breeders

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Broiler breeders (BB) are an integral part of the commercial broiler industry, yet there are few researchers who currently conduct trials focused on them. Research on BB health and performance is crucial as egg production and fertility continue to be challenges. Choline has long been known to be an essential nutrient due to its involvement in numerous bodily functions including cell structure formation, fat metabolism, and methyl donor contributions. However, little work has been conducted to determine the optimal levels in the modern BB. For this trial, various levels of supplemental choline were tested using 960 Ross 708's females placed with Yield Plus Males. Birds were randomly divided into one of three treatments with 8 replications including 34 females and 4 males. Treatments were allocated as follows; A) 500 mg/kg (industry standard), B) 1,000 mg/kg, or C) 1,500 mg/kg of choline chloride. Birds were fed the same levels of choline from day of age throughout the lay phase. Overall egg production, incubation and egg quality were examined. Eggs were collected and sampled at weeks 40 and 64 for egg quality evaluation. JMP One-way ANOVA Student's T test with α set at 0.05 was used to analyze data. Fertility was evaluated at ages 41 and 45. With differences ($p=0.0198$) being observed in average fertility with group C having the highest (99.18%) followed by A and B (97.94% and 96.24%) No statistical differences were observed in the hatch of fertile eggs. There were no statistical differences observed at the 40 week sampling; however, at the 64 week sampling as the birds aged numerous statistical differences were observed. Egg weights were highest ($p=0.0367$) in group C (71.15g), group A being intermediate (69.92g), and B (68.982g) lowest. Statistical differences were also observed in yolk color scores ($p=0.0114$) with the group C having the highest score (7.81) followed by groups B and A (7.34, 7.33) respectively. Vitellin force scores also differed significantly ($p=0.0317$) with group B having the highest scores followed by A and C. Vitellin elasticity also differed ($p=0.0224$) with group B having the highest score (1.856), followed by C (1.56) and A (1.55). Our results indicate a positive dose response when choline chloride at 1500mg/kg is added in broiler breeder feed that may improve fertility and egg quality, supporting the need for more in depth research studies.

Keywords: Choline, broiler breeders, fertility, egg quality, metabolism

109 Lipid biomarkers associated with egg storage and early embryonic mortality in broiler breeder chickens

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Early embryonic mortality (EEM) occurs within the first week of incubation, peaking between days one and three, and poses a significant challenge for the poultry industry by reducing hatchability and flock productivity. This study explores the yolk lipidome of Cobb broiler breeder embryos, comparing lipid profiles from EEM and healthy, no embryonic mortality (NEM) eggs during early embryonic development to identify potential lipid biomarkers associated with early embryonic mortality. A previous discovery-phase lipidome trial in our laboratory utilizing Multiple Reaction Monitoring (MRM) detected a total of 3,706 lipid species across 10 lipid classes in broiler breeder embryonic day 7 yolk samples. For the current trial, yolk samples were collected on days one, two, or three of incubation from fertilized broiler breeder eggs subjected to (1) either 2 or 10 days of storage (n=4/day/storage duration) and (2) with normal development (NEM) or succumbing to EEM (n=3/day; only collected on days 2 and 3 of incubation). Collected yolk samples were screened for the previously detectable lipid species using MRM profiling. Total abundance of each lipid class as well as relative ion intensities for each lipid species were compared by t-test between experimental groups. Significant effects ($p<0.05$) were detected for the phosphatidylserine (PS) lipid class with respect to storage at day

one and for the PE lipid class with respect to EEM at day three. Metaboanalyst 6.0 identified a total of 134 lipid species that were significantly affected by storage and 46 by mortality. Lipid ontology enrichment analysis (LION) highlighted changes in energy storage pathways, particularly lipid droplet formation and triacylglycerol metabolism. The enrichment of lipid storage-related terms suggests a crucial role for lipid reserves in supporting early embryonic development. This study provides the first MRM-based lipidomic profiling of broiler breeder egg yolks, revealing storage-related lipidomic shifts and identifying lipid profiles differentiating NEM from EEM samples. These findings offer insights into early embryonic development and mortality mechanisms, supporting lipid biomarker discovery as an approach to improve poultry production. Further research is needed to determine how storage impacts yolk composition as well as to determine if lipidomic shifts are subsequent to embryonic mortality, or if yolk composition is contributing to early embryonic mortality.

Keywords: early embryonic mortality, lipidome, broiler breeder, biomarker, yolk

110 Antibiotic growth promoters appear to influence hepatic regulation of glucose metabolism during heat stress in modern and legacy broilers

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Selective breeding of broilers for rapid muscle accretion has resulted in a decreased tolerance to high temperature due to increased metabolic heat production associated with robust growth. Antibiotic growth promoters (AGPs) improve broiler performance during heat stress (HS) via mechanisms not fully understood. The liver is crucial for glucose metabolism and regulating whole body growth through somatotropic axis signaling. This study evaluated the effects of bacitracin methylene disalicylate (BMD) at AGP levels on growth performance, blood glucose, and related hepatic gene expression in modern and legacy broilers under high temperature or thermoneutral (TN) conditions. Day (D) of hatch male chicks were randomly assigned into 8 groups (n=6) with two lines [Ross 708 (ROSS) or Athens-Canadian Random Bred (ACRB)], two diets [antibiotic free (ABF) or BMD-supplemented], and two temperatures [TN (75°F) or HS, 95°F for 8h/day]. Birds were fed treatment diets for the entire experiment and subjected to temperature treatments from D32-38. Birds and feed were weighed on D0, 14, 32, and 38. On D32 & 38, whole blood was collected from one bird/pen and analyzed via i-STAT to assess effects of acute and chronic HS, respectively. Liver was analyzed via RT-qPCR for mRNA levels of glycogen phosphorylase (*PYGL*), insulin receptor (*INSR*), glucagon receptor (*GCGR*), glucose transporters (GLUT) 1, 2, 5, 11, & 12, insulin like growth factor 1 (*IGF1*), IGF-binding proteins (IGFBP) 1, 2, 3, 4, 5, & 7, and acid labile subunit (ALS). Data were analyzed using ANOVA and Fisher's LSD test (P≤0.05). Final body weight (BW) and average daily gain (ADG) from D32-38 were lower in HS-ROSS, while BMD-supplementation during HS improved final BW and ADG compared to ABF-ROSS (P≤0.05). Blood glucose during chronic HS was higher in ABF groups of both lines during HS, whereas BMD-HS groups were not different than TN (P≤0.05). During acute HS, *PYGL* and *GCGR* expression decreased in ROSS, but BMD-supplementation during HS increased *PYGL* expression compared to ABF-ROSS (P≤0.05). Expression of *INSR* was higher in ROSS during acute HS (P≤0.05). Levels of *GLUT2* & *5* decreased in acute HS ROSS (P≤0.05), while *GLUT12* expression was lower in ABF-ROSS but not BMD-ROSS during chronic HS (P≤0.05). Relative mRNA levels of *IGF1*, *IGFBP5*, and *ALS* were lower, and *IGFBP1* and *IGFBP2* higher only in ROSS during acute HS (P≤0.05). Results indicate that selection has resulted in altered hepatic response HS, as evidenced by decreased glycogen breakdown and glucose release and altered somatotropic signaling in modern broilers. Notably, BMD-supplementation partially reversed the effects of HS on glucose metabolism, indicating potential targets for novel AGP alternatives.

Keywords: Blood glucose, Glycogen phosphorylase, Glucose transporters, Insulin-like growth factor, Insulin-like growth factor binding proteins

111 Effects of oral nanoplastic exposure on intestinal absorption, tissue distribution, and gut health in broiler chickens

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Nanoplastics (NPs) are emerging environmental contaminants, yet their interactions with the intestinal epithelium, particularly in agricultural animals, remain poorly understood. This study investigates whether ingested NPs cross the intestinal barrier, identifies primary absorption sites, maps tissue distribution, and assesses their impact on intestinal health. Fourteen-day old broiler chickens were orally gavaged with two fluorescently labeled polystyrene (PS) nanoplastics with size 50nm and 100nm (PS50, PS100) at a dose of 100 mg/kg body weight (n = 4). Blood samples were collected at 3, 8, 24, 36, and 48 hours post-gavage, while tissue samples (spleen, liver, kidney, duodenum, jejunum, ileum, and cecum) were harvested at 24 and 48 hours to assess NP distribution and tissue damage. Gene expression of tight junction proteins (CLDN1, CLDN2), inflammatory cytokines (TNFA), and antioxidant enzymes (SOD1, SOD2) was analyzed using qRT-PCR. Additionally, ex vivo Ussing chamber assays were conducted to evaluate NP absorption across four intestinal segments in untreated birds (n = 4). Data was analyzed using Student's *t*-test to compare NP-treated and control samples at each time point. Results revealed that PS50 was primarily absorbed in the ileum ($P < 0.05$), with detection in serum as early as 3 hours post-gavage, peaking at 8 hours, and declining after 24 hours. PS50 significantly accumulated in all tissues at 24 hours ($P < 0.05$) but was largely cleared by 48 hours. In contrast, PS100 showed no significant differences in absorption among intestinal segments, suggesting an alternative uptake mechanism. Serum detection of PS100 was delayed until 24 hours, peaked at 36 hours, and declined by 48 hours ($P < 0.05$). Tissue accumulation of PS100 was also delayed, with detection in all organs only at 48 hours ($P < 0.05$). NP exposure upregulated CLDN1 at 24 and 48 hours ($P < 0.05$), indicating compensatory repair of epithelial cells. CLDN2 expression increased at 48 hours in the ileum and cecum suggesting barrier damage and a compensatory effort to expel the NPs. SOD1 expression was upregulated at 24 hours ($P < 0.05$) but not at 48 hours, reflecting an initial oxidative defense, while expression of SOD2 did not increase until 48 hours post exposure, implying subsequent mitochondrial oxidative stress as cellular damage progressed. TNFA was elevated in the ileum at 24 and 48 hours ($P < 0.05$). These findings demonstrate that nanoplastics are rapidly absorbed, systemically distributed, and induce oxidative stress and inflammation in the intestinal epithelium. Particle size

influences absorption and clearance kinetics, underscoring potential risks to poultry gut health.

Keywords: Nanoplastics, Broiler chickens, Gut health, Oxidative stress, Intestinal absorption

112 Not Presented

113 Orange corn diet decreased serum corticosterone and improved gait, egg production, egg quality and fertility of breeder Pekin ducks exposed to heat stress

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Heat stress (HS) negatively affects poultry making it essential to find mitigation strategies. Carotenoids improve antioxidant defenses, decrease oxidative stress and consequently improve heat tolerance. Therefore, we hypothesized that a high carotenoid orange corn (OC) diet would alleviate the effects of HS on breeder Pekin ducks compared to a yellow corn (YC) diet. 224 hens and 56 drakes were assigned to OC or YC diets across 8 pens, with each diet fed for 3 weeks before and during 3 weeks of cyclic HS exposure (35°C for 10 hours, reduced to 29°C for 14 hours/day). Blood samples (n = 8/sex/treatment/timepoint) were collected at weeks -3 and -2 prior to the onset of HS and at 4 hours, 48 hours, 1 week, 2 weeks, and 3 weeks of HS. Body weight (BW, n = 40 hens and 28 drakes/treatment), feed intake (FI, n = 4 pens/treatment), egg production, egg quality (n = 40 eggs/ treatment/timepoint), and transect walk assessment (n = 4 pens/treatment) were collected weekly before and during HS. Fertility and hatchability were assessed at the end of HS. Data were analyzed with ANOVA, t-tests, or hi-squared tests as appropriate, with significance set at $p \leq 0.05$ and trends at $p < 0.1$. Hen serum corticosterone levels were higher in the YC than in the OC treatment at 4 hours ($p < 0.05$) and 48 hours ($p < 0.01$) of HS. At week 2 of HS, serum cortisol was higher in hens ($p < 0.05$) on the OC diet compared to YC. No differences in the drakes' glucocorticoids were observed. Diet had no effect on the heterophil to lymphocyte ratio (HLR), but HS increased HLR in the OC hens and drakes at 48 hours ($p < 0.001$) compared to 4 hours, 2 and 3 weeks of HS. Diet did not affect BW, although HS decreased BW in OC and YC hens ($p < 0.001$) at week 3 compared to baseline, with no effect on drakes. FI was similar between diets, but HS led to a decrease ($p < 0.001$) compared to baseline. Transect walk assessment showed a higher percentage of ducks exhibited poor gait in the YC treatment at weeks -1 ($p < 0.05$), 2 ($p = 0.07$), and 3 ($p = 0.07$) compared to the OC treatment. Egg production was higher in the OC treatment during the first week of HS ($p < 0.05$) compared to YC treatment. Egg weight was higher at week -1 ($p = 0.056$), Haugh unit values were higher at weeks -2 and 3 ($p = 0.06$), yolk weight was higher at week -1 ($p < 0.01$), and yolk color was darker at weeks -2, -1, 2, and 3 ($p < 0.001$) in the OC treatment compared to YC treatment. Fertility was higher in the OC ($p < 0.05$) compared to YC treatment, although hatchability was not different. Our results suggest that OC diet may help alleviate some of the effects of HS in breeder hens.

Keywords: Orange corn, Carotenoid, Heat stress, Poultry, Ducks

114 Effect of cyclic heat stress and bird strains on blood gas parameters in laying hens

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The increasing global temperatures pose a significant challenge to poultry production by disrupting birds' physiological balance, reducing productivity and overall well-being. The objective of this study was to investigate the simple and main effects of cyclic heat stress and bird strain on blood gas response variables in laying hens and to understand their physiological responses to cyclic heat stress. A total of 240 fifteen wk-old pullets (120 brown and 120 white) were assigned to treatment in a randomized complete block design in a 2 (white vs. brown hens) x 2 (thermoneutral vs. cyclic heat stress) factorial arrangement of treatments with 6 hens per replicate, and 10 replicates per strain in each of the two rooms. Blood samples were taken from the first 6 replicates in each of the two rooms. All birds were fed the same corn-soybean meal-based diet that met or exceeded their nutritional requirements throughout the duration of the study. All hens were on the same thermoneutral temperature (23.9 ºC) throughout the duration of the study except the hens on cyclic heat stress that were maintained at 33.9 °C for 8 hr per day (8.00 am to 4.00 pm, Monday to Friday). The response variables measured using an *i-STAT* blood gas analyzer were the rectal temperature, pH, pCO_2, pO_2, HCO_3, Na, K, glucose, hematocrit, and hemoglobin (at wk 18 and 25 of age). The heat-stressed hens were sampled after 4 hr exposure to heat stress. The data were analyzed using the MIXED procedure of SAS appropriate for a factorial arrangement of treatments. No significant interaction was observed between breed and temperature at both ages. For the main effect of breed at wk 18, rectal temperature, hematocrit, and hemoglobin were higher ($p<0.05$) in the white strain, while serum sodium higher ($p<0.05$) in the brown strain. Serum-ionized calcium levels decreased ($p<0.05$) while rectal temperature increased ($p<0.05$) with heat stress. At wk 25, rectal temperature increased ($p<0.05$) while hematocrit and hemoglobin levels decreased ($p<0.05$) with heat stress. Partial pressure of O_2, hematocrit, and hemoglobin were higher ($p<0.05$) in the white strain, while serum levels of sodium and glucose were higher ($p<0.05$) in the brown strain. In conclusion, these results suggest that breed differences influenced blood gas responses to exposure to cyclic heat stress with white hens showing higher oxygen transport parameters and brown hens exhibiting greater sodium and glucose levels at wk 25. Heat stress increased rectal temperature at both wk, indicating a disruption in thermoregulation, while its effect on lowering ionized calcium observed only at wk 18 suggests a temporary imbalance in electrolyte homeostasis

Keywords: birds strain, blood gas, cyclic heat stress, laying hen

115 Effects of different levels of wheat on *Histomonas meleagridis* and *Eimeria* challenge turkey poults on intestine inflammation and gut microbiota

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Histomonas meleagridis (HM) and *Eimeria* (EM) are protozoan parasites known to cause severe necrosis in Turkey's gastrointestinal tract. The role of wheat inclusion and varying viscosity in developing parasitic infections is poorly understood. This study aimed to evaluate the impact of different levels of wheat in the diet on the development of *Eimeria* and histomoniasis in turkey poults, focusing on intestine health. A total of 540 one-day-old male turkey poults were randomly allocated into nine treatments with five replicated cages of 12 birds/cage during 22 days of the experiment. A 3×3 factorial design consisted of three dietary wheat inclusion levels (0%, 15%, and 30%) and three disease challenge groups (No disease challenge (NC), HM, and EM). Birds in the HM-challenge group got 10^5 *Histomonas meleagridis* on day 12, while the EM-challenge group received 8,000 *Eimeria meleagrimitis* and *Eimeria adenoeides* oocysts on day 16. Bird mortality and feed intake were recorded daily. On day 22, jejunum and ceca were collected for cytokines mRNA abundance and gut microbiota analysis, respectively. The data were analyzed using two-way ANOVA in JMP Pro 18, with Tukey's test for multiple comparisons. Histomonas challenge groups showed a significant increase in mortality compared to the NC and EM groups ($P<0.0001$). Body weight significantly decreased in EM (17.3%) and HM (34.7%) compared to the NC ($P<0.0001$); additionally, the inclusion of wheat increased BW compared to No-wheat diets ($P<0.05$). Histomonas-challenge groups had significantly higher FCR than the EM and NC ($P<0.0001$). Ileal digesta viscosity of wheat-included groups significantly increased compared to No-wheat diets ($P=0.04$). Jejunum *IL-6* and *IL-18* were highly upregulated in EM-challenge groups compared to the HM and NC ($P<0.0001$). Alpha diversity metrics significantly decreased in the HM and EM compared to the NC ($P<0.0001$). Proteobacteria phylum significantly increased in the HM (74.6%) compared to the EM and NC ($P<0.0001$). The disease and wheat interaction was insignificant in any parameters ($P>0.05$). In conclusion, the present data suggest that adding wheat to the diet may increase the growth performance of turkey poults but have NO ameliorative effect on *Histomonas meleagridis* and *Eimeria* infections.

Keywords: Histomonas, Eimeria, Wheat

116 Colonization efficacy and safety of Monovalent Anti-Flagellin C nanobody-secreting *Bacillus subtilis* in broiler birds

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Genetically modified probiotics targeting specific intestinal pathogens can effectively reduce enteric pathogen loads. This study evaluates the colonization efficacy of a live probiotic *Bacillus subtilis* (Bs^{FliC}) strain, genetically engineered to secrete monovalent neutralizing nanobodies (Nbs) against the key virulence factor Flagellin C (FliC). The genetic code for Abi-Se07, a potent neutralizing Nb against FliC, was inserted into *B. subtilis* using the NBP3510 promoter and a chloramphenicol resistance selection marker. The resulting Bs^{FliC} strain secreted Abi-Se07 into the supernatant at a yield of approximately 100 mg per liter—approximately one thousand times higher than the binding constant of Abi-Se07 (KD = 16.2 nM). The secreted anti-FliC from Bs^{FliC} recognized four *Salmonella enterica* serovars *in vitro*. A *B. subtilis* strain (Bs^{GFP}) engineered to express green fluorescent protein (GFP) served as a control. Twenty-four day-old Cobb broiler chicks were randomly assigned to three groups: Control, Bs^{GFP} and Bs^{FliC} in eight replications. The Bs^{GFP} and Bs^{FliC} group received an oral gavage of engineered Bs^{GFP} or Bs^{FliC} at 1×10^8 CFU per day from days 0 to 5 post-hatch. The control group received PBS gavage. Data were analyzed using one-way ANOVA. At 28 days of age, no significant differences ($P > 0.05$) in body weight gain (BWG) or feed conversion ratio (FCR) were observed between groups, indicating that genetically modified *Bs* supplementation did not impair production performance. On day 7, 14, 21, and 28 the Bs^{GFP} and Bs^{FliC} groups showed an 9.7 and 5.6 -fold higher ($P < 0.05$), 3.0 and 4.9 -fold higher ($P < 0.05$), 2.7 and 2.4 -fold higher ($P < 0.05$), and 3.3 and 2.8 -fold higher ($P < 0.05$) *B. subtilis* load compared to the control group. These results suggest that the engineered bacteria successfully colonized the broiler gut without affecting key production parameters. As *B. subtilis* can form stress-resistant spores, engineered Bs^{FliC} strains offer a shelf-stable, cost-effective, and feed-stable solution, facilitating their broad use in the poultry industry.

Keywords: Probiotics, Gut health, Broilers, Salmonella, Nanobody

117 Evaluation of a botanical feed additive enriched in polyphenols and saponins in stress-challenged broilers

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Environmental and immunological stressors often impair broiler performance and health in commercial production settings. In this study, a commercial feed additive (Micro-Shield™; DPI Global, Porterville, CA) was evaluated for its effects on growth performance and immune responses. 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 Micro-Shield™/kg diet in the starter phase + 500 mg of Micro-Shield™/kg diet in the grower phase (MSLOW), and 4) 1,000 mg of Micro-Shield™/kg diet in the starter and grower phases (MSHIGH)]. Therefore, 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-MSLOW, 4) BC-MSHIGH, 5) FP-NC, 6) FP-PC, 7) FP-MSLOW, 8) FP-MSHIGH. Treatments raised in battery cages were allotted with 10

birds to each of the 10 replicate cages, and treatments raised in floor pens were allotted with 10 birds to each of the 8 replicate pens. To impose a multiple mild stressors challenge, birds underwent a 12-h feed withdrawal prior to a coccidiosis vaccine (Coccivac-B52) 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 $P < 0.05$. During the grower phase, the FP-NC treatment had the highest ($P < 0.05$) BWG compared with all other treatments. Additionally, NC birds had the highest ($P < 0.05$) FI and FP birds had the lowest ($P < 0.05$) FCR during the grower phase. On 6 DPI, jejunal gene expression of pro-inflammatory cytokines IFN-γ, IL-1β, and IL-10 was highest ($P < 0.05$) in FP-MSHIGH birds compared with all other treatments, which could suggest a more robust immune response in these birds. On 15 DPI, jejunal gene expression of IFN-γ, IL-1β, and IL-10 was higher ($P < 0.05$) in FP birds compared with BC birds. While relative liver weights were higher ($P < 0.05$) in birds exposed to stressors compared with NC birds on 3 and 6 DPI, MSLOW and MSHIGH birds maintained higher ($P < 0.05$) relative liver weights compared with NC and PC birds on 9 DPI, which may indicate the maintenance of an immune response. Overall, these results suggest that Micro-Shield™ may benefit the health of birds experiencing multiple mild stressors. Further, these results could indicate that housing context influences growth performance and immune response outcomes in experimental settings.

Keywords: broiler, coccidiosis, environmental stressors, polyphenols, saponins

118 Evaluation of dietary propolis as feed supplement in broilers coinfected with *Salmonella Typhimurium* and *Eimeria tenella*

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This study aimed to evaluate the effect of dietary propolis supplementation on growth performance, *Salmonella* colonization and immune response in broilers coinfected with *S. Typhimurium* (**ST**) and *Eimeria tenella* (**ET**). A total of 630 one-day-old male Cobb 500 broilers were randomly allocated into six treatments with seven replicates (15 birds per cage) for 28 d. The treatments consisted of non-challenge control (**NC**), ST challenge control (**STC**), and ST and ET coinfection groups comprising different dietary propolis supplementation levels (0, 125, 250, and 500 mg/kg diet). On d 0, all birds in STC and coinfection groups were orally challenged with 0.5 mL of ST suspension (10^9 CFU/mL per bird), and the coinfection groups were orally challenged with 1.0 mL of ET (25,000 sporulated oocysts/mL per bird) on d 7. Mortality and feed intake (**FI**) were monitored daily. Body weight (**BW**), BW gain (**BWG**), and feed conversion ratio (**FCR**) were measured on d 7, 14, 21, and 28. ST loads and prevalence in different organs and gene expression in the cecal tonsil were examined on d 14, 21, and 28. All quantitative data were analyzed by one-way ANOVA with Tukey's post-hoc test, and the orthogonal polynomial contrast was performed in the coinfection groups to evaluate the propolis dose effect. ST prevalence was assessed using Fisher's exact test with Bonferroni adjustment. ST and ET coinfection significantly decreased BWG and FI and increased FCR from d 7 to 28 ($P < 0.05$). However, dietary propolis supplementation dose-dependently improved BWG and FCR in coinfection groups from d 7 to 28 (Linear and quadratic, $P < 0.05$). In addition, dietary propolis linearly reduced cecal ST loads on d 14, 21, and 28 among the coinfection groups ($P < 0.05$). Dietary propolis decreased ST prevalence in the liver and spleen on d 21 and 29 compared to the coinfection control without propolis ($P < 0.05$). On d 14, dietary propolis supplementation levels linearly or quadratically downregulated the expression of TLR4, NF-κB, TNF-α, IL-1β, and IL-10 in the cecal tonsil among the coinfection groups ($P < 0.05$). In addition, dietary propolis decreased TLR2, NF-κB, and IFN-γ expression in the coinfection groups on d 21 in a dose dependent manner (Linear and quadratic, $P < 0.05$). Results of the present study revealed that dietary propolis supplementation improved growth performance, inhibited ST colonization, and modulated NF-κB signaling pathway-related gene expression, exhibiting dose-dependent effects in broilers coinfected with ST and ET. Therefore, the present study suggests that dietary propolis has potential as a dietary intervention against *Salmonella* colonization in broilers under ST and ET coinfection.

Keywords: Coinfection, Salmonella Typhimurium, Eimeria tenella, Propolis, Broiler

119 Dietary Seaweed supplementation partially improves intestinal health of broiler chickens under dexamethasone-induced stress

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Seaweed (SW) has been reported to improve broilers performance and modulate beneficial microbes that could potentially improve gut integrity. Dexamethasone (DEX), a synthetic glucocorticoid, is commonly used as a model to induce intestinal permeability in chickens, which can disrupt gut barrier function. In this study, we evaluated the effects of SW supplementation on performance, intestinal permeability, and the expression of jejunal genes related to gut health in broiler chickens subjected to DEX-induced stress from d 14 to 21 post hatching. A total of 384 chicks were assigned to 3 diets containing graded levels of SW (0%, 1%, and 2%) from d 0 to 14 post hatching. Thereafter, the birds were reallocated within each of the three diets in a randomized complete block design and split into 3 dietary groups with or without DEX, resulting in a 3×2 factorial arrangement. Fluorescein isothiocyanate-dextran (FITC-D) was used as a marker for intestinal permeability. Data from d 1 to 14 were analyzed using the MIXED procedure of SAS. Data from d 14 to 21 were analyzed as a 3×2 factorial using the MIXED procedure to estimate the main effects. ANCOVA was applied to adjust for differences in body weight on d 14. The diets were considered fixed effects, while the block was treated as a random effect. Contrasts were used to estimate the linear and quadratic effects of SW supplementation. Results showed that SW tended to linearly increase ($P = 0.09$) feed intake (FI) and increased ($P < 0.05$) the gain-to-feed ratio (G: F) on d 14. A quadratic effect of SW was observed for body weight gain (BWG) on d 21, with no influence on FI and G: F. Dexamethasone significantly reduced BWG, FI, and G: F ($P < 0.01$). No differences were found in villus height (VH) and crypt depth (CD) in broiler chickens fed the SW diets, whereas DEX reduced both VH and CD ($P < 0.01$). Additionally, DEX increased serum FITC-D concentration, while SW supplementation tended to decrease FITC-D concentration ($P = 0.08$). The mRNA expression of Occludin, Interleukin-6, and Interleukin-1 Beta (IL-1B) were not influenced by SW supplementation, whereas DEX increased the expression of Occludin, Zonula Occludens-1 (ZO-1), IL-1B, and Mucin2. Seaweed supplementation showed a quadratic increase in the mRNA expression of ZO-1, Interleukin-10,

Sodium-Glucose Cotransporter 1, Glucose Transporter 2, Superoxide Dismutase 1, and Glutathione Peroxidase 1. Sodium-Glucose Cotransporter 1 expression was decreased by DEX (P = 0.05). Overall, DEX impaired broilers performance and gut integrity, while the supplementation of SW showed potential protective effects on broiler intestinal health.

Keywords: broiler, dexamethasone, health, Prebiotics

120 Differential effects of direct-fed microbial blends on the mRNA abundance of host defense peptides in broiler chickens during a *Salmonella* Typhimurium challenge

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Salmonella Typhimurium presents a major challenge to the poultry industry as contaminated products can lead to foodborne human salmonellosis. Avian β-defensins (AvBDs) and liver-expressed antimicrobial peptide 2 (LEAP2) are host defense peptides (HDPs) with anti-bacterial activities as part of the innate immune response. This study investigated the effects of supplementing two blends of probiotic strains with prebiotic enhancer to a low-energy diet on the mRNA abundance of HDPs in the cecal tonsils of broilers during a *S.* Typhimurium challenge. Day (d) of hatch Ross 708 male chicks (n=256) were randomly divided into 4 dietary treatment groups (8 birds/cage) and raised for 28 d. Treatments consisted of a standard energy diet (PC; 2,975 kcal/kg ME); a 100 kcal/kg reduced energy diet (NC); and the NC diet supplemented with either blend 1 (EFS) or blend 2 (BAX). All birds were orally inoculated with 1 × 10^7 CFU of nalidixic acid-resistant *S.* Typhimurium (STNR) on d 7. On days 7, 10, and 17, 5 birds per group (20 birds/d) were humanely euthanized to collect the cecal tonsils. RNA was extracted, cDNA was synthesized, and RT-qPCR was performed to determine the mRNA abundance of AvBD8, AvBD12, AvBD13, AvBD14, and LEAP2. mRNA levels were analyzed using 18 S ribosomal subunit as an endogenous control and average mRNA abundance relative to the PC group was calculated using the $2^{-\Delta\Delta Ct}$ method. The data were analyzed using one-way ANOVA and significance ($P \leq 0.05$) between groups was determined by LSD test. Prior to inoculation with *S.* Typhimurium (d 7), BAX and EFS had lower mRNA abundance of AvBD13 ($P = 0.037$) compared to PC. Additionally, BAX showed lower mRNA abundance of AvBD8 ($P = 0.037$), AvBD12 ($P = 0.046$), AvBD14 ($P = 0.033$), and LEAP2 ($P = 0.048$) compared to PC. On d 10, there were no significant differences between groups in the mRNA abundance for any of the targets. While there were no significant differences for EFS or BAX when compared to PC on d 17, AvBD14 ($P = 0.038$) and LEAP2 ($P = 0.024$) mRNA abundances were significantly higher in BAX compared to NC. The supplementation of blend 2 (BAX) to the reduced energy diet decreased the mRNA abundance of HDPs prior to inoculation with *S.* Typhimurium but tended to increase their abundance post-inoculation. These results, coupled with previous findings of reduced STNR colonization in the ceca of BAX birds, highlight the positive influence of these blends on the anti-bacterial activities of HDPs against *S.* Typhimurium. Insight into these host-defense mechanisms could be used to improve pre-harvest mitigation strategies aimed at reducing *Salmonella* loads in broilers and subsequent foodborne cases of human salmonellosis.

Keywords: Salmonella Typhimurium, mRNA, probiotic, broiler, B-defensins

121 Effect of *in-ovo* administration of bovine milk Osteopontin on hatchability, chick quality, performance, and immune system development in hatched chicks

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Osteopontin (OPN), a pleiotropic cytokine, is a glycoprotein known for its involvement in various physiological processes, including tissue development and remodeling. OPN comprises approximately 2.1% of the total protein content in human milk, underscoring its importance in infant growth and development. In the mouse and human intestines, OPN is primarily expressed by innate CD8αα cells, contributing to the maintenance of intraepithelial lymphocytes (IELs) balance by promoting their survival and proliferation, as well as fostering a healthy intestinal microbiome. However, the precise role of OPN in chicken intestinal development has not been investigated compared to other animal species. Our hypothesis is that the *in-ovo* administration of bovine milk-derived OPN (bmOPN) has no adverse effects in newly hatched chicks but enhances their intestinal development and performance. On day 18 of embryonation, 197 fertilized specific-pathogen-free eggs were randomly divided into six groups, where each group was *in-ovo* inoculated with 200 µl of PBS per egg, containing either 0, 0.1, 1, 10, 25, or 50 mg of bmOPN. Another set of eggs was left without any injection. The *in-ovo* injection was repeated 8 times to obtain % hatchability. At hatch, chick quality parameters including navel condition, chick length, and hock condition, were evaluated. Additionally, we measured body weight, residual yolk, and intestinal length on the day of hatch and every week. At day 7 of age, peripheral blood mononuclear cells (PBMCs) and Ileal IELs were isolated and stained for flowcytometry analysis. Data were analyzed using generalized linear mixed model, one-way ANOVA, and Kruskal-Wallis test and level of significance was set at $p<0.05$ followed by Tukey HSD test for mean separation. Our preliminary results indicate that eggs receiving 1 mg of bmOPN had the highest hatchability (95.06%), while the eggs receiving 50 mg of bmOPN had the lowest hatchability (75.84%) ($p<0.05$). Chicks from eggs treated with 50 mg bmOPN displayed increased chick length, improved navel condition, and greater ileal length ($p<0.05$). Likewise, eggs receiving 25 mg of bmOPN had higher body wt. and lower residual yolk wt. ($p<0.05$). No post-hatch mortality or morbidity were observed between groups. Eggs administered with 1 mg bmOPN had no effect on PBMCs, while significantly increased the number of IEL subsets ($p<0.05$). These preliminary findings suggest a potentially beneficial role of bmOPN in egg hatchability and early chick development. Further studies are warranted to explore the potential effects of bmOPN on intestinal development, immunity, bone health, and microbiome composition.

Keywords: Hatchability, Chick Quality, Immunity, Intestine, Osteopontin

122 The effect of different doses of isoquinoline alkaloids (IQs) on the performance of broilers exposed to *clostridium perfringens* challenge

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The current investigation aimed to evaluate the potential benefits of Isoquinoline Alkaloids (IQs)-based phytobiotics to maintain performance and intestinal health in broilers recovering from necrotic enteritis (NE). Different 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*). A total of 360, 0-day-old Ross-308 chicks were randomly allocated to six treatments, each group was further divided into 10 replicates with six birds per replicate. On arrival, all chicks will be confirmed for the absence of *Salmonella* and *C. perfringens*. In a CRBD, the 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. On d 7, all birds except the control group (T1) were orally inoculated with *C. perfringens* as oral gavages using a 10-fold dose of anticoccidial vaccine (Coccivac-D) and a cocktail containing *C. perfringens* at the rate of 4× 10^8 CFU/ml. A one-way ANOVA was used to analyze data applying general linear model approach (version 9.4, SAS Institute Inc., Cary, NC, USA). Fisher's protected test was used when the ANOVA revealed significant differences at $P<0.05$. The data revealed that during the cumulative period (0-35 d), the -ve control (T1) had a similar cumulative average daily gain (ADG) to all other treatments except T2. Chicks that were subjected to the bacterial challenge without any additive T2 (+ve control) had the lowest ADG ($P<0.001$). The unchallenged group (-ve control) and challenged groups with Maxus (T3) or IQ (T4, T5, and T6) had better FCR and performance efficiency factors (PEF) when compared to the +ve control groups (T2) ($P<0.001$) regardless of the dose of IQ. Villous height VH, crypt depth (CD), and VH: CD ratio improved in IQ-supplemented groups like the -ve control group regardless of the dose. Pathogenic bacteria, *C. Perfringens* were reduced significantly, and probiotic bacteria, *Lactobacillus*, significantly improved because of IQ supplementation. Based on all previous observations, it can be concluded that the lower dose of IQ can be used as an alternative to AGP without a negative effect on FCR.

Keywords: Phytobiotics, Isoquinoline Alkaloids (IQs), Broilers Performance, Gut health

123 Effects of chromium propionate supplementation during stress events on turkey performance

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Turkey production involves multiple stress-inducing events that negatively impact growth performance and overall production efficiency. Chromium, an essential trace element, plays a crucial role in glucose metabolism, insulin sensitivity, and stress reduction which can help mitigate the impacts of stress during production of turkeys. This study evaluated the effects of dietary chromium propionate supplementation (KemTRACE® Chromium, Kemin Industries, Inc., Des Moines, IA) on growth performance, mortality, and kilograms of body weight (BW) per square meter (kg/m2) in turkeys over a 19-week period. Poults (Nicholas Select, Aviagen Turkeys, Inc., East Lewisburg, WV) were assigned to either a control diet (CON) or a chromium propionate supplemented diet (KemTRACE Chromium 0.04%; CHR) at 0.5 kg of ton (200 ppb chromium inclusion) from placement through 19 weeks. Each dietary treatment had 8 replicate pens in the brooder and 16 replicate pens in the grower. Body weight was recorded at multiple time points (d 0, 35, 56, 77, 98, 119, and 133), along with daily mortality. Data were analyzed using a mixed linear model using PROC Glimmix in SAS (SAS Institute Inc., Cary, NC) with dietary treatment (CON or CHR) fit as a fixed effect. Turkeys fed diets supplemented with CHR were heavier at 35d ($P < 0.03$), 98d ($P < 0.01$), and 119d ($P < 0.05$). It should be noted that from d 98 to d 133, turkeys experienced acute thermal stress. It is also during this same period, when turkey mortality began to rise for birds fed the unsupplemented feed (d98 ($P = 0.13$), d119 ($P < 0.01$), and d133 ($P < 0.02$)). Due to this increase in mortality, BW kg/m2 was examined at the conclusion of the study. Turkeys fed CHR supplemented feed had a 5.9 kg/m2 greater yield ($P < 0.010$). Results from this study suggest CHR supplementation may improve livability of grower turkeys, particularly when stressful events, such as heat associated with summer, occur.

Keywords: Turkey, Chromium

124 Colonization efficacy and safety of a novel divalent anti-flagellin C nanobody-secreting *Bacillus subtilis* in broiler birds

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Flagellin-C (FliC) is a virulence factor of Salmonella that mediates attachment and colonization in the chicken gut. Genetic engineering of a spore-forming probiotic, namely *Bacillus subtilis*, to secrete a divalent form of anti-FliC (biFliC) offers a potential tool to control salmonellosis in chickens. This study evaluates the colonization efficacy and safety of orally administered bivalent anti-Flagellin C nanobody-secreting *B. subtilis* (Bs^{biFliC}) in broilers. Genetic code of Abi-Se07, a potent neutralizing nanobody against FliC, was genetically fused as two copies with a flexible peptide linker (3×GSSSS) to create a bivalent anti-FliC strain (Bs^{biFliC}). The biFliC construct was inserted into the *AmyE* locus of *B. subtilis* using the NBP3510 promoter and a chloramphenicol resistance selection marker. A *B. subtilis* strain (Bs^{GFP}) engineered to express green fluorescent protein (GFP) served as a control. The bivalent anti-FliC nanobody exhibited ~100-fold higher binding affinity to FliC antigen than the monovalent form. Additionally, in a functional assay quantifying inhibition of bacterial motility, the bivalent nanobody from Bs^{biFliC} supernatant showed ~100-fold greater efficacy than the monovalent form. A total of 24 one-day-old Cobb 500 chicks were randomly allocated into three treatment groups: 1) Basal diet, 2. Basal diet + Bs^{GFP}, and 3) Basal diet + Bs^{biFliC}, with eight replicates per group. Bs^{biFliC} and Bs^{GFP} spores were orally gavaged from days 1 to 5 post-hatch at 1×10^8 CFU/bird. The control group received PBS gavage. Production performance was assessed on day 21, and cecal B. subtilis loads were analyzed using qPCR on days 7 and 21. Data was analyzed using one-way ANOVA. On day 21, no significant differences in body weight gain (BWG) or feed conversion ratio (FCR) were observed between treatment groups ($P > 0.05$). On days 7 and 21, cecal *B. subtilis* loads of Bs^{GFP} and Bs^{biFliC} group were 1.4 and 3.3-fold higher and 2.4 and 3.05-fold higher than the

control group, respectively (P > 0.05). It can be concluded that *Bs*^{biFliC} successfully colonized the broiler gut and had no adverse effects on production performance.

Keywords: Nanobody, Bacillus, Genetic engineering, Broilers, Production

125 Spleen metabolome reveals immune modulatory mechanisms induced by onion peel extract in *Salmonella*-challenged broiler chicks

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The spleen is a secondary lymphoid organ that is involved in initiating immune response to pathogens. Onion peel extract (**OPE**) is rich in polyphenolic compounds known to enhance antimicrobial efficacy and modulate immune mechanisms. *Salmonella* infection in young chickens is associated with severe morbidity and impaired growth rate, and consequent gastroenteritis in humans that consume contaminated poultry meat. The objective of this study was to investigate the effect of dietary OPE on the spleen metabolome in broiler chicks infected with *Salmonella* Enteritidis (**SE**). Day-old chicks (Ross 708 male; n = 128) were randomly assigned to 4 treatments, each consisting of 4 replicate pens (8 chicks / pen). The treatments included **CON** (no SE challenge), **CON-SE** (SE-challenged), **OPE** (OPE at 6 g/kg diet, but no SE challenge) and **OPE-SE** (OPE at 6 g/kg diet with SE challenge). At 2 d of age, chicks in CON and OPE treatments were orally gavaged with sterile tryptic soy broth, while those in CON-SE and OPE-SE were gavaged with 2.25 x 10^8 CFU SE/mL. At 5 and 12 d post infection (**dpi**), 6 chicks/treatment were euthanized, and spleen samples were aseptically collected and subjected to metabolomic analysis by high performance liquid chromatography (**HPLC-MS**). Data analysis was performed using MetaboAnalyst 6.0 and volcano plot analyses between CON and each of the CON-SE, OPE, and OPE-SE. Results revealed several differentially (P<0.05 and absolute fold change ≥2.0) abundant metabolites. Partial least square discriminant analysis showed distinct metabolites profiles. In comparison to CON, energy generating metabolites (6-phosphogluconic acid, methylmalonic acid) were upregulated (P<0.05), whereas bacteriostatic and bactericidal (2-(1H-Benzimidazol-2-yl)-3-(5-nitro-2-furyl) acrylonitrile) and anti-inflammatory (13,14-dihydro-15-keto-prostagladin D2) metabolites were downregulated (P<0.05) in CON-SE on 5 dpi. Also, by 12 dpi kynurenic acid (an endogenous metabolite of tryptophan metabolism) was downregulated (P<0.05). Notably, in OPE and OPE-SE, immune modulating and antioxidant enhancing metabolites (L-Val-Gly, Leu-Gly, Gly-Gly-Leu and Asn-Leu) were upregulated (P<0.05) on 5 dpi, while energy (ATP) producing metabolites (3,6-di-O-methyl-beta-D-glucose and 3-O-beta-D-galactosyl-sn-glycerol) were downregulated (P<0.05). Similarly, the energy (ATP) producing metabolites and S-beta-D-glucosyl-L-cysteine (glucose metabolism intermediate) were downregulated (P<0.05) on 12 dpi. It was concluded that OPE modulated metabolic pathways including tryptophan metabolism and biotin metabolism suggesting immune activation, reduced oxidative stress and inflammation, unlike CON-SE with unregulated immune response to *Salmonella* infection.

Keywords: Spleen metabolome, Dietary onion peel extract, Broiler chicks, Salmonella Enteritidis, Immune response

126 Not Presented

127 Impact of amino acid levels during rearing on feed intake and body composition

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The objective of this study was to determine the impact of diets containing various amino acid (AA) levels on feed intake and body composition in two laying hen strains during the rearing phase. The experiment evaluated six isocaloric diets containing varying levels of recommended dietary balanced AA levels relative to digestible Lysine (dLys): 60, 70, 80, 90, 100 (control), and 110%, based on the ideal protein concept. Two choice treatments enabled birds to select from either 60, 80, and 100% (6810), or 70, 90, and 110% (7911) diets. Bovans Brown and Babcock White pullets (n=128; 8 birds per strain × dose level) were reared in floor pens and fed with multi-feeder precision feeding stations that allocated the assigned diet to each bird and measured individual feed intake, thus each bird was an experimental unit. Treatments were applied from 0 to 18 weeks of age, after which all birds received common commercial layer diets until the end of trial at 30 weeks of age. Data collection included feed intake and body composition via dual energy x-ray absorptiometry every four weeks from 12 to 30 weeks of age. All birds were euthanized at 30 weeks of age and weights of *Pectoralis major, minor*, liver, and abdominal fat weight were recorded. The effect of AA level, strain, age, and their interactions were analyzed using ANOVA. Tukey's multiple range test was used to separate means. Overall, feed intake and body fat increased with age ($P < 0.05$). Brown pullets consumed more feed than White pullets after 16 weeks of age (average 89.8 vs 76.5 g/bird/d, $P < 0.001$), and had greater absolute *Pectoralis major* and liver weights at 30 weeks of age (164.7 vs 153.9 g, $P = 0.004$; 39.9 vs 36.2 g, $P = 0.007$). White pullets had greater body fat level than Brown pullets at 12, 16, and 28 weeks of age (9.4 vs 7.6 %, 11.2 vs 9.2 %, and 15.2 vs 13.2 %, respectively, $P < 0.05$), consistent with relative abdominal fat pad at 30 weeks of age (3.19 vs 2.66 % live BW, $P = 0.001$). Within strain, AA levels did not affect body fat level in the White pullets. Brown pullets fed 60% AA levels had greater body fat level than those fed 70, 90, and 100% AA levels (13.3 vs 10.3, 10.5, and 10.9 %, respectively, $P < 0.001$) despite no differences in feed intake. In summary, body fat level of Brown pullets was more influenced by AA levels than White pullets, which points to intrinsic metabolic differences between strains.

Keywords: laying hens, dietary balanced amino acids, body composition, precision feeding, dual energy x-ray absorptiometry

128 Evaluating a growth model to predict amino acid requirements for commercial turkey toms with and without performance enhancing feed additives

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Estimating amino acid requirements by empirical dose-response methods is resource intensive and often do not include performance enhancing feed additives. We hypothesized that a feeding program of dietary amino acid specifications generated by a recently developed mechanistic growth model would outperform the 1994 NRC (NRC) recommendations, and that additives would enhance growth performance under various management conditions. Nicholas Select toms from the same hatch were raised in two housing locations and assigned to a 2x2 factorial arrangement of two feeding program specifications (Model vs. NRC), with or without feed additives (phytobiotics, enzymes, yeast, etc.). Both locations placed birds at 0.374m2/bird density (site 1: 2,976 poults in 32 pens (93/pen); site 2: 2,176 poults in 32 pens (68/pen)), and used feeding phases (days 0–21, 22–42, 43–63, 64–91, 92–111, and 112+). At 35 d poults were moved to the finishing barn. At 140 d market age, 30 birds per treatment per location were evaluated for carcass parts yield. The NRC feeding program included 1.6%, 1.6%, 1.5%, 1.3%, 1.0%, and 0.8% total lysine, and 2800, 2900, 3000, 3100, and 3200 kcal/kg ME. The Model feeding program was estimated using a modified Gompertz-Richards model. Lysine and FI specifications (with fixed ME consistent with modern feeding programs) were generated from simulating current large white turkey target weights. This provided 1.75%, 1.41%, 1.23%, 1.09%, 1.05%, and 1.0% digestible lysine and 3000, 3085, 3200, 3325, 3400, and 3450 kcal/kg of ME. Production metrics were analyzed using a mixed model (PROC GLIMMIX, SAS), with location, nutrition, additive, phase and all their interactions as fixed effects, and pen as a random effect across phases. Location effects were significant: best FCR at site 1 ($P < 0.0001$) and best weight gain at site 2 ($P < 0.0001$). The Model feeding program outperformed the NRC with .6% heavier BW and significantly lower FCR ($P<0.05$). Significant nutrition by additive interaction showed that additives reduced breast meat yield by 1% ($P=0.017$) on Model nutrition, with no effect on NRC. Significant nutrition by additive interactions affected FCR ($P=0.01$), and feed cost ($P<0.05$) and lysine conversion ($P=0.055$), with Model nutrition showing improved outcomes. Additive effects varied by nutritional program, showing benefits with Model diets but neutral or negative effects with NRC diets. This study supports the use of a growth simulation model for estimating feeding program specifications in modern turkey toms, showing advantages in BW, FCR and feed cost as compared to the 1994 NRC standards. Future research should explore site-specific practices, and additive optimization for enhanced performance.

Keywords: Turkey, Growth Model, Amino Acid Requirements, Additives, Management

129 Influence of dietary protein reduction with feed-grade amino acids on performance, body parts weights, and liver transcriptome of broilers

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This study evaluated the effects of dietary CP reduction through feed-grade amino acid incorporation on the performance, body parts weights, and liver transcriptome 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, all cages were standardized to 4 birds/cage with uniform body weights and were 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, whereas the low (19%) CP diet contained Met, Lys, Thr, Val, Ile, Arg, Trp, Gly, His, and Phe to meet minimum specifications for these amino acids. Feed intake, body weight, and feed conversion ratio were determined at 14, 28, and 35 d. On d 35, liver tissue was collected from 1 bird/cage from the high and low CP treatments for transcriptomics analysis (Illumina, poly-A enriched, 150PE, 20 M reads/sample). On d 36, the weight and yield of body parts (liver, fat pad, *pectoralis major* and *minor*) were determined on 2 birds/cage from all treatments. Performance and parts data were analyzed by one-way ANOVA and means were separated using a Tukey's test. Linear and quadratic contrasts were also performed. Statistical significance was set at $P \leq 0.05$. For transcriptomics data, the DeSeq2 package of R was used to assess changes in gene expression with differentially expressed genes (DEG). There were 312 DEG ($P<0.05$) included in the Quantitative Pathway Activation algorithm (BIOFRACTAL, Portugal) to obtain pathway activation scores. No differences in performance ($P > 0.05$) were observed during the experimental period from 14 to 35 d of age. At 36 d, a linear increase ($P = 0.051$) in fat pad weight was observed as CP concentration decreased, but no differences were observed ($P > 0.05$) for weights and yields of the other body parts. Pathway activation indicated changes in energy and carbohydrate metabolism with the low CP diet, including activation of glycogen and fatty-acid metabolism. Additionally, pathways associated with oxidative stress were activated and innate immune response was inhibited in birds fed the low CP diet compared to those fed the high CP diet. These results indicate that feeding broilers reduced CP diets through the inclusion of additional feed-grade amino acids can influence fat deposition even when growth performance and breast meat yield are maintained. Ongoing analyses of dietary ME and body composition of birds from this trial will provide further insights into the relationship between hepatic nutrient metabolism and overall energy balance when broilers are fed reduced CP diets.

Keywords: crude protein, amino acids, broilers, performance, gene expression

130 Assessment of the interactive effect of dietary fat and feedstuff types on their standardized ileal amino acid digestibility and the morphology of small intestine in broiler chickens

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Apparent ileal digestibility (AID) of amino acids (AA) is usually corrected for basal endogenous amino acid losses (BEL) to yield standardized values (SID) because the latter is preferred due to its additivity in diet formulation. Fat digestibility depends on the physical and chemical properties of fat sources, which influence the digestibility of other dietary components. This complicates the comparison of the results of SID assays using animal or plant fats. We previously reported that BEL was not significantly different for broiler chickens that received nitrogen-free diets in which tallow or soybean oil was used, but it is unclear if the same will hold in SID determination. A total of 320 male broiler chicks were allocated to 64 cages with four treatments (2 × 2 factorial) and eight replicates per treatment to study the influence of fat types (soybean oil added at 15g/kg) or (tallow added at 20g/kg) on SID of AA of two feedstuffs varying in AA profile (corn and SBM) and on the morphology of small intestine. All the birds were fed a basal corn-soybean meal diet for 16 d and experimental diets from d 16 to 21. On day 21, all the birds were euthanized, and the digesta were collected from the distal ileum to calculate the AA digestibility. Sections of the jejunum and ileum were collected to measure villi height (VH), crypt depth (CD), and their ratio (VH: CD). Data were analyzed as a two-way ANOVA using JMP, and significance was set at $P < 0.05$. There were no feedstuff × fat-type interactions for any of the responses. The coefficients of SID were significantly greater ($P < 0.01$) for SBM than for corn, except for Leu, Met, Ala, Cys, and Pro. Although not significantly different, except for Ala, the SID values were numerically greater for birds receiving semi-purified diets with soybean oil as the fat type. The jejunal and ileal VH and jejunal VH: CD were significantly greater ($P < 0.01$) for the birds that received the semi-purified SBM-based assay diets. Our studies on fat-type effects on BEL and SID consistently showed numerically higher values for birds that received soybean oil as dietary fat. However, these differences reflected a minimal, non-statistically significant effect of fat type within the inclusion levels used in the current experiment. In conclusion, using soybean oil or tallow as fats in the diets had only marginal effects on BEL and SID of AA, and the morphological characteristics of the small intestine in the experiment were protein feedstuff- rather than fat type-dependent. Therefore, comparing SID values determined with assay diets using these fat types should not lead to erroneous conclusions.

Keywords: Standardized digestibility, basal endogenous losses, gut morphology, soybean oil, tallow

131 Standardized ileal digestibility of amino acids in differently processed sunflower meals fed to broiler chickens

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Sunflower meal (SFM) is a climate-resilient protein source; however, its high fiber content limits its use in poultry diets by reducing crude protein (CP) and amino acid (AA) digestibility. This study evaluated the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA in four processed SFM products: completely dehulled cold-pressed (SFM_CP), solvent-extracted (SFM_SE), high-protein electrostatically separated (SFM_SEP), and its high-fiber, low-protein co-product (SFM_SEF). Each ingredient was added at 40% in a cornstarch-based diet, ensuring uniform composition so that digestibility differences reflected only the protein source. A nitrogen-free diet (NFD) was also tested. Titanium dioxide was added as an indigestible marker. A total of 240 one-day-old Ross 708 broilers were assigned to five treatments (SFM_CP, SFM_SE, SFM_SEP, SFM_SEF, and NFD) after 14 days on a common starter diet (n=8; six birds/replicate). Birds were fed the experimental diets for seven days before being euthanized for ileal digesta collection. Samples were analyzed for CP, AA, and Ti content. Data were analyzed using Proc GLIMIXX (SAS), with protein sources as fixed factors and digestible CP or AA as dependent variables. Among the ingredients, SFM_CP had the highest CP content (524.4), followed by SFM_SEP (484.2), SFM_SE (393.5), and SFM_SEF (228.4) g/kg DM. Compared to SFM_SE, dietary fiber was reduced by 46.0% in SFM_CP and 28.9% in SFM_SEP, while it increased by

44% in SFM_SEF. The feed intake was highest (P < 0.05) in SFM_CP and SFM_SEP and lowest in SFM_SEF compared to SFM_SE (P <0.001). Compared to SFM_SE, the AID of lysine, and methionine increased (P<0.05) by 9.1%, and 1.3%, in SFM_CP, respectively, in SFM_SEP by 4.7%, and 1.4%, respectively. In contrast, SFM_SEF had the lowest AID values. The SID of CP and lysine in SFM_CP was higher (P<0.001) than for SFM_SE but similar (P>0.05) to SFM_SEP products. The SFM_CP had the highest SID of histidine (P <0.05). Compared to SFM_SE, among indispensable AA (g/kg, DM), the SID of lysine increased from 11.9 to 17.3 in SFM_CP and 14.9 in SFM_SEP, while methionine increased from 10.4 to 14.6 and 12.7 in SFM_CP and SFM_SEP, respectively. The mean SID of indispensable AA increased from 16.5 to 23.3 in SFM_CP and 20.6 in SFM_SEP, and total AA digestibility increased from 19.5 to 27.9 and 24.6 in SFM_CP and SFM_SEP, respectively (P <0.05). Overall, complete dehulling with cold-pressing of oil (SFM_CP) and electrostatic separation of crude fiber (SFM_SEP) improves the CP and AA concentration. These products can act as an alternative to conventional protein sources in broiler diets. Further research should explore their effects on broiler growth performance.

Keywords: high protein sunflower meal, broiler chickens, processing, amino acid digestibility, Alternative protein sources

132 The effects of Arginine, Threonine, and Valine supplemented throughout a 20-day starter phase on broilers challenged with coccidiosis

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An experiment was conducted to evaluate the effects of amino acid (AA) supplementation on broiler performance and intestinal health during a 20-day growout following administration of a coccidia vaccine containing Eimeria acervulina (EA), E.tenella (ET) and E.maxima (EM). A corn-soy diet was used for 7 treatments (trts) with 4 trts containing increases in individual AA ratios (threonine,valine and arginine) via free AA inclusion. The negative control (NC) contained Salinomycin (60g/ton) and was void of coccidia vaccine. Positive control 1 (PC1) birds received a 1X dose of coccidia vaccine via spray cabinet on d0 and positive control 2 (PC2) birds received a 10X dose on d8 via oral gavage. Both PC diets were non-medicated and had standard AA levels (69% dThr:dLys, 79%dVal:dLys, and 107% dArg:dLys). Four trts had a 5% increase in dArg:dLys ratio (Arg), a 3% increase in dThr:dLys ratio (Thr), a 5% increase in dVal:dLys ratio (Val), and a trt with an increase in all 3 AA (A+T+V). On d8, birds fed PC2 and increased AA diets were given 0.5ml of 10X coccidia vaccine via oral gavage. A total of 1,848 day-old Ross 708 x YPM males were placed in 12 replicate floor pens (22 birds/pen; 0.076 m2/bird). Average body weight (BW), BW gain (BWG), feed intake/bird (FI), and feed conversion ratio (FCR) were measured on d0, 8, and 20. Samples were collected from the jejunum for histological measurements on d18. Additionally, 3 birds/pen were scored for macroscopic lesions of EA, EM, and ET. Data was analyzed using PROC GLM in SAS 9.4, with 2-way ANOVA and Tukey's test for means separation (P≤0.05). NC (d9-20) had higher BWG (P=0.0009) compared to PC2, Arg, or A+T+V, with other trts performing similarly. Comparably, d9-20 and overall NC (P=0.0071;P=0.0051) had the lowest FCR compared to PC2 and Val, with other trts intermediate. Birds fed NC had higher d20 BW and d0-20 BWG (P=0.0050;P=0.0009) compared to Arg or A+T+V, while other trts showed similar results. PC2 had the highest average EA lesions (<0.0001) compared to A+T+V and the NC with other trts performing similarly. Although not statistically significant, the addition of Thr and Arg reduced FCR by 4 and 2 points, respectively, compared to PC2, with both showing performance similar to NC diet in the mean groupings. Histology confirmed Arg increased crypt depth (P=0.0409) compared to NC, with other trts. showing intermediate effects. In conclusion, birds fed the NC showed improved BW, BWG, and FCR; however, Thr and Arg trts had similar performance compared to the PC groups, suggesting potential for improving resilience to coccidia challenges. Further exploration of these AA inclusion levels could offer practical benefits in managing poultry health and performance.

Keywords: amino acids, intestinal health, broilers, growth performance, coccidia

133 Effects of different leucine levels with *Eimeria* challenge on growth performance and intestinal health in broilers

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Poultry performance can be influenced by a variety of factors including the amino acid content of the diet. Branched-chain amino acids (**BCAAs**; leucine, valine, and isoleucine) play an important role in protein synthesis, muscle growth, and immunity. Leucine (**Leu**) has been proven to influence immune response and muscle growth. This study aimed to evaluate the effects of different Leu levels on growth performance and intestinal health of *Eimeria*-challenged broilers. A total of 640 thirteen-day-old male Cobb 500 broilers were allocated in a 2 × 4 factorial arrangement (four diets, each with or without *Eimeria* challenge), into eight treatments, each with eight replicates. There were four different Leu to lysine (Leu:Lys) ratio corn-soybean meal-based diets: 1) 130% Leu:Lys ratio (L130); 2) 150% Leu:Lys ratio (L150); 3) 170% Leu:Lys ratio (L170); and 4) 190% Leu:Lys ratio, (L190), which were fed to the birds from d 13 to 26. On d 13, the challenged groups were orally inoculated with mixed sporulated *Eimeria* spp. (25,000 *E. acervulina* oocysts, 10,000 *E. maxima* oocysts, and 10,000 *E. tenella* oocysts). From d 13 to 26, feed intake was recorded daily, and body weight were recorded on d 20 and 26. The data were analyzed using orthogonal polynomial contrasts to evaluate linear and quadratic responses, and a two-way ANOVA was performed. Means were separated using Tukey's HSD test if *P*-value of ANOVA less than 0.05. A significant interaction ($P < 0.05$) between dietary leucine levels and *Eimeria* challenge was observed for body weight gain (BWG) during d 13-26. During d 21-26, increasing dietary leucine levels resulted in a significant linear decrease ($P < 0.05$) in body weight gain (BWG) and feed efficiency. The L190 group had a negative effect on overall BWG and FCR compared to the other Leu groups. The challenged groups showed significantly lower BWG, and feed efficiency compared to the non-challenged (**NC**) groups ($P < 0.001$) from d 13-26. During the acute challenge period (d 13-20), the challenged chickens reduced feed intake (**FI**) compared to the NC ($P < 0.001$). In the recovery phase (d 21-26), the chickens fed the L190 diet had lower BWG than L130, L150, or L170. The challenged groups showed increased intestinal permeability

compared to the NC ($P < 0.001$). However, there were no significant differences in intestinal permeability among the Leu groups. Coccidiosis lesions in the duodenum, jejunum, and ceca were reduced in L150 than the L130, L170 and L190. In conclusion, the *Eimeria* challenge shows detrimental effects on growth performance and gut health, and different Leu levels further influence growth performance and intestinal health in broiler chickens under coccidiosis conditions.

Keywords: branched-chain amino acid, Leucine, coccidiosis, growth performance, intestinal health

134 The inclusion rate of soybean meal and valine supplementation affect growth performance, carcass weight, body composition, and feed intake regulation in Ross 708

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Changing the soybean meal (**SBM**) content in broiler diets to promote efficient growth is likely to alter the proportion of non-bound amino acids (**AAs**) and leucine, potentially leading to negative consequences such as reduced feed intake (**FI**) and feed efficiency. Supplementing with additional valine may help address imbalances in branched-chain AAs and mitigate reduced feed consumption. This study employed a 3 × 2 factorial arrangement, consisting of two main effects: SBM inclusion rate and valine supplementation. A total of 960 zero-d-old male Ross 708 broilers were assigned to six treatments with eight replicates each. The six dietary groups were as follows: (1) low crude protein (**LCP**; reduced SBM inclusion rates, -1.5% CP levels compared to the medium CP group); (2) medium CP (**MCP**; 22.5/20.5/19.0% CP for starter/grower/finisher phases, respectively); (3) high CP (**HCP**; increased SBM inclusion rates, +1.5% CP levels compared to the MCP); (4) 150% valine added to the LCP (**LCP+V**); (5) 150% valine added to the MCP (**MCP+V**); and (6) 150% valine added to the HCP (**HCP+V**). The levels of all indispensable AAs in the LCP and HCP groups were kept the same as in the MCP. The digestible valine:lysine ratio for the LCP, MCP, and HCP groups was set at 0.76. All data were statistically analyzed using a 2-way ANOVA, with Student's t-test or Tukey's HSD test applied if $P < 0.05$. Interactions were observed in the grower phase (d 15-28) body weight gain (**BWG**) and FI, with the LCP+V group showing the lowest BWG among all groups ($P < 0.05$). The MCP groups had the highest BWG and FI in both the finisher and overall phases compared to other groups ($P < 0.01$). The LCP groups showed significantly lower BWG, FI, feed efficiency, chilled carcass weight, total tissue weight, lean weight, and lean percentage compared to the MCP groups ($P < 0.01$). However, the LCP groups had significantly higher abdominal fat pad weight and fat percentage compared to the MCP and HCP groups ($P < 0.001$). During the finisher and overall phases (d 29-42 and d 0-42), the 150% valine groups had significantly lower BWG and FI compared to the 100% valine groups ($P < 0.05$). In addition, the 150% valine groups had significantly lower carcass weight, litter ammonia levels, and footpad dermatitis scores compared to the 100% valine groups ($P < 0.05$). The 150% valine groups also significantly upregulated hypothalamic appetite-related gene expression levels compared to the 100% valine groups ($P < 0.05$). In conclusion, using appropriate SBM levels without altering dietary CP level showed optimal growth performance in broilers. Additional valine reduced growth performance, likely due to reduced feed consumption and changes in appetite-related gene expression levels.

Keywords: Soybean meal, Feed intake, Valine, Carcass weight, Hypothalamus

135 Interactive effects of branched-chain amino acids (Leucine, Isoleucine and Valine) on performance in peak Hy-Line W-36 hens

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Branched-chain amino acids (BCAAs), including isoleucine, leucine, and valine, play a crucial role in the health and productivity of laying hens. The objective of this study was to investigate the relationship among BCAA on the performance of Hy-Line W-36-laying hens during peak production. For the peak phase, a total of 800 hens were housed in 200 conventional A-frame cages according to a 2^3 full factorial circumscribed central composite design (CCD) with 20 treatments (8 factorial, 6 star, and 6 center points). The experiment was conducted from 20 to 35 weeks of age. Each treatment consisted of varying dVal:Lys (64 to 104), dIle:Lys (55 to 95), and dLeu:Lys (120 to 220) ratios; dLys was formulated at 0.80% per the Hy-Line W-36 management guide. Eggs were collected daily to determine hen-day egg production (HDEP). Feed and eggs were weighed weekly to assess feed intake (FI), feed conversion ratio (FCR), and hen-day egg mass (HDEM), while birds were weighed at 20 and 35 weeks of age to determine body weight (BW). Data were analyzed as a CCD using the surface response option of JMP v. 18, and means were considered significant at $P < 0.05$. Feed intake was significantly increased (103.63g/bird/day; $P = 0.0122$; $R^2 = 0.80$) at moderate levels of Val:Lys (87) and Ile:Lys (75) and the lowest level of Leu:Lys (120) ratios. However, when Val:Lys was lowered keeping Ile:Lys and Leu:Lys constant, FI decreased. Hen-day egg production was significantly increased (91.8%; $P = 0.0050$; $R^2 = 0.70$) at high ratios of Val:Lys (91), moderate levels of Ile:Lys (75) and Leu:Lys (184). As Val:Lys and Ile:Lys decreased HDEP also decreased. Similarly, HDEM was significantly increased (51.84 g; $P = 0.0030$; $R^2 = 0.85$) at moderate levels of Val:Lys (84) and higher levels of Ile:Lys (90) and the lowest Leu:Lys (120) ratios. Egg weight was also significantly increased (57.77 g; $P < 0.0001$; $R^2 = 0.97$) at high levels of Val:Lys (93) and Ile:Lys (91) and low Leu:Lys (120) ratios. Body weight significantly increased (1668.02 g; $P = 0.0275$; $R^2 = 0.76$) at a moderate Val:Lys (86) ratio, high levels of Ile:Lys (80) and low Leu:Lys (120) ratios. A reduction in BW was observed as Val:Lys decreased in the diets. Both BW and FI were negatively influenced as Val:Lys ratio decreased in the diet; additionally, when both Val:Lys and Ile:Lys decreased, HDEP, HDEM, and egg weight were negatively influenced. These data demonstrate that adjusting the Val:Lys and Ile:Lys ratios accordingly can help to maximize performance in peaking hens, particularly when feeding diets with increased Leu:Lys, common in corn and soybean-based diets.

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

136 Effects of varying levels of dietary tryptophan and leucine on performance, gut health, and plasma serotonin levels in broilers challenged with mixed *Eimeria spp*

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Supplementation of amino acids is one of the nutritional strategies to mitigate the detrimental effects caused by coccidiosis in broilers. Tryptophan (Trp) is an essential amino acid & a precursor of serotonin, which influences feed intake, gut integrity, and immune response. Leucine (Leu), a branched-chain amino acid, interferes with Trp metabolism. A study was conducted to investigate the effects of different levels of Trp and Leu on growth performance, gut integrity, and bone health in broilers with/without *Eimeria* challenge. A total of 900 one-day-old Cobb500 broiler chicks were allocated into a 3x2x2 factorial arrangement, with each treatment replicated five times (n=5). The birds were fed six grower diets from d14 to 28: Trp deficient, 80% (T_d), Trp control, 100% (T_c) & Trp excess, 150% (T_e) of the actual requirement in combination with two leucine: lysine levels, 1.42 (moderate, L_m) & 1.63 (high, L_h) with © and without (NC) *Eimeria* challenge. The birds were inoculated with mixed *Eimeria* solution containing 25,000 *E. maxima*, 25,000 *E. tenella*, and 1,25,000 *E. acervulina* oocysts per mL by oral gavage on day 14. The birds were sampled on 6 & 14 days post inoculation (dpi). The data were analyzed by three-way ANOVA, and the means were separated by Tukey HSD and Student's t-test (a = 0.05). Intestinal lesion scoring was analyzed using the non-parametric Kruskal-Wallis test (a = 0.05). On 6 & 14 dpi, Body weight gain (BWG) & average daily feed intake (ADFI) results showed an interaction between Trp levels and challenge. The T_eNC showed significantly higher BWG and ADFI than the other groups (P<0.05). On 14 dpi, Trp & Leu levels showed an interaction where the T_eL_m had significantly higher BWG (P<0.05) compared to T_dL_m, whereas the T_dL_m group had a poorer feed conversion efficiency (P<0.05) compared to all the treatment groups. Body composition was measured using dual-energy x-ray absorptiometry (DEXA). On 6 & 14 dpi, the bone mineral density (BMD) and bone mineral content (BMC) were unaffected by any dietary treatments but significantly decreased by the *Eimeria* challenge (P<0.05). However, the T_e showed a numerically higher BMD & BMC than T_c. The T_e showed significantly lower lesions in the duodenum region than in the T_d & T_c groups (P<0.05). On 6 dpi, plasma serotonin had a significant interaction effect with the highest and lowest levels in the T_eL_mNC and T_dL_hC group, respectively (P<0.05). In conclusion, the T_e and L_m levels improved performance, BMD, BMC, and plasma serotonin levels, and reduced intestinal lesions. Therefore, optimizing the dietary Trp and Leu levels is important to minimize the negative effects of coccidiosis and maintain the performance and gut health of broilers.

Keywords: Tryptophan, Leucine, Serotonin, Gut health, Broilers

137 Exploring optimal digestible histidine to lysine ratio for Ross -708 male broilers in finisher phase (d 24 – 42)

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Histidine is an indispensable amino acid and can be limiting when crude protein reduction in diets is achieved with the inclusion of feed-graded amino acids. Thus, this experiment was aimed to understand the optimum digestible Histidine:Lysine (dHis:dLys) ratio for Ross-708 male broiler performance during the finisher phase (24 – 42 d). Ross-708 male broilers were raised in an environmentally controlled house under standard conditions and fed a common starter (0-10 d) and grower (11-23 d) diet as per 2022 Ross-708 broiler nutrition specifications. On 24 d, 1,323 broilers were redistributed on a body weight basis in 63 pens (7 treatments x 9 replicates x 21 birds) and fed experimental finisher diets until d 42. A basal His deficient diet with a dHis:dLys ratio of 28% was formulated using protein-containing dietary ingredients (corn, wheat, soybean meal, meat and bone meal, and peanut meal) and feed-graded amino acids (L-lysine, DL-methionine, L-threonine, L-arginine, L-isoleucine, L-valine, L-phenylalanine, L-leucine, glycine, L-tryptophan, L-alanine and L-glutamic acid). Later, L-histidine-HCl was added at the expense of an inert filler to produce diets with targeted ratios (32, 36, 40, 44, 48, and 52%). At d 42, body weight (BW) was recorded, and body weight gain (BWG), feed intake (FI), and mortality-corrected FCR were calculated. Data were analyzed using one-way ANOVA with Tukey's HSD for mean separation ($P < 0.05$). Quadratic regression (QR; 95% asymptote), linear broken line (LB), and quadratic broken line (QB) models were used to determine the optimal dHis:dLys ratio. Significance was accepted at $P \leq 0.05$, and R^2 and the sum of squares error (SSE) were used to assess the best-fitting model. The broiler fed dHis:dLys 48% diet showed better BWG than dHis:dLys 28% (1.839 vs 1.746 kg, $P = 0.0371$). An increase in dHis:dLys ratio from 40 to 48% improved FCR, while a further increase in dHis:dLys to 52% reduced the positive effect ($P < 0.0001$). Optimal dHis:dLys ratio was determined for BWG (43, 39, and 43%) and FCR (40, 37, and 41%) using QR, LB, and QB, respectively ($P < 0.05$). In conclusion, the optimum dHis:dLys ratio for BWG and FCR was 43 and 40% using the best fitting models, indicating optimizing BWG required a slightly higher dHis:dLys ratio compared to FCR.

Keywords: Amino acids, Broilers, Carcass yield, Growth performance, Histidine

138 Not Presented

Student Competition: Animal Well-Being and Behavior I

139 Comparing beak treatment head holders on chick stress and welfare

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At the hatchery, leghorn chicks undergo infrared beak treatment (IRBT) and s.c. vaccination on automated machines (Nova-Tech Poultry). The Poultry Services Processor (PSP) has head holders (HH), where the jaw rests and two plastic 'feet' (F1) close around the head with the body extending down. A newer Hatchling Support System (HSS) has a modified HH with amended feet (F2) and more head space, reduced feet load pressure, a cradle with keel support and a back band. This study tested three HH configurations: PSP HH with F1 (PSP-F1), PSP HH with F2 (PSP-F2), or HSS HH with F2 and cradle (HSS), at a commercial hatchery. Day-of-hatch chicks (120 Lohmann LSL-Lite (W) and 120 Lohmann Brown (B)) underwent standard hatchery processing before placement on the PSP/HSS. To isolate HH effects, birds did not receive IRBT or vaccination. Heart rate (HR) was measured on 15 chicks/HH/strain (bird as replicate) pre- and post-treatment (trt), and blood was collected post-trt to assess heat shock protein (HSP60, 70, and 90), TNFα, and Transient Receptor Potential Vanilloid 4 (TRPV4) expression via quantitative PCR. Thermal imaging assessed surface body temperature (BT) post-trt. Another 15 chicks/HH/strain underwent a tonic immobility test post-trt. Bird behaviour was recorded when released from the PSP/HSS to assess righting time (disorientation measure). All birds were euthanized then incised to expose the *M. complexus* (*M.c.*) and fascia at the occipital crest for head bruise (HB) scoring. Data were analyzed as a 2x3 factorial with a two-way ANOVA and HB scores were analysed using a chi-square contingency table test. Post-trt, HR was highest in the PSP-F2 vs PSP-F1 trt (P=0.01) and in B birds (P<0.01). Interactions occurred for HSP60, 90, TNFα, and TRPV4 expression. HSP60 was higher in B-PSP-F2 vs B-PSP-F1 and B-HSS (P<0.01). HSP90 was highest in W-PSP-F1 vs W-PSP-F2 and W-HSS (P<0.01). TNFα expression was highest in the W-PSP-F1 trt (P<0.01). TRPV4 was highest in W-PSP-F1 vs B-PSP-F2 and W-PSP-F2, with intermediate effects in other trt (P=0.01). Righting time was longest in W-PSP-F1 compared to W birds in the other trt (P<0.01), and shortest in the B-HSS trt (P<0.01). Chicks in PSP-F1 had a greater fear response compared to those in HSS (P<0.01), and fear was higher in W birds (P=0.02). Surface BT was higher in W-PSP-F1 vs W-HSS (P=0.02). The percent of birds with HB did not differ by HH trt but was higher in W birds for fascia (P<0.01) and B birds for the *M.c.* (P<0.01). No HB was observed in untreated chicks. HB may be due to head-size differences between strains. W chicks were more fearful and disoriented, especially in the PSP-F1 trt. The HSS configuration had the least negative impact on chick stress and welfare.

Keywords: hatchery, automation, heat shock proteins, fear, heart rate

140 Evaluating the impact of feeder design and management on feed spillage related behavior in broiler chicks

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Feed wastage due to feed being spilt into the litter can have economic consequences for the producer. Bird activity at the feeder is one factor that can cause feed spillage. This study aimed to evaluate the impact of commercially available feeders on feed spillage related behaviors of broiler chicks. A total of 720 male broiler chicks (YPxRoss708) were randomly placed into 24 floor pens (30 birds/pen) and assigned one of four feeder treatments; C2 plus feeder (C2), C2 with supplemental turbo grow (C2TG), C2 with supplemental feed tray (C2FT), and Konavi feeder (KON). Behavior was recorded on days 1, 4, 7, and 14 measuring the frequency of behavior performance as counts with 5-minute observations taken every 30 minutes. Behaviors analyzed were eating whilst outside the feeder, eating from the edge of the feeder, eating whilst inside the feeder, entering-exiting the feeder, attempts to enter the feeder, moving inside the feeder, litter pecking within the feeder, feed scratching, feed selection, jostling at the feeder, displacing other birds from the feeder, and repetitive feeder pecking. A one-way ANOVA (PROC GLIMMIX, SAS 9.4) assessed the effect of feeder treatments on behaviors with means separation via Tukey-Kramers. Birds ate more from outside C2TG and KON (9.98; 9.68) than C2 and C2FT (6.79; 6.78). More birds ate inside C2FT (6.10), then C2 (3.34), followed by C2TG (0.97), and least in KON (0.11). Birds ate from the edge of the feeder most in C2 (0.44), and least in KON (0.1). Birds had the most successful entering-exiting feeder with C2FT (4.12) and most unsuccessful attempts with KON (0.78). Movement inside feeder was seen more in C2FT (2.10), then C2 (0.74), followed by C2TG (0.29) and least in KON (0.01). Litter pecking, feed scratching, and feed selection were highest in C2FT (P<0.01). Both jostling at feeder and displacement of chicks from feeder occurred least with C2 and most with KON (P<0.01). Repetitive feeder pecking occurred more with KON (1.17) than the other feeders. Feeders, which chicks can enter successfully (C2FT and C2), had a higher frequency of eating inside, moving inside the feeder, and explorative behaviors. This may have led to spillage when feed particles spill out due to birds scratching or repeatedly entering and exiting the feeder. In feeders where chicks could not readily enter (C2TG and KON), more birds were eating from outside, and there were higher occurrences of jostling and displacement at the feeder. These behaviors frequently led to the feeder swinging or shaking, forcing out the feed particles along with further spillage. Overall, the study suggests that birds adopt different feeding and feed-seeking strategies across feeder treatments that cause feed spillage.

Keywords: behavioral observation, feeding, feed-seeking, feed wastage, feeder management

141 Evaluating the influence of natural and artificial light on broiler behavior across growth stages

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In recent years, the addition of windows to broiler houses to provide natural light during rearing has increased; however, the effects of natural light on bird behavior remain largely unknown. To evaluate the impact of raising broilers under natural or artificial light on bird behavior, mixed-sex Ross 708 chicks (N=720) were housed in 16 rooms (44 birds/room), with 8 rooms per light treatment and raised until 56 d of age. Chicks were randomly assigned to one of two light treatments: artificial light provided via a 5000K LED (AL), or natural light provided via 1 ft^2 window and supplemented by a 5000K LED bulb (NL). Behavior frequency was assessed every 30 minutes over a 24-hour period via scan sampling on d 12, 33, and 54, for behaviors of; body shaking, drinking, dustbathing, eating, environmental pecking, foraging, frolicking, other active behaviors (jumping, flight, shuffle gait and adjusting posture), preening, resting (sitting and sleeping), running, self-scratching, standing, stretching, walking, and wing-flapping. Data were analyzed as a repeated measures ANOVA (PROC GLIMMIX, SAS 9.4) for main effects of light treatment and age, and means were compared via the Tukey-Kramer test. A p-value of <0.05 was considered statistically significant. An interaction between treatment and age was found for body shaking, frolicking, and resting. Body shaking occurred more on d 54 in NL (1±0.4), then in NL on d 12 (0) and 33 (0), and AL on d 12 (0) and 54 (0). Birds frolicked more on d 12 in NL (2.5±0.9) than on d 12 (0), 33 (0.25±0.25), and 54 (0) in AL, and d 54 in NL (0). Rest was highest on d 54 for both AL (1698±8.8) and NL (1658.5±7.5); followed by d 33 in AL (1572.25±13), then d 33 in NL (1535±25), and d 12 in NL (1472±5.7) and lowest on d 12 in AL (1430±19.4). Results showed that NL birds performed more drinking and active behaviors (60.6±2.4 and 26.2±2.7 respectively) compared to those raised under AL (51.6±2.4 and 17.2±1.6, respectively). Running (0.6±0.2), standing (87.1±7.7), and dust bathing (7±1.3) were performed more on d 12 than on either d 33 or 54. Whilst eating (139±8.5) and walking (57.5±3.2) were performed most on d 12, then d 33, and least on d 54. Foraging was performed more on d 12 (65±10.1) and 33 (74.9±4.5) than on d 54 (40.1±2.8). Birds preened most on d 33 (73.2±2.7). Stretching was most frequent on d 33 (40.9±3.4), followed by d 12 (15.9±1.1), with the least amount observed on d 54 (6.7±0.9). Overall, this study suggests that the NL treatment influences various behaviors in birds, including nutritive, play, and other active behaviors. Birds raised under NL conditions exhibited more frequent drinking, active behaviors and frolicking compared to those raised under AL conditions.

Keywords: light source, husbandry, management, welfare, broiler

142 Laser enrichment device with increased 8-minute duration improved broiler activity without sacrificing welfare outcomes

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Modern commercial broilers have been selected for rapid growth and increased breast muscle mass, which has been associated with leg lameness and reduced mobility as birds approach market age. Previous research investigating alternative environmental enrichments has shown that leg lameness can be mitigated using novel laser enrichment devices without negatively impacting broiler welfare. The study objectives were to investigate the impacts of increased laser enrichment duration on behavior and welfare assessments across the performance period. A total of 1,360 day-old straight run Ross 708 broilers were housed in floor pens (20 pens/trt) and randomly assigned to laser enrichment or control upon placement for a 7-wk grow-out cycle. Each laser enrichment device projected 4 randomly moving red dots across 2 pens and was programmed to run for 8-min periods, 4 times daily for the study duration (5:30, 11:30, 17:30, and 23:30). Eighty birds from 8 laser and 8 control pens were randomly selected as focal birds (5 focal birds/pen). Broiler behavior (feeder, active, and inactive) and walking distance were continuously collected during laser-active periods for all video timepoints on d 0-9 and weekly on d 9, 16, 23, 30, 37, and 42 (wk 2-7). Behavior measures were analyzed using one focal bird/pen/behavior. Welfare assessments included gait score (0-2), breast blister presence (A/P), and footpad dermatitis presence were assessed on all focal birds weekly. All data were analyzed using the GLIMMIX procedure with fixed effects of treatment (laser versus control), day (0-8) or week (1-7), and their interactions (SAS 9.4, $P \leq 0.05$). Across all measures, no interactions were found to be significant ($P \geq 0.05$). Laser exposure increased bird activity and distance walked while decreasing time spent at the feeder from d0 to d9 (33.7%, 33.0%, and 33.2%, respectively; $P \leq 0.04$). During laser periods, laser-enriched birds were 13.8% more inactive and spent 42.0% less time at the feeder than control birds throughout the study ($P \leq 0.02$). Distance walked decreased over time in both treatments, with the greatest distance walked during week 1 compared to all other weeks (45.3-79.8%, $P<0.0001$). No differences were observed in gait score, breast blister presence, and footpad dermatitis between laser-enriched and control birds or by week ($P>0.05$). In conclusion, laser enrichment stimulated broiler activity and walking distance during the first week. Over the entire study, laser-enriched birds were more inactive and spent less time at the feeder, but this did not compromise welfare assessments. Further investigation is needed to determine the optimal laser program duration and frequency settings and its long-term effects on broiler welfare.

Keywords: broiler, behavior, environmental enrichment, gait score, walking distance

143 Evaluating the impact of age, sex, and lameness on the problem-solving ability of broiler chickens

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Problem-solving abilities help individuals analyze, understand and solve challenges effectively. These abilities are important for adaptability and environmental changes. The objective of this study was to evaluate how sex, age, and lameness affect problem-solving abilities of broiler chickens. A total of 88 broiler chickens (Ross 708), 44 females (F) and 44 males (M) were randomly distributed in four pens of 2.78 m^2. Three different problem-solving tasks were developed (A, B, C), each bird was randomly assigned to a different task at 14 and 28 days of age (doa). Task A consisted on a clear cylinder with a string attached to a food reward, task B were three clear cups with lids, one of the cups with a food reward and task C was a clear box with food inside and bars above it. Birds were tested individually and in groups. Birds were presented the task and given five minutes to solve the task. Latency of the first movement, latency of the first interaction with the task, duration to solve the task, number of attempts and their success rate were measured. Gait score was determined for each bird after they finished their respective task. Data was analyzed using a generalized linear mixed model with the fixed effects of age, gait, sex and group with task as a covariant (PROC GLIMMIX; SAS 9.4). For individual birds, birds took longer at 14 doa (8.8s) to make their first movement than at 28 doa (6.95s). Birds with gait

score of 2 (9.7s) and 1 (12.9s) took more time to make their first movement compared to birds with gait score of 0 (4.6s). Latency to interaction and task duration were both longer for F (188.0s; 221.6s, respectively) than M (145.8s; 193.1s, respectively), and at 14 doa (188.9s; 220.6s, respectively) compared to 28 doa (145.8s; 194.06s, respectively). Twenty eight day old birds with a gait score of 0 (129.7s) or 1 (151.1s) had a shorter latency to interaction than 14 doa birds with a gait score of 1 (210.7s). Birds on 28 doa with LS of 1 (6.24) attempted to solve the tasks more compared to birds at 14 doa with LS of 1 (0.75). At 28 doa (0.41) birds were more successful at solving the tasks than at 14 doa (0.28). Birds presented the task in groups (0.88) were more successful then when presented individually (0.54). In groups, birds at 28 doa (0.88) were more successful to solve the tasks than at 14 doa (0.54). Males were quicker at interacting with and solving the tasks. Gait influences time taken to start moving, interact with the task and the number of attempts. Overall, these results indicate that as birds age their problem-solving abilities improve and are more successful at solving task in groups.

Keywords: Cognitive abilities, cognitive function, development, gait score, welfare

144 A new approach to identifying severe woody breast in market age broilers

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Woody breast (WB) is a myopathy that economically impacts the broiler industry. WB is typically assessed post-mortem due to a lack of validated non-invasive in vivo techniques. Impaired muscle functionality of the *Pectoralis major* muscle is associated with WB which has been proposed to restrict wing extension and possibly affect broilers' ability to upright themselves when on their backs. We hypothesized that these physical limitations could serve as indicators of severe WB in market-aged broilers. At 47-48 d of age, 28 broilers were selected from two commercial flocks. Sampling was initially conducted randomly. Subsequently, broilers were selected for inclusion based on a qualitative assessment of wing extension: the ability or inability to extend their wings over their backs. We measured the distance between the glenohumeral joints when the broilers' wings were extended backwards and equated partition of ≥ 6 cm as indicative of limited wing extension. We also performed a flip over test by recording the time it took for each bird to upright itself when placed on its back. Broilers were either able to turn themselves upright within 4 s or were unable to upright before the end of the 60 s trial. Post-mortem palpation was used to identify severe WB, recognized as a hardening in the cranial and caudal regions of the *Pectoralis major* muscle. Accuracy, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each of the in vivo measures. The wingspan test had an accuracy of 92.8%, a sensitivity of 87.5%, a specificity of 100%, a PPV of 100%, and a NPV of 85.7%. An accuracy of 71.4%, a sensitivity of 41.7%, a specificity of 93.8%, a PPV of 83.3%, and a NPV of 68.2% was calculated for the flip over test. We concluded that the wingspan test shows promise as a non-invasive method for detecting severe WB in live birds. The results also point to a possible animal welfare implication of severe WB. Of the 12 birds with severe WB, 5 were unable to upright themselves within the 60 s limit. In comparison, 1 out of 16 broilers in the moderate or no WB category failed to upright itself, while all others did so in under 1.5 s.

Keywords: broiler, myopathy, woody breast, assessment, welfare

145 Evaluation of mild woody breast meat's contact stiffness via beam buckling mechanism

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An experiment was conducted to 1) evaluate contact stiffness differences between normal and mild WB based on the indentation depth, and 2) identify the optimum needle type and indentation depth to accurately detect mild WB. Inspired by the monofilament-needle technique for diabetic neuropathy, we propose a novel approach based on contact mechanics and beam buckling theory to differentiate normal and mild woody breast meat. The proposed method employs a monofilament needle to indent the meat, with buckling as an indicator of WBM. In this study, a total of 27 normal breast meat (control group) and 18 mild woody breast meat(test group) samples were selected by a trained specialist for testing. In this experiment, a total of 6 needle types with the highest differentiation potential were selected for testing. To avoid penetration, needle tips were coated with food-grade beeswax. A programmable indentation system was developed to indent a needle at the cranial region with each indentation step set at 0.15 mm. Each needle was initially lowered to contact the carnival surface without exerting compression force. We visually observed the needle for buckling as it indented step by step. The indentation depth at first buckling was recorded. The experiment was replicated three times to ensure the repeatability of the proposed method. One-way ANOVA tests were conducted to compare the differences in indentation depth between the two groups for each needle type. Support vector machine (SVM) was used to identify the optimum indentation depth. Results found significant differences ($p<0.05$) in indentation depth between the control and test groups for all six needle types. No significant difference ($p>0.05$) in indentation depth was found among the replications. Two out of the six needle types (Needle A: Energy Flo J type 0.14mm x 30mm and Needle B: J type 0.1mm x 15mm) were particularly good at separating the two groups (no overlap in indentation depth). The indentation depth of Needle A was 2.21±0.18 for the control group and 1.24±0.18mm for the test group. Based on SVM, the optimum indentation depth for identifying the mild WB was 1.72mm. The indentation depth of Needle B was 2.67±0.24mm and 1.84±0.14mm for the control and test groups respectively, and the optimum indentation depth was 2.22mm. Based on these findings, indentation depth was set to 1.8mm for Needle A and 2.15mm for Needle B. In conclusion, mild WB has significantly higher contact stiffness than normal breast meat, which can be quantified via needle indentation depth. This characteristic allows mild WB to be accurately, objectively and inexpensively detected using the presented method.

Keywords: Woody Breast, Contact Stiffness, Sensor, Processing

146 Impact of controlled atmosphere and electrical stunning with water immersion and air chilling on muscle-specific meat quality

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Due to a shift towards controlled atmosphere stunning (CAS) as an alternative to electrical stunning (ES) a study was conducted to compare two stunning methods (CAS, ES) combined with two chilling methods (immersion-IC, air-AC) on breast fillet and thigh pH, color, lipid oxidation, drip loss, and shear force. Sixty broilers were processed using either CAS or ES. Following evisceration half were IC and half were AC using typical industry parameters, resulting in four treatments, CAS/IC (n=15), CAS/AC (n=15), ES/IC (n=15), and ES/AC (n=15). Following chilling, carcasses were deboned and breast fillets and boneless thighs were analyzed for pH and color. At 24 h post-deboning, pH, color, lipid oxidation, drip loss, and shear force were measured. A completely randomized design with factorial arrangement using the PROC GLIMMIX procedure was used. Significant t tests were separated using least significant differences. Both stunning and chilling methods impacted fillet and thigh pH and color after deboning. CAS fillets and thighs had higher pH than ES fillets and thighs ($P<0.001$). For fillets, hue was lower and chroma was higher for CAS than ES ($P\leq0.034$). However, CAS thighs were lighter, more yellow, and higher hue than ES thighs ($P\leq0.044$). IC fillets had higher pH than AC ($P<0.001$). For thighs, IC resulted in higher pH, lightness, and hue than AC ($P<0.001$). Both fillets and thighs had interactions between stunning and chilling methods for redness. Fillets from CAS/IC were the most red ($P=0.004$), while thighs from ES/AC were the most red ($P<0.001$). After 24 h, AC fillets had higher chroma than IC ($P=0.018$). There was an interaction between stunning and chilling for fillet pH where ES/AC pH was the highest and CAS/AC pH was lowest ($P=0.001$). ES thighs were lighter than CAS thighs ($P=0.025$) and both redness and hue had stunning and chilling interactions. CAS/AC thighs were redder than ES/AC thighs ($P=0.025$) while CAS/IC had a higher hue than CAS/AC ($P=0.016$). Fillets from CAS had less lipid oxidation than ES ($P=0.013$), but thighs had more lipid oxidation from CAS than ES ($P=0.033$). Thighs also had higher lipid oxidation from IC compared to AC ($P=0.001$). Drip loss in thighs was higher for CAS than ES ($P=0.019$) with no difference in fillets. ES fillets had lower shear force ($P<0.001$) compared to CAS, whereas IC had lower shear force than AC ($P=0.023$). There were no differences in shear force among thighs, whereas CAS/IC thighs had lower shear energy ($P=0.016$) than CAS/AC. These results showed that stunning and chilling methods significantly influenced meat quality and can interact to further impact pH, color and shear energy, making decisions regarding processing methods important for subsequent product quality.

Keywords: controlled atmosphere stunning, meat quality, electrical stunning, air chilling, immersion chilling

147 Effect of stunning methods and deboning time on broiler breast fillet quality

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Commercial poultry facilities typically utilize Electrical Stunning (ES) during processing, however Controlled Atmosphere Stunning (CAS) is quickly gaining popularity. Broiler breast meat quality is known to be affected by stunning and deboning time, although the combined impact of CAS with deboning time is less clear. The aim

of this study was to assess the relationship between the stunning method (CAS and ES) and various deboning periods (1, 2, and 4 hours post-mortem) on the quality of broiler breast meat. A 2x3 factorial design was used to allocate 60 broilers (42 days of age), with 30 for each stunning method and 10 per deboning time subgroup. The six treatment groups included CAS1hr, CAS2hr, CAS4hr, ES1hr, ES2hr, and ES4hr. After immersion chilling, breast filets were assessed for pH and meat color at the three deboning periods and 24-hour postmortem, and their weights were recorded to quantify drip loss. Lipid oxidation and shear force were assessed at 24 h. Results were assessed using SAS's PROC GLIMMIX technique in a completely randomized design with a factorial layout. For significant t tests ($P<0.05$), least-squares means were produced, and least significant differences were used for separation. Initially after deboning there were significant ($P<0.05$) effects of time of deboning on pH value. There was a notable interaction between various deboning durations and stunning techniques. The four-hour deboning time for both CAS (6.02) and ES methods (6.03) had a lower pH as compared to deboning times of one (6.38) or two (6.19) hours. At two hours, breast fillet pH from CAS birds (6.26) was higher than breast fillet pH from ES birds (6.13). No significant variations were seen in L*, a*, and b* across either stunning methods or various deboning times. No notable changes in drip loss were observed. After 24 hours there was a stunning time effect on pH. Birds stunned using CAS and deboned at two hours had lower pH ($P<0.05$) values than others. Whereas there were no significant differences in color values or shear force. These results showed that CAS and ES stunning methods did not have a significant impact on the influence of deboning times on subsequent meat quality. Further research should explore the potential impacts on retail display shelf life, sensory attributes, and consumer preferences.

Keywords: controlled atmosphere stunning, electrical stunning, deboning time, meat quality

148 Effect of peracetic acid dipping on the quality and microbial load of chicken tenderloins

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This research aimed to evaluate the impact of peracetic acid (PAA) at varying dip frequency on both meat quality and microbial growth of *Pectoralis minor* (tenderloin). Tenderloins were randomly separated into five treatments: negative control - no dipping (NC), one water dip control (WC), one dip in PAA (PAA1), three dips in PAA (PAA3), and five dips in PAA (PAA5), using n=36 per treatment over 3 replications. Each group was dipped for 15s in 750ppm PAA or water, according to treatment, and when multiple dips were used a 90s wait period was used between dips. The tenderloin pH, color L*, a*, b*, % pickup, % drip loss (after storage), % cook loss, and Meullenet Owens Razor Shear Force (MORSF) were assessed. Microbial count for aerobic (AC) and lactic acid bacteria (LAB) were taken on day 0 and 2, each after initial treatment and after storage. Data was subjected to analysis of variance one-way ANOVA ($P<0.05$) where treatment method served as the main effect. Treatments using multiple dips of PAA (PAA3 and PAA5) exhibited a significant ($P<0.05$) reduction in AC and LAB compared to the control and water dip treatment on Day 1 whereas all PAA treatments has lower APC and LAB after storage (Day 2). There was no difference ($P>0.05$) among treatments initial attributes of pH, L*, a* or b* or for pH and b* value after storage. However, dipping tenderloins multiple times in PAA resulted in higher ($P<0.05$) L* value after storage than those dipped once in PAA or water, or the negative control which were similar after storage ($P>0.05$). treatments containing PAA (PAA3, and PAA5) The a* values after storage decreased in tenderloins dipped multiple times in PAA (PAA3 and PAA5 < WC and NC, $P<0.05$). PAA5 was higher ($P<0.05$) for % drip loss compared to all other treatments. Cook loss generally increased with dipping multiple times in PAA; PAA5 had greater ($P<0.05$) cook loss than WC, but all other treatments were intermediate ($P>0.05$). There was no significant difference for MORSF values ($P>0.05$) among treatments. Results indicated that varying frequency of PAA dips had a significant effect on both microbial count and meat quality. These results suggest that different quantities of peracetic acid dips can decrease microbial growth but can have negative impacts on color and water holding capacity when dipped multiple times.

Keywords: Peracetic acid, Chicken tenders, Meat quality, Microbial load, Water holding capacity

149 Effects of duck breeds and chicken production types on egg functionality and sensory evaluation through fourteen days of egg storage

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In recent years, duck egg consumption in the US has increased. Duck eggs are sought out for their larger size and better baking functionality. The goal of this study was to evaluate how egg functionality and sensory characteristics differ between duck breeds. These breeds were compared to two retail chicken sources and evaluated for changes during storage. Duck eggs from 3 breeds (Duclair-D, Fawn and White Runner-FWR, Silver Appleyard-SAY) were obtained from commercial duck breeder hens, while chicken eggs were conventional white shelled (CW) and pasture-raised brown shelled (PB) and were obtained locally. The eggs were kept at 3°C/93% RH and divided into 3 sampling days (0,7,14). Egg functionality tests were performed using angel food cake (AFC) volume and custard height, texture, and weep. On each sampling day, 3 batches of AFC and custard were evaluated. Three products were obtained from each batch (N=9). Sensory evaluation of AFC and custard was completed using an electronic nose and tongue. Statistical analysis was performed using the General Linear Models procedure with means separated by LS Means. AFC volume was higher in eggs from chickens than ducks ($P<0.0001$) and cake volume did not change due to egg storage ($P=0.4218$). Custards made with duck eggs were taller than those made with chicken eggs ($P<0.0001$) and as eggs were stored, custard height increased ($P<0.0001$). Custard weep was not different between breeds ($P=0.4675$) or during storage ($P=0.7309$). Custards made with chicken eggs were firmer than those made with duck eggs ($P<0.0001$) and as eggs were stored, custards became softer ($P=0.0053$). AFC contained volatile compounds from aldehydes, alkanes, ketones, alkyl halides, acids, haloalkenes, amines, furans, alcohols, terpenes, esters, nitriles, and aromatic compounds. Alcohols (n-butanol, 2-hexanol) were present at d0, 7, 14 in D and FWR and only d14 in CW. Alcohols are a product of lipid oxidation and create a rancid aroma. Furans (furfural) created an almond, baked aroma through the Maillard reaction and were present in all samples except d0 in SAY and

CW. Amines (trimethylamine) were present in all samples except d7 in SAY, d14 in CW and D0, 14 in PB. Amines add an ammoniacal odor. There were volatile differences for custards. Electronic tongue for AFC and custard could discriminate between duck breeds and chickens, generally, but not between the chicken production types. Electronic tongue could discriminate between different duck breeds for AFC but not custards. These observed differences between duck egg breeds and retail chicken eggs may influence consumer egg purchases.

Keywords: functionality, sensory, duck, chicken, egg

150 Changes in eggshell translucency and its relationship with egg quality during 28 days of cold storage

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Eggshell translucency has gained attention in the poultry industry due to its impact on consumer perception and egg shelf life. This study aimed to evaluate changes in eggshell translucency scores and their relationship with overall egg quality during a 28-day storage period at 4°C. A total of 521 eggs were collected from individual 55-week-old Hy-Line W-36 hens within a 24-hour laying period. Eggs were graded for translucency using a three-level scoring system (TS 1 = few translucent spots, TS 2 = several small spots, TS 3 = many large spots). External quality parameters (weight, L* color score, thickness, and translucency score) were measured on days 0, 7, 14, 21, and 28, while internal quality parameters (albumen height, Haugh unit, breaking strength, egg grade, and yolk color) were measured on days 1, 14, and 28. Statistical analyses were performed using SAS (Version 9.4) with significance at $P \leq 0.05$. PROC Mixed Repeated Measures, Chi-square, and PROC GLM ANOVA with Tukey's HSD were used to analyze various quality parameters. Results showed a significant increase in eggshell translucency scores over time ($P < 0.0001$). Egg weight decreased by 1.36 g from day 0 to day 28 ($P < 0.0001$), with no notable differences between TS 3 and TS 1 categories. L* scores rose until day 14 before declining by day 21 ($P < 0.0001$), indicating that shells became lighter initially before darkening; however, this parameter was not affected by translucency. Breaking strength and eggshell thickness remained stable throughout storage, but egg thickness varied by translucency score, with TS3 being 12 µm thicker than TS1 ($P = 0.0196$). For internal quality, significant interaction effects were observed. On day 1, TS 3 eggs exhibited the highest albumen height (7.44 mm, $P < 0.0001$) and Haugh unit (84.30 HU, $P < 0.0001$). However, internal quality decreased across all groups by day 28. Yolk color ($P = 0.0450$) and egg grade ($P = 0.0456$) were influenced by storage period but not by translucency score. These findings suggest increasing eggshell translucency over time is associated with decreased internal egg quality, particularly after extended storage.

Keywords: Trasnlucency, Egg quality, Storage period, Internal quality, External quality

151 Not Presented

152 Disinfection of poultry processing wastewater using advanced oxidation processes

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Water is an essential resource used in poultry processing, generating large volumes of pathogen-rich wastewater. Treating poultry processing wastewater (PWW) is crucial for recycling to reduce operational costs and promote sustainability. While conventional disinfection methods are effective, they impose significant capital on producers. Advanced Oxidation Processes (AOPs) present a sustainable alternative for wastewater recycling and reuse by integrating electrochemical (EC) and photochemical (PC) treatments to enhance microbial safety through the generation of reactive oxygen species (ROS). Based on our previous studies on the effectiveness of AOPs in disinfecting simulated wastewater using a 7L benchtop setup, this study further demonstrated the effectiveness of combined EC-PC AOP treatments for disinfecting PWW obtained from the scalder and picker of a poultry processing plant. The experiment employed a combination of EC voltage load of 70V and 100V for scald water only combined with PC treatment using 1.6% curcumin-coated spheres as a photosensitizer to generate ROS under a light source. Adding ammonium sulfate as an electrolyte, the wastewater was treated for 300 min, with aliquots collected every 30 min for microbial analysis including, *Campylobacter* and *Salmonella* detection. Samples were plated on Petrifilms™ and incubated at 37°C for 48h to quantify reductions in aerobic bacteria (AC), coliforms (CC), *Enterobacteriaceae* (EB) and *E. coli* counts. The results were analyzed using ANOVA ($P \leq 0.05$) in SAS 9.4. Complete disinfection of *Campylobacter*, *Salmonella*, CC, EB and *E. coli* was observed at both EC levels in scald and picker water within 150 and 90 min, respectively. At 70V, there was a respective 44% and 34% reduction of AC. Increasing the EC to 100V accelerated microbial reduction in the scald PWW, eliminating CC, EB, and *E. coli* within 90 minutes. There was also a 62% reduction of AC in 300 minutes, which is below the 500 CFU/mL required USDA regulation for poultry wastewater reuse. Our results showed a significant difference between EC levels ($P < 0.0001$). These findings demonstrated that combined EC and PC treatments effectively reduced pathogens including *Campylobacter* and *Salmonella* in processing wastewater. This further suggests that AOPs are viable and sustainable disinfection strategies for poultry wastewater, reducing environmental pollution while promoting safe wastewater reuse in poultry processing operations. Further studies will evaluate wastewater disinfection at 100V using a larger AOP setup and analyze changes in microbiological and chemical properties for effective wastewater reuse.

Keywords: Poultry Processing Wastewater, Advanced Oxidation Processes (AOPs), Electrochemical Treatment, Photochemical Treatment, Pathogens

153 Dealing with big data using restricted maximum-likelihood model (REML) to increase the precision of estimates and generate a better set of results for poultry industry decision makers

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Carcass condemnation is a relevant problem for the industry, but analyzing industrial big data to identify its causes can be challenging. First, there is often no scientific rigor in big data collection, leading to a significant number of outliers and lack of comply with statistical assumptions. Second, multiple factors

influence condemnation, complicating results interpretation and practical applicability. We hypothesized that rather than using original big data, as tabulated, to perform ANOVA, grouping it by similarity between possible influencers of condemnation (moderators) and analyzing it by restricted maximum-likelihood model (REML) would generate a more precise, accurate and useful set of results. Thus, a 3-year historical big data of carcass condemnation from a Brazilian broiler company, with 6489 lines of information, was evaluated. Each line presented total number of slaughtered and condemned broilers, separated by moderators such as processing plant location, producer class, slaughter date, broiler house type, bird gender and stocking density. For ANOVA, condemnation percentage was calculated within each line by dividing the number of condemned birds by the total slaughtered. This variable was analyzed by one-way ANOVA plus Tukey test, using moderators as fixed factors. Alternatively, same dataset was grouped by similarity between moderators, pooling total number of slaughtered (sample size) and condemned broilers (event frequency) into 163 lines. This binomial structure was analyzed using REML, assigning different weights to each line according to its precision (number of birds). Heterogeneity was investigated by meta-regression and subgroup analysis. ANOVA identified all six moderators, except producer class ($p=0.525$), as influencers ($p<0.001$) of condemnation (overall mean = 0.74 ± 0.01%), which was lower in the center west plant (0.36%), at all seasons but winter (0.84%), in both female (0.32%) or as hatched (0.37%) birds raised in negative pressure houses (0.42%). Surprisingly, stocking densities below 25 kg/m2 condemned more (1.36%) than any higher density. In contrast, REML only identified plant location ($p<0.001$), bird gender ($p=0.002$) and stocking density ($p=0.006$) as significant moderators (overall mean = 0.67 ± 0.001%). They explained 11.34, 5.30 and 3.78% of condemnation differences. Center west plant (0.57%) and females (0.47%) showed lower condemnation rates. Stocking density increase was positively associated with condemnation, ranging from 0.62% to 0.78%. In conclusion, REML allows a more precise identification and hierarchical assessment of carcass condemnation influencers, better assisting decision-makers in establishing protocols to reduce carcass losses in the industry.

Keywords: big data, carcass condemnation, random-effects model, REML, statistical inference

154 Assessing the interaction of resistant starch and dietary crude protein concentrations on growth performance, nutrient digestibility, and digesta pH in broiler chickens

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The objective of the 35-d study was to investigate the interaction between dietary crude protein (CP) and resistant starch (RS) levels on growth performance, nutrient digestibility, and digesta pH in broiler chickens. A total of 1,056 day-old Cobb 500 male broilers were allocated to a 2 × 3 factorial treatment arrangement in a randomized complete block design, with 8 replicates (22 birds/pen). All birds were fed a common starter diet (d0-7) before introduction to experimental diets on d7. Six dietary treatments were formulated: one factor consisted of 3 CP concentrations – a corn-soybean meal-standard protein (SP) diet with 200 g/kg CP during the grower phase (d7-21) and 180 g/kg CP during the finisher stage (d21-35), and the second and third diets with 15 and 30 g/kg CP reduction from SP, respectively. The second factor was two dietary levels of raw potato starch (RPS) at 20 or 40 g/kg. Birds and feed were weighed on d7, 21, and 35. On d21, the jejunal and ileal digesta of 5 birds/pen were sampled for digestibility. Samples from 1 bird/pen on d21 and 35 were collected for digesta pH and gene expression. Data were analyzed using the PROC MIXED in SAS. Orthogonal polynomial contrasts were applied to assess 1) RS × linear effect of CP, 2) RS × quadratic effect of CP, 3) linear main effect of CP, 4) quadratic main effect of CP, and 5) main effect of RS. For the overall period (d7-35), the weight gain (WG) and feed intake (FI) showed RS × quadratic CP effect (P=0.015 and 0.044). Diet with 40 g/kg RPS showed reduced WG and FI; decreasing dietary CP levels in diets with 20 g/kg RPS diets linearly decreased WG and FI, but dietary CP reduction in 40 g/kg RPS diets showed a quadratic effect, wherein intermediate CP showed the highest WG and FI. On d21, apparent ileal digestibility of starch or Gly and Ser increased linearly or quadratically (P<0.05) with decreasing CP levels. Gene expression of protein synthesis genes in liver (S6KB1, 4EBP1) increased linearly (P<0.05) as dietary CP level decreased on d21. On d35, jejunal pH was lower (P=0.014) in birds fed 40 g/kg RPS and decreased linearly (P=0.019) with decreasing CP levels. The peptide and amino acids transporters and tight junction protein gene expression in jejunum increased (P<0.05) with the dietary CP reduction on d35. In conclusion, a 30 g/kg reduction in dietary CP level may adversely affect growth performance of broilers in the presence of dietary RPS. Impaired growth may be associated with alteration in jejunal pH. Increased gene expression related to protein utilization, nutrient transporter, tight junction protein suggests a potential compensatory effect to mitigate the negative effects of CP reduction on broilers growth.

Keywords: resistant starch, crude protein, raw potato starch, digesta pH, broiler chickens

155 Evaluation of metabolizable energy in ultra-high protein, low oligosaccharide soybean meal compared with conventional soybean meal in precision-fed roosters and ab libitum-fed broilers and turkeys

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The objective of this study was to determine the metabolizable energy content in ultra-high protein, low oligosaccharide soybean meal (**UHPLO-SBM**) compared with conventional SBM (**C-SBM**). In Experiment 1, a precision-fed rooster assay was conducted using Leghorn roosters with intact ceca to determine nitrogen-corrected true metabolizable energy (**TME_n**). Birds were fasted for 26 h and then precision-fed (crop intubated) with 25 g of UHPLO or C-SBM. Excreta were then quantitatively collected for 48 h. In Experiments 2 and 3, nitrogen-corrected apparent metabolizable energy (**AME_n**) was determined using ad libitum-fed male broiler chickens (Experiment 2) and female turkey poults (Experiment 3). Birds were provided a nutritionally complete corn-soybean meal-based starter diet until 5 d-of-age. From 6 to 9 d-of-age, birds were provided 1 of 3 experimental diets ad libitum and excreta were collected during the final 48 h. After excreta collection, birds were placed back on the common starter diet until d 17. From 18 to 21 d-of-age, birds were provided experimental diets ad libitum, and excreta were collected during the last 48 h. Diet 1 was a nutrient-adequate corn-soybean meal-based diet. Diets 2 and 3 were the same as diet 1, except 30% of the basal diet was replaced by UHPLO or C-SBM. The AME_n of the test ingredients were calculated by difference using the basal substitution method. Data from Experiment 1 were analyzed using a 1-way ANOVA for a completely randomized design, whereas data from Experiments 2 and 3 were analyzed using a 2-way ANOVA for a 2 × 2 factorial arrangement of treatments with main effects being age and SBM type. The significance value for all analyses was $P < 0.05$. The UHPLO-SBM contained 57.2% CP and 0.8% crude fat (DM basis), compared with 50.2% CP and 3.0% crude fat in C-SBM. Furthermore, the oligosaccharide content in UHLPO-SBM was reduced by approximately 80% compared with C-SBM. In Experiment 1 with precision-fed roosters, there was no difference ($P > 0.05$) in TME_n values between C-SBM and UHPLO-SBM (3057 and 3011 kcal/kg DM, respectively). In Experiment 2 with ad libitum-fed broiler chickens, there was no age × SBM interaction ($P > 0.05$) and UHPLO-SBM contained a greater AME_n value compared with C-SBM (2688 and 2410 kcal/kg DM, respectively at 21 d-of-age). In Experiment 3 with ad libitum-fed turkey poults, there was no age × SBM interaction ($P > 0.05$) and UHPLO-SBM contained a greater AME_n value compared with C-SBM (2714 and 2567 kcal/kg DM, respectively at 21 d-of-age). In conclusion, UHPLO-SBM contained a greater AME_n compared with C-SBM in both ad libitum-fed broiler chickens and turkeys, indicating UHPLO-SBM has increased nutritional value compared with C-SBM for poultry.

Keywords: metabolizable energy, soybean meal, oligosaccharide, chicken, turkey

156 Determination of metabolizable energy in corn fermented protein and conventional distillers dried grains with solubles using precision-fed roosters and ad libitum-fed broiler chickens

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The objective of this study was to determine the nitrogen-corrected true metabolizable energy (TMEn) in precision-fed roosters and

nitrogen-corrected apparent metabolizable energy (AMEn) in ad libitum-fed broiler chickens of corn fermented protein (CFP) and distillers dried grains with solubles (DDGS). In Experiment 1, the TMEn of 8 samples of CFP obtained from POET and 2 conventional DDGS samples were determined using precision-fed Single Comb White Leghorn roosters. Roosters were fasted for 26 h prior to being precision-fed (crop intubated) with 30 g of either 50% CFP or 50% DDGS mixed with corn. Excreta were quantitatively collected for 48 h. The TMEn values for feedstuffs were calculated by difference. In Experiment 2, the AMEn values for the same feedstuffs (8 CFP and 2 DDGS samples) were determined in ad libitum-fed broiler chickens. Birds were provided a nutritionally complete corn-soybean meal starter diet from 0 to 18 d-of-age. From d 18 to 21, birds were provided experimental diets ad libitum and excreta were collected during the final 48 h. Diet 1 was a nutrient-adequate corn-soybean meal-based diet. Diets 2 through 11 were the same as diet 1, except that CFP or DDGS samples were added at the expense of 30% of the basal diet (diet 1). The AMEn was calculated by difference. Statistical analyses were conducted using a one-way ANOVA for a completely randomized design and pairwise treatment comparisons were conducted using the least significant difference test. The probability value for the ANOVA and pairwise treatment comparisons was $P < 0.05$. The CFP contained a mean CP content of 55% and a mean crude fat content of 3.7% on a DM basis, compared with 30% CP and 8.5% in DDGS. In Experiment 1, there were few differences ($P < 0.05$) in TMEn values among CFP samples. Further, the TMEn of CFP samples was generally greater compared with DDGS, where the mean TMEn for CFP was 3,305 kcal/kg DM compared with 3,152 kcal/kg DM for DDGS. In Experiment 2, the mean AMEn of CFP on a dry matter basis was 2,777 kcal/kg, compared with 2,568 kcal/kg in DDGS. Results were in good agreement with Experiment 1, where the mean difference between CFP and DDGS was 153 kcal/kg in Experiment 1 compared with 209 kcal/kg in Experiment 2. In conclusion, the relative difference between feedstuffs was in good agreement between bioassays. Further, the TMEn and AMEn content of CFP was generally consistent among samples and CFP contained greater ME values compared with conventional DDGS.

Keywords: True Metabolizable Energy, Apparent Metabolizable Energy, Distillers dried grains with solubles, Corn fermented protein, Fractionation

157 Comparing broiler mobility and foot health between two breeds in silvopasture and conventional environments

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Silvopasture is an outdoor access environment integrated with orchards or forests. There is limited information as to how silvopasture affects key welfare indicators of broilers (i.e., mobility and footpad condition), particularly considering breeds differing in growth rate. We aimed to evaluate the effect of housing environment and breed on the mobility and prevalence of footpad dermatitis in broiler chickens. A total of 500 Ross 708 (fast-growing; R) and 500 SASSO (slow-growing; S) male chicks were randomly placed in either indoor conventional (I) or outdoor silvopasture housing (O; n=5 pens/breed/environment, 50 chicks/pen). To achieve a similar target weight, R were reared to 42d and S to 56d. Gait scoring was conducted at 41-42d, while footpad scoring was performed after processing at 42d for R and 56d for S. Both scoring methods utilized 3-point scales from the National Chicken Council Welfare Guidelines. Data were analyzed with general linear models in R software. More S broilers displayed a normal gait score of 0 (i.e., walk at least 5 ft with a balanced gait) than the R birds ($p<0.0001$; S:99.9±0.02%, R:93.2±0.4%, mean±SE). More R broilers displayed an abnormal gait score of 1 (i.e., walk 5 ft but with uneven, awkward steps) compared to S broilers ($p<0.0001$; Slow:0.01±0.01%, Fast:5.6±0.4%). The percentage of birds scored as 2 (i.e., unable to walk 5 ft) did not differ between breeds (p=0.09). Location did not affect the percentage of broilers within any gait score category ($p>0.05$ for all). S broilers had a higher prevalence of normal footpads with a score of 0 than R broilers (p=0.009; Slow:52.6±8.9%, Fast:13.7±7.0%). Regardless of breed, more broilers reared in I had normal footpads compared to broilers reared in O ($p<0.001$; I: 66.4±7.4%, O: 8.2±4.6%). There was an interaction between breed and environment for a footpad score of 1 (i.e., lesions covering less than half of the footpad). S broilers reared in I had the lowest prevalence of score 1, while S broilers reared in O had similarly high prevalences as R broilers in both environments (p=0.04; S, I:16.7±7.4%, S, O:46.7±9.9%, R, I:48.3±9.9%, R, O:43.1±9.0%). A footpad score of 2 (i.e., lesions covering more than half of the footpad) was not affected by breed (p=0.32) but was affected by environment (p=0.002). Broilers reared in O displayed a higher prevalence of score 2 than broilers reared in I (I:1.7±1.1%, O:36.4±9.4%). The S broilers demonstrated better mobility than R broilers, whereas footpad dermatitis was exacerbated by the environment. A weather event caused higher ground moisture content in O. Footpad health was best with the S breed reared in I, but mitigating ground moisture in O environments warrants further investigation.

Keywords: gait, footpad dermatitis, broiler, growth rate, silvopasture

158 Warmed perches for mitigating cold stress in laying hens: productivity impacts

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Cold stress is a critical condition that compromises animal welfare and productivity once ambient temperatures deviate from an animal's thermoneutral zone. The aim of the current experiment was to investigate if providing warmed perches can improve the performance of cold stressed laying hens. A total of 216 15-wk-old Lohmann Selected Leghorn Lite (LSL) pullets were used in this study. The pullets were randomly allocated to 1 of 3 treatments across 6 cage banks (2 banks/treatment) within an environmental-controlled room: 1) conventional cages without perches (NP), 2) cages fitted with galvanized-steel perches at ambient temperature (AP), and 3) cages fitted with galvanized-steel perches warmed by circulating water at 30°C (WP). Each bank consisted of 6 cages (6 birds/cage) arranged in 3 deck levels (2 cages/deck). The WP system was incorporated with a single-loop design circulating warm water. Cold stress was induced at wk 26 by setting the room temperature to 10°C. The WP was automatically controlled by a multi-zone controller to be activated when the ambient temperature dropped below 12°C. Feed intake (FI), feed to egg conversion ratio (FCR), egg production, and egg quality parameters were measured over 3 days at wk 25 (pre-cold stress), 30, and 34 of age during the 2024-2025 winter. Rectal temperature (RT) and body weight (BW) were recorded (n = 12/treatment). Both WP and AP hens reached 50% egg production at wk 21, a week earlier than NP hens. Body weight and RT were analyzed using PROC MIXED (SAS 9.4). For FI, egg production, and egg quality parameters, hen age was included as a repeated measure. Overall, BW (p = 0.15), FCR (p = 0.19), eggshell breaking force (p = 0.90), eggshell weight (p = 0.24), eggshell percentage (p = 0.18), and hen-day egg production (p = 0.12) did not differ among treatments. However, NP hens had higher FI than AP hens (NP = 154g, AP = 129g; p = 0.02). Hens in the WP treatment tended to have higher RT than NP hens (WP = 41.1°C, NP = 40.9°C; p = 0.09). Egg weight of WP hens was higher than AP hens' and tended to be higher than NP hens' (WP = 60.6g, NP = 59.4g, AP = 59.1g; p = 0.02). Hens in the WP treatment had thinner shell thickness compared to both AP and NP hens (AP = CP = 0.39 mm, WP = 0.38mm; $p < 0.01$) and tended to have higher yolk weight than AP hens (WP = 15.0g, AP = 14.6g; p = 0.09). These preliminary data suggest that providing a warmed perch system may be beneficial to laying hens under cold conditions. Additional analyses are ongoing to investigate immune, endocrine, and neurophysiological responses of laying hens undergoing cold stress.

Keywords: warmed perches, cold stress, laying hens, egg quality, cage

159 Warmed perches for mitigating cold stress in laying hens: welfare impacts

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Environmental stressors, such as cold stress, pose a challenge to the welfare of laying hens. Exposure to low temperatures could cause physiological and behavioral changes that negatively impact health and productivity. This study aimed to evaluate the use of a warmed perch for improving laying hen welfare during cold temperatures. White laying hens (n=216) were randomly assigned

to 36 cages (6 hens per cage) at 15 WOA. Cages were assigned to one of three treatment groups: WP (warmed perches); AP (ambient perches); and CON (control, no perch). The room temperature was decreased to 10°C from 26 WOA to 33 WOA to induce cold stress. Body weight (BW) and welfare assessments were conducted at 18, 22, 25, 29, and 33 WOA for three focal hens per cage to assess hens' beak, comb, and feather (neck, back, wings, tail, belly) condition, footpad health, toe damage, and keel bone fractures and deviations based on the Welfare Quality® Protocol. Data from BW and welfare scores were analyzed using PROC MIXED and PROC LOGISTIC (SAS 9.4), respectively. Results were modeled as the odds of having a score of 0 (no damage). Hens in the CON group had higher odds of having no footpad issues (score of 0) compared to WP hens (OR = 79.54, 95% CI: 9.64 – 656.40, $P < 0.0001$) and AP hens (OR =11.67, 95% CI: 1.39-98.04, $P = 0.0235$) starting at 25 WOA. Compared to WP hens, CON and AP hens (OR = 18.09, 95% CI: 1.27–258.40, $P = 0.0329$) had higher odds of having no wing feather damage. Hens in the AP group had higher odds of having no belly feather damage compared to CON (OR = 2.96, 95% CI: 1.06-8.27, $P = 0.0380$) and WP (OR = 4.21, 95% CI: 1.51-11.74, $P = 0.0061$) hens. The odds of having no keel bone damage were higher for CON vs. AP (OR = 2.46, 95% CI: 1.43-4.35, $P = 0.0011$) and WP (OR = 3.90, 95% CI: 2.29-6.67, $P < 0.0001$) hens. Welfare parameters worsened with age, regardless of treatment. While body weight increased with age, no differences were observed among treatments. Overall, both age and treatment significantly affected most welfare variables, with the WP being associated with increased risks of footpad issues and keel bone and feather damage over time. This study was funded by USDA NIFA grant 2023-67015-39679.

Keywords: laying hen, welfare, cold stress, perch, age

160 Comparative effects of isolation during early-life and pullet stage on behavior, immune response and cecal microbiota in Hy-Line Brown chickens

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Psychological stress can elicit physiological and behavioral responses in poultry, yet its effects may vary across developmental stages. We conducted two trials to compare isolation stress response in Hy-Line Brown chickens at distinct life stages: Experiment 1 (E1) with 1-wk-old chicks and Experiment 2 (E2) with 12-wk-old pullets. Birds were randomly assigned to either Control (CO) or Stress (ST) rooms, each with 6 floor pens (≥ 12 birds/pen). Twelve anechoic chambers (18 x 18 x 22 cm^3) equipped with cameras were used for isolation. In E1, ST chicks experienced 1-hr daily isolation for 5 consecutive days, while in E2, ST pullets alternated between individual isolation and group restraint over 8 days. After isolation, birds were returned to their home pen and their behaviors were monitored. Post-stress challenge (d6 for E1 and d8 for E2), 3 chicks/pen (n=18/treatment) were euthanized to collect cecal tonsils and mucosal scrapings. Cecal tonsils were analyzed for mRNA abundance of IL-1β, IL-4, IL-10 and IFN-γ. Cecal mucosal scrapings were used for microbial DNA extraction and 16S rRNA sequencing. Gene sequences were analyzed using QIIME2 (ver. 2024.2). Vocalization frequency and activity duration were recorded for each bird in the isolation chamber, while maintenance and comfort behaviors were recorded using scan sampling within home pens. Kruskal-Wallis test was used to analyze the data and $P \leq 0.05$ was deemed significant. Isolation induced vocalizations and increased activity during the first 10 min in both experiments ($P \leq 0.001$), declining with repeated tests. In E1, ST chicks showed more sleeping and dustbathing ($P=0.004$), and less foraging in their home pens than CO chicks ($P=0.002$). In E2, ST pullets exhibited less foraging and sitting than CO pullets ($P \leq 0.001$). Isolation resulted in higher mRNA abundance of cytokines (IL-1β, IFN-γ, IL-10, and IL-4) in E1 chicks, but only IL-4 in E2 pullets. Stressed E1 chicks had altered gut microbiota, with enrichment of Erysipelothrichaceae and Peptostreptococcaceae ($P \leq 0.05$) families and predicted upregulation of branched-chain amino acid metabolic pathways ($P \leq 0.01$). In E2 pullets, isolation enriched Ruminococcaceae ($P=0.004$) and Rikenellaceae ($P=0.03$), while decreasing Lachnospiraceae, Erysipelatoclostridiaceae and Christensenellaceae families ($P \leq 0.01$). In E2, pathways related to energy metabolism and co-factor/vitamin synthesis were predicted to be upregulated in the ST pullets ($P \leq 0.01$). These results highlight pronounced stress responsiveness in chicks, likely due to developmental plasticity, versus a relatively muted response in older pullets. Further, changes in cecal microbiota possibly mediated energy homeostasis during stress in these chickens.

Keywords: isolation, psychological stress, behavior, cecal microbiome, immune response

161 Parting the feathers: Examining the effects of northern fowl mite infestation level on cage-free laying hen welfare

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Northern fowl mites (NFM) are the most prevalent ectoparasite found on North American laying hens, negatively affecting productivity and hen welfare. This study examined the relationship between mite infestation level and hen welfare. Pedigreed laying hens (n=600) were randomly assigned to a floor pen (n=40 hens/pen, 5 pens/room, 3 rooms) containing nest boxes and wood shavings. At 24 weeks of age (wk), all hens were infested with 50 NFM applied directly to the skin anterior to the cloaca. Prior to infestation, at 24 wk, the number of feathers present on a 4x6cm skin area anterior to the cloaca were counted on all hens. At 20, 24, 26, and 30 wk, the following were assessed for all hens: body weight (BW), welfare measures [condition of the beak, footpads, feathers (neck, back, left wing, right wing, tail, belly), toe damage, keel bone fractures and deviations], and mite-associated measures (mite infestation level score, skin inflammation, skin lesions). Mite-associated measures were only assessed within the 4x6cm area where feather counts were previously completed. Based on their mite score during peak infestation at 30 wk, hens were classified as either HIGH (>100 mites) or LOW (≤100 mites). Welfare measures and skin lesions were analyzed using PROC LOGISTIC (SAS 9.4). Feather count, skin inflammation area, and BW were analyzed using PROC MIXED (SAS 9.4). Footpad condition and toe damage were unaffected by age and infestation level. At 24 wk, HIGH hens (43.14±0.56) had more feathers on the vent area than LOW hens (41.42±0.55, P=0.029). HIGH hens were 3.32 times more likely to have healed inflammation (95% CI: 2.43-4.55, P<0.001) and less likely to have no inflammation (OR=0.3, 95% CI: 0.22-0.41, P<0.001) compared to LOW hens during peak infestation at 30 wk. HIGH hens (4.63±0.26cm^2) had a larger skin inflammation area than LOW hens (2.47±0.35cm^2, P<0.001)

during peak infestation at 30 wk. HIGH hens were 5.41 times more likely to have worse skin lesion scores than LOW hens (95% CI: 3.82-7.66, P<0.001). BW did not differ between clusters, but hens progressively weighed more at each age (20 wk: 1.74±0.007kg, 24 wk: 1.95±0.007kg, 26 wk: 2.02±0.007kg, 30 wk: 2.07±0.007kg; P<0.001). Neck, back, wings, tail, and belly feather condition worsened with age (P<0.001). Keel bone damage and keel tip fractures were more prevalent in older hens (P<0.001). Initially having more feathers in the vent area was associated with having higher levels of mites, and higher mite levels were associated with skin lesions and inflammation. Future research will examine whether infestation level differentially affects laying hen behavior as well as its genetic background. This work was supported by USDA-NIFA 2023-67015-39683.

Keywords: ectoparasites, northern fowl mites, laying hen, welfare, cage-free

162 Fowl moods: Age- and environment-related differences in vocalizations and brain serotonin activity in laying hens during an isolation fear test

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The isolation fear test is a common welfare-based test measuring the latency and frequency of vocalizations. Despite its commonality, there has yet to be research conducted on the impact of isolation on a chicken's neurochemistry. This study examines how isolation in the light or dark affects vocalizations, central dopamine (DA) activity, and serotonin (5-HT) turnover. Vocalizations are associated with the isolation fear test while DA and 5-HT activity are closely associated with the chicken's affective state. 36 W-36 hens at 25 weeks of age (early lay) and 36 W-36 hens at 70 weeks of age (geriatric) were used. During photophase, two treatments (n=12/group) were isolated in an anechoic chamber for 90 minutes and vocalizations were recorded. One treatment was isolated with light (Light) and the other with no light (Dark). After 90 minutes, both groups were euthanized using intravenous pentobarbital (FatalPlus 390 mg/kg). Controls (Con) were removed from their home cages and euthanized immediately. Brains were collected and microdissected into the caudal (CM) and rostral mesencephalon (RM), diencephalon (DI), and lateral (LT) and medial telencephalon (MT). Mass spectrometry was used to measure DA and 5-HT with their metabolites. The ratios of metabolites to respective neurotransmitters were calculated for DA and 5-HT turnover as a relative measure of synaptic activity. Analysis of neurotransmitter data used an ANOVA, and vocalizations a Mann-Whitney U Test. Early lay Light hens had a shorter duration to ($p = 0.0005$) and frequency ($p = 0.0039$) vocalizing than early lay Dark, which did not vocalize. Geriatric Dark hens also did not vocalize, while geriatric Light hens vocalized infrequently but more than Dark ($p=0.037$). No differences were observed among the groups for DA turnover. We found that early lay hens isolated with light had higher 5-HT turnover in the CM ($p = 0.0004$), RM ($p = 0.01$), and LT ($p=0.0002$) compared to Dark and Con groups. In contrast, geriatric hens isolated with no light had a significantly higher turnover in the LT ($p = 0.017$) and the MT ($p = 0.017$) compared to Con. These data reinforce the fact that neurochemically, birds are in a negative affective state with isolation. The vocalizations associated with this negative affective state may differ by age and environment.

Keywords: Neurochemistry, Stress, Layers, Isolation

163 Characterizing the development of the behavioral repertoire of turkey poults during early-life

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During early-life, the behavioral repertoire of turkeys expands as experiential learning shapes innate behaviors. However, knowledge on the ontogeny of turkey behavior is limited. This study aimed to characterize the normal development of turkey poult behavior by analyzing patterns across age, time-of-day (TOD), and sequence. Seventy-five Nicholas Select male turkey poults (25 birds/pen) were placed on D0 and three poults per pen were selected as focal subjects (n=9). Behaviors of locomotive, standing, sitting, sleeping, comfort, nutritive, explorative, social, individual play, and social play were assessed by 24-hour continuous sampling on D1 to 7. Behavior durations were analyzed by age and TOD, and the Shannon diversity index quantified diversity. A sequence probability matrix was generated to assess the probability of a target behavior (TB) occurring after an antecedent behavior (AB). The effect of age on duration and diversity was assessed by regression analyses (PROC REG, SAS 9.4). An ANOVA was used to assess the effect of TOD on duration (PROC MIXED) and age and behavior on sequence (PROC GLIMMIX–beta distribution). Means were compared via Tukey-Kramers. The time poults performed locomotive, standing, comfort, nutritive, explorative, social, individual play, and social play (P<0.01; social play and explorative–linear, remaining–quadratic) increased with age. Sitting peaked on D2 and then plateaued (P<0.01; quadratic). Poults slept less as they aged (P<0.01; quadratic). The TOD affected all behaviors (P<0.01). Performance of locomotive, standing, and nutritive behaviors was greatest during the morning (3.81%; 1.88%; 4.99%) and mid-day period (3.56%; 1.92%; 5.18%, respectively). Sitting and comfort behaviors were greatest in the pre-dark (2.57%; 0.72%) and evening periods (2.50%; 0.73%, respectively). Poults slept the most in the dark period (15.04%). Explorative behavior was greater in the mid-day (0.45%), afternoon (0.58%), and evening (0.56%) periods than the pre-dark (0.25%) and dark periods (0.05%). Social behaviors were performed more in the photophase periods than the dark (0.01%). Individual play was greater mid-day (0.09%) than the dark period (0.00%), whereas social play occurred more in the afternoon (0.24%) and evening (0.20%) than the dark (0.00%). Behavioral diversity increased (P<0.01) with age. Regardless of age (P<0.01) or AB (P<0.01), the most probable TB for poults to perform were active behaviors. Overall, as poults aged, the decrease in sleep along with the increased performance of other behaviors led to greater behavioral diversity. These results demonstrate the importance of active behaviors and the impact that age and TOD have on the early development of turkey behavior.

Keywords: behavioral diversity, behavioral sequence, diurnal activity, focal sampling, continuous sampling

164 Using a preference test to evaluate the relationship between feather pecking and foraging in turkey toms

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Foraging behavior in farmed poultry is an example of contrafreeloading (CF), a phenomenon when an animal prefers to

work for food rather than obtaining it from a freely available source. With suboptimal foraging conditions, laying hens redirect foraging efforts to feather pecking flock mates, which could become injurious. Injurious pecking is prevalent in turkey toms, but its relationship with foraging is unclear. Our objective was to determine if an individual's prevalence of feather pecking predicted their preference to perform foraging. A total of 176 turkey toms from one commercial strain were randomly assigned to 8 pens (22 birds/pen). Within each pen, 8 focal birds/pen were colored for individual identification. At 14 wks, the total bouts of body/wing/tail (BWT) pecking for each focal bird were determined using continuous focal animal sampling (6 h total over 4 d/pen). Two focal birds/pen with the highest sum of BWT pecks were deemed High Feather Peckers (HFP; n=16 total, 343.1 ± 248.8 pecks/6 hr, mean ± SD), while two focal birds/pen with the lowest total sum of BWT pecks were classified as Low Feather Peckers (LFP, n=16 total, 59.2 ± 31.7 pecks/6 hr). At 15 wks, the HFP and LFP turkeys underwent a conditioned place preference test. Individuals were conditioned to associate wall patterns with either the presence of dried mealworms and feed mixed into bedding substrate to promote foraging (CF side), or a pan feeder that contained dried mealworms and feed for birds to freely access and no feed was mixed into the bedding substrate (FF side). Conditioning occurred for three consecutive days, where birds were exposed to the CF and FF side separately for 5 min/day. Foraging and feeding durations were coded from video files for each conditioning exposure, and data were analyzed with linear mixed effect models in R software. All turkeys spent more time foraging on the CF side than the FF side (p=0.027; CF: 32.66 [23.15, 43.81], FF: 23.87 [15.86, 33.51] sec, mean [95% CI]). Only 10 of the 32 toms fed on the FF side. Also during conditioning, HFP and LFP turkeys spent a similar amount of time foraging (p=0.70; HFP: 29.67 [18.42, 43.61], LFP: 26.55 [15.96, 39.82] sec). On the fourth day, birds were tested for their preference for CF or FF for 2.5 min. The proportion of time a bird chose each side of the arena during testing was analyzed with Wilcoxon signed-rank tests in R software. During testing, HFP and LFP turkeys did not display a preference greater than chance for the CF or FF side (p>0.05). To our knowledge, this is the first CF preference test conducted in turkeys. Individual turkeys vary widely in their feather pecking behavior, so further investigation of the underlying causes is warranted.

Keywords: turkey, feather pecking, preference test, behavior, foraging

165 Effect of time of day preference and male-male competition on broiler breeder mating behavior and fertility during the early production period

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Broiler breeder mating success and interactions can be influenced by factors like time of day, male-male competition, and frequency of matings, which impact fertility. The objective of this study was to assess 1) daily patterns in mating behavior and mating attempts and their evolution with age, 2) the influence of male competition on mating behavior throughout the day, and 3) the relationship between fertility and mating attempts. At 20 weeks of age (woa), a total of 472 broiler breeders, 408 females (F; Ross 308AP) and 64 males (M; Ross 344) were randomly distributed into two treatments: 8 Small pens (SM) and 8 Large pens (L). Both treatments maintained a ratio of 1M: 9F, with L having 4 M and SM having 1 M. At 25 and 30 woa, mating behavior attempts were recorded during the lighting period (7AM- 9PM), and were coded as Successful (S, with cloacal contact), Unsuccessful (U, without cloacal contact), and Not Visible. Fertility was assessed *via* egg breakouts followed by 3 days of incubation. Mating behavior was analyzed *via* a three-way ANOVA for effects of age, treatment, and time of day (PROC MIXED) with means separation via Tukey-Kramer. Spearman correlation (PROC Corr) was used to establish correlations between fertility, S, and U attempts. Age and treatment had an interactive effect on total attempts, with fewer attempts in SM at 30 woa, compared to 25 woa. Interestingly, while U attempts were higher at 25 woa (18.03) than 30 woa (9.03), and in L (15.5) than in SM (11.57), S attempts did not differ with age or treatment. Time of day influenced S, U, and the total mating attempts ($P<.0001$). Regarding behavior distribution throughout the day, total mating behavior was highest at 7 AM hours (18%) and 7 PM (18%; $P<0.001$). S was highest at 19 hours (18%) and lowest at 11 hours (2%; $P<0.001$). U was highest at 7 hours (25%) and lowest at 8, 10, 11, 12, and 15 hours (2%, 2%, 2%, and 3%, respectively; $P<.0001$). As anticipated, fertility showed a negative correlation with U (-0.55, $P=0.0025$) and a positive correlation with S (0.54, $P=0.0025$). The presence of competition translates into an increased frequency of total mating attempts but does not result in more S mating attempts. As birds aged, the number of U and total attempts decreased, and S attempts stayed consistent. Mating activity differed throughout the day, with birds showing a preference for mating after the lights went on and for a few hours before the lights turned off. Additionally, higher S attempts and lower U attempts are positively correlated with fertility, leading to the production of more fertile eggs. In conclusion, male-male competition and age did not alter S mating attempts but did impact U, reflecting on the total mating attempts.

Keywords: Mating success, mating attempts, reproductive behavior, Broiler breeder, sexual competition

166 Serotonin and dopamine activity associated with aggressive feather pecking in Pekin ducks

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Feather pecking and picking are both unwanted behaviors within the Pekin duck industry. Feather pecking is the act of mutilation to a conspecific's feathers, while feather picking is the act of automutilation. Aggressive feather pecking is associated with altered brain dopamine (DA) and serotonin (5HT) activity in chickens. There is no literature about the neurochemistry that drives feather pecking in Pekin ducks. Thus, we set out to determine if feather pecking is associated with altered 5HT and DA activity. We collected brains from 6 ducks that had a body condition score of 2 (BCS; peckees), and brains from 6 ducks with a BCS of 0 (peckers) at 30 days of age. The state designation based upon BCS was confirmed by video analyses of feather pecking and BCS. Brains were dissected on the midsagittal plane. Each hemisphere was microdissected into caudal mesencephalon (CM), rostral mesencephalon (RM), diencephalon (DI), lateral telencephalon (LT) and medial telencephalon (MT). The hemispheres were used for mass spectrometry to determine the neurotransmitter concentration (ng/mg). We applied these concentrations to determine serotonin and dopamine turnover as the ratio of metabolite to neurotransmitter. After log transformation, a Student's t-Test was run for each brain region with $p \leq 0.05$ considered significant. No differences in serotonin turnover were observed between peckers and peckees, however a

trend was observed for increased 5HT turnover in peckees. Decreased dopamine turnover was observed for the peckers in the CM ($p<0.0001$), RM ($p=0.0002$), LT ($p=0.07$), and MT ($p=0.0001$), suggesting increased DA synaptic activity. Increased brain DA activity is related to aggressive behaviors in numerous species, including chickens. Our data shows that increased DA activity is associated with feather pecking in ducks and suggests that feather pecking directed at wings and tails is an aggressive behavior.

Keywords: feather pecking, serotonin turnover, dopamine turnover, Pekin ducks

167 Transportation elicits a decrease in serotonergic activity, but not dopaminergic activity, in 5-day-old grow-out Pekin ducklings

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Previous studies in our lab have shown clear sex differences in ducks' responses to stress, including transportation stress. The sex difference in adults with transportation stress was particularly pronounced in brain serotonin activity associated with affective states. Sex differences in physiology suggest the involvement of gonadal steroids. Thus, we tested the hypothesis that immature ducks would not have sex differences in response to transportation stress, given the near-absence of gonadal steroids. Five-day-old ducklings were randomly assigned to one of three treatment groups (n=10 per group): ducklings immediately euthanized in their home pens (Con), caught, crated, and kept in the home pen for 90 minutes (Crate), or caught, crated, and transported for 90 minutes under similar environmental temperatures as their home pen (Transport). Brains were collected and microdissected into the caudal mesencephalon (CM), rostral mesencephalon (RM), and diencephalon (DI). We used mass spectrometry to evaluate serotonin, dopamine, and their metabolites, and respective turnovers were calculated. We utilized an ANOVA to analyze data. No sex differences were observed in any variable measured; thus, the sexes were combined for final analyses. No significant differences in dopamine turnover were observed among the groups in any brain area. An increase in serotonin turnover, thus reduced synaptic activity, was observed in the CM (Con: 0.323 ± 0.020; Crate: 0.363 ± 0.045; Transport: 0.384 ± 0.032; $p= 0.0030$) and the RM (Con: 0.291 ± 0.019; Crate: 0.369 ± 0.039; Transport: 0.359 ± 0.016; $p = 0.0031$) in both the Crate and Transport groups compared to controls. No differences were observed in the DI among groups. These results agree with our previous report on adult ducks in that transport elicits a reduction in the affective state but differ in that there is a lack of sex difference in immature ducklings. These data strongly suggest a gonadal hormone component in adult duck stress response, emphasizing the importance of considering sex and age when evaluating stress responses. Further research is required, however, to evaluate the full role of gonadal steroids in stress and welfare in birds.

Keywords: Affective state, stress response, brain chemistry, welfare

168 Dietary flaxseed oil modulates metabolites crossing the blood-brain barrier and enhance growth performance in broiler chickens

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Dietary unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs), are important for maintaining membrane integrity and function, playing a vital role in brain health and cognitive function. The dorsal raphe nucleus (DRN) is a major source of serotonin in the brain, which plays a key role in regulating mood and behavior, while the hypothalamus (HYP) regulates energy homeostasis. Unlike poultry fat (CON), flaxseed oil (FLAX) is rich in omega-3-PUFA and is known to influence brain serotonergic signaling and behavioral traits in mice and humans. Therefore, this study aimed to investigate FLAX's influence on metabolites' composition, crossing the blood-brain barrier (BBB) to the serotonergic-rich sections of the brain and growth performance. In a 3-week experiment, day-old broiler chicks (n=160, Ross 708 male) were weighed and randomly assigned to 2 dietary treatments, consisting of 5 replicate pens of 16 chicks each. The experimental treatments included a basal diet of corn-soybean meal with a 3% dietary inclusion of two fat types; CON and FLAX. Growth indices: feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were recorded and analyzed using T-test. DRN, HYP, and Plasma were collected and subjected to the HPLC-MS metabolomics approach. This was followed by multivariate and principal component analyses (PCA) to discern distinct metabolites. The BWG was significantly ($P<0.05$) higher (0.8725 kg/bird) in FLAX compared to the CON (0.8131kg/bird). Furthermore, FLAX had a significantly ($P<0.05$) better FCR than CON. A univariate analysis using contrasts was used to identify significantly ($P< 0.05$) different metabolites between treatments, while KEGG and HMDB databases were used to annotate the metabolites. Results generated 2,271 metabolites, of which 650 ($P<0.05$) were annotated. Out of the 35 differentially expressed metabolites in the plasma, 6 metabolites crossed the blood-brain barrier and were all upregulated in the plasma of the FLAX group. In the brain region of the FLAX group, dethiobiotin (energy generation) was upregulated in the HYP, while Corydaline and Galanthamine (Neuroprotective function) were upregulated in the DRN and HYP, respectively. Xanthoxyletin (anti-inflammatory) and Gamboic acid (antioxidative) were downregulated and upregulated in the HYP, respectively, while Tryptophan, the primary precursor of serotonin, responsible for mood and behavior, was upregulated in the DRN. The upregulation of tryptophan and other neuroactive metabolites in serotonergic-rich brain regions suggests a potential link between dietary FLAX and enhanced cognitive function and adaptive behavior in broilers.

Keywords: Dorsal Raphe Nucleus, Hypothalamus, Plasma metabolites, Dietary fat type

169 Reference point indentation correlates with bone mechanical properties in chicken tibia

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Reference point indentation (RPI) is a minimally invasive technique developed to assess bone mechanical properties in vivo. RPI metrics have been implicated as indicators of bone mechanical properties in humans and mice; however, RPI usage as an alternative method to 3-point breaking is limited in poultry. The objective of this study was to investigate whether microindentation-induced cracks affect bone mechanical properties. In this study, a total of 100 clean and excised tibiae

were obtained from 45-week-old laying hens. Eighty bones were indented with RPI at different force levels (2N, 4N, 5N, 6N, 7N, 8N, 9N, and 10N), with 10 bones per force and 20 bones as a control group (0N). Following RPI, all bones underwent a 3-point bending test (BREAK) to assess bone stiffness and breaking strength. ANOVA was conducted to determine differences in BREAK metrics across indentation force levels, and correlation analysis was conducted to examine associations ($P < 0.05$) between RPI and BREAK parameters at each RPI force using Pearson's (r) and Spearman's (ρ) correlation coefficient for data that followed normal and non-normal distribution, respectively. ANOVA indicated that there was no difference ($P > 0.05$) in bone break metrics across indentation force levels, which suggests that indentation force did not affect postmortem bone mechanical properties. However, our previous RPI analysis found significant differences between different indentation forces, raising questions about how RPI metrics relate to bone mechanical properties. Correlation analyses indicated that there are differences ($P < 0.05$) in the association of RPI and BREAK metrics across indentation force levels. There was no association ($P > 0.05$) between RPI and BREAK metrics at 2N and 10N, which suggests that these forces may not be appropriate in capturing meaningful bone mechanical properties. There were positive correlations between bone breaking strength and RPI metrics of loading and unloading slopes ($r = 0.740$ to 0.806), and indentation distance increase ($\rho = 0.754$) at 6N and 4N, respectively. There were negative correlations between bone breaking strength and RPI metrics of creep indentation distance ($r = -0.783$ to -0.952) at 5N, and indentation distance ($\rho = -0.709$ to -0.733) at 7N and 9N, respectively. These findings suggest that higher loading and unloading resistance reflects stronger bones, while higher creep indentation and indentation distance increase is linked to reduced bone strength. Together these findings suggest RPI could be used as an alternative tool to 3-point bending. However, further studies should examine RPI impact on bone microarchitecture in live birds, a key utility not offered by traditional breaking tests.

Keywords: bone, correlation, indentation force, mechanical test, reference point indentation

170 A computer vision method to detect foraging behavior in cage-free hens

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Foraging behavior in hens is an important indicator of welfare status. It involves searching for food and exploration of the environment, which provides enrichment. In addition, it has been inversely linked to damaging behaviors such as severe feather pecking. Conventional studies rely on manual observation to investigate foraging location, duration, and frequency. However, the manual approach is labor-intensive, time-consuming, and subject to human bias. This study developed a computer vision-based method to automatically detect foraging hens in a cage-free pen and compared the performance of different deep learning models. A cage-free room was divided into four pens, two measuring 3 m X 2.3 m with 32 hens each and two smaller pens measuring 2.3 m X 1.7 m with 16 hens each. Cameras were positioned vertically, 3 m above the floor, recording the videos at 15 frames per second. A total of 4,886 images were used for this study: 70% for model training, 20% for model validation, and 10% for testing sets. We evaluated multiple YOLO (You Only Look Once) detection models, including YOLOv9, YOLOv10, and YOLO11 series, trained at 100 epochs each. All the models had precision, recall, and mAP@0.5 (mean average precision at 0.5 intersection over union) of 80% or higher. YOLOv9c achieved the highest precision (83.9%), YOLO11x achieved the highest recall (86.7%), and YOLO11m achieved the highest mAP@0.5 (89.5%). These results demonstrate the potential of computer vision to make the welfare assessment such as foraging behavior monitoring more efficient and objective.

Keywords: Laying hen, Cage-free housing, Animal welfare, Animal behavior, Computer Vision

Student Competition: Metabolism and Nutrition: Vitamins and Minerals

171 Graded levels of riboflavin effects on growth performance during the summer months in turkey hens

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Riboflavin is an essential water-soluble vitamin which is a cofactor that works as an electron carrier in the electron transport chain and the TCA cycle. Commonly, riboflavin is active as flavin adenine dinucleotide or flavin mononucleotide. These are key physiological processes to energy production for the body. Currently, there is a wide range of recommendations to follow for feeding riboflavin in turkeys. Resources recommend anywhere from 4 to 21 mg of riboflavin per kg of finished feed for the first six weeks of age. Summer months are challenging for turkeys with literature reporting negative impacts in body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) during heat challenges. To better understand the riboflavin needs of starter turkey hens during the summer months, we conducted a study aimed at evaluating inclusion level of riboflavin during July and August. Day-old hens (Hybrid Genetics, $N = 992$) were randomly assigned to one of thirty-two pens (31 birds/pen) which were randomly assigned to one of four dietary treatments within location block. Dietary treatments were defined by inclusion of riboflavin at either, 11.24, 16.86, 22.48 and 28.10 mg/kg. One basal diet per phase was made and equally divided into four batches for the inclusion of the respective riboflavin treatment. BWG, FI, and FCR were collected and calculated for day 0-21, 21-43 and 0-43. Daily temperature (T) and humidity (RH) were recorded hourly and were used to calculate the average daily T and RH. The daily high, low, and optimal temperature are also reported. It is worthy to note only 4 days of the 43-day trial had an average RH below 55% with the RH ranging from 58-77% for d 21-43. Average daily T was approximately optimal for days 0-21 but for days 22-43 average daily T was above optimal and never went below 75 degrees Fahrenheit. There were no differences in BWG, FI or FCR for the pre-starter period of d 0-21 ($P > 0.05$). There was a quadratic response ($P < 0.05$) for the starter phase (d 21-43) and tendency for a quadratic response ($P < 0.061$) overall (d 0-43) for BWG with increasing riboflavin inclusion in the diet. Hens had similar BWG when fed up to 22.4 mg/kg riboflavin and BWG increased in hens fed 28.1 mg/kg. These data suggest that including riboflavin at 28 mg/kg in the diet may optimize BWG during the summer months, but not FI or FCR. Further research is needed to better understand how riboflavin improves BWG and the economic benefits it may offer.

Keywords: turkeys, riboflavin, growth, summer months

172 Role of 25-hydroxyvitamin D_3 on inflammatory and biochemical responses in coccidiosis-vaccinated broilers challenged with *Eimeria* spp

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Coccidiosis impairs growth performance, intestinal and bone health. A previous study from our lab reported that *Eimeria* challenge negatively affected vitamin D_3 status, bone quality, and growth at 28 days. However, 25-hydroxyvitamin D_3 ($25OHD_3$) mitigated these adverse effects while reducing cecal lesions of *Eimeria tenella* and increasing serum concentration of $25OHD_3$. To understand the mechanism of these responses, the current study investigated the effects of $25OHD_3$ (Hy-D®, dsm-firmenich, Switzerland) on biochemical serum parameters and cytokines in coccidiosis-vaccinated chickens challenged with *Eimeria* spp. A total of 900 one-day-old Cobb 500 male chicks, vaccinated with Coccivac®-B52, were raised in floor pens and challenged on D22 with *E. acervulina* (250,000 oocysts/bird), *E. maxima* (50,000 oocysts/bird), and *E. tenella* (50,000 oocysts/bird). Birds were assigned to four treatments (25 birds × 9 replicates): NCC, non-challenged control; CC, *Eimeria*-challenged control; DD, challenge + 2,760 IU/kg vitamin D_3; and 25D, challenge + 69 µg/kg $25OHD_3$ (bioequivalent to 2,760 IU vitamin D_3/kg). All basal diets contained 1,400 IU/kg vitamin D_3. Gut lesions were evaluated, and blood biomarkers were analyzed by using VetScan, iStat, and iCheck at D28. Blood cytokine concentration (MILLIPLEX®) was measured at D28 and 42. Data were analyzed using one-way ANOVA with Tukey's HSD ($P < 0.05$). The *Eimeria* challenge significantly reduced total protein, albumin, carotenoids, Ca, P, and CO_2 ($P<0.05$), and tended to decrease bicarbonate (HCO_3; $P=0.07$), and to increase uric acid (UA; $P=0.08$). Additional $25OHD_3$ increased blood pH ($P=0.02$) when compared to the CC group, and tended to restore CO_2, HCO_3 and UA level similar to NCC. During the acute challenge phase (6 dpi), coccidiosis challenge numerically increased IL-10 and significantly reduced IFN-a, potentially as response to reduce inflammation for coccidia survival and replication. Adding $25OHD_3$ mitigated these changes and brought IL-10 ($P=0.004$) and IFN-a ($P=0.016$) back to a similar level to NCC. At D42, $25OHD_3$ lowered IFN-a compared to CC, which might contribute to the recovery from infection ($P=0.046$). In summary, *Eimeria* challenge impaired blood biochemical concentrations, reduced carotenoid absorption, and may have impacted kidney function (CO_2, HCO_3, pH, UA level). By adding $25OHD_3$, these effects were mitigated, which shows that this metabolite may possess additional benefits on the health of the chickens, apart from what is traditionally known. It also showed effects on the regulation of the inflammatory response, which might contribute to the overall health of the birds.

Keywords: 25-hydroxivitamin D3, broiler, cytokines, coccidiosis challenge, vaccine

173 Egg quality and hatchability response to canthaxanthin and 25-Hydroxyvitamin D3 in broiler breeder hens

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Egg quality is a key determinant of embryonic development and hatchability in broiler breeder production. Parameters such as eggshell quality, albumen quality, and yolk composition play essential roles in protecting the embryo, regulating moisture loss, and facilitating gas exchange during incubation. In recent years, a decline in hatchability has been linked to compromised egg

quality. Our study evaluated the effects of dietary supplementation with 25-hydroxyvitamin D3 (HyD), canthaxanthin (Cx), copper (Cu), and gluco-oligosaccharides (GO) on egg quality and hatchability in broiler breeders from 30 to 50 weeks of age. Using a completely randomized design, 210 hens and 21 males were allocated to 21 floor pens (10 females and 1 male per pen), with seven replicates per treatment. A basal diet consisting of a standard broiler breeder layer diet formulated with 0.5 kg/MT sand as a filler served as the control (C). Maxichick® (MC; containing HyD and Cx) and Maxirepro™ (MR; containing HyD, Cx, Cu, and GO) were included in the basal diet by replacing the sand at equivalent levels. Birds were adapted to the basal diet from 25 to 30 weeks, after which treatment diets were fed for 20 weeks. Egg quality was assessed at weeks 30, 35, 41, 45, and 50, while hatchability was evaluated at weeks 30, 41, and 50. Data were analyzed using one-way ANOVA, with significance set at $p \leq 0.05$. Both MC and MR significantly improved yolk color throughout the study. Maxirepro™ enhanced albumen height and Haugh units, while MC improved shell thickness, shell percentage, and shell weight at 45 weeks. Maxichick® also yielded higher hatchability compared to MR and control groups. Although some egg quality traits were negatively correlated with hatchability, overall, results indicate that Maxichick® and Maxirepro™ supplementation from 30 to 50 weeks can enhance egg quality and improve hatchability in broiler breeder hens.

Keywords: Egg quality, Hatchability, Broiler breeders, 25-hydroxyvitamin D_3, Canthaxanthin

174 Effects of multiple 25 Hydroxyvitamin D sources on the performance and eggshell quality of 95-week-old white laying hens

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The objective was to investigate the effects of various sources and doses of 25-hydroxyvitamin D3 (25OHD3) on the performance and eggshell quality of caged 95 to 101-week-old white laying hens. Laying hens were sourced from a commercial egg facility and were allowed a 2-week adaptation period (initial egg production = 74.8%; P = 0.98) before experimental diets were provided. The dietary treatments included a control diet with no 25OHD3, 0.5 kg/MT of a commercially available, synthetically derived 25OHD3 (25OHD3-1S), 0.5 kg/MT of a commercially available naturally derived 25OHD3 (25OHD3-1N), and the same naturally derived 25OHD3 fed at 1 kg/MT (25OHD3-2N). Experimental laying hens were control fed 105g/d of feed to 20 replicate cages of 3 laying hens for each treatment. Hen day egg production (HDEP), egg weight (EW), and egg mass (EM) were determined daily and analyzed every 2 weeks. Feed intake was determined every 2 weeks and used to calculate feed conversion ratio (FCR) every 2 weeks. Eggs were collected weekly for external egg quality determination. At the end of the experimental feeding period all remaining hens were euthanized, and the right tibia was collected for fat-free tibia ash determination. Performance and egg quality data were analyzed using repeated measurements using ANOVA and initial egg production. Tibia ash was analyzed using ANOVA. Significance was accepted at $P \leq 0.05$. There were no differences in HDEP (66.8% - 71.8%; P = 0.20), but 25OHD3-2N improved FCR by 8 points over the control and significantly improved FCR in comparison to the 25OHD3-1S and 25OHD3-1N treatments (P = 0.01). Shell thickness, shell weight, shell breaking strength and egg specific gravity were all numerically increased with the 25OHD3-2N in comparison to the other treatments, but only shell thickness and specific gravity reached significance. The 25OHD3-2N increased eggshell weight by 2.0 percentage points and specific gravity 0.002 units in comparison to the control, 25OHD3-1S and 25OHD3-1N treatments ($P \leq 0.05$). All 25OHD3 treatments increased tibia ash and the 25OHD3-2N treatments maximized tibia ash on both a weight and percentage basis. Overall, 25OHD3 metabolites appear to effectively enhance shell quality and skeletal mineral reserves, with the 25OHD3-2N yielding the greatest response.

Keywords: Laying hen, egg quality, vitamin D, metabolite

175 Comparison of relative Ca bioavailability based on bone ash and apparent ileal Ca digestibility with and without phytic acid in broiler chickens

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The object of this study was to determine the effect of phytic acid on Ca availability using relative Ca bioavailability based on bone ash and apparent ileal digestibility (**AID**) of Ca and to compare the Ca availability estimates obtained from the 2 bioassays. In Experiment 1, relative Ca bioavailability of reagent-grade calcium carbonate (**$RCaCO_3$**) and $RCaCO_3$ + phytic acid was determined in ad libitum-fed broiler chickens using the slope-ratio method. Dietary treatments were corn-soybean meal-based diets and birds were provided feed ad libitum from 7 to 21 d-of-age. Diet 1 was Ca-deficient (0.30% Ca; 0.45% non-phytate P), while diets 2 through 5 were the same as diet 1, except that 2 increasing levels of $RCaCO_3$ or $RCaCO_3$ + phytic acid were added in place of Solka Floc to increase the dietary Ca level to 0.45 or 0.60%. Relative Ca bioavailability was determined by regressing bone ash content (mg/tibia) and concentration (%) against supplemental Ca intake. In Experiment 2, AID of Ca in $RCaCO_3$ with and without phytic acid was determined in broiler chickens using corn-based diets (0.83% Ca and 0.45% non-phytate P), where $RCaCO_3$ was the main Ca source in the diet. There were 2 dietary treatments that were identical, except diet 1 contained $RCaCO_3$, while diet 2 contained $RCaCO_3$ + phytic acid. Birds were provided experimental diets ad libitum from 19 to 21 d-of-age. In Experiments 1 and 2, there were 5 and 8 replicate pens of 5 chickens per treatment, respectively. Data were analyzed using a one-way ANOVA and slopes generated from regression of bone ash against supplemental Ca intake in Experiment 1 were compared using slope contrasts. The significance value for all analyses was $P < 0.05$. In Experiment 1, tibia ash (% and mg/tibia) increased linearly ($P < 0.05$; $R^2 = 0.89 – 0.94$) as supplemental Ca intake from $RCaCO_3$ and $RCaCO_3$ + phytic acid increased. Relative Ca bioavailability obtained from multiple linear regression of tibia ash content (mg/tibia) against supplemental Ca intake was reduced ($P < 0.05$) from 100 to 80% when phytic acid was added. In Experiment 2, phytic acid reduced AID of Ca in $RCaCO_3$ from 38.1 to 30.4% ($P < 0.05$). Further, the relative AID of Ca was reduced from 100 to 80% when phytic acid was added. In conclusion, phytic acid reduced both relative Ca bioavailability based on bone ash and AID of Ca in broiler chickens. Furthermore, the relative comparison between the 2 test ingredients in both bioassays was similar, indicating that both assays can potentially be used to assess Ca availability and that Ca digestibility values may be able to be predicted from relative bioavailability values as long as the AID of Ca in the standard reference Ca source is known.

Keywords: Calcium, bioavailbility, digestibility, phytic acid, bone ash

176 Effect of different limestone sources, solubility and inclusion of phytase on apparent ileal digestibility of Ca and P in broiler chickens

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A study was conducted to determine the effect of 18 limestone sources varying in solubility from 10 different countries, and inclusion of phytase on apparent ileal digestibility (AID) of Ca and P. The 1,728 day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery and raised in battery cages. Birds were fed a common starter diet in mash form from days 1 to 19 post-hatch. Each limestone source was then added at 1.5% in experimental diets without and with phytase (HiPhorius™, dsm-firmenich, Switzerland) at 1000 FYT/kg, which were provided ad libitum to 2 replicate cages of 6 birds from day 19 to 21. Titanium dioxide (0.5%) was added as an indigestible marker for calculations of AID. This protocol was repeated 4 times to a total of 8 replicate cages per treatment. At the end of the experiment, birds were euthanized using CO_2. Digesta were collected from the distal half of the ileum. Samples of digesta and diets were analyzed for titanium, P and Ca. The statistical model was an 18 x 2 factorial arrangement for 18 limestone sources and 2 inclusions of phytase; in addition, a 3 x 2 factorial arrangement for 3 limestone solubilities (slow, medium, fast) and inclusion of phytase was analyzed. Experimental repetition was set as a block for each analysis and significance was determined at $P < 0.05$. There was a significant interaction between limestone source and phytase for AID of Ca ($P = 0.006$) and P ($P = 0.001$), with phytase increasing AID of Ca and P more in some limestones than others. Likewise, there was a significant interaction between limestone solubility and phytase for AID of Ca ($P = 0.008$) and P ($P = 0.009$). The medium and fast solubility limestones showed higher AID of Ca, but lower AID of P compared to slow, whereas the improvements in AID of Ca and P by phytase were greater for medium than for slow and fast solubility. These results indicate that AID of Ca, P, and the magnitude of the increase associated with phytase, varied among limestone sources and limestone solubility. This emphasizes that the *per se* Ca and P value of diets, and the effect of phytase, may need to be tailored according to specific limestone characteristics.

Keywords: Calcium, Phosphorus, Digestibility, Limestone, Phytase

177 Evaluating the additivity of digestible calcium in a mixed diet formulated from organic and inorganic dietary calcium sources

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The digestible calcium **(Ca)** system allows for a more accurate estimation of how phytase influences Ca availability, better aligning the Ca-to-P ratio and preventing negative interactions like reduced phosphorus availability due to excess free Ca. A study was designed to determine digestible Ca additivity in broiler chickens when fed mixed diets formulated with limestone, meat and bone meal **(MBM),** and canola meal **(CM)** without or with exogenous phytase. A total of 444 male broiler chickens were used in the trial from d 18 to 21 post hatching with 6 replicates per treatment. Dietary treatments were arranged as 4 × 2 factorial with 4 diets based on limestone or MBM or CM as the sole contributor of Ca, or a combination of limestone, MBM, and CM, and phytase supplementation at 0 or 1,000 FYT/kg. A Ca-free diet **(CFD)** was formulated with yellow corn and potato protein to evaluate the basal endogenous Ca losses **(BEL)**. The estimated BEL of Ca in birds fed the CFD at the ileum and total tract level were 0.369 and 0.456 g/kg dry matter intake, respectively, with numerically greater endogenous Ca output observed at the total tract level compared to the ileum. A significant interaction effect ($P < 0.05$) between Ca sources and phytase supplementation was observed for the apparent ileal digestibility **(AID)** of P, where phytase only improved P digestibility in the canola meal-based diet, while no such improvement was observed in other Ca sources. Phytase only improved the AID and standardized ileal digestibility **(SID)** of Ca in the single diet formulated with CM but not in the other Ca sources, leading to an interaction effect ($P < 0.05$). The predicted SID of Ca was different ($P < 0.05$) from the determined values in the mixed diet when without or with supplemental phytase. The expected SID of Ca in the mixed diet was predicted to be 52.6% without phytase and 50.4% with phytase, but the determined values were lower, at 37.6% and 34.0%, respectively. Therefore, the ileal digestibility of Ca was not additive in the mixed diet in the absence or presence of exogenous phytase. The results demonstrate the variations in the utilizable Ca from different feed ingredients and enhance our understanding of the dynamic impact of phytase in improving Ca utilization in a mixed diet based on the unique combination of feed ingredients.

Keywords: calcium, canola meal, meat and bone meal, limestone, phytase

178 Phosphorus requirements of laying hens in the presence of a mega dose of phytase

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This study aimed to evaluate the effects of graded increases of available phosphorus (Av. P), with or without phytase supplementation. A total of 324 Bovans White laying hens (18 weeks old) were individually housed in cages following a completely randomized design. From weeks 18 to 25, the hens were fed a common commercial diet and then fed the experimental treatments from 26 to 57 weeks in eight 28-day periods. Treatments were diets containing 0.09% (no supplemental phosphate), 0.16%, 0.23%, 0.30%, 0.37%, and 0.44% Av. P, with or without phytase (4,000 FTU), in a 6 × 2 × 8 factorial arrangement. Each treatment had 27 replicates. Egg production was evaluated daily from 26 to 57 weeks. Egg quality was evaluated once per period by assessing egg weight, yolk, albumen, and shell percentage and quality, as well as breaking strength, specific gravity, and eggshell thickness. At 57 weeks of age, 15 tibiae per treatment were collected to determine bone-breaking strength and calcium and phosphorus content. Additionally, blood samples were collected to evaluate circulating alkaline phosphatase levels. Data were analyzed as repeated measures in a three-way ANOVA (phytase, Av. P, and period) using SAS with significance at $p \leq 0.05$. Results indicated that egg production, eggshell quality, feed conversion ratio, and feed intake were significantly influenced by available phosphorus levels, phytase

supplementation, and experimental period ($p < 0.05$). Phytase supplementation increased egg production by 23% in hens fed a diet without supplemental phosphate ($p < 0.05$). Tibia mineral content increased as dietary Av. P levels increased ($p < 0.05$). Furthermore, levels of circulating alkaline phosphatase varied significantly among treatments ($p < 0.05$). Phytase supplementation improved egg production in hens fed a non-phosphate-supplemented diet, indicating its role in enhancing phosphorus bioavailability. These findings highlight the potential of phytase to reduce dietary phosphate dependency while maintaining production performance and bone integrity.

Keywords: Phytase, Performance, Laying hens, Available phosphorous

179 Evaluating the effects of different supplemental Selenium sources on Pekin duck growth performance and tissue deposition

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This 35-d duck study evaluated the effects of feeding different Selenium (Se) sources on the performance and Se deposition in various tissues of Pekin ducks. A complete randomized design was used to distribute 6 dietary treatments, each consisting of 10 replicate pens and 25 straight-run day-of-hatch Pekin ducks/pen. The treatments were: Negative control (T1, no SE supplementation); Positive control (T2, 0.3ppm Sodium Selenite); Selenomethionine Hydroxy Analogue (SMA) at either 0.2 ppm (T3), 0.3 ppm (T4), or 0.6 ppm (T5); Selenium Yeast (0.3ppm, T6). Body weight (BW) and feed consumption (FC) of birds from 0-35 d-of-age were measured for each pen; mortality corrected feed conversion ratio (FCR) was calculated based on these measurements. On d 35, 1 duck/pen had blood collected for Glutathione Peroxidase activity (GPX) and then was euthanized to collect liver, pectoralis major, skin, abdominal fat, and kidney samples to determine Selenium deposition. Data were analyzed using the ANOVA and means were separated using Tukey's Procedure ($P<0.05$). No differences ($P > 0.05$) were observed between treatments in BW, FC, FCR or mortality during the study. However, differences in GPX and Selenium deposition were observed ($P <0.05$), especially as the level of SMA increased. The GPX activity was lower in the NC than all other treatments ($P < 0.05$). In the liver, pectoralis major, skin, and kidney T5 had the highest ($P < 0.05$) Se deposition compared to all other treatments. In the fat T4 was highest (P 0.05) in Se deposition compared to all other treatments. These results indicate that although Se supplementation had no effect of production under a stress-free grow out, it was demonstrated that the duck is able to effectively and safely store organic Se when compared to Se from inorganic and seleno yeast sources.

Keywords: Selenium, Selenomethionine Hydroxy Analogue, Selenium Yeast, Pekin duck, Performance

180 The interaction of dietary copper levels and litter conditions on broiler growth performance

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Restrictions on antibiotic usage in poultry production have led to increased supplementation of copper in broiler diets due to its growth-promoting and antimicrobial effects. This study evaluated the impact of copper levels (CuSO4 5H2O) on the growth performance of broilers raised under different litter conditions up to 49 days of age. A total of 1,776 one-day-old male Cobb 500 chicks, vaccinated with Coccivac-B52®, were randomly assigned to a 4×2 factorial arrangement (6 replicates*37 birds), with four copper levels (5, 125, 250, and 500ppm) and two litter conditions (used and new). At 14, 28, 42, and 49d, body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated. Blood copper levels were measured at 28, 42, and 49 d, and gizzard lesions were assessed at 49d. Data were analyzed using two-way ANOVA with Tukey's HSD for mean separation ($P<0.05$). The broken line model was used for marginal safety dosage. During 0-14d, the interaction effects showed 125 ppm Cu resulted in higher BW, BWG, and FI in new litter than used litter, while 500 ppm Cu has the lowest BW, BWG, and FI ($P<0.0039$) regardless the litter type. Furthermore, main effects of Cu showed 500 ppm had higher FCR than 5 and 250 ppm ($P=0.0016$). During 15-28d, interaction effects showed 250 ppm Cu did not significantly affect performance in either litters, except 125 ppm showed higher BW in new litter than used litter. Similar to the previous phase, 500 pm Cu resulted in lowest BW, BWG, and FI ($P< 0.0072$). In addition, main effects showed 250 ppm had better FCR than the rest of treatments ($P<0.0001$), and new litter has better FCR than used litter ($P=0.0014$). There is no interaction for the rest of phases, however, the main effects consistently showed the 500 ppm Cu had lowest performance parameters ($P<0.0001$). Meanwhile, the main effects on litters exhibited opposite effect to 0-28d, with used litter presented higher BWG ($P=0.0058$), FI (29-42d) ($P=0.0467$), and higher FI (43-49d)($P=0.0051$) than new litter. From the broken line model, the margining level to cause adverse effects on BWG is 250 ppm for both new and used litter up 28d. However, in the later stage, the new litter has lower safety margining than used litter, which resulted in 234 ppm for new litter, and 250 ppm for used litter ($P<0.05$) during 0-49 d. No significant difference was found in mortality, blood copper levels, or gizzard erosion. In conclusion, 500 ppm Cu showed the worst performance in broilers regardless of litter conditions. And new litter has lower safety margining for Cu than the used litter.

Keywords: Copper Sulfate, Broilers, Growth performance, Gizzard erosion, Mineral

181 Reducing copper requirement with organic supplementation in pullets

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Copper (Cu) is a trace mineral (TM) needed as a cofactor for several enzymes in metabolic processes, playing key roles in antioxidative and immune function. Inorganic Cu has low bioavailability because of its interactions with other minerals and ligands in the feed, while Cu proteinate has been proved to be more bioavailable. This study was designed to investigate the productive performance, oxidative profile, and mineral composition of laying pullets fed inorganic and proteinate Cu sources during the growing phase. Five hundred and thirty-two 1-d old Novogen white chicks were distributed into a completely randomized design with 2 treatments consisting of 100% of the Cu requirement using copper sulfate (CuS) and 50% of the requirement using Cu proteinate (CuP, Bioplex Copper, Alltech Inc.). Diets were prepared according to requirements of 11.18 mg Cu /kg from 1-4 weeks and 9.48 mg Cu/kg from 5-16 weeks. Animals were analyzed from 1 to 4 weeks, from 5 to 14 weeks, and from 1 to 14 weeks for feed intake (FI), feed conversion g/g (FC), body weight (BW), and weight gain (WG). Lipid peroxidation of serum (week 15) and liver (week 16) were also observed. Tissue and excreta (week 16) mineral concentrations including copper, iron (Fe), and zinc (Zn) were quantified. The data were analyzed by ANOVA, and the means were compared by Tukey test using SAS v.9.0. Results were considered significant at $p \leq 0.05$. The productive performance variables were similar among the two treatments ($p>0.05$) in all periods. From 1 to 14 weeks, pullets weighed on average 1069.06 g BW, FI was 4032.51 g/bird, FC was 3.58, and WG was 1031.21 g. The malondialdehyde concentration (MDA) of serum from birds fed CuP diet was lower ($p=0.04$) than that from birds fed CuS diet (10.50 mg/ml vs. 12.00 mg/ml). However, there was no statistical difference for MDA in the liver, with an average of 9.11 mg/mL of MDA for CuS and 8.45 mg/mL for CuP. Pullets fed the CuP diet had a higher concentration of Zn in the spleen ($p=0.02$) and Cu in the heart ($p=0.01$) compared to those fed CuS diet. The Cu sources had no significant effect ($p>0.05$) on the deposition of Cu, Fe, and Zn in the oviduct, breast muscle, and liver, or in the excreta at 16 weeks of life. Under the conditions of this experiment, providing Cu at 50% of requirement with Cu proteinate can be used in the diet of laying pullets during the growth phase (1 to 14 weeks) without negative impact on productive performance and may lower lipid peroxidation in the serum.

Keywords: antioxidant activity, copper proteinate, copper sulphate, mineral deposition, performance

182 Use of two selenium sources in the diets of heavy breeder hens and their effect on the small intestine of embryos at 17 days of incubation

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The present study aimed to histologically evaluate the intestines of embryos at 17 days of incubation fed different sources of selenium in breeders' diet. Forty-eight breeder hens and eight roosters of the Cobb 500® lineage with 53 weeks of age were fed diets containing different sources of selenium for 28 days: selenomethionine (0.2%) and sodium selenite (0.011%). A total of 167 eggs from these breeders were incubated, and on the 17th day of incubation, 10 eggs with embryonic development from each treatment were selected for embryo euthanasia by refrigeration. The small intestines of the embryos were collected because selenium is expected to exert its antioxidant action directly on intestinal cells, contributing to the development and integrity of this tissue. The small intestine is the primary site of amino acid absorption, making it essential for evaluating the action of selenomethionine. Since this form of selenium is chelated to an amino acid, its absorption occurs predominantly in this portion of the digestive tract. This effect may enhance nutrient absorption efficiency during the animals' growth and production phases. Histological slides of each segment (duodenum, jejunum, and ileum) were prepared and stained with Mayer's Hematoxylin and Eosin. The slides were analyzed using a LEICA DM4B bright-field microscope with an attached camera (DFC700 T), determining villus length, villus width, crypt depth, and crypt width in three distinct villi from each structural segment at magnifications of 5x, 10x, and 20x using LAS X software. Additionally, the villus-to-crypt ratio and absorptive surface area were calculated. Data was analyzed using a completely randomized design (CRD) through ANOVA with resources available at R® software. Parametric means were compared using the t-test (5%). Preliminary results of the histomorphometric evaluation of the small intestine of embryos showed no statistically significant differences in villus length, villus width, crypt depth, villus-to-crypt ratio, or absorptive surface area in the duodenum, jejunum, and ileum segments. However, a statistically significant difference was observed in crypt width in the duodenum and jejunum segments, with the selenomethionine treatment showing a smaller crypt width. This finding suggests a higher density of villi in these segments of embryos at 17 days of incubation. Based on these preliminary results, it is possible to infer that the selenomethionine treatment promotes denser villi in the duodenum and jejunum segments, which may indicate that as these cells mature, these animals could have a greater capacity for nutrient absorption.

Keywords: Antioxidant, Histology, Mineral

183 Effect of trace mineral sources on antioxidant response and chick quality under heat stress

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This study aimed to evaluate the effects of different trace mineral sources on the antioxidant response of heat-stressed embryos and the quality of chicks at hatch. A total of 420 Cobb 500 hens and 40 Cobb broiler breeder males were allocated in a completely randomized design. From weeks 25 to 45, the hens received two different diets: one containing only inorganic trace minerals (Zn, Mn, Cu, Fe and Se) and the other containing chelated trace minerals plus chromium (Zn, Mn, Cu, Fe, Se and Cr), with 10 replicates for each diet. At 45 weeks of age, eggs were collected for incubation. After seven days of storage, the eggs were categorized according to their eggshell translucency score (low, medium, and high). From day 1 to day 15 of incubation, the eggs were incubated at 37.5°C with 65% humidity. On day 16, the eggs were subjected to a heat stress stimulation at 38.9°C for 7.5 hours. The eggs were weighed before incubation and at transfer. At hatch, liver samples were collected from 30 chicks per treatment, exclusively from eggs with high translucency scores. Oxidative stress was assessed using the following markers: gene expression related to Nrf2, glutathione peroxidase, carbonylated proteins, superoxide dismutase, thiobarbituric acid reactive substances, 8-hydroxy-2-deoxyguanosine, and heat shock protein 70. The chicks

were weighed both with and without the yolk sac, and their length was measured. Data were analyzed using ANOVA in the GLM procedure of SAS, with significance set at $p \leq 0.05$. A two-way ANOVA (2 mineral sources × 3 translucency scores) was performed. The results showed higher levels of carbonylated proteins and gene expression related to Nrf2 in the chelated trace mineral treatment. In contrast, the inorganic treatment resulted in higher expression of heat shock protein 70 ($p < 0.005$). Other oxidative stress parameters were not affected by the treatments ($p > 0.005$). Eggs from hens fed chelated trace minerals exhibited lower weight loss in the low translucency score group ($p < 0.005$). Additionally, chicks from high translucency score eggs showed higher length and lower yolk sac weight ($p < 0.005$). This study suggests that chelated trace minerals, particularly those with chromium, enhance the antioxidant response, improve chick quality, and reduce egg weight loss under heat stress, especially in eggs with low translucency scores.

Keywords: Trace minerals, Antioxidant response, Heat stress, Eggshell translucency, Chick quality

184 Zinc oxide adversely impacts broiler development starting at moderate supplemental levels

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This study aimed to investigate the effects of Zn oxide (ZnO) supplementation on carcass yield, growth performance, and Zn metabolism in broiler chickens. A total of 960 slow-feathering Cobb × Cobb 500 male chicks were allocated in 64 pens and randomly assigned to one of four dietary treatments. The control diet was supplemented with 100 mg/kg of zinc sulfate heptahydrate (ZnSO4-7H2O) had 300, 600, or 1,200 mg/kg of ZnO, in addition to the basal 100 mg/kg of ZnSO4-7H2O. Throughout the experiment, performance parameters such as water intake, feed intake (FI), feed conversion ratio (FCR), and body weight gain (BWG) were monitored. Additionally, serum biomarkers, metallothionein (MT) concentrations, and liver Zn content were examined, along with excreta moisture, nutrient retention, and Zn excretion. Data were analyzed using ANOVA and linear regression models. The results indicated that Zn supplementation did not significantly improve overall broiler performance ($p < 0.05$), on the contrary, increasing ZnO levels led to linear reductions in BWG, FCR, water intake, and carcass yields, including yields of commercial cuts. Serum Zn and MT concentrations, as well as liver Zn levels, increased in a dose-dependent manner, while plasma Cu-Zn-superoxide dismutase (SOD) activity declined with higher ZnO inclusion ($p<0.05$). Moreover, diets with higher ZnO content were associated with increased dry matter content and Zn excretion, indicating lower mineral retention efficiency and raising environmental concerns ($p<0.05$). Hepatic biomarkers ALT and AST also showed significant increases in broilers fed the 1,200 mg/kg ZnO diet. Overall, these findings highlight the complexity of ZnO supplementation in broiler nutrition, emphasizing both its metabolic effects and environmental implications, and suggesting that excessively high ZnO levels may be detrimental to overall production efficiency and broiler growth performance.

Keywords: Mineral homeostasis, Performance, Overdose, Toxicity, Zinc oxide

185 Making a splash: Environmental enrichment has no effect on brain dopamine and serotonin levels or synaptic activity in Pekin ducks

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Little is known about what produces a positive affective state in ducks. Open water sources have been suggested to improve ducks' ability to perform normal behaviors, however this has been controversial. Brain serotonin and dopamine activity have been associated with affective states for decades and in many mammalian and avian species. Thus, we set out to determine if water-based enrichment can impact the affective state in grow-out Pekin ducks through the lens of brain neurotransmitter levels and activity. Our study investigated the link between feather pecking with levels and activity of brain serotonin (5-HT) and dopamine (DA). To accomplish this goal, we evaluated the effect of preening cups on 5-HT and DA turnover via mass spectrometry. The goal of our study was to determine if environmental enrichment would have an affect on brain neurotransmitter activity that has been associated with a positive affective state. Grow-out Pekin ducks (n = 525) were housed at Purdue and raised per industry standards. On day 16, brains were collected from ducks in pens before the enrichment were placed (PRE, n = 20) and, again on day 44, in pens with wiffle balls (n = 5), a preening cup (PC = 5), Pekino (PEK = 5) and ducks without any enrichment (CON = 5). Brains were dissected into right and left halves, then further microdissected into 4 brain areas: caudal mesencephalon (CM), rostral mesencephalon (RM), diencephalon (DI), and telencephalon (T). The right hemisphere was used for mass spectrometry to determine the neurotransmitter concentration (ng/mg of tissue) and those concentrations were applied to neurotransmitter turnover equations. Data were analyzed at the duck level using ANOVA (SAS JMP) with $p < 0.05$ considered significant. Our results revealed no significant differences in neurotransmitter levels or turnover. These data suggest that the presence of the enrichments had no impact on affective state after 26 days of exposure. This project however, is part of a larger study that incorporates behavioral analysis via video footage to quantify actual duck behavior. In order for this section of data to be conclusive, we will need to relate the neurotransmitter data with behavior in order to develop a complete picture.

Keywords: welfare, brain, environmental enrichment, affective state

186 The impact of floor hatching on hatchability and performance of broiler chicks

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Several broiler companies have indicated interest in hatching eggs on the floor of poultry houses rather than in the incubator. Three pen trials were conducted to evaluate hatchability and bird performance between hatching eggs on the floor (FH) and traditional incubator. The studies were conducted in 2.3 m^2 pens in 3 rooms, each containing 6 pens. On Incubation Day 18, eggs were randomly distributed into FH or hatchery-hatched (HH) groups. FH eggs were placed on the pine shavings in the pens, and HH eggs were set in a traditional incubator. On the day of hatch, HH chicks were placed into the remaining 9 pens. A different number of eggs and breeder flock ages were used in each study: In trial 1, 468 eggs from 43 wk-old breeders; Trial 2, 502 eggs from 34-wk-old breeders; and Trial 3, 597 eggs from 30-wk-old breeders. The room target temperature was 33.9°C until chick placement and then gradually reduced to 25.5°C by D21. Relative humidity (RH) was kept below 60%. Temperature and RH were recorded every 5 minutes. Individual body weight (BW) and feed consumption were recorded on 0, 3, 7, 14, and 21 d. Mortality was recorded daily. Data were analyzed by one-way ANOVA ($P \leq 0.05$). In the first trial, FH hatchability (95.3%) was 2.7% higher than HH chicks (92.6%). BW was lower for the FH chicks compared to HH chicks on D3 ($P \leq 0.05$), and BW gain was lower for the FH chicks on D3 and D14 ($P \leq 0.05$). The feed intake per bird was lower for the FH chicks on D3 and D7 ($P \leq 0.05$), while the feed-to-gain ratio was higher for FH chicks on D3 ($P \leq 0.05$) but lower on D7 ($P \leq 0.05$). In the second trial, FH hatchability (98.4%) was 0.8% lower than HH chicks (99.2%). BW was lower for the FH chicks compared to the HH chicks ($P \leq 0.05$), and on D3 FH chicks gained less BW than HH chicks ($P \leq 0.05$), while FCR was higher ($P \leq 0.05$). In the third trial, the FH chick's hatchability (97.6%) was 0.7% lower than HH chicks (98.3%); however, no statistically significant differences were observed for any of the performance parameters measured. No differences were observed in any of the parameters evaluated on D14 and D21 for any of the trials. Numerically higher mortality was observed in the FH chicks compared to the HH chicks in all trials, probably due to the removal of cull chicks traditionally done at the hatchery. While further studies will be conducted looking at the impact of hatching eggs on the floor, these data suggest that hatching chicks on the floors with pine shavings does not impact broiler performance through 21 days of age.

Keywords: Hatchery, Environmental Control, floor hatch, Hatchability

187 Thermal responses to feed intake in newly hatched chicks

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Maintaining an appropriate body temperature is vital for neonatal poultry, as their thermoregulatory capacity is immature during the first 72hr of the post-hatch period. At this stage, chicks rely on external heat sources and metabolic processes to stabilize their internal temperature, which is essential for early survival. This study evaluated the influence of feed intake on internal and surface body temperatures in newly hatched broiler chicks. Twenty broiler chicks underwent a total of ten temperature measurements, five prior to and five after feeding. Internal temperatures were recorded subcutaneously and in the crop via transponders and by digital vent thermometry. Surface temperatures were captured using infrared images from the front, left side, and top of the birds. Data were analyzed using generalized linear mixed models (PROC GLIMMIX, SAS 9.4) with gamma distribution and log link; means were compared using Tukey-Kramer tests. Subcutaneous temperature rose significantly after feeding, with values in rounds 1 to 5 (11.1°C, 11.2°C, 11.1°C, 11.1°C, 11.3°C) lower than rounds 6 to 10 (11.6°C, 11.8°C, 11.9°C, 11.8°C, 12.1°C), and round 6 lower than round 10. Crop temperature followed a similar trend, with rounds 1 to 4 (11.1°C, 11.2°C, 11.1°C, 11.1°C) lower than

rounds 6 to 10 (11.7°C, 11.7°C, 11.9°C, 11.8°C, 11.9°C). Vent temperature rose from 10.3°C to 12.0°C ($P < 0.01$), with rounds 1 to 4 (10.3°C, 10.2°C, 10.0°C, 10.1°C) lower than rounds 6 to 9 (11.0°C, 11.1°C, 11.2°C, 11.6°C), and round 10 the highest (12.0°C). Surface temperatures, although significantly different, showed no clear relationship to feed intake. Front maximum temperature varied across rounds, with round 2 (11.2°C) being higher than rounds 1 (10.1°C), 6 (10.2°C), and 10 (10.7°C), with no difference between rounds 1 and 10. Left side maximum temperature was lower in round 1 (11.2°C) than rounds 2 to 10 (11.2°C, 11.7°C, 11.7°C, 11.7°C, 11.6°C, 11.6°C, 12.1°C, 12.3°C, 12.2°C, 12.1°C). Round 6 was also lower than rounds 7 and 8, and round 8 was higher ($P < 0.01$) than all rounds. Top of body maximum temperature was lower in round 1 (7.2°C) than rounds 2 to 10 (7.2°C, 8.1°C, 7.9°C, 8.0°C, 8.7°C, 8.5°C, 8.9°C, 9.1°C, 9.2°C, 9.3°C), with the most significant temperature measurement seen in round 8. A temperature drop occurred in round 6 across many surface temperature variables, coinciding with water ingestion. Internal temperatures consistently rose in post-feeding, confirming the thermal effect of feed intake that occurs during early development. These findings show that postprandial metabolic heat raises internal body temperature in neonatal chicks, though this is not fully reflected in surface temperatures via infrared thermography.

Keywords: neonatal chicks, internal temperature, surface temperature, metabolic heat production, infrared thermography

188 Impact of feeding regimens during rearing on reproductive parameters in broiler breeder hens through sexual maturity

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Increased consumer demand for poultry has led to intensive genetic selection for broiler growth, requiring restricted feeding regimens to regulate body weight (BW) and flock uniformity in broiler breeders prior to sexual maturity. Thus, this study aims to evaluate the impact of pullet feeding regimens on physiological alterations. At 3 weeks of age (woa), 216 Cobb 500 FF pullets were randomly placed into individual cages pre-assigned one of three treatments: (1) daily control diet (CON), (2) skip-a-day control diet (SKIP) or (3) daily high fiber diet (ALT). SKIP birds transitioned to a daily allocation at 19 woa, while ALT continued for the remainder of the trial. Rearing photoperiod was maintained at 8L:16D. Birds were photostimulated at 21 woa. All birds were weighed weekly, and eggs were collected and weighed daily, noting any softs and cracks. Egg quality measuring egg shape index (ESI), eggshell thickness (EST), egg weight (EW), eggshell breaking strength (EBS), and Haugh Units (HU) was performed weekly from 24 to 29 woa and then every 5 weeks from 30 to 60 woa. An ANOVA was conducted using the MIXED procedure SAS v9.4, with age and treatment as the fixed effects when appropriate, and relationships determined using Pearson correlation coefficients. A treatment effect on age at first egg (AFE) (P=0.007) was found with ALT and CON hens entering lay earlier than SKIP hens. Additionally, ALT hens weighed more than CON and SKIP hens at the time of AFE (P=0.001), demonstrating a strong positive correlation (r^2=0.703; P<0.001) and indicating that an earlier onset of lay is associated with higher BW independent of treatment. However, BW at AFE did not influence the weight of the first egg. Effects of age, treatment, and their interaction regarding weekly BW (P<0.001) were found. Overall, ALT and CON hens were heavier than SKIP hens. Egg production was affected by age (P<0.001), increasing rapidly from 27 to 30 woa before gradually declining, with significant declines at 35, 44, and 57 woa. Age, treatment, and their interaction (P<0.05) influenced settable eggs, with ALT producing the most settable eggs overall, especially during peak production. Correspondingly, ALT hens laid fewer cracked eggs than CON and SKIP hens (P<0.001), but no differences in soft egg production were found. EST increased with age (P<0.001), and treatment effects (P=0.006) showed that, overall, ALT eggs had the thickest shells and CON eggs the thinnest. Similarly, EW, EBS, and HU increased with age, regardless of treatment (P<0.001). In conclusion, based on the evaluated parameters, ALT feeding regimens demonstrated positive impacts on reproductive physiology, leading to an earlier onset of lay and higher-quality settable eggs.

Keywords: broiler breeder, reproduction, physiology, feeding regimen, rearing

189 Effect of breeder age and storage temperature on eggshell translucency of Cobb eggs

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Storage conditions affect internal egg traits; however, the effects of breeder age and storage temperature (ST) on the distribution of egg translucency scores (TS) remain poorly understood. This study evaluated the effects of ST on TS and their correlation with shape, volume, surface area, and shell thickness in eggs from Cobb breeders of two age periods. Eggs (N = *510*) were collected from one Cobb breeder flock at 245 and 340 d of age, with egg weights of 61.6 ± 0.2 g and 70.5 ± 0.3 g, respectively. Eggs were stored at 14 ± 1 °C and 19 ± 1 °C, for 6 d. Egg weight loss % (EWL) was calculated. The TS (1 - 3) were assessed daily using the BlueBox. The eggshell thickness was measured using an ultrasonic gauge, and egg length and width were measured using an electronic caliper. The effects of ST and storage duration on TS were evaluated using contingency analysis and regression analysis. Results indicated TS was affected by an interaction effect ($P <$ 0.001) of breeder age and ST, with differences ($P <$ 0.001) observed over storage time. Eggs from 35-wk-old breeders stored at 19 °C had EWL of 0.91%, and 49.7% of eggs were TS1, 44.6% TS2, and 5.7% TS3 after 1 d of storage, with the proportion increasing steadily ($P < 0.001$) to 64% TS2 and 7.1% TS3, while only 28.9% remained TS1 by d 6. Conversely, 59.9% of eggs stored at 14 °C were TS1, 37.6% TS2, and 2.5% TS3 on d 1, and by d 6, TS1 decreased ($P < 0.001$) to 41%, while TS2 stabilized at 54%, with TS3 increasing slightly to 5%. The EWL was 0.24%. In 48-week-old breeder eggs, the TS changed linearly ($P < 0.001$), particularly at 14 °C. Eggs at 14 ºC had EWL of 0.55%, were 63% TS1 after 1 d of storage, and went to 3.51% at 5 d and none at 6 d. The TS2 increased progressively from 37% on d 1 to 93% by d 6. No eggs were initially TS3 but reached 7% at 6 d. At 19 °C, the EWL was 1.18%, and the changes in TS were slower ($P < 0.001$), with TS1 declining from 55% to 20.9%, TS2 went from 45% on day 1 to reach 73% on d 6. The TS3 showed a gradual increase,

reaching 6.1% by d 6. Comparing ST, it was evident that 14 °C resulted in a slower initial increase in TS, but a more dramatic shift was observed in this study for eggs laid by 48-wk-old breeders. Eggs from 35-wk-old breeders had slower ($P < 0.001$) increase in TS as storage progressed than eggs from 48-wk old breeders. The TS was weakly correlated ($P < 0.001$) only with egg surface area ($r = 0.29$) and eggshell thickness ($r = 0.12$). Egg ST and days of storage changed the TS of 96 or 97% of eggs at 35 and 48 wk, indicating changes in eggshell properties that may have implications for egg contamination and egg weight loss during incubation. In conclusion, the distribution of TS might change with breeder age in Cobb eggs.

Keywords: Eggshell translucency, Storage temperature, Eggshell quality, Breeder age, Cobb breeders

190 Evaluating the biofilm formation of *Salmonella* and *E. coli* in poultry drinking water system

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Quality drinking water is essential for poultry health, but microbial contamination remains a challenge, especially due to biofilm formation in the drinking water system. Biofilm protects pathogens and makes sanitation less effective. While several antimicrobials are used at the preharvest level, their effects on biofilm formation are less studied. This study evaluated the ability of cocktail strains of *Salmonella enterica* (Se) and *E. coli* (Ec) individually and in mixed cultures to form biofilm on polyvinyl chloride (PVC) pipes, a material widely used in poultry farms. Furthermore, the efficacy of three antimicrobials: acetic acid (AA), lactic acid (LA), and peroxide (PB) to reduce the planktonic bacteria were determined. Poultry drinking water (PDW) obtained from a commercial farm was used to prepare the cultures (Se, Ec, and mixed) as sterilized to assess biofilm formation without interference and unsterilized to simulate natural farm environments. Biofilm formation was assessed in beakers containing PVC rings over a 12-day period. The minimum inhibitory concentrations (MIC) of AA, LA, and PB against the cultures were assessed in a 96-well microtiter plate. The data was analyzed using ANOVA with Tukey's HSD (SAS 9.4). Biofilm formation varied significantly ($p < 0.0001$) between the cultures in both sterile and non-sterile PDW. In sterile water, Se exhibited the highest biofilm formation (4.57 – 4.88 Log CFU/mL), peaking on Day 10, while Ec remained lower (2.90 – 3.39 Log CFU/mL) peaking on Day 12. The mixed culture followed a similar trend, with Se reaching 5.17 Log CFU/mL on Day 10 and Ec staying below 3.20 Log CFU/mL. Interestingly, biofilm formation in non-sterile water significantly declined over time, with Se decreasing by 3.18 Log CFU/mL from 4.69 to 1.51 Log CFU/mL and Ec by 2.86 Log CFU/mL from 3.48 to 0.62 Log CFU/mL by Day 12. A similar trend was observed in the mixed culture, where Se dropped from 4.47 to 1.46 Log CFU/mL and Ec from 3.27 to 0.25 Log CFU/mL. Overall, biofilm formation was significantly lower in non-sterile water, with all isolates showing a progressive reduction. The tolerance of the cultures to AA, LA, and PB differed significantly ($p < 0.0001$). AA inhibited Se and Ec at 640 ppm, while LA had a higher MIC of 2560 ppm across all isolates. PB had the lowest MIC of 23.3 ppm for Se and 46.5 ppm for Ec. The MICs were similar for all antimicrobials against mixed cultures, except for PB, which was 46.5ppm. This data suggests that while Se and Ec formed biofilm in PDW, the microbiome of water significantly reduced the biofilm formation. Further analysis will identify this microbiome and assess the ability of biological-based antimicrobials to eliminate biofilm in PDWS.

Keywords: Antimicrobials, biofilm, Salmonella, E. coli, polyvinyl chloride (PVC)

191 Does exposure to blue light from 0-15 or 0-17 weeks of age affect aggressive behaviours, fear, and stress levels of egg-laying pullets reared to 17 weeks of age?

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Few studies have adjusted light intensity for poultry vision when studying the effects of light wavelength. This study examined the effects of 3 light color treatments (L) standardized to 15 gallilux: white light to 17wk (W17), blue light (peak at 455 nm) to 17wk (B17), and blue light to 15wk followed by 2wk of white light (B15) on the aggression, fear, and stress levels of 2 Lohmann strains (S), (Brown-Lite (LB) and LSL-Lite (LW)) from 0-17wk. The experiment used a 3x2 factorial arrangement (B15 and B17 combined before 15wk) in a randomized complete block design (trial as block, n=2). Pullets (n=900/S) were reared in light-tight single-level perchery-type rooms (2 rooms/L) with 6 floor pens per room (3 pens/S/room). Behaviour was recorded over 24h at 16wk (1 pen/S/room), with continuous sampling performed for two 15-min periods (10:15-10:30 and 14:15-14:30) to identify all occurrences of pecking behaviour between conspecifics. At 14 and 17wk, tonic immobility (TI) tests were performed (2 birds/pen) to assess fear. At 1, 2, 3, 4, 14 and 17wk, novel object (NO) tests were performed in all pens as an additional measure of fear. Data were analyzed using Proc Mixed (SAS 9.4) with Tukey's range test to separate means with differences significant when $P \leq 0.05$. A LxS interaction was noted where LW pullets reared under W17 performed a lower percentage of total gentle pecks than other LxS combinations, except the intermediate B17 ($P=0.03$). Neither S or L affected the percentages of severe pecks. Pullets reared under W17 performed a higher percentage of total aggressive pecks than those reared under B15 or B17 ($P<0.01$). An increased percentage of total aggressive pecks was also performed by LW pullets ($P<0.01$). At 14 and 17wk, pullets reared under W17 had reduced latency to peck at the NO compared to those reared under blue light or B15 and B17 ($P <0.01$, <0.05). Similarly, at 17wk, pullets reared under W17 had reduced latency to peck at the NO when compared to B15 and B17 ($P<0.05$). Differences in S were also noted, as LW pullets demonstrated reduced latency to peck compared to LB at 1, 3, and 14wk ($P<0.01$). An interaction between LxS in NO test was identified at 2wk, when LW pullets reared under blue light had reduced latency to peck compared to LB with the same L ($P=0.02$). As well, at 4wk, a LxS interaction was observed where LW pullets in W17 showed reduced latency to peck compared to all other LxS combinations ($P<0.01$). Neither L or S affected latency to rise during TI at 14 or 17wk. These results highlight the genetic differences between LB and LW pullets. Further, these data demonstrate a decrease in aggression in LB and LW pullets when reared under blue light, which could be related to higher fear levels.

Keywords: light color, behavior, fear, stress, aggression

192 The impact of hatching method on growth performance in broiler chickens

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This study aimed to compare the impact of on-farm and conventional hatching methods on growth performance and feed efficiency in straight-run broiler chickens. A total of 560 straight-run Ross 308 birds were divided into two treatments of hatching methods. Each treatment had 8 replicates with 35 birds per replicate. Assuming an 87% hatchability rate, 360 eggs were procured from a commercial hatchery on day 18 of incubation and transported via a climate-controlled truck to University of Sydney Poultry Research Facility in Camden. Upon arrival, the eggs were placed into a floor pen with 6cm deep litter. The temperature of the room was maintained at 34°C with relative humidity above 35%. On day 21 of incubation, 280 day-old chicks – hatched in a conventional commercial hatchery from the same breeder flock – were also procured. The hatching window for on-farm hatched chicks was considered complete upon the arrival of the commercially hatched chicks. All chicks were sorted by hatching method and randomly allocated to one of 16 floor pens. A standard wheat and soybean meal-based diet was offered across three feeding phases: days 0-14, 14-28 and 28-39 days post hatch. Body weight and feed intake were recorded on days 14, 28 and 39. FCR and body weight gain was calculated for each feeding period. Each replicate pen was considered as an experimental unit. Growth performance data each phase was analysed using two-way ANCOVA with male to female ratio in each pen as a covariate. Despite having heavier BW at day 0 ($P < 0.001$) and eating more feed from day 0 to 14 ($P = 0.021$), birds hatched on-farm were not significantly heavier at day 14 ($P = 0.091$). At day 28, on-farm hatched birds had higher BW ($P = 0.044$) but cumulative performance from day 0 to 28 showed no significant differences in growth rate (0.089). Hatching method had no significant impact on BW or BWG at day 39 however, on-farm hatched birds had significantly higher FCR. Despite the early advantage on BWG at day 0, the benefits to growth performance with on-farm hatched birds were not sustained until the end of the production, consequently, on-farm hatched birds generated higher FCR. Acknowledgment: The study was supported by the AgriFutures Chicken Meat Consortium (PRO-016111).

Keywords: Broilers, Growth Performance, Hatching system, On-farm hatching, Production

193 Production performance and egg yolk omega 3 fatty acids in laying hens fed ahiflower seed and its press cake during peak and late production phases

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The effect of ahiflower seed (AS) and presscake (APC) inclusion in the diets of Lohmann LSL-lite laying hens during their peak and late production phases, on production performance and yolk enrichment with omega 3 fatty acids (n-3FAs), over 12 weeks (4 weeks/period), was evaluated. Eight dietary treatments were randomly assigned to 48 replicate cages (5-6 hens/cage) in a completely randomized design. Dietary treatments included a control diet (CD), CD supplemented with 10% flaxseed (FS), 1, 5, or 10% AS, and 5, 10, or 15%APC. Hen day egg production (HDEP) was determined every period, while egg yolk n-3FAs profile was determined at the end of the trial. Data were analyzed by SAS v9.4 Proc Mixed and the Tukey range test was used for mean separation. In the older hens, HDEP was reduced (P=0.000) by 10%FS compared to all AS and APC inclusion levels. Contrarily, in the younger hens, 10%FS did not affect production performance. The 10% AS increased (P<.0001) stearidonic acid in both production phases. The 10%AS and 10%FS diet increased (P<.0001) eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA) in both phases of production compared to CD. In younger hens, docosahexaenoic acid (DHA) was increased (*P<.0001*) by 10% AS (5.39mg/g) compared to CD (2.82mg/g) which did not differ from 5%AS (4.58mg/g) and 5%APC (4.42mg/g). In contrast, in the older hens, DHA was increased (P<.0001) by 10% AS (2.23mg/g) and 10% FS (2.04mg/g) compared to CD (1.25mg/g/g). There was no significant effect (P>0.05) of treatment on total omega3 (n-3) and 6 (n-6) fatty acids, n-6/n-3 ratio in younger hens, but in older hens 10% FS increased (P<.0001) total n-3 FAs, and reduced (P<.0001) n-6/n-3 (2.11) compared to CD (9.80) and all APC inclusion levels. The 10% AS also reduced (P<.0001) n-6/n-3 (2.78) compared to CD (9.80) in older laying hens but not in younger ones. Likewise, in the older hens, the total n-6 FAs was increased (P<.0001) by 15%APC (28.64 mg/g) compared to CD (22.55mg/g), 10%FS (19.98mg/g), 1%AS (21.69mg/g) and 10%AS (21.35mg/g). Furthermore, in both younger and older hens, linoleic acid was increased (*P<.0001*) by 15%APC (24.1mg/g in older hens and 33.95mg/g in younger hens) compared to CD (26.63mg/g in younger hens and 19.1mg/g in older hens), while 10% FS (33.62mg/g) and 5% APC (33.59mg/g) also increased (*P<.0001*) linoleic acid in younger hens compared to CD and all AS inclusion levels. It was concluded that 10%AS increased n-3FAs in egg yolk and can serve as an alternative to FS for n-3FAs egg yolk enrichment. AS and APC did not affect production performance negatively.

Keywords: laying hens, ahiflower, omega 3 fatty acids, nutrient digestibility, production performance

194 Supplementation of novel probiotics improves layer performance while reducing egg-borne *Salmonella* transmission

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Probiotics are widely used feed supplements in the poultry industry. Besides its ability to support performance, several strains are also reported to reduce *Salmonella* Enteritidis (SE) colonization in chickens. In an attempt to develop a comprehensive, one-stop solution, in the present study we evaluated the potential for novel probiotics that can support layer performance while also reducing SE transmission to shell eggs. Sustained in feed supplementation of *Lactobacillus paracasei* DUP-13076 (LP) and *L. rhamnosus* NRRL-B-442 (LR) promote layer performance while improving egg safety. Two separate trials were conducted to evaluate the effect of probiotics on layer performance and *Salmonella* challenge using four dietary treatments which include corn-soybean meal-based diet without probiotics (Con) and feed supplemented with 9 log CFU/kg of LP, LR, and probiotic cocktail of LP and LR (PR). In the performance study, 19-week-old Lohmann lite layers were randomly allocated to 4 treatments with 5 replicate pens of 15 birds each. Besides egg production and FCR, at 48 weeks of age cecal contents were

processed for microbiome analysis and jejunum samples were collected for histomorphology and gene expression assays. For the *Salmonella* trial, 40-week-old birds were assigned to the 4 feed treatments as in the performance trial with replicate pens of 8 birds each for 60 days. Birds were challenged with SE (10^{10} log CFU/bird) by crop gavage. The eggs were tested daily for 5 weeks for SE presence on the outer shell (OS), inner shell (IS) and internal contents (IC) of the eggs. Both studies were set out as completely randomized design and data was analyzed using one-way ANOVA using GraphPad with $P \leq 0.05$ considered significantly different. In-feed supplementation of LP and LR significantly improved layer performance and reduced SE transmission to eggs ($P \leq 0.05$). Specifically, we observed a 3 – 5% increase in egg production an associated improvement in FCR following probiotic (FCR: 2.94) supplementation when comparted to the control (FCR: 3.22). Further, we recorded an increase in jejunum villi height and crypt depth and upregulated expression of *PEPT1, GLUT1,* and *MUC13*. In addition, we observed higher cecal Lactobacilli populations and reduced coliforms in LP, LR, and PR when compared to control. Along these lines, supplementation of LP and LR significantly reduced SE contamination in eggs by > 25% on the OS and in the ICs when compared to the control. Collectively, LP and LR in-feed could be a comprehensive approach to improve layer performance while promoting egg safety.

Keywords: Layers, Probiotics, Feed efficiency, Salmonella control, comprehensive approach

195 Food safety and Salmonella ecology in poultry production: Current perspectives

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In the United States as well as globally foodborne salmonellosis remains one of the more prevalent diseases of public health concern. Poultry meat and eggs continue to be one of the more prominent sources of foodborne Salmonella. Extensive research efforts have been made to develop better detection systems including quantitation and delineation of individual serovars and strains. These efforts have been greatly aided by rapid research advances in molecular and genomic technologies. Such advancements have also led to a more in-depth understanding of Salmonella ecology in poultry production environments. As more is known about Salmonella ecology, opportunities to develop interventions both in poultry flocks as well as processing plants have been explored. There have been numerous intervention approaches for limiting Salmonella occurrence in poultry flocks and processing plants that have been developed and implemented over the years. In addition to vaccines, preharvest interventions have included feed additives such as prebiotics, probiotics, postbiotics, synbiotics, organic acids, bacteriophage, and botanicals to prevent establishment and/or reduce Salmonella in the gastrointestinal tract of the birds. Once birds arrive at the processing plant an array of antimicrobials has been applied to reduce Salmonella on poultry carcasses. Successful mitigation of Salmonella in poultry and poultry products continues to be a challenge but more recent advancements in Salmonella genetics such as whole genome sequencing have led to extensive databases that provide for a better understanding of the genetics of Salmonella. In-depth genomic databases provide an opportunity for developing specific research strategies to study Salmonella. and identify factors that influence Salmonella persistence during poultry production. Identifying these factors can potentially improve mitigation efficacy by implementing more targeted interventions in both preharvest and postharvest production.

Keywords: Salmonella, ecology, poultry production, food safety, interventions

196 Comparing broiler microbiome profiling methods for practical application

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Gut microbiome profiling provides insights into poultry development, gut health, and potential food safety risks. The microbiome is also used to assess differences between treatments in poultry research, contributing to understanding mode of action. 16S rRNA gene amplicon sequencing is the most frequently employed method to characterize poultry microbiota although other methods are available. A microarray platform containing selected biomarkers for performance, health and food safety has been proposed as an alternative for routine microbiome profiling. This study objective was to compare results between these two methods and highlight their pros and cons. Fecal swabs were collected from six replicate pens where broilers were fed three distinct diets at 6, 20, 34 and 41 days of age. After DNA extraction, 51 out of the 72 samples had enough DNA for 16S sequencing while all 72 samples could be used for microarray analysis. Sequencing was performed on an Element AVITI platform and microarray analysis was done by Galleon (Cargill proprietary fluorescence bacteria microarray). Results showed that 16S sequencing identified 124 genera while microarray identified 75, with 27 overlapping genera. Although the number of identified features was higher for the 16S platform, the 27 array features that overlapped accounted for 86% of all abundance, thus covering the majority of the high abundant genera. Comparing relative abundance and relative fluorescence showed high correlation between platforms for most genera like Lactobacillus, Streptococcus, Staphylococcus and Escherichia-Shigella and lower for others such as Enterococcus. ANOVA analysis showed treatment effects explained 39% of variance with 16S sequencing and 53% with microarray data. Diet, age, and their interaction were fixed effects, while block was considered a random effect. The number of significant bacteria by FDR ($p<0.05$) due to diet increased from 16 to 82 moving from 16S sequencing to microarray dataset. Advantages of 16S analysis included looking into a larger number of bacteria genera compared to the array, and it allowed the estimation of microbiome diversity. Microarray analysis was less restricted by DNA amount and resulted in a larger number of significant bacteria due to higher biological and technical replication. The array also supported evaluation of presence (relative fluorescence signal) of undesirable pathogens and food safety related bacteria even when they were not identified by 16S analysis. Sequencing of 16S sRNA gene is a better option for data exploration and discovery as it covers a larger possible number of taxa, but microarray analysis is a suitable option for routine monitoring focusing on commercially relevant biomarkers.

Keywords: broiler, microbiome, method, 16S Sequencing, microarray

197 Broiler litter physicochemical properties and microbiome can be used for predicting pre-harvest *Salmonella* contamination

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Broiler litter characteristics could be useful to predict foodborne pathogens such as *Salmonella* on the farm. This study investigated the physicochemical properties and microbiome of broiler litter from two commercial houses. A total of 235 broiler litter samples were collected during early (4-14 days) and late (32-38 days) periods of grow-out of four successive flocks, and before and after alum application during downtime. Commensal bacteria (*E. coli* and *Staphylococcus*) and pathogens (*Salmonella* and

Campylobacter) were detected using culture methods, and microbiome composition was determined via 16S rRNA gene sequencing. Caked and friable litter moisture was determined gravimetrically by drying samples for 24 hours; pH was measured in litter eluate using a pH meter; and nutrients (e.g., nitrogen, phosphorus, potassium) were quantified via dry combustion, dry-ashing, and spectrophotometry. A random forest (RF) model incorporating bacteria concentration, microbiome, and physicochemical properties was developed to predict *Salmonella* presence. Model hyperparameters were optimized with 10-fold cross-validation, and feature importance was assessed based on the mean decrease impurity (Gini). A total of 8 models were constructed based on the combination of subsets of data (i.e., whole dataset or dataset from grow-out samples only), and type of data (i.e., whole data, microbiological data, or physicochemical properties). Overall, model performance was higher when whole datasets were used (average AUC = 0.9091) compared to using only grow-out dataset (average AUC = 0.7986). Regardless of the models, moisture of litter, concentration of *E. coli* and *Staphylococcus*, and total Enterobacteriaceae gene abundance were among the top predictors of *Salmonella* presence. Seven bacterial taxa classified as family *Bacillaceae*, *Cerasibacillus spp*., *Aeromicrobium spp*., *Saccharopolyspora aidi ngensis*, *Aerococcus spp*., and *Nocardiopsis spp.* were identified as important litter microbiota that influenced *Salmonella* predictions and warrants further investigation. These findings indicate that litter physicochemical and microbiological properties have the potential to be used as on-farm pre-harvest pathogen indicator.

Keywords: Microbiome, Salmonella, Litter, Machine learning

198 Effect of chicken breed on Campylobacter load and prevalence in pastured poultry management systems

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Pasture-raised farms mainly raise Cornish Cross for broiler production, but increasing consumer demand for stronger flavor and texture is prompting a shift to heritage breeds like Freedom Ranger. With the increased environmental interaction and the potential increase in exposure to foodborne pathogens in these settings, a better understanding of the prevalence and load of foodborne pathogen populations inherent within pastured poultry breeds is needed. To achieve this, 300 Cornish Cross birds and 300 Freedom Ranger were obtained from the same hatchery and brooded indoors until 3 weeks of age. In the third week, 150 birds from each breed were allocated into moveable coops to ensure daily access to fresh pasture. Pooled broiler samples (ileum, ceca, and broiler feces) and environmental samples (feed and water) were collected on days 0 and 7. On days 21, 35, 49, 56, and 77, pooled broiler samples (ileum, ceca, broiler feces, pasture feces) and environmental samples (flies, feed, water, and wild bird feces). Campylobacter load was determined by cultural methods and 542 presumptive Campylobacter isolates were saved and characterized by BAX® PCR. One-way ANOVA with breed or sample type as the main effect, followed by Tukey's multiple comparison method for pairwise comparison, was performed using R software. Campylobacter was not detected in any samples in the first 3 weeks, except in the flies at day 21. Campylobacter load was significantly higher in the Cornish Cross breed compared to Freedom Ranger during the mid grow-out period (6.3 vs. 4.7 log10CFU/ml) and end grow-out period (4.04 vs. 2.79 log10CFU/ml) ($p < 0.05$). The final product from the Cornish Cross had a higher Campylobacter load compared to the Freedom Ranger (2.47 vs. 0 log10CFU/ml) ($p < 0.05$). All characterized isolates were Campylobacter jejuni. These results indicate that the Cornish Cross breed can potentially harbor a higher load of Campylobacter, and that flies can serve as a potential source for transmission of Campylobacter within a pasture-raised flock. By leveraging these findings, farmers and industry leaders can implement targeted strategies, such as improved fly control and breed selection, to decrease Campylobacter load in pastured raised poultry.

Keywords: Campylobacter , Pasture-raised farms

199 Temporal and regional differences in antibiotic-resistant *Salmonella* populations in chicken across the United States

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Antimicrobial resistance in foodborne pathogens is a public health concern due to the risk of consumers being exposed to resistant pathogens. Chicken is the most consumed animal protein in the United States and has been implicated in many foodborne outbreaks. This study aimed to investigate the prevalence of antibiotic-resistant *Salmonella* and resistant profiles in chicken carcass rinse data published by the U.S. Department of Agriculture-Food Safety Inspection Service (FSIS) and compare with isolates from retail chickens. National data from FSIS monitoring program of processing plants across the U.S. from 2015 to 2023 was evaluated along with *Salmonella* isolates from retail chicken from conventional (CON) and raised without antibiotics (RWA) sources. Chi-square test and ANOVA were used to analyze the data using Excel and SAS (v9.4, SAS Institute). A total of 3,645 *Salmonella* was isolated by the FSIS from chicken carcass rinses from 2015 to 2023, with a higher prevalence ($p<0.0001$) in the South (66%) followed by Midwest (15%) and Northeast (12%) regions. Prevalence was lower in 2015 due to low sample size and highest in 2017 and 2023. From these positives, 63% were resistant to at least one antibiotic with similar prevalence across all regions ($p=0.31$) and years except 2015-2016 ($p<0.05$). Of the resistant *Salmonella*, 30% were multi-drug resistant and this varied ($p<0.05$) across all regions with the highest incidence in the South (33%) and Northeast (31%) regions; while temporal patterns reflect three peaks: 2015, 2018-2022, and 2023. Serotype Kentucky was the most prevalent across all regions and the South had the highest diversity with 57 distinct serotypes. Generally, resistance patterns showed high levels of streptomycin and tetracycline across all regions with a distinct pattern for penicillins, cephems, and quinolones in the South. For retail chicken, a total of 81 isolates was evaluated from CON (72%) and RWA (28%) sources. Of these, resistant *Salmonella* was similar ($p>0.05$) between CON (84%, 49/58) and RWA (78%, 18/23). Moreover, 48% (28/58) CON and 30% (7/23) RWA were multi-drug resistant, and the isolates were mostly Infantis from both sources. Like the national data, resistance to quinolones and tetracyclines was high for both sources. Interestingly, no resistance to antibiotics commonly used to treat foodborne illnesses like

azithromycin was observed in the retail isolates, however, resistance to quinolones from the FSIS data is concerning. This temporal data provides a critical insight into the circulation of antibiotic-resistant *Salmonella* in chicken from processing plants and at retail level to consumers, which further emphasizes the importance of proper handling of chicken products.

Keywords: Salmonella, Antimicrobial resistance, Chicken, Retail market, Multidrug resistance

200 Effect of environmental conditions on survivability of *Campylobacter jejuni* in used broiler litter

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The objectives of this experiment were to determine the survivability of a *Campylobacter jejuni* marker strain in used broiler litter under varying environmental conditions including temperatures ranging from 4-60°C, moisture content ranging from 15%-35%, and acidification of litter using sodium bisulfate. Previous research has shown that sufficient downtime between flocks can prevent cross-contamination from reusing litter between flocks, highlighting its importance in controlling *C. jejuni* in broilers. However, the required duration of downtime and the influence of environmental conditions during this period are still unclear. To explore this question, used litter was collected from the Miller Center research farm at Auburn University. Portions of this litter (650 g) were placed into 24, 32 cm x 36 cm boxes and inoculated with a 48 h culture of a ciprofloxacin-resistant marker strain of *C. jejuni* to give a final concentration of 6.91 Log_{10} CFU/g of litter. Litter in each of these boxes was adjusted with sterile distilled water to 15, 25, or 35% moisture such that four temperatures (4°C, 22°C, 42°C, 60°C) could be tested with each moisture level in addition to sodium bisulfate amendment (110 kg/93 m^2 or none) for a total of 24 treatments (4 temperatures x 3 moisture levels x 2 sodium bisulfate levels). Boxes were then sealed with aluminum foil and incubated at the designated temperatures and tested for the *C. jejuni* marker strain every 24 h by spread plating 10 g composite litter samples diluted in phosphate buffered saline onto Campy Cefex agar with 1 mg/L ciprofloxacin (Cefex) and enriching in NCEB before streaking onto Cefex. This experiment was repeated 3 times and average CFUs were calculated. After 24 h of incubation, only 1 treatment had recoverable *C. jejuni* (incubation temperature of 4°C, litter moisture at 35%, and no sodium bisulfate amendment) at an average population of 3.70 Log_{10} CFU/g of litter. After 48 h, this same treatment showed reduced recoverable *C. jejuni* of 2.54 Log_{10} CFU/g. After 72 h, *C. jejuni* was only recoverable from this sample by enrichment and was no longer recoverable at 96 h. These results show that *C. jejuni* can survive for several days in used litter under certain conditions, namely low temperature and high moisture content. In addition, amendment with sodium bisulfate has a negative impact on *C. jejuni* survival. These results suggest that proper management of litter moisture, application of sodium bisulfate, and adequate downtime between flocks are all potentially important factors in preventing *C. jejuni* cross-contamination via litter at low temperatures.

Keywords: Broiler, Campylobacter, litter, survivability, environment

201 Not Presented

202 Assessing *Salmonella* survival and invasion on shell eggs during refrigeration

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Salmonellosis, caused by infection with *Salmonella*, is the most common foodborne illness linked to table eggs. Shifts in commercial egg production, driven by consumer preferences, favor cage-free production and other alternative methods over conventional cages. This study compared the eggs from cage and aviary systems over the lay cycle on *Salmonella* survival and its penetration during refrigeration. Forty-eight unwashed fresh eggs were collected from commercial caged and aviary Hyline W36 hens at 22, 32, 52, and 82 weeks of age, then inoculated with 10^6 CFU/mL *Salmonella* and stored at 4°C. Sampling by shell rinse, egg contents, and air cell was conducted the day after inoculation and each of the following three weeks with direct plating for *Salmonella* enumeration and enrichment to identify *Salmonella* presence. All enumeration values were log-transformed prior to statistical analysis, performed using JMP Pro 18 with α=0.05. A generalized linear mixed model was used to analyze *Salmonella* presence with hen's age, housing, and refrigeration duration as fixed effects with interactions. A chi-squared test with an odds ratio was used to analyze inoculated *Salmonella* presence related to housing environment. A parametric survival analysis was also used to analyze the relationship between egg refrigeration and the amount of *Salmonella* on the eggshell. *Salmonella* was less likely to survive on eggs from older hens ($p<0.0001$). During refrigeration, *Salmonella* kept decreasing over time ($p<0.0001$). Overall, more inoculated *Salmonella* were detected on the shell of cage-free eggs than caged eggs ($p=0.0002$), with the interaction of hen's age and housing environment reflecting that less *Salmonella* was retained as the hens aged ($p=0.0110$). Parametric survival analysis revealed that hen's age, housing environment, and egg refrigeration were significantly associated with differences in the amount of *Salmonella* recovered ($p<0.0001$). Inoculated *Salmonella* was not recovered from the air cell or egg contents throughout the entire experiment, indicating the important role refrigeration plays in ensuring food safety. This study showed that there was a higher propensity for cage-free eggs to retain *Salmonella* on eggshells compared to caged eggs, the mechanisms of which could lead to future studies.

Keywords: Salmonella, Caged eggs, Cage-free eggs

203 Whole genome sequencing of *Pseudomonas* isolates from fresh ground turkey reveals the presence of *Pseudomonas aeruginosa* virulence and resistance genes

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Pseudomonas is a common spoilage organism in poultry meat. It can harbor antibiotic-resistance genes, potentially transferring to other bacteria in various environments. This study aimed to isolate and identify the spoilage organisms in ground turkey meat during refrigerated storage using traditional culturing methods and whole genome sequencing. Fresh ground turkey meat samples were purchased from a local market. Twenty-five g samples of ground turkey were stored at 4 degree Celsius for 0, 2, 4, 6, 8, 10, and 12 days. Homogenized meat samples on these days underwent macro-

dilution assays, and appropriate dilutions were spread-plated on Trypticase Soy Agar (TSA), Plate Count Agar (PCA), and *Pseudomonas* Isolation Agar (PIA) and incubated. Three unique bacterial colonies with similar colony characteristics isolated over multiple assays were randomly selected for DNA extraction using DNeasy PowerSoil kit. The DNA library preparation and whole-genome sequencing were done using NexteraXT and the Illumina NovaSeq platform. Sequences were trimmed using Trimmomatic and assembled using Shovill software, and the assembly quality was checked using QUAST software. Nucleotide BLAST was performed on the contigs files of the sequences to identify the bacterial isolates. Resistance genes, virulence genes, and plasmids in the isolates were detected using MEGARes, VFDB, and PlasmidFinder databases in ABRicate. Two isolates were mapped to *Pseudomonas fragi* (strains DBC and A13BB), and the third was mapped to *Pseudomonas shahriarae* (strain SWRI52) with a percentage of identical positions above 94% and e-value of zero. Six resistance genes, including resistance to the tetracycline class and multidrug-efflux pump mechanisms, were found in *P. fragi* strains. In addition to the genes above, aminoglycoside resistance genes were found in *P. shahriarae*. Fourteen virulence genes associated with type IV pili and flagellar motility of the human opportunistic pathogen *Pseudomonas aeruginosa* PA01 were identified in *P. fragi*, whereas 30 virulence genes were identified in *P. shahriarae*. No plasmids were found in any of the isolates. The presence of virulence and antibiotic resistance genes of the human opportunistic pathogen, *P. aeruginosa* PA01, in fresh local ground turkey, demands vital steps to investigate the source, distribution, and outcomes of these genes in the food supply (MIN-16-071; MIN-16-141).

Keywords: Meat spoilage, Pseudomonas, Bacterial whole genome sequencing, AMR, Food safety

204 Assessment of poultry transport coop microbiota post cleaning and disinfection

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Broiler transport coops are a potential fomite for human and poultry pathogens. Implementing robust cleaning and disinfection measures are critical to mitigate transmission of infectious agents. The objectives of this pilot study were to evaluate the effects of three disinfectants on reducing transport coop microbial loads and determine the impacts on the microbial ecology. Over 2 trials, coops were power washed for 120 s each, and disinfectants were applied with garden sprayers for 90 s, left for a 10 m contact, then rinsed for 60 s. Treatments were applied to single coops and included a sterile tap water rinse control (WR), 0.25% glutaraldehyde (G), a quaternary ammonium compound (Q), and a G – Q blend (GQ). Samples were collected using sterile gauze in BPW with metal 5 x 5 cm templates from each coop compartment before and after disinfection (n = 10, N = 160) and enumerated for total aerobes. Diluent from Trial 2 was extracted for genomic DNA and submitted for 16S amplicon sequencing targeting the V4 region of the rRNA gene (N = 80). Plate counts were analyzed via a mixed effects model for the interaction of treatment x time ($\alpha \leq 0.05$). Microbiota diversity and composition were analyzed with QIIME2-2024.10 for richness, evenness, and Analysis of Composition of Microbiome (ANCOM) with and without bias correction ($P \leq 0.05$, $Q \leq 0.05$). There was no difference between WR loads pre- and posttreatment (5.25, 5.07 $\log_{10}$ cfu/cm^2, respectively). Loads on coops treated with G, Q, and GQ (3.22, 3.02, 3.48 $\log_{10}$ cfu/cm^2, respectively) were significantly decreased compared to pretreatment levels (5.24, 5.23, 5.62 $\log_{10}$ cfu/cm^2, respectively; $P<0.01$). No differences were observed between posttreatment loads. Evenness and richness were greatest on coops treated with G and WR, but lowest on pretreatment G, Q, and GQ coops ($P < 0.05$, $Q < 0.05$). There were 11 differently abundant genera per ANCOM, including *Staphylococcus*, *Acinetobacter*, *Deinococcus*, *Exiguobacterium*, and *Psychrobacter*, along with taxa not previously described. When correcting for bias, WR depleted the abundance of *Psychrobacter*, Q depleted *Exiguobacterium*, *Acinetobacter*, and an *Enterobacterales*, and GQ depleted *Staphylococcus*. Treatment with G, Q, and GQ enriched 5, 6, and 3 taxa, respectively. Many of the differentially abundant taxa have been implicated in meat spoilage. The current results mirror previous evidence that general disinfectant use reduces microbial loads. However, impacts on the microbial ecology vary by chemistry type, including blended chemistries. Future replicates will evaluate population-wide genomic shifts to help producers tailor disinfectant use and mitigate consumer risks.

Keywords: transport coops, Biosecurity, Microbiota, Microbial ecology, Disinfection

205 Dynamic prevalence and distribution of *Salmonella enterica* in chicken rinses across the United States

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Salmonella remains a pathogen of concern in the U.S. and consumption of poultry products is one of the contributing factors to salmonellosis. The objective of this study was to determine the dynamic change in *Salmonella* prevalence and predominant serovars in the United States from the chicken carcass rinses collected by USDA-FSIS from routine sampling of slaughter facilities between 2015 and 2023. Data was downloaded from the USDA-FSIS website and sorted by year, region, and serovar. The data was analyzed using R 4.0.2 (R core Team, 2021). Chi-square test was used to test the independence of *Salmonella* prevalence by year and region. Time series regression was used to identify the seasonality effect of *Salmonella* prevalence. A total of 80,285 chicken carcass rinses were collected, with an average *Salmonella* prevalence of 4.5%. Prevalence trends varied significantly across three distinct periods: 2015, 2016-2018, and 2019-2023 ($P < 0.001$). In 2015, 5164 samples were collected and had the lowest prevalence (2%) than subsequent periods. From 2016 to 2018, an average of 9002 samples per year were collected with an average prevalence of 4.9% that decreased to an average of 4.61% by 2019 to 2023 with an average of 9623 samples per year. There were significant ($P < 0.001$) differences for prevalence between the regions with the South (66%) being the highest and the West (7%) being the lowest while the Midwest (15%) and Northeast (12%) were similar. Furthermore, *Salmonella* prevalence followed a moving average time series model with a time lag of 0.25 (one season) and a coefficient on residual of 0.54 ($P = 0.014$), suggesting that prevalence stabilized at 5%. The prevalence patterns across all regions were consistent with the overall trends. Serovars Kentucky, Enteritidis, Typhimurium, and

Schwarzengrund were consistently over 5% in positive samples. Among these serovars, the incidence of serovar Infantis surged from < 0.1% in 2015 to 14% in 2023. This followed an autoregressive integrated moving average model (ARIMA) with a significant autocorrection of 7 lags ($P < 0.001$), suggesting an increasing trend in the incidence of serovar Infantis from chicken carcass rinses. The ARIMA model also suggests that the increasing rate of serovar Infantis started to decrease over time. This data showed the temporal differences in *Salmonella* prevalence in chickens processed across the United States, revealing dynamic trends in regional serovar distribution. Identifying lags in pathogenic serovars over time can critically help discern peaks for certain serovars and the emergence of others. This knowledge could help industry make informed decisions on a robust intervention strategy to control *Salmonella*.

Keywords: Salmonella, salmonellosis, food safety, poultry, chicken

206 Benefit of genomic breeding values to evaluate egg quality traits in layers

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Egg quality encompasses both optimizing environment for embryo development and nutritional value for consumers. For both aspects both external qualities, recorded without breaking an egg, and internal quality are important and new technologies being developed to capture different phenotypic aspects of the egg. Egg quality traits are under partial genetic determination and are characterized by low (egg defects 0.1-0.3, albumen height 0.2-0.3) to moderate heritability (egg weight ~0.3-0.6, shell color 0.4-0.6, shell strength 0.5-0.7, yolk weight ~0.4) but also affected by non-genetic factors such as management, nutrition, age of birds, health status, storage length and storage conditions. Some egg quality traits (in particular egg weight and shell strength) tend to be unfavorably genetically correlated with each other and with egg production therefore balanced selection indices have to be applied to improve them simultaneously. New methods including vision technology are being developed to further advance accurate phenotyping of egg quality. For this study 2 traits were selected because of their high economic importance: egg weight around 40wks of age and breaking strength around 70wks of age. Data of 37,814 White Leghorn hens with 66,092 breaking strength and 47,505 egg weight records were used to evaluate impact of genotyping on persistency of estimated breeding values. Number of genotypes included was 52,422 including both males and females across 12 generations. First 5 generations of data (plus two additional generations in pedigree and genotypes) were used for training and 5 that followed for validation in a forward prediction. AIREMLF90 was used to estimate variance components and EBVs, Single Step method implemented in MixBLUP was used to estimate GEBVs and LR method was applied to evaluate accuracy. Persistency of GEBVs was a lot better than that of pedigree EBVs resulting in average benefit of genomic information increasing from 64% in progeny to 463% for prediction 5 generations forward for egg weight with respective numbers of 63% to 570% for breaking strength. Genomic information has improved understanding of egg quality through identifying genomic regions associated with these traits and increasing accuracy and persistency of estimated breeding values thus enhancing genetic progress.

Keywords: genomics, egg quality, layers

207 CT imaging for poultry breeding: A novel CT-derived lung trait for turkey (*Meleagris gallopavo*) genetic improvement

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Although productivity in turkey production has improved significantly, pre-slaughter mortality remains a major concern. This issue could stem from disproportionate growth of internal organs relative to increased meat yield. Consequently, there is growing interest related to incorporating traits related to organ health and function into breeding programs to enhance turkey welfare and overall health. One promising strategy involves computed tomography (CT) imaging. This non-invasive technology utilizes X-rays to generate cross-sectional images of internal structures, offering potential novel phenotypes for genetic selection. Using CT-derived traits to assess and improve livability traits, organ traits in particular, could be a key step toward optimizing both efficiency and welfare. This study aimed to investigate a novel CT trait to describe lung size and its potential as a novel livability phenotype. The lung liveability trait, lung pixels ($Lung_{px}$), was derived from the number of pixels from each slice of the CT scan where lungs were detected. $Lung_{px}$ records from 5,367 purebred male turkeys from a reproduction-focused line (Line A) and 6,774 purebred male turkeys from a meat-yield focused line (Line B). $Lung_{px}$ and breast meat yield derived from CT (BMY_{ct}) were measured, allowing for genetic evaluation without sacrificing the bird. A multi-trait linear animal model for $Lung_{px}$, BMY_{ct}, and breast weight from cut up data (BRMT) was implemented in ASREML 4.2, a statistical software, to estimate genetic parameters of each trait and examine their genetic variance of each line and covariances between traits. The model structure included age at scan and a location factor (barn and farm) as fixed effects with animal as the random effect. Average heritability for $Lung_{px}$, BMY_{ct}, and BRMT in line A was 0.29 ± 0.03, 0.55 ± 0.04, and 0.50 ± 0.04, respectively. Unfavorable genetic correlations were estimated between $Lung_{px}$ and BMY_{ct} (-0.27 ± 0.07) and $Lung_{px}$ and BRMT (-0.16 ± 0.08), whereas favorable genetic correlations were estimated between BMY_{ct} and BRMT (0.68 ± 0.04). Average heritability for $Lung_{px}$, BMY_{ct}, and BRMT in line B were 0.16 ± 0.02, 0.46 ± 0.03, and 0.33 ± 0.03, respectively. Unfavorable genetic correlations were estimated between $Lung_{px}$ and BMY_{ct} (-0.16 ± 0.08) and $Lung_{px}$ and BRMT (-0.09 ± 0.09), whereas favorable genetic correlations were estimated between BMY_{ct} and BRMT (0.72 ± 0.03). These results show potential for selection on $Lung_{px}$ and provide insight into the relationship between potential lung traits and meat yield traits in two lines. By integrating CT-based selection criteria, the turkey industry could enhance sustainability, reduce mortality, and improve the overall robustness of flocks.

Keywords: breast meat yield, lungs, genetic parameters, computed tomography, mortality

208 Role of mitochondrial network dysregulation in broiler woody breast myopathy

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The woody breast (WB) myopathy poses significant economic and welfare concerns to the poultry industry, however, there is no effective mitigation strategy due to its unknown etiology. Previously we have shown that hypoxia is a key factor in WB progression, and as mitochondria is a major target of hypoxia and primary site of oxygen consumption, we sought to determine its role in WB myopathy. For *in vivo* studies, breast muscle from 56d-old broilers were hand scored by a well-trained person for WB on a scale of 0-3. Breast muscle from score 0 (normal, n=6) and 3 (severe WB, n=6) were snap-frozen and stored at -80°C for RNA and protein analysis or fixed for electron microscopy. For *in vitro* studies, primary myoblasts were isolated from breast muscle

of E18 embryos and cultured on collagen-coated culture dishes in a humidified atmosphere at 37°C. Hypoxia was induced using a gas-tight hypoxic chamber (1% O_2/5% CO_2/94% N_2) for 24h (n=6). Control cells (n=6) were maintained at normoxic conditions (5% CO_2/95% O_2). Gene and protein expression were determined by real-time qPCR and Western blot, respectively. Data were analyzed by Student's *T*-test. Electron microscopy showed that gross myofiber structure and mitochondrial morphology were disrupted in WB, with wave-like patterns present in the myofibers and loss of cristae structure within the mitochondria. Increased levels of calcium ($P<0.01$) in both WB-affected tissues and hypoxic myoblasts suggest that mitochondrial Ca^{2+} overload is likely a leading cause for mitochondrial dysfunction. Genes associated with endoplasmic reticulum-mitochondrial contact were differentially expressed in both WB and hypoxic myotubes. Markers of fission and fusion were dysregulated, with lower expression of the mitofusins (MFN, $P<0.05$) and increased gene expression of dynamin-related protein 1 (DNM1, $P<0.01$), indicative of increased mitochondrial fragmentation. Protein expression of Complex I and Complex IV were increased ($P<0.05$) in WB compared to normal muscles, and Complex II and Complex V were increased ($P<0.05$) in hypoxic compared to normoxic cells. Gene expression of components of Complex I, Complex III, and Complex IV were significantly downregulated ($P<0.05$), while Complex II was upregulated in both WB muscle ($P<0.01$) and hypoxic myotubes ($P<0.05$) as compared to controls. Mitochondrial oxygen consumption rate, as measured by Seahorse metabolic analyzer, indicated lower basal respiration ($P<0.01$) and ATP production ($P<0.005$) in hypoxic primary myotubes. These findings provide fundamental insights into the underlying molecular mechanisms of WB and the interplay between calcium, mitochondrial (dys)function, and avian muscle health.

Keywords: woody breast, broiler, mitochondrial dysfunction, hypoxia, bioenergetics

209 Collagen characterization in broiler *Pectoralis major* muscles affected by wooden breast myopathy

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The present study aimed at characterizing the composition and expression level of Collagen type III (COL3) in chicken *Pectoralis major* muscles (PMs) affected by wooden breast (WB), to ultimately deepen the knowledge on its potential implication in the onset of this myopathy by specifically addressing the hypothesis of a different COL3 biosynthesis and composition in WB breasts. To this purpose, 10 PMs (5/group) were selected 3 hours *post-mortem* at a commercial broiler abattoir, grouped according to their phenotype as unaffected (N) or severe WB, and collected for subsequent analysis. From each sample, an aliquot was subjected to COL3 extraction and amino acid composition analysis. An additional subsample (flash-frozen in liquid nitrogen; stored at -80°C) was used to perform gene expression analysis. More in detail, COL3 amino acid composition was assessed by HPLC method, and the normalized gene expression level of its coding gene (*COL3A1*) was quantified by means of qRT-PCR. Furthermore, normalized expression of *Prolyl 4-hydroxylase subunit alpha 1* (*P4HA1*) and *Lysyl oxidase* (*LOX*) mRNA were investigated in view of their post-translational role in collagen synthesis and stabilization. For gene expression analyses, *GAPDH* has been used as normalizing gene. After testing the normality of data distribution (Shapiro-Wilk test), differences between groups were assessed by using the non-parametric Mann-Whitney U test. Statistical analyses were performed in R (v.4.3.2) and all differences were considered significant at a level of $P\leq0.05$. Results from the COL3 amino acid composition evidenced that WB-affected PMs had a higher ($P<0.05$) content (g/100g of COL3) of glycine (14.6 vs 10.5), proline (7.2 vs 4.9), alanine (5.6 vs 4.1), hydroxyproline (7.9 vs 5.7) and hydroxylysine (1.2 vs 0.7) when compared with N. As for the gene expression results, *COL3A1* normalized expression was significantly higher ($P\leq0.05$) in WB compared to N samples. Analogously, both *LOX* and *P4HA1* normalized levels of expression were significantly higher ($P\leq0.05$) in WB than N. Overall, results achieved in this study corroborated previous evidence that the occurrence of WB is strongly related to alterations in connective tissue components of PMs. Besides, in view of its association with muscle regeneration, the higher expression level of COL3 in WB muscles might be associated with the pathological events occurring in abnormal muscles. However, extensive alterations in COL3 composition together with profound changes in gene expression levels of players involved in its *post*-translational modifications might suggest its involvement in the WB underlying mechanisms. Research supported by NextGenerationEU, National Grant PRIN2022 (Prot. n. 2022EPWEPW).

Keywords: Growth-related abnormalities, Collagen type III, Gene expression analysis, Amino acid composition, Post-translational modification

210 Liver RNA-seq analysis of meat-type chickens supplemented with or without glucose and reared under chronic heat stress

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Heat stress (HS) results from unsuccessful thermoregulation where animals produce more heat than they can dissipate. The negative balance between net amount of heat produced and its capacity to lose heat to the environment leads to reduced energy intake and growth. We hypothesize that supplementing glucose will reduces the negative effects of HS by improving energy metabolism, enhancing antioxidant defenses and growth performance. Glucose was supplemented to the drinking water of chickens reared under HS. Liver transcriptome was studied to delineate the molecular mechanisms that underlie glucose supplementation of chickens reared under HS. There were two temperature levels (25°C [thermoneutral, TN]) and 35°C (8.00 AM to 8.00 PM, [Heat Stress, HS]), and two glucose levels (0% and 6%). A total of 456 four-week-old broilers were distributed to 4 treatment (TN_0, TN_6, HS_0, and HS_6). After 7 days, post HS, 6 chickens per group were sampled for their liver tissues. RNA samples were sequenced using NGS Illumina platforms. Reads were processed and hit counts were used to identify differentially expressed genes (DEGs) for comparisons with fold change ≥1.2 and FDR≤0.05. There were 8 downregulated and 7 upregulated DEGs between TN_0 and TN_6 and the significant gene ontology (GO) were cellular to fatty acids, regulation of glucose metabolism, insulin receptor signaling,

and reactive oxygen metabolic processes. There were 43 downregulated and 98 upregulated DEGs between TN_0 and HS_0. The MAPK signaling, Toll-like receptor signaling were upregulated while alanine-aspartate-glutamate, tryptophan and nucleotide sugar were downregulated. There were 82 downregulated and 108 upregulated DEGs between TN_0 and HS_6. Arginine, proline, PPAR signaling were upregulated while cysteine, glycine-serine-threonine and dicarboxylate metabolisms were downregulated. MAPK signaling, Toll-like receptor, insulin signaling pathways were downregulated and biosynthesis of unsaturated fatty acids, steroid hormone biosynthesis and p53 signaling pathways were upregulated in HS_6 compared to HS_0 (37 down- and 2 up-regulated DEGs). Changes in cysteine metabolism and p53 signaling suggest that glucose supplementation mitigated some of the metabolic stress associated with HS. Cysteine is a precursor to glutathione, an antioxidant that protects cells from oxidative stress. During HS, oxidative damage can increase, and cysteine aids in the maintenance of antioxidant status of immune cells. The p53 protein plays a critical role in cellular stress responses, including heat stress by maintaining energy balance and supports cellular functions under stress conditions.

Keywords: Heat stress, Glucose supplementation, RNA-seq, Liver, MAPK signaling

211 Optimization of culture conditions for chicken tracheal epithelial cells to investigate avian influenza virus infection

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Avian influenza virus remains a significant threat to the U.S. poultry industry, with the current outbreak of H5N1 virus resulting in the loss of over 160 million birds. The virus initially targets epithelial cells of the respiratory system which serve as the first line of defense; however, in vitro models to study viral interactions with these cell types are currently limited. This study aimed to optimize culture conditions for chicken tracheal epithelial cells and assess their susceptibility to avian influenza virus. Tracheal epithelial cells were harvested from two-week-old White Leghorn chickens and cultured in three different media formulations to identify optimal growth conditions. After selecting the most suitable medium based on cell growth and cryopreservation tolerance, the cells were cultured with transforming growth factor beta (TGF-β; A8301), bone morphogenetic protein (BMP; DMH-1), or rho-kinase (ROCK; Y-27632) inhibitors at concentrations of 0.5 μM or 1 μM to determine conditions for maintenance of self-renewing basal cells. Cultures treated with 0.5 μM BMP or ROCK inhibitors showed enhanced cellular proliferation that enabled passaging over 20 times, whereas control cells underwent senescence after 10 to 15 passages. Additionally, cells cultured with BMP or ROCK inhibitors retained epithelial characteristics, as confirmed by cytokeratin type II staining at passage 15. To evaluate the cells as a model for viral infection, tracheal epithelial cells were infected with the recombinant H5N1/-PR8 or avian H7N1 viruses at a multiplicity of infection (MOI) of 0.01. At 48 hours post-infection, visible cytopathic effects, such as cellular detachment and dealth, were observed, and viral nucleic acid abundances measured by qRT-PCR in the infected cells were comparable to those seen in DF-1 fibroblasts, a commonly used in vitro model for avian influenza virus infection studies. In summary, the optimized culture system for chicken tracheal epithelial cells enables extended maintenance and provides a novel in vitro model for studying avian influenza virus infection.

Keywords: avian influenza virus, cell culture, respiratory, epithelial, optimization

212 Assessing population dynamics and AMR trends in *Salmonella* infantis

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Foodborne *Salmonella* infection poses a significant public health concern. *S.* Infantis is one of the key serovars accounting for 2% of human infections, with a higher potential to carry antimicrobial resistance (AMR) compared to other serovars. This study aims to analyze the population dynamics and risk of AMR genes in *S.* Infantis. Sequences and metadata information of 16,156 *S.* Infantis were obtained from the NCBI and Pathogen Finder database (Nov. 15, 2024). PubMLST analysis of obtained genome sequences revealed that 96.56% of *S.* Infantis isolated and sequenced worldwide fall under the ST-32. Additionally, while analyzing the ST type based on the source of isolation, 96.4% from animals, 99.31% from chicken, and 93.4% from humans were ST-32. *S.* Infantis from ST-32 was further analyzed for the presence of AMR genes using the ResFinder database in the Abricate platform. Population dynamics and odds ratio of AMR genes across sources, time of years, and continent were analyzed using logistic regression. Tukey's HSD test was used to further separate the difference within sources, time of years, and continent. The significance level was set at 0.05. The odds of obtaining AMR genes in *S. Infantis* was impacted by the source of isolation. The risk of harboring most of the AMR genes in *S.* Infantis isolated from animal sources was significantly lower compared to chicken and human, despite variations in the number of genes and magnitude of odds ratio. The odds of harboring AMR genes *(aac (3)-IId, aadA, ant (2)-Ia, cmlA, InuG, qnrs1*, and *sul2)* in *S.* Infantis were higher in chicken sources, compared to humans. Additionally, within chicken sources, the *S.* Infantis isolated from chicken carcasses had lower odds of harboring most of the AMR genes compared to *S.* Infantis obtained from chicken ceca and environmental samples. Ceca had lower odds for (*aph(3)-Ia*, *blaCTX.*M, *dfrA*) compared to environmental isolates from chicken houses and processing plants. While analyzing the odds of harboring AMR genes between the years of isolation (2010-2014, 2015-2019, and 2020-2024), the odds of harboring AMR genes were lower in isolates between 2010-2014 compared to 2015-2019 and 2020-2024. However, the odds of harboring AMR genes decreased between 2020-2024 compared to 2015-2019, suggesting a decline in the spread of AMR genes in recent years. While analyzing the odds of harboring AMR genes in *S.* Infantis isolated from different geographical locations (Africa, Asia, Europe, North America, and South America), Australia had notably lower odds of finding AMR genes. In conclusion, this study contributes to the understanding and risk factors for harboring AMR genes in *S.* Infantis isolated throughout the world and over time.

Keywords: Salmonella Infantis, Odds ratio, AMR genes, Source, Location

213 Potential of shotgun metagenomic analyses for in-depth taxonomic characterization of microbial communities in egg wash and poultry litter from a laying hen facility

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Microbial contamination in poultry environments, including egg wash and litter, influences both food safety and environmental health. However, the microbial composition of these matrices is not well-characterized. The objective of this pilot study was to compare the microbial communities of poultry litter and egg wash using shotgun metagenomic sequencing. We hypothesized that microbial diversity and composition would differ between the two sample types due to their distinct environmental exposures. One composite litter sample and one composite egg wash sample were collected from a commercial laying hen facility. The egg wash was obtained by washing 120 eggs in a commercial tabletop washer; litter was collected from the corresponding pen. DNA was extracted using chemical lysis, and shotgun metagenomic sequencing was performed. Library preparation and sequencing were completed on the Illumina platform. Due to the low DNA yield from the diluted egg wash, comparing DNA quality between sample types prior to sequencing was impractical. Taxonomic classification using Kraken2 showed that a higher proportion of reads were classifiable in the egg wash (>80%) compared to the litter sample (~20%). *Escherichia coli* comprised <10% of reads in the egg wash and ~30% in the litter. Species richness was higher in the egg wash (~10,000 taxa) than the litter (<2,000 taxa), though adjusted diversity measures were comparable. These findings highlight shotgun metagenomics as a useful tool for profiling poultry-associated microbiota and suggest that egg wash may capture a broader range of microbial taxa than litter alone.

Keywords: Poultry environment, Egg wash, Poultry litter, Shotgun metagenomics, Biosecurity

214 Longitudinal analysis of broiler cecal microbiome: unraveling the species level dynamics across age and sex

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Understanding the microbial dynamics at species level is essential to better implement microbiota-based interventions. Therefore, this research aimed to compare the composition of cecal microbiome of broilers between age and sex. For this study, a total of 70 day of hatch (35 male and 35 female) broilers were obtained and raised up to d42 following commercial industrial guidelines, with ad libitum access to feed and water. Cecal samples were collected on d0, 14, 28, and 42 from 6 birds per sex. DNA extracted from cecal samples was PCR amplified for full-length 16S rRNA V1-V9 hypervariable region, followed by sequencing using the Oxford Nanopore platform. MinKnow was used for base calling and demultiplexing, and the data were analyzed using EPI2ME and R-Packages. The Phyloseq package was used for pre-processing and structuring microbiome data, and background noise was removed. The normality of data was analyzed using the Shapiro-Wilk test, and either ANOVA or Kruskal-Wallis test was done, followed by Tukey's HSD or Dunn's multiple comparison test. There was no significant difference in alpha diversity metrics (Shannon, richness, Pielou's evenness, and Chao1) between the sexes with the same age group. However, comparing the alpha diversity metrics longitudinally within the sex, there was a significant difference between the ages, specifically, d0 had significantly ($p < 0.05$) lower diversity metrics compared to d14, 28, and 42. Assessing the beta diversity (Jaccard and Bray-Curtis), there was no significant difference between the sexes (male and female) within the same age group. However, there was a significant difference for both Jaccard (adonis $p = 0.001$) and Bray-Curtis (adonis $p = 0.001$) due to age, specifically, the community of d0 was completely different compared to the d14, 28, and 42, for both males and females. Differential abundance analysis using DESeq2 shows significant changes in the bacterial abundance between males and females within the same age group. Additionally, there was significant upregulation and downregulation of the bacterial species between ages for both sexes. While comparing the bacterial species between the ages of both males and females, the number of bacterial species present in the ceca plateau at d14 with approx. 400 bacterial species, which remained constant over the age up to d42 in our study. In conclusion, the results indicates that both sex and age were the major drivers of the cecal microbiome of the broiler, however impact of sex was minimal compared to age. Additionally, the shifts in the microbial composition of broilers between d0-14 were much higher compared to shifts between d14 up to d42.

Keywords: Species Level Identification, Oxford Nanopore, Microbiota, Differential Abundance, Broiler

215 Breed-specific variations in tracheal microbiome of broiler chickens

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The respiratory route is one of the primary routes for transmission of bacterial and viral diseases in poultry. Various bacterial pathogens including foodborne *Salmonella* may be transmitted via the respiratory route. Therefore, understanding the microbial composition of trachea is essential to implement microbiota-based interventions. The objective of this study was to compare the composition of tracheal microbiome of three broiler genotypes: a heritage New Hampshire × Columbian cross (**NHC**), Ross 308 (**R308**), and Ross 708 (**R708**). Tracheal samples were collected on d56 from 8 birds per genotype raised in floor pens. Tracheal samples were used to extract DNA followed by PCR amplification of full-length 16S rRNA. Thus, amplified PCR product was sequenced using MinIon (Oxford Nanopore). Minknow software was used for base calling and demultiplexing the raw reads, while phylogenetic assignment was performed using EPI2ME with the NCBI 16S database. The derived OTU table was processed using Phyloseq package in R. Shapiro-Wilk test was conducted to test the normality of the data and either ANOVA or Kruskal-Wallis test was conducted followed by Tukey's HSD or Dunn's multiple comparison test. There was no significant difference in alpha diversity metrics among genotypes. The microbial communities in abundance were significantly different among genotypes as beta-diversity matrix Bray-Curtis ($p = 0.005$) was significantly different; however, overall presence/absence of bacterial species was not impacted by genotype according to Jaccard distance ($p = 0.052$). In comparing the core microbiome, 33% (403) bacterial species were common among the three genotypes, whereas NHC had the highest number of unique bacterial species in trachea compared to R308 and R708. Differential abundance analysis using the DESeq2 revealed changes in the bacterial abundance among genotypes. Compared to NHC, in R708 the potentially pathogenic bacteria such as *Shigella sonnei, Streptococcus pluranimalium*, and *Streptococcus cohnii* had significantly lower representation, however, *Pseudoflavonifractor* gallinarum and

Lachnoclostridium urinimassiliense had significantly higher representation. Similar trends of bacterial species were observed while comparing between NHC and R308. Comparing between the R308 and R708 showed that some of the opportunistic pathogen or pathogenic bacteria such as *Streptococcus pluranimalium* and *Staphylococcus chromogenes* had representation in R708. Compared to NHC, both R308 and R708 had significantly lower opportunistic pathogens in the trachea. Overall, these results indicate that bacterial abundance in trachea was impacted by genotype but there were no genotype specific changes in bacterial taxa.

Keywords: Trachea, Oxford Nanopore, Microbiota, Species Level Identification, Broiler genotypes

216 Development of universal real-time PCR tests for the detection and quantification of fowl aviadenoviruse

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Fowl aviadenoviruses (FAdVs) are responsible for several economically significant poultry diseases, including inclusion body hepatitis (IBH), gizzard erosion (GE), and hepatitis-hydropericardium syndrome (HHS). This study aimed to develop and validate two universal real-time PCR assays using TaqMan and SYBR Green chemistries, targeting a conserved region of the 52K gene, for the detection and quantification of all FAdV serotypes. The experimental design included assay development, analytical validation, and clinical comparison with a reference real-time PCR assay. Positive controls derived from known FAdV strains and viral DNA from all 12 reference FAdV serotypes were used for analytical evaluation. clinical performance was conducted on 47 clinical samples representing four circulating serotypes. Reactions were performed using standard cycling conditions, and specificity was tested against common avian pathogens. Both assays showed excellent amplification efficiency (~99%), Strong linearity (correlation coefficient = 0.983), and a detection limit of less than 10 copy/reaction. Both assays successfully amplified all FAdV serotypes, confirming their universality, with no cross-reactivity observed with other avian pathogens. The intra- and inter-assay coefficients of variation were <0.02% and <0.01%, respectively, with no statistically significant differences in Cq values between serotypes ($P > 0.05$). Clinical performance showed 100% detection across samples with strong agreement to the reference assay (correlation coefficient = 0.987). In conclusion, the developed universal real-time PCR assays are rapid, sensitive, and specific tools for detecting all FAdV serotypes. Their strong analytical and clinical performance makes them well-suited for routine surveillance and outbreak response in poultry health programs.

Keywords: FAdV, Real-time PCR, TaqMan, Syber Green, Diagnosis

217 Enzymes in poultry nutrition: Where do we stand?

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The use of enzymes in poultry feed has been the main significant step forward for nutritionists in the past couple of decades. In practice, the main enzymes used in poultry feed are in order of importance phytase, xylanase, glucanase, protease. Phytase became commercially available in the 90's but it was limited in use to areas where manure phosphate levels were regulated. When phytase prices dropped and inorganic phosphate costs soared in the mid 2000's, its use skyrocketed and it is now a common component of monogastric feeds. Today, there are multiple reasons beyond phosphorus release for which phytase is used due to the indirect implications it has for the animals' overall metabolism. Recent innovative work has been conducted to include the use of phytase in nutrient requirement recommendations in mechanistic model based on a multifactorial approach. Benefits from significant phytate hydrolysis by using high doses of phytase was developed and is well documented when used in practice to increase either animal efficiency and/or help in specific phases where the digestion capacity is not yet mature. More recently, this concept has been shown to facilitate the release of inositol via the intestinal phosphatase alkaline. Inositol has is involved in many metabolic pathways and one of them relates to better oxygen release from red blood cells that has helped in reducing the incidence of chicken breast myopathies. Another type of enzyme to increase the value of raw ingredients are the carbohydrases. There were 3 proposed mechanisms as mentioned by Bedford, 2000 based on 1) viscosity reduction 2) cell wall degradation and 3) prebiotic effect. The first one depends mainly on the cereal type, the second has been controversial while the third hypothesis has been increasingly supported by recent publications. Our recent learnings on fibre carbohydrate composition and its role in poultry called into question the fact that fibre is an inert component used as a filler. In fact it may even bring benefits beyond the physiology aspect in optimising gut function and productivity. Unfortunately, fibre is not a simple substrate as "phytate" can be for phytase and further research would need to deliver answers regarding the best way to optimise fibre use and carbohydrase. Some recent results have pointed out the need to test/validate carbohydrase enzyme benefits considering its influence on fibre fermentation, which is a change from the traditional methods of their evaluation used to this point. Understanding how enzymes can optimise animal performance is important but with recent trends and market changes we also have to consider welfare and profit, both of which may not coincide with maximal performance.

Keywords: enzymes, poultry

218 Evaluation of different commercial brands of phytase enzymes on the growth responses and tibia mineralization of broiler chickens

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Several commercial phytase enzymes have demonstrated improved availability of phosphorus (P), calcium (Ca), and energy. Pecozyme is a new phytase enzyme that has not been evaluated, but it has high potential to match the capabilities of available commercial enzymes. The objective of the study was to evaluate the productive performance, carcass yield, and tibia mineralization of broilers fed low metabolizable energy (ME), low (Ca), and low available P (avP) diets added with known phytase enzymes and Pecozyme. A total of 1,248 one-day-old male chicks were housed in groups of 26 in 48 floor pens. There were eight treatments with six replicates per treatment: 1) Positive control (PC) formulated to cover the nutrient requirements of the strain; 2) Negative control (NC) with reductions of 130 kcal ME, 0.15% Ca and 0.18% avP compared to PC; 3) NC + 1000 FYT of Brand 1 phytase; 4) NC + 1000 FYT of Brand 2 phytase; 5) NC + 1000 FYT of Brand 3 phytase; 6) NC + 1000 FYT of Brand 4 phytase; 7) NC + 1000 FYT of Pecozyme™ phytase; and 8) NC with reductions of 130 kcal of ME, 0.17% Ca and 0.20% avP compared to PC + 2000 FYT of Pecozyme™ phytase. A xylanase enzyme was added to diets 3 to 8 as is usually done in commercial formulations. Corn and soybean meal diets were used in three feeding phases: 1–14, 15–28 and 28–42 days. On day 21, three chicks per pen were sacrificed to obtain the left and right tibias. On the right tibia, the weight, length, and diameter of the epiphysis and diaphysis were registered; the dry matter, ashes, Ca and P content were also determined. Left tibias were used to determine the breaking strength at a load of 2 mm/min. At day 42, five broilers per pen were slaughtered for carcass measurements. Results were subjected to ANOVA using a completely randomized design. The final body weight (BW), weight gain (WG), tibia length and dry matter, ashes, Ca and P content and breaking strength were lower ($P < 0.01$), and the feed conversion ratio (FCR) was higher in NC broilers compared to those in the other treatments. The carcass and breast weight were highest ($P < 0.01$) with Pecozyme added at 2000 FYT, Brand 1 phytase and PC, and were lowest in NC, while the rest of the treatments had intermediate values. In conclusion, the reduction in BW, WG, carcass and breast weight, impaired FCR, and decreased responses of tibia mineralization observed in NC broilers were restored with the addition of different brands of commercial phytases, including Pecozyme. Broiler fed reduced ME, Ca, and avP diets supplemented with various phytases, including Pecozyme, showed similar productive performance and tibia mineralization compared to bids fed the PC diet.

Keywords: Broiler chickens, Phytase enzymes, Performance, Carcass yield, Tibia mineralization

219 Effects of xylanase and phytase supplementation on cecal microbiota composition and short-chain fatty acid production in laying hens

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The cecal microbiota and blood short-chain fatty acids (SCFAs) of laying hens fed an enzyme product with both phytase and xylanase activities were analyzed. 400 45-week-old Hy-Line W36 laying hens were distributed into a completely randomized design with 5 treatments consisting of a positive control (PC), a negative control (NC), and three levels of inclusion of an enzyme product (75, 150, and 225 g/ton of Allzyme®Spectrum). The PC was formulated with corn, soybean, and wheat meal to meet the layers' requirements.

The NC and enzyme inclusion treatments had a reduction of 90 kcal/kg ME, 0.15% Ca, and available P. On the 61-week-old, the cecal contents (n=7) were analyzed for microbiota composition using 16S rRNA gene sequencing, and the blood samples (n=10) were analyzed for SCFA concentrations by gas chromatography. Data were analyzed by the non-parametric Kruskal-Wallis test, followed by Dunn's test for multiple comparisons. Results were considered significant at $p \leq 0.05$. No significant difference in evenness, richness, and overall diversity ($p > 0.05$) of the microbiota in the cecum was observed among treatments, but differential abundance analysis showed taxa with significant differences ($p < 0.05$) for different families and genera. The family *Bacteroidaceae* had the highest relative abundance, regardless of treatment (26.3%), and there were differences among treatments for the families *Lachnospiraceae* (p=0.01), *CAG-508* (p=0.02), and *Gastranaerophilaceae* (p=0.04). The abundance of the *Lachnospiraceae* was lower in birds feed NC+75 compared to NC and NC+225, the *CAG-508* was greater in NC compared to PC, NC+75 and NC+150, and the *Gastranaerophilaceae* was lower in birds feed NC+75 compared to other treatments. The most abundant genus observed was *Phocaeicola* (15.8%), with effects among treatments for the genera *Mediterraneibacter* (p=0.04), *Faecalibacterium* (p=0.03), and *Prevotella* (p=0.03). The NC+75 group had a lower abundance of *Faecalibacterium* and *Prevolela*, compared to PC and NC+225, and the NC+225 had a greater abundance of *Mediterraneibacter* compared to PC, NC+75 and NC+150. The SCFA analysis revealed the presence of acetate (39.6 ppm), propionate (20.8 ppm), butyrate (17.3 ppm), isobutyrate (16.3 ppm), valerate (11.7 ppm), isovalerate (14.1 ppm), heptanoic (11.2 ppm), and lesser amounts of hexanoic (1.4 ppm) and methylvalerate (1.4 ppm) in the serum samples, without significant differences ($p > 0.05$). In conclusion, using a multiactivity enzyme product in laying hen diets resulted in some changes in the cecal microbiota without differences in serum levels of SCFA, but the overall microbiome structure remained unchanged, similar to that of the PC diet.

Keywords: Xylanase, Phytase, Short-chain fatty acid, Microbiota, Phosphorus

220 Sfericase protease: Improving protein solubility in diverse soybean meal varieties

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Soybean meal (SBM) is a protein-rich ingredient largely used in animal feed diets for livestock. SBM nutrient composition, amino acid digestibility, and energy content varies depending on several factors such as soybean genetics, cultivation, region, weather and bean processing. Using an exogenous feed protease allows optimized protein digestion leading to cost savings, since animals achieve the same performance with less protein inclusion in the diet. In this study, eleven samples of SBM were treated with exogenous sfericase protease (500 ppm) and evaluated by *in vitro* hydrolysis. A negative control without enzyme treatment was included. Sample analyses were carried out in triplicates. SBM and protease were incubated in 50 mL tubes, containing 15% total solids, pH 6.5 at 40°C for 4 h under 25 rpm agitation. Soluble protein (SP) was measured from processing of dried pellets in nitrogen analyzer by Dumas methodology. Data was analyzed as one-way ANOVA and means were individualized by SBM sample, comparing treatments (control versus protease) using Student`s t test on JMP 16 software with significance set at $P < 0.05$. SBM-06 presented SP levels of 14.28% ± 1.46% without the presence of protease, while SP released by the effect of the protease was 26.12% ± 1.48%, with statistical significance ($P = 0.0006$). This represents an increase of 83% in SP over control. For SBM-02, (7.47% ± 2.55% of SP on control treatment), the exogenous protease was able to bring SP levels to 24.01% ± 0.11%, delivering a statistically significant increase of 221% over control ($P < 0.0004$). Analysis for all 11 samples shows the sfericase protease delivers consistent and significant increase in SP when compared to the control, ranging from 83 to 221%. Our findings indicate that the protease tested can be used as an effective tool to handle different varieties and qualities of SBM. Additionally, we selected 6 out of the 11 SBM samples tested and compared the sfericase protease to two other commercial proteases (marked A and B). Using Tukey-Kramer test, it is observable that SBM-06, treated with proteases A and B showed increased SP levels at 17.49 ± 0.34% and 17.91% ± 0.66%, respectively, with no statistical significance between enzyme A and B ($P = 0.6531$), but statistically different from the SP released by sfericase protease ($P < 0.0001$). Increases in protein solubility delivered by the sfericase protease in comparison to the other enzymes tested were significantly more expressive in all 6 SBMs.

Keywords: soybean meal, sfericase protease, protein solubility, animal feed, enzyme hydrolysis

221 Protease supplementation can ameliorate negative effects of high trypsin inhibitor in broiler diets

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Exogenous enzymes have been used in monogastric animal nutrition for improving nutrient digestion as well as ameliorating the negative effect of the antinutritional factors present in feed ingredients. This study was conducted to investigate the effect of exogenous protease on performance of broilers fed diets containing high levels of trypsin inhibitor (TI). A total of 1632 Ross 308 male broiler chicks were randomly allocated to one of four treatments with 12 replicate pens per treatment and 34 birds per replicate: All treatments were formulated using soybean meal (SBM), full fat soy (FFS) with low TI levels (3.8 and 4.7 mg/g, respectively) and 3% of raw soy with 34.0 mg/g of TI during grower and finisher phases; T1 (positive control) was formulated according to the genetic requirements. T2 (negative control) was formulated with 5% lower levels of digestible amino acids compared to T1. T3 & T4 were the same as T2 but with the addition of exogenous protease at 250 g and 500 g/MT, respectively. The calculated level of TI for starter diet was 1,38 mg/g, grower diet was 2.33 mg/g and 2.19 mg/g for the finisher. Mash diets were provided over 3 dietary phases: starter (0-7d), grower (8-21d), and finisher (22-35d). Body weight and feed intake were determined at 14, 21, and 35 d of age. Data was analyzed as one-way ANOVA, and means were separated using Tukey HSD test ($P \leq 0.05$). No differences in BWG were observed among treatments ($P = 0.207$), while significant differences were observed for feed intake ($P = 0.0495$) and FCR ($P = 0.015$) between the T2 (negative control) and T4 (diets with 500 g/MT protease inclusion) for the overall period from 0-35d, T1 (Positive control) and T3 (diets with 250 g/MT protease inclusion) being intermediate. Supplementing protease at 500 g/MT decreased feed

intake by 3.14% and FCR by 5 points compared to the negative control (P≤0.05), and 2.35% and 1.5 points beyond the PC for feed intake and FCR, respectively. Based on the results, supplementation of exogenous protease can improve growth performance in broilers through mitigating the negative effect of high levels of TI as well as compensating for 5% of amino acid reduction.

Keywords: Broilers, Trypsin Inhibitor, Protease, Raw Soy

222 Evaluation of the use of different levels of Arabinoxylans insolubles in broiler diet

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Dietary fiber plays a crucial role in the nutrition of monogastric animals, particularly in poultry diets, regarding gastrointestinal tract development, gut health, and fermentation. The understanding of non-starch polysaccharides (NSPs), as a better criterion, to characterize total dietary fiber has evolved significantly; this better understanding combined with the application of NSP-degrading enzymes have revolutionized the possibility to use diverse feedstuff. Arabinoxylans are the main fermentable constituents in a feed based on corn and soybean meal and it can be increased in the diet by including other cereal sources, such as wheat bran. The current research evaluated the effect of increasing levels of insoluble arabinoxylans (A/Xins) with inclusion of wheat bran (0-5%) on performance of broilers. One experiment was run out tested four diets: 1) positive control diet (PC) based on corn and soybean meal (A/Xins =3.6%) + 100g/ton of a ß 1-4-endoxylanase (Econase XT 25P, AB Vista) considered equivalent to 100 kcal/kg ; 2) Diet 1 including 1% of wheat bran (A/Xins =3.8%); 3) Diet 1+ 3% wheat bran (A/Xins =4.1%) ; 4) Diet 1 + 5% wheat bran (A/Xins= 4.4%). Each treatment had 10 pens of 40 birds per pen, and dietary treatments were evaluated from 8 to 38 days, and performance from 0 to 38 days. A one-way ANOVA was performed (JMP Pro 15, SAS) and means were separated using a Tukey test with significance accepted when P≤0.05. Treatments did not affect the intake of broilers (P=0.299). Body weight gain was not different among treatments (P=0.110). Nevertheless, the results of body weight gain showed that the highest A/Xins diet (4.4%) was numerically lower than lowest A/Xins diet (2213g vs 2318g respectively, 105 g difference at 38d). By consequence, FCR was different (P <0.001) and observed to be the lowest for the broilers fed the 3.8% A/Xins (1.642) and the highest for broilers fed the 4.4% A/Xins (1.754). The 3.6% and 4.1% fed broilers showed similar and intermediate performance (1.664 and 1.685, respectively). The livability percentage was not affected by experimental treatments (P>0.05). The economic analysis based on price of feed formulation per broiler showed being 0.2 cents (USD) cheaper with the inclusion of 1% wheat bran (3.8% of A/Xins) compared to the diet without wheat bran. The incorporation of 3% and 5% wheat bran with A/Xins =4.1% and 4.4% increased the cost by 0.25 cents (USD) and 0.45 cents (USD), respectively compared to the level of 3.8% of A/Xins. This study shows the possibility to increase to intermediate level, the incorporation of fibrous ingredients without impairing poultry producer productivity.

Keywords: Dietary fibre, broiler performance, Insoluble arabinoxylan, xylanase

223 Not Presented

224 Reduction in ribonucleotide reductase subunit RRM2 links to mitochondria dysfunction and altered gut health of woody breast chicken

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The woody breast (WB) condition impairs the texture of chicken breast meat causing a financial loss for the poultry industry. Recent studies suggest that mitochondria may play a role in muscle and gut health by regulating inflammatory mediators. Among the three intestinal sections, the duodenum plays a crucial role in gut health as it is the primary site where food is mixed with digestive enzymes from the pancreas, initiating the process of nutrient absorption. However, there is limited information available on gut and pancreas health as they relate to the WB condition. Ribonucleotide reductase, subunit RRM2, is involved in DNA synthesis and mitochondria function. Inhibition of RRM2 increases gastrointestinal disturbances and apoptosis in pancreatic cells by disturbing inflammatory mediators and mitochondrial homeostasis. This study aimed to investigate links between RRM2 expression, pancreas and gut health in broilers exhibiting WB. Breast muscle, duodenum and pancreas samples were collected from 10 birds showing severe WB and 10 normal (N) birds. Gene expression was measured using qRT-PCR with SYBR reagents. Mitochondrial total protein content and functionality were measured in mitochondria isolated from the tissues. Data were analyzed using Prism V.9 and t-tests. Results indicated that WB chickens had greater hemorrhage scores (P=0.05). Compared to N, WB had lower expression of RRM2 in muscle (P=0.03), pancreas and duodenum (both P=0.04). Expression of cytochrome b (mitochondrial gene that helps convert food into energy) and mitochondrial uncoupling protein 3 (regulates energy metabolism in mitochondria) were reduced in muscle (P=0.05 and P=0.002) and pancreas (P=0.009 and P=0.002) from WB, while only cytochrome b was reduced in duodenum (P=0.04). Total mitochondria protein (cellular energetics and metabolism) and mitochondria complex V activity (produces ATP), which are indicators of mitochondrial dysfunction, were reduced for WB in muscle (P=0.0006 and P=0.004), pancreas (P=0.01 and P=0.002) and duodenum (P=0.001 and P=0.002), respectively. In conclusion, the present data suggest that in birds with WB reduced RRM2 expression in the duodenum and pancreas may alter tissue function by increasing mitochondria abnormalities and oxidative damage while reducing energy production.

Keywords: woody breast, gut health, ribonucleotide reductase enzyme, mitochondria

225 Evaluation of different inclusion levels of novel phytase on broiler growth performance

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The study evaluated the effects of dietary supplementation with a novel phytase on broiler growth performance and feed efficiency, aiming to determine the optimal inclusion level for the phytase. A total of 420 one-day-old Cobb broilers were assigned to seven

groups in a completely randomized design, with six replicates of 20 broilers each. The control group (CON) received a corn-soybean meal-based diet without phytase, with aP:Ca of 0.32:1 for 1-21d and 0.23:1 for 22-42 days. Five experimental groups were supplemented with varying levels of novel phytase at 250, 500, 1000, 1500, and 2000 U/kg, while an additional group received 500 U/kg of a commercial phytase (C). The trial spanned 42 days, with growth metrics and feed conversion ratios evaluated at multiple stages. Broilers were weighed at day 1, 21 and 42, and feed intake was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Data were analyzed using one-way ANOVA, and regression models were applied to determine the optimal phytase inclusion level. Results revealed significant improvements in ADG and FCR for broilers receiving 500–2000 U/kg of novel phytase compared to CON group ($P < 0.05$) at day 42 ; Increasing novel phytase from 250-2000 U/kg, linearly increased body weight (2891.8, 3040.6, 3106.9, 3113.7, 3213.7, 2993.3, $P< 0.001$), ADG (67.84, 71.39, 72.96, 3106.9, 73.14, 75.51, $P< 0.001$) and linearly reduced FCR (1.56, 1.50, 1.49, 1.49, 1.46, $P < 0.002$) at day 42; ADFI during 0-42 days was not affected by treatment ($p=0.52$). Notably, phytase supplementation improved ADG with novel phytase only at 1000–2000 U/kg outperforming the C group at day 22-42 (101.92, 102.33,105.79, vs 95.31). Quadratic regression analysis determined the optimal inclusion level of the novel phytase to be 1911.6 U/kg, achieving the highest ADG (75.51, $P <0.001$) while minimizing FCR (1.46, $P <0.005$). These findings demonstrate the efficacy of the novel phytase in improving growth performance in broilers, supporting its potential as an effective tool for enhancing production efficiency. Furthermore, the study highlights the advantages of the novel phytase over existing commercial alternative, particularly in the later growth stages, which is crucial for sustainable poultry production. In conclusion, supplementing broiler diets with the novel phytase at appropriate inclusion levels significantly benefits growth performance, confirming the safety and compatibility of the novel phytase with typical broiler diets.

Keywords: Broilers, Novel Phytase, Weight Gain, Feed Efficiency

226 Determination of phosphorus release value of a novel *E.coli* phytase from 250 to 2,000 FTU/kg in broiler chickens fed a corn and soybean meal based diet

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Supplementation of phytase has become a general practice to facilitate hydrolysis of phytate naturally occuring in plant ingredients thus to reduce content of inorganic P in feed. A key consideration in phytase application is its P-release value from targeted feed ingredients. The objective of this study was to determine P-release value of a novel *E. coli* phytase in broiler chcikens fed corn and soybean meal diets. A total of 2,060 day old (male) Ross 708 broiler chicks were fed a common starter diet in crumble form from d 0 -7. For the grower (d 8–21) and finisher (d 22–35) phases, 10 experimental diets were allocated in two experiments (Exp.). In Exp.1, floor pen growth performance was evaluated (n = 8; 22 birds/pen), and in Exp. 2, metabolism cages were used (n = 6; 5 birds/cage). Diets 1-5 were formulated to meet specifications with 5 levels of NPP (Grower: 0.42, 0.37, 0.32, 0.27 and 0.22%; Finisher 0.375, 0.325, 0.275, 0.225 and 0.175%) while keeping the Ca/NPP ratio constant, without using phyase. Diet 5 (NPP 0.22/0.175%) was used as a basal diet to add phytase at 250/500/1000/1500/2000 FTU/kg to form Diets 6-10. Data were analyzed as a completely randomized block design with pen as the experimental unit, and treatment as a fixed effect using Proc GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Proc IML procedures of SAS was used to generate polynomial contrast for the response for increasing dietary npp or phytase. Results were considered significant with $P\leq0.05$. Growth performance, bone traits, apparent retention (AR) of Ca and P were evaluated using index marker. At the end of trial, 2 birds per cage were scarified for left tibia and femur for determining breaking strength (BS), weight and ash content. Results showed that increasing NPP (Diets 1 to 5) linearly improved ($P<0.01$) growth and FCR. The supplementation of phytase linearly ($P<0.01$) increased BWG and FCR (Day 8-35), with a cubic response in FCR in finisher phase. Similarly, the addition of phytase linearly increased tibia and femur attributes as well as AR of Ca and P. Based on the responses detected, P-release were calculated for BWG: 0.07, 0.12, 0.20, 0.19 and 0.28%; FCR 0.11, 0.22, 0.31, 0.36 and 0.45%, corresponding to the levels of phytase at 250, 500, 1000, 1500 and 2000 FTU/kg. The average P-release of tibia and femur attributes were estimated as 0.08, 0.13, 0.18, 0.19 and 0.21%, respectively. In conclusion, the current study confirmed 1) the novel *E. coli* phytase is effective in liberating P from phytate in the corn and soybean meal diet; 2) the P-release values go up along with increases in phytase dosage; and 3) the P-release values differ depending on phytase dosage and evaluation parameters used.

Keywords: E. coli phytase, P release, performance, bone traits, Ca and P retention

227 Can phytase substitute the entire dietary inorganic phosphorus for broiler?

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Early studies suggest phytase at 500 FTU/kg feed can serve as an effective dose, and higher doses could lead to more P-release and none-P benefits, such as enhanced digestibility of minerals and amino acids. The recent advancements in biotechnology and fermentation seem to have contributed to a new generations of phytase with superior heat stability and catalyzing strength. The objective of this study was to determine whether a novel phytase can support high level of growth performance of modern broiler fed on inorganic P free diets. A total of 500 day-old Ross 308 male broiler chicks were allocated to 5 dietary treatments, each treatment consisted in 10 pen replicates of 10 birds per pen, fed on corn, soybean meal, with lower inclusions of cassava, rice bran, wheat bran, DDGS and rapeseed meal. A positive control group (PC) was formulated to provide standard levels of NPP by inclusion of MCP at 20.4/14.5/11.4/8.6 kg/t, resp. for starter (0-10d), grower (10-21d), finisher 1 (21-35d) and finisher 2 (36-42d). A negative control (NC) diet contained only avP 0.22% for starter diet to prevent the birds from high mortality. Diets 3-5 contained no inorganic P, but supplemented with three phytase brands (QB, HT and RK) at the same pattern of 3000/2000/1000/1000 FTU/kg for the four growing phases. Feed intake (FI), body weight (BW) and mortality were recorded, and the data were analyzed by one-way ANOVA, and $P\leq 0.05$ was used as the criterion for statistical significance. The results showed that for NC group, the decrease of dietary avP to 0.22% in starter feed followed by MCP removal in the subsequent feeds significantly depressed FI and BW with increased mortality (21%), confirming the impact of P deficiency.

In contrast, supplementating three phytase successfully prevented high mortality yet restored growth rate to the level of PC, but these birds tended to consume more feeds than the PC group, leading to increased FCR (p<0.05). No significant differences were observed among the three phytase brands despite the birds on phytase QB and HT gained numerically more live weight. The study results suggest that it is feasible to use phytase to completely substitute inorganic P in diets, for the birds grow as well as those receiving normal level of dietary P. We need further investigate why the birds on phytase schemes tended to consume more feeds that led to poorer FCR.

Keywords: phytase, super dose, broiler, MCP-free, performance

228 Where is the poultry production sector going with behavior, range area, nutrition, and land areas in the US

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The US egg industry is changing dramatically with the major component of that growth focusing on alternative production systems such as cage-free or range egg production because of concerns about the impact of the cage environment on laying hen wellbeing. This shift has contributed to the increase in egg prices but has also increased the number of households raising chickens. The most positive result of this is that more people understand what it takes to produce eggs. We forget that the World Health Organization indicates that approximately 90% of all layers in the world are kept in cages and with an increasing world population, production efficiency is very important. Constituents of growth in the layer industry in the US, Canada and Europe involves the exclusion of intensive egg production, which creates uncertainty in relation to the future. We fail to consider that cage free and free-range (pasture) operations would place an additional level of demand on the land utilization. This will impact the environment and the conflicts between urban and rural uses associated with zoning. However, one of the current issues is that our knowledge base of how these alternative production methods influence egg performance and quality characteristics is limited to research studies that were conducted in the late 1940s and early 1950s. The 41st NCLP&MT showed that shifting from cage to free-range reduced the amount of eggs produced for every kg of feed by 13.5 and 2.1% in white and brown egg strains, respectively. This translates to an increase in feed consumption by 4.9 and 4.6% in white and brown egg strains, respectively. This increased demand for feed will require an additional 4.4 mil acres of corn to be planted as well as added acres of soybeans and other ingredients. Where are the resources needed to plant these acres and where are these acres going to be coming from? Research on range or cage-free production done in controlled settings are growing, but, additional studies relevant to egg producers wishing to expand cage-free and range egg production are needed. This movement towards extensive, free-range animal production represents an irreconcilable use of our limited resources needed to feed a hungry and growing world population. Therefore, an examination of alterative laying hen husbandry practices in the context of the current knowledge base would provide beneficial information to identify how these husbandry and feeding practices translate to modern strains of laying hens under cage-free or range production.

Keywords: Production systems, cage-free, Free-Range, land usage, Nutrition

229 Impact of plastic huts and metal ramps as environmental enrichment on welfare and performance in Ross 308 broilers to 32 d of age

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Environmental enrichment can enhance broiler welfare through physical and mental stimulation, but its effectiveness depends on type, design, quantity, and combination. This study assessed the impact of plastic huts and metal ramps on the performance and welfare of Ross 308 broilers up to 32 days. A single trial was conducted with 7,256 broilers housed in eight rooms (9.75 × 6.50 m). Each room contained 907 birds, with a final estimated stocking density of 31 kg/m2. Four rooms contained a plastic hut and a metal ramp (E), while four were unenriched controls (C). Data were collected on basic health and functioning (body weight, feed intake, mortality, and scores for foot pads, hocks, gait, and plumage cleanliness), affective states (novel object, tonic immobility, inversion tests, and heterophil-to-lymphocyte (H/L) ratios), and natural living (enrichment use, activity). A completely randomized design was used for analyses, with normality tests and log+1 transformations applied as needed. Initial body weight tended to be higher in E than C (P=0.06), but no effect was found at 7, 21, or 32 d. A trend toward higher average gain from 21-32 d was observed for E (P=0.08), but no differences occurred from 0-7, 7-21, or 0-32 d. Feed intake was higher for E than C between 21 and 32 days (P=0.04) but not in other periods. No differences were found for gain-to-feed, feed-to-gain ratios or mortality. E broilers tended to have higher mortality due to metabolic issues (P=0.06), while skeletal mortality diagnoses tended to be higher in C (P=0.08). From 7-21 d, infection-related mortality was lower in E than C (P=0.01), with a trend for higher polyserositis in C (P=0.07). No enrichment effects were found for foot pad, hock, gait, or plumage cleanliness scores (d 28), weekly novel object, or tonic immobility (d 20). In the inversion test, C broilers tended to move their heads more than E birds on d 28 (P=0.09). H/L ratios were lower in E than C on d 30 (P=0.01), suggesting reduced chronic stress. Enrichment use (%) was calculated as the average number of birds on or under enrichment, adjusted for available space and bird size. Enrichment-by-day interactions (P<0.01) showed that broilers used more space under the hood than the ramp on d 4, 11, 18, and 25. Hood use remained stable, while ramp use underneath declined from d 4 to 11 before increasing until d 31. Ramp use on top peaked at d 11 but was lower at d 18, 25, and 31. Hood use on top at d 11 matched ramp use at d 25 and 31, but overall, birds used the top of the hood less than the ramp at d 4, 18, 25, and 31. No enrichment effect was found on activity. In summary, enrichments did not affect final production performance but were linked to reduced chronic stress.

Keywords: Environmental Enrichment, Broiler Welfare, Broiler Behaviour, Broiler Production, Broiler Management

230 Effect of rearing by sex or as-hatched on growth performance, body composition, and carcass yield of Ross 308 broilers from 0 to 43 days of age

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The objective of this study was to evaluate the effect of rearing broilers by sex or as-hatched on growth performance, body composition, and carcass yield from 0 to 43 d of age. In total, 1,440-day-old Ross 308 chicks were separated by sex and placed in 36 pens according to the rearing strategy: by sex (40 females or 40 males) or as-hatched (mixed-sex broilers; 20 females + 20 males). All the birds received the same commercial diets, based on cereals and soybean meal and there were three feeding phases: starter (0 to 10 d), grower (11 to 28 d,) and finisher (29 to 43 d). The growth performance was determined by phase and cumulatively. Body composition, by bioelectrical impedance (at 9, 28, and 41 d of age), and carcass yield (at 43 d) of the birds were measured in two birds per pen. Data were analyzed as CRD with

three rearing strategies (female, male, or as-hatched). Each treatment was replicated 12 times, and the experimental unit was the pen. The Gompertz equation was applied to model the genetic growth potential of the broilers. From 0 to 43 d of age, the males consumed more feed, grew faster, and had better FCR ($P < 0.001$) than females, with the as-hatched birds being intermediate. On a % fresh basis, male birds had higher water and body fat content but less protein and ash content than female birds at 43 d of age ($P < 0.05$). No differences were observed in the carcass yield of the birds ($P > 0.05$). When each gender within each rearing strategy, male and female reared by sex vs. as-hatched) was compared, no differences in any of the variables studied were observed ($P > 0.05$). In summary, males exhibited the best performance throughout the experiment, followed by as-hatched and then female birds. Under the conditions of this experiment, both sexes exhibited the same responses across the rearing strategies.

Keywords: broiler, growth performance, as-hatched, rearing strategy, sex

231 Manure accumulation and removal study for raised flooring operation in broiler houses

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A significant challenge faced by poultry farmers is maintaining an optimal environment to raise broiler chickens. Controlling moisture and temperature is complex, and uses a substantial amount of energy. Moisture in the house comes from three main sources: 1) Water vapor entering the house from outside, which represents about 10-15% of the total moisture. 2) Breathing, skin evaporation, and manure from the chickens contribute approximately 75-80% of the moisture. 3) The remaining 10-15% of moisture comes from water on the ground, walls, sink surfaces, equipment in the house, and bedding. To address the largest contributor to the poultry house moisture content, researchers from the Georgia Tech Research Institute (GTRI), in collaboration with University of Georgia (UGA), have initiated a trial of the Broiler House Integrated Guided-Motion Excreta Saturation System (BHIG-MESS) at UGA's Poultry Experimental Center. The BHIG-MESS is a raised floor system designed to manage manure and moisture by automatically and regularly removing poultry waste from the house. This could help reduce ventilation rate and lower energy consumption. In traditional flooring, litter is the primary method for absorbing moisture and fecal material while also providing insulation. The BHIG-MESS system utilizes a system of trays occupying the space below the raised flooring to collect and remove chicken manure efficiently. To study the manure collection process, the BHIG-MESS flooring system was installed in two environmentally controlled bird rooms. The trial ran for 49 days, with each room containing pens with a traditional wood shaving floor and an experimental raised flooring system separated by a fenced enclosure. Each coop started with 110 broiler chicks and had a total area of 60 square feet. The research team recorded weights of the manure and generated a 3D depth map to characterize volume and spatial distribution of the manure on a weekly basis. The weight of broilers in both pens was recorded weekly throughout a 7-week trial. The weights of broilers on traditional flooring and raised flooring were statistically similar. The final weights of the 7-week broilers were 4 kg for those on wood shavings flooring and 3.9 kg for those on raised flooring with p value of 0.35. The flooring system did not have a negative impact on bird performance. Manure production per bird per day was approximately 5.7, 24.7, 72, 121.6, 139, 152, and 132 grams for week 1 though week 7 respectively. The measurements will provide engineering requirements for GTRI and UGA to design an automatic manure removal system.

Keywords: Manure, Raised flooring, Broiler house, moiture, ventilation

232 Influence of different litter regimens on ceca microbiota profiles in Salmonella-challenged broiler chicks

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Litter management is a critical component in poultry production, with implications for health, and productivity of the birds. A 14-day study was conducted to evaluate the effect of litter type (dirty litter, **DL**; fresh litter, **FL**) and *Salmonella* Enteritidis (**SE**) challenge (no challenge, **NC**; challenge, **SE**) on performance and cecal microbial profile of neonate chicks. Broiler chicks (day-old; n=240, Ross 708 male) were allocated to 2 x 2 factorial design consisting of four treatments: chicks raised on dirty litter (**CONDL**), chicks raised on fresh litter (**CONFL**); and chicks raised on litter type similar to CONDL and CONFL but inoculated with 7.46×10^8 CFU SE /mL at d 1 (**CONDLSE** and **CONFLSE**). Performance indices evaluated include body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), feed conversion ratio (**FCR**) and mortality (%). Cecal SE was enumerated on d 3 and 14, after which DNA was extracted and subjected to microbiome analysis of the V3–V4 region of the 16S rRNA gene. The sequencing yielded 75246 ± 11068 reads per sample. Data were analyzed by 2-way ANOVA while cecal SE data by t-test ($P < 0.05$). Cecal SE was lower in DL at d 3 and 14 respectively ($P<0.05$), suggestive of positive action against SE. Chicks reared on DL had higher ($P<0.05$) BW, BWG and FI compared to FL, while those in SE group had poorer FCR ($P<0.05$) compared to NC. The interaction of litter type and SE challenge influenced ($P<0.05$) FCR, FI and Mortality. Specifically, CONDL had the poorest FCR (1.832), FI was highest in CONFLSE, CONDL and CONDLSE (0.655, 0.692 and 0.677) respectively, and mortality (1.67%) was lowest in CONDLSE. Alpha diversity (chao1 and Shannon index) was higher ($P < 0.05$) in DL compared to NC indicating greater species evenness and richness, while the beta diversity revealed a significant separation ($p < 0.05$) between microbial communities in DL and FL. Comparative heat tree analysis revealed 21 differential genera ($P < 0.05$) with 14 in DL and 7 in FL. Some of these genera include *Lactobacillus, Clostridia_vadinBB60_group, Lachnospira, Oscillospiraceae UCG_005*, *Eubacterium ventriosum* and *Marvinbryantia* in DL, all known to play significant roles in improving growth performance, metabolic homeostasis within the gut, energy metabolism, and short chain fatty acid (SCFA) utilization. Representative genera identified in FL include *Lachnospiraceae_NK4A136_group*, *Ruminococcus_gauvreauii_group*, *Oscillibactor*, *Butyricicossus*, and *Colidextribacter* which are associated with inflammation regulation, pathogen defense abdominal fat deposition and glycemic control. This study indicated that the ceca microbiome of broiler chicks reared on used litter contain bacteria species that enhance their resistance to colonization by SE.

Keywords: Gut microbiota, Salmonella Enteritidis, Microbiome-host interaction, Neonate chicks, Litter regimen

233 Unlocking probiotics recovery in poultry feed production

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Poultry farming is a predominant sector in animal protein production, supported by advances in genetics, health, nutrition and equipment technology. These advancements intensify global competitiveness and the continuous need for improvements in zootechnical practices. Key objectives include reducing health challenges, optimizing feed conversion, accelerating body weight gain, and decreasing antimicrobial use. Probiotics have emerged as a viable alternative to antimicrobials, enhancing immunomodulation, establishing eubiosis and improving feed efficiency. However, thermal processes such as extrusion—essential for increasing digestibility and inactivating pathogens—compromise the viability of probiotic strains. To address this challenge, a formula (POTENBAC® AVESUI SE TR, PolySell Produtos Químicos Ltda, Brazil) was developed, composed of inulin, amylase, protease and probiotic strains (*B. subtilis, B. licheniformis, B. coagulans, L. acidophilus,* and *S. cerevisiae*), with a total concentration of 1.12×10^9 CFU/g. This formulation was designed to withstand the thermal challenges of pelletization and extrusion. To achieve this, a proprietary "shielding technology" was developed, combining theoretical concepts of covalent bonding, melting points and mixing processes to provide thermal protection to strains. To validate the shielding technology, the formula, at a recommended inclusion rate of 200 g/ton of feed, was mixed with feed ingredients for 15 minutes to ensure homogeneity and then subjected to the following thermal treatments: i) Negative control (NC): no thermal processing; ii) Pelletization process (PP): steam at 80°C for 30 sec; iii) Extrusion process (EP): Steam stages: 85°C for 1 min, plus 85°C for 1 min and 85°C for 1 min; Extrusion die: 110°C for 30 sec. Ten samples were collected to assess microbiological recovery of sporulated strains (SS), lactic acid bacteria (LA), and yeast (Y) according to methodologies described in the Brazilian Pharmacopoeia 6th edition and the Chi-square test was applied for statistical evaluation. The expected minimum guaranteed recovery levels were: 2.1×10^5 CFU/g for SS, 1×10^4 CFU/g LA, and 4×10^3 CFU/g Y. The microbiological recovery results were: i) NC: 2.2×10^9 CFU/g for SS, 1.8×10^7 CFU/g LA, and 1.1×10^7CFU/g Y ($p<0,0001$); ii) PP: 5.4×10^6 CFU/g for SS, 1.4×10^5 CFU/g LA, and 1.4×10^5 CFU/g Y ($p<0,0001$); iii) EP: 1.8×10^7 CFU/g for SS, 3.9×10^6 CFU/g LA, and 3.7×10^6 CFU/g Y ($p<0,0001$);. The results demonstrate the effectiveness of the shielding technology in the synbiotic-enzyme formula in ensuring probiotic strain recovery after exposure to the thermal challenges of pelletization and extrusion, exceeding the expected minimum guarantee values.

Keywords: Probiotics, Poultry feed, Pelleting, Extrusion, Feed Conversion

234 Litter amendments drive distinct compositional and functional shifts in the chicken gut microbiome

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Objective: Litter amendments are used in poultry production primarily to reduce ammonia and improve litter quality. However, potential effects on the chicken gut microbiome remain understudied. Since chickens do ingest litter during feeding, amendments may influence gut microbial composition and function, with possible implications for foodborne pathogen carriage. Common litter amendments such as alum and sodium bisulfate were selected to investigate potential links between litter management practices and gut microbiome. This study sought to determine how different litter amendments may affect the functional potential of the chicken gut microbiome. Experimental Design: Male chicks (n=12 chickens/group/age) were randomly divided into pens containing poultry litter with 1) 200lbs alum/1,000 ft^2, 2) 200lbs alum/1,000 ft^2 + 5lbs Ca nanoparticles/1,000ft^2, 3) 200lbs $NaHSO_4$/1,000ft^2, 4) 200lbs alum mud litter amendment (AMLA)/1,000 ft^2, or 5) 200lbs $NaHSO_4$/1,000 ft^2 + 5lbs Ca nanoparticles/1,000 ft^2. All birds were raised under otherwise standard conditions including stocking density, with feed and water provided *ad libitum*. At 3 and 6 weeks of age, cecal and ileal contents, along with tissues of each gut region were collected. Norepinephrine concentrations and total metal concentrations were measured in ileal samples. Shotgun metagenomic sequencing was performed using Illumina NexteraXT methods (NovaSeq platform), with sequencing quality control and host contamination removal conducted using Kneaddata. Functional profiling of the microbiome was carried out using the HUMAnN3 pipeline. Statement of statistical analyses: Microbial taxonomic complexity was analyzed using multivariate statistical approaches, with significance assessed using PERMANOVA. Differential abundance of microbial taxa and functional pathways was determined using ANCOM-BC, while co-occurrence network analysis was performed to identify associations between microbial taxa, gut metal concentrations, and norepinephrine levels. Results: Litter amendments altered ($p<0.05$) microbial community structure, leading to changes in microbial communities associated with nutrient metabolism, gut mucosal integrity, and immune regulation. Functional profiling revealed shifts ($p<0.05$) in pathways related to amino acid biosynthesis, carbohydrate metabolism, and nucleotide metabolism, indicating microbial adaptation to different litter conditions. Conclusions: Co-occurrence network analysis identified associations between major bacterial taxa, gut metal concentrations, and norepinephrine levels. Together, these findings demonstrate how litter management strategies influence gut microbial communities, with potential implications for poultry health and food safety.

Keywords: Litter, Microbiome, Neurochemical , Catecholamine, Gut

235 Investigation of poultry fecal removal efficiency and volume estimation on grooved-floor panels

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An experiment was conducted to 1) evaluate the feasibility of removing feces from grooved floor panels designed for cage-free broiler production and 2) develop an image processing algorithm to estimate fecal weight for PLF applications. A total of 14

grooved floor panels (1×6 ft) were produced and placed underneath 14 cages (1×1.67 ft) to collect feces produced by 2 (n=6) & 3 (n=8) 20-week-old layers within 24-hour windows for two days. A mobile platform equipped with an electric cylindrical brush was developed to remove feces with a walking speed of 6m/s. To mimic the birds walking on the grooved floor panels in a cage-free broiler production system, the fecal matter was compressed against the floor surface before the fecal removal operations. The fecal removal efficiency was measured by weighing the grooved panels before placement, and before and after fecal removal operations. The removed fecal weight was documented. A 3D LiDAR camera was held 25cm above the floor to scan each floor panel 3 times to acquire the A) original fecal surface, B) compressed fecal surface, and C) floor surface after the fecal removal operation. Fecal volume was computed by subtracting the C surface from the A and B surfaces, respectively. Comparisons of fecal removal efficiency under different stock densities and collection windows were analyzed via one-way ANOVA tests. The relationship between fecal volume and weight was evaluated using linear regression analysis. Results indicated that the average fecal removal efficiency was 90.4±1.97% for the 24-hour collection window on Day 1 and 89.47±2.32% for the 18-hour window on Day 2. No significant difference ($p > 0.05$) in cleaning efficiency was found between two or three birds per cage. In addition, linear regression analysis showed a strong correlation between fecal weight and fecal volume ($r = 0.97$) collected in an 18 & 24-hour window before compression. However, after compression, the correlation weakened ($r = 0.84$) due to changes in fecal density. ANOVA tests showed significant differences ($p < 0.05$) in fecal volume (both 24-hour and 18-hour windows) between cages with 2 and 3 birds ($p<0.05$). For uncompressed fecal volume, the significant difference between 2 and 3 birds suggests that evaluating fecal volume may help determine the number of live birds in each cage. An increase in variation of the compressed fecal density was observed when stock density was increased. Therefore, evaluating fecal volume near the feed/water lines in cage-free broiler systems may not provide informative insights into birds' health status. In conclusion, this study showed that daily produced feces can be effectively removed from the grooved floor and fecal weight can be accurately estimated.

Keywords: Precision Livestock Farming, Broiler production, Fecal Inspection, Robotic, Machine Vision

236 Evaluating draft Environmental Protection Agency emissions models for broiler operations

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In August 2021, the U.S. Environmental Protection Agency (EPA) released draft models to estimate daily NH_3, H_2S, $PM_{2.5}$, PM_{10}, and total suspended particulate emissions from U.S. broiler operations using inputs of daily mean ambient temperature, relative humidity, and live animal weight. The EPA developed these models using datasets collected between 2005 and 2007. It is important to note that over 15 years have passed since the original data were collected, and broiler genetics, nutrition, and management have improved considerably. These models, if adopted, could have substantial impact on the U.S. broiler industry regarding air emission management and regulation. The objectives of this work were to 1) evaluate the draft EPA emissions model robustness and practicality, and 2) assess the estimated annual emissions for broiler operations in representative U.S. climates using the draft EPA models. The draft EPA emissions models and coefficients were coded and checked against example calculations provided in the draft EPA report, with discrepancies noted. The draft EPA models with log transforms were found to be overly complicated for deployment by most laypersons intending to estimate daily annual emissions from a house or a site with multiple houses. Substantive, fundamental model challenges were uncovered due to narrow model input ranges. For example, lower bird inventory (<20,000 birds per house) generally resulted in much greater emission factors per bird, but should be relatively constant for different inventories at the same environmental conditions; all particulate matter models predicted negative emissions at lower bird inventories; and of the 5 air pollutant models presented, only ammonia was found to be potentially limiting for larger farms in terms of exceeding the threshold of 100 tons per yr. We conclude that the current draft EPA emission models are not appropriate for accurately or reasonably estimating emissions for a realistic range of broiler operations in their current forms. Revisions are suggested to accommodate the range of climates encountered within the U.S. broiler industry and to better reflect relations found in the scientific literature.

Keywords: air quality, emissions models, ammonia, hydrogen sulfide, particulate matter

237 A method for successful autonomous navigation by a robot in broiler-breeder flocks

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Our objective is to develop a method for navigation of a robot in a commercial poultry farm while not adversely affecting welfare. We developed a method where a robot interacts with the chickens by detecting their position relative to the robot, determining if it is blocking the robot's desired path, then nudging them to encourage them to move. A robot with cameras and lidar was used. Live experiments were performed in a commercial broiler-breeder flock with 12,000 chickens 18 to 20 weeks of age. The aggressiveness of the nudge was optimized and the navigation with nudging was compared against a sense-and-avoid only method. Experiments were carried out to determine animal reaction as a function of nudging speed of the robot. This was achieved via gradually increasing the velocity of the robot during a nudge interaction resulting in the robot impacting the chicken with greater force. Three levels of velocity were tested: 0.3, 0.45, and 0.6 m/s. This test was conducted 10 times at each speed and the robot was given as much time as needed to perform 30 nudges. Positions of chickens relative to the robot were recorded both before and after a nudge. Second, the navigation method was tested with both the nudge interaction and a sense-and-avoid-only method. The robot was given 10 minutes to traverse the house utilizing both methods and the total distance travelled was recorded. Each method was repeated 10 times. Finally, end-to-end navigation was demonstrated where the robot was given a goal at the opposite side of the house, and allowed to attempt to traverse it for 3 attempts. It was allowed as much time as necessary to traverse, and the time required for traversal was recorded. For the 30-nudge tests, a one-way ANOVA showed there is a significant difference in the

displacement of the closest chicken when nudged at a speed of 0.45 m/s (p = 0.0274) and 0.60 m/s (p = 0.0088) compared to 0.3 m/s on average. No significant difference was observed when the bird was nudged at a speed between 0.45 and 0.60 m/s (p = 0.9460). When comparing navigation methods, for each of the 10 tests performed, it was clearly shown that the navigation via avoid-only method was unsuccessful. With nudging, the robot was able to navigate 49.8 meters on average (σ = 8.05) in 10 minutes. Finally, 3 attempts to navigate the entire facility (with nudging) resulted in 100% success. The robot travelled 109 meters to the goal in an average time of 21.6 minutes (σ = 4.5). There were 0 failures during attempts to navigate the entire facility. In summary, we have demonstrated a method for enabling a robot to autonomously navigate in a flock of broiler-breeder chickens in a commercial house setting via physical interaction with the birds.

Keywords: Robotics, automation, navigation, interaction, behavior

238 Breeder age and egg storage temperature affect the eggshell translucency scores of Ross breeders

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Translucency is a practical method to evaluate eggshell quality. Storage conditions influence egg internal traits, and recent reports indicated effects on eggshell properties. However, the effects of breeder age and storage temperature (ST) are not well understood. This study evaluated the effects of ST on translucency scores (TS) in eggs from Ross broiler breeders at three age phases. Eggs were collected from a Ross breeder flock at 245 (N = 510), 340 (N = 510), and 392 days (N = 310) of age with an average egg weight of 58.7 ± 0.2, 66.8 ± 0.2, and 70.5 ± 0.3 g. Eggs were stored at 14 ± 1°C and 19 ± 1°C for 6 days. Egg weight loss % (EWL) during storage was calculated. The TS (1 to 3) were determined daily using the BlueBox®. Data were analyzed using contingency and regression analyses. Results indicated that breeder age and ST interacted ($P < 0.001$) on TS. In eggs from 35-wk-old breeders stored at 19°C with EWL 0.86%, TS1, and TS2 decreased from 89.9 and 9.6% on day 1 of storage to 32 and 56%, respectively, by day 6, TS3 increasing from 0.5 to 12% ($P < 0.001$). In contrast, no variation ($P > 0.05$) in TS distribution was observed in eggs stored at 14°C with EWL 0.36%. Eggs with TS1 decreased from 93.5 to 84.5%, TS2 rose from 6.5 to 14%, and TS3 went from 0 to 1.5% when comparing 1 to 6 days of storage. Eggs with TS1 from 48-wk-old breeders stored at 14°C represented 53.9% of the sample at day 1 and remained at 53.5% at day 6 ($P < 0.001$). Eggs with TS2 decreased from 44 to 42% and TS3 from 2 to 4.5% ($P < 0.001$) on days 1 to 6, and their EWL was 0.23%. Translucency increased ($P < 0.001$) in eggs stored at 19°C, with TS1 decreasing from 21.2 to 12.6%, TS2 from 74.3 to 70%, and TS3 increasing from 4.4% at day 1 to 17.4% at day 6, and their EWL was 0.95%. Eggs from 56-wk-old breeders stored at 14°C had a EWL of 0.77% and rapidly shifted in translucency ($P < 0.001$), with TS1 decreasing from 50 to 15.7%, and TS2 changing from 50 to 55%. TS3 had the most significant increase, progressing from 0% at day 1 to 29% at day 6. At 19°C, EWL was 1.34%. The proportion of eggs with TS1 decreased from 75 to 22.1%, TS2 increased from 25 to 29.8%, and TS3 increased from 0 to 48.1% ($P < 0.001$). Comparing the two temperatures, eggs stored at 14°C exhibited a slower initial increase in TS but a more dramatic shift over time, especially in eggs from 56-week-old breeders. These results demonstrated that both breeder age and ST changed the TS of 91.2 to 96.3% of eggs. Eggs stored at 19°C exhibited a more pronounced and consistent increase in translucency. In conclusion, temperature during egg storage plays a crucial role in maintaining eggshell quality, particularly for extended storage periods and older broiler breeder flocks.

Keywords: Translucency, Storage temperature, Eggshell quality, Eggshell properties, Broiler breeders

239 Recent advances in probiotic research

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Early establishment of healthy microbiota improves bird health and contributes to enhanced responses to challenges. Probiotics are defined as live microorganisms able to confer a benefit to their host by various means. Once established in the intestinal microenvironment, they competitively exclude pathogens by competing for resources, occupying attachment sites in the intestines, lowering gut pH, and producing bacteriocins, all of which reduce a pathogen's capacity to colonize the host. Probiotic application in poultry has been thoroughly studied, yet with differential outcomes based on the health status of birds, their age, administration methods, etc. Probiotics can be given via feed or water, alone or coupled with other additives, in ovo or post-hatch, pre-emptively or post challenge manifestation, and at any age of the flock considering there is no withdrawal period. Considering their role in enhancing the host intestinal microbiota, their impacts under enteric challenges are vastly studied. Across a plethora of recent research trials, performance, intestinal integrity, host immune responses, microbiome, pathology and stress response, and overall health all constituted key assessment variables. More recently, probiotics evolved into key components of precision feeding strategies as well as preventive measures often in the form of synbiotics, but also in combination with postbiotics, enzymes, and organic acids. Numerous studies reported benefits of feeding probiotics or synbiotics to poultry during disease challenges; yet, early in ovo or hatchery application may give additional benefits of establishing healthy microbiota prior to bird placement on the farm that eventually translates into longer-lasting protection. One of the misrepresented measures has been that of the immune response. A major challenge lies in the fact that immune responses are variable among birds and treatments as they often tend to be more circumstantial to each research setting and field conditions. How or when we assess their effectiveness is key to achieving expected outcomes. While timing is key in terms of when samples are collected and assessed, which pertinent parameters and how to measure them are more critical. Equally important, data interpretation must remain contextual and proportional to actual results without further exaggerations. Comprehensive understanding of complex host immune responses continues to present challenges awaiting more adequate, feasible, and representative tools. Until then, ongoing research will report findings in the context of concurrent challenges and non-drug supplements, which are well documented to impact intestinal immunity, integrity, and physiology.

Keywords: probiotics, immune response, intestinal integrity, non-drug additive, host defense

240 The effects of yeast cell wall and probiotic (*Bacillus* spp.) combination products on growth performance and mortality of broiler chickens

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An experiment was conducted to determine the effects of different strategies of use of a novel blend of yeast cell wall and probiotics (YWP) (Actigen BAC [Alltech, Nicholasville, KY]) on the performance parameters of coccidiosis vaccinated broiler chickens, specifically total weight gain (WG), adjusted feed conversion ratio (FCR), and mortality. A total of 3,000 1-d old male Cobb chicks were used in a 42-day experiment. Floor pens of 50 chicks each were randomly assigned to 1 of 5 dietary treatments, with 12 replications per treatment. Dietary treatments consisted of: i) negative control (non-YWP supplemented), ii) positive control (a generic yeast cell wall and probiotic blend product) included at 0.05%), iii) YWP step-down program (0.08, 0.04, and 0.02% in starter, grower, and finisher, respectively), 0.04% YWP, and 0.02% YWP. The diets were corn – soybean meal based, and the feeding program comprised of a starter (d 0 - 14), grower (d 15 - 28), and finisher (d 29 – 42). Feed was offered *ad libitum* in crumble, day 0 -14, and pelleted form, day 15 - 42. Pens of chickens were weighed and feed disappearance was measured on day 0, 14, 28, and 42 to calculate feed intake, average weight gain, and adjusted feed conversion ratio. Data was analyzed using the PROC GLIMMIX procedure in SAS 9.4, with pen as the experimental unit and location as a blocking factor. Dietary treatment was the fixed effect and block served as the random effect in the analysis. From day 0 to 28, birds fed diets supplemented with 0.02% YWP increased ($P = 0.050$) weight gain compared to birds fed unsupplemented diets. In addition, birds fed diets with YWP or positive control improved ($P < 0.001$) adjusted FCR compared to birds fed unsupplemented diets. From day 0 – 42, birds fed diets supplemented with the YWP step-down program or YWP at 0.02% increased ($P = 0.027$) weight gain compared to birds fed unsupplemented diets. In addition, birds fed diets with YWP or positive control improved ($P < 0.001$) adjusted FCR compared to birds fed unsupplemented diets. Furthermore, birds fed diets with the positive control marginally increased ($P = 0.094$) mortality compared to the birds fed YWP supplemented diets or unsupplemented diets. Results from this experiment suggest that the supplementation of YWP in a step-down program or at 0.02% inclusion improved growth performance, similar to the positive control at 0.05% inclusion.

Keywords: broiler, Yeast cell-wall, probiotics, performance

241 Next-Gen in ovo probiotic programming: Computational modeling integrates meta-analysis, Monte Carlo simulations, and machine learning

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This study aimed to evaluate and predict the efficacy of in ovo probiotic programming in poultry production using a next-generation computational modeling framework. Several studies suggest that in ovo feeding of probiotics benefits perinatal development and growth; however, a comprehensive evaluation is lacking to validate this technology. The impact on body weight (BW) and feed conversion ratio (FCR) were assessed using integrated meta-analysis, probabilistic simulations, and machine learning. A quantitative meta-analysis was performed using data from 93 peer-reviewed trials comparing in ovo probiotics to non-

treated controls. A pooled standard deviation (SD) was derived from the combined SEM and total sample size across trials. Meta-analysis estimated SMDs for BW and FCR, while Monte Carlo simulations forecasted the probability of treated birds outperforming controls. Trials varied in probiotic strains, broiler ages, and evaluation intervals. A random-effects model was applied, and machine learning assessed prediction performance using classification metrics. Meta-analysis showed probiotics significantly increased BW overall (SMD = 0.307; 95% CI: 0.188–0.427; $p < 0.001$), with strong effects at 7 days (SMD = 0.662), 14 days (SMD = 0.745), and 35 days (SMD = 0.431). Monte Carlo simulations estimated ≥99.99% probability of improvement at these intervals. FCR was significantly improved overall (SMD = –0.100; 95% CI: –0.187 to –0.014; $p < 0.023$), with a 99% probability of benefit. Time-point analysis showed modest improvements in FCR at 14–21 days (SMD = –0.028; 86% probability) and 21–28 days (SMD = –0.352; 81% probability). The AI model achieved 96% accuracy with high precision and recall for predicting BW improvement. Integrating statistical and AI-based modeling tools provides a powerful, scalable framework for predictive health and performance optimization in poultry production, though its power depends on consistent data reporting and standardized study formats.

Keywords: In ovo, Probiotics, meta-analysis, Monte Carlo simulation, Machine learning

242 Effect of dietary *Bacillus amyloliquefaciens* CECT 5940 supplementation on the performance and intestinal microbiota of laying hens during 25 - 41 weeks of age

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Bacillus amyloliquefaciens CECT 5940 (BA) is used in probiotics in animal feeds and has been shown to benefit the performance, immunity, intestinal morphology and microflora in broilers. However, studies on BA supplementation in laying hens are relatively scarce. Thus, this study aims to evaluate the effect of BA supplementation on the performance and intestinal microbiota of laying hens during weeks (wk) 25-41 of age. Seventy-two 25-wk-old healthy Bovans Brown hens, with ≥ 90% egg production (EP) and body weight (BW) within one standard deviation of the mean population BW, were randomly assigned to 2 groups (12 pens/group, 3 hens/pen). The hens received basial diet (control group, 17.87% crude protein, 2750 kcal/kg AMEn) or basial diet plus additional BA (BA group, 1g/kg feed, 1×10^6 CFU/g feed) for 112 days (d, wk 41 of age). Feed and water were provided *ad libitum*. On d 84 of study (wk 37 of age), each hen received 1 ml of *Salmonella enterica* serovar *enteritidis* (1×10^8 CFU/ml, local field strain) *via* oral gavage. We measured BW, mortality rate, BW gain (BWG), feed consumption (FC), EP, egg weight (EW), egg mass (EM, per hen per day), feed efficiency (FE, per kg egg mass) and egg quality during the study. On d 112 of study, intestinal counts of *Lactobacillus* spp., *Escherichia coli* and *Salmonella* spp. were determined using classical methods with selected growth media, due to their important roles in gut health and food safety in egg production. Data was analyzed using Student's t-test in R (v 4.2.2). Significances were considered if P<0.05. During d 0-84 of study, hens in the BA group had significantly (P<0.05) better FE (-5.2%), EW (+3.6%), EM (+5.5%) and EP (+1.9%) than those in the control group. During d 84-112 of study, FE (-4.3%), EW (+2.4%) and EM (+4.4%) of hens were significantly (P<0.05) better in BA vs. control group, although EP (mean 90.58%) did not differ significantly. Egg quality, including shell weight, yolk weight, albumen weight and Haugh units, were significantly (P<0.05) higher in BA vs. control group during the two periods. There were no significant differences in BW (d 0, 84, 112 of study), BWG, FC and mortality rate between the groups during the two periods. On d 112 of study, the counts of *Lactobacillus* spp. in ileum and cecum content were significantly higher (P<0.01, +13.1%, +14.7%, respectively), while the counts of *Escherichia coli* and *Salmonella* spp. in ileum and cecum content were significantly lower (P<0.01, -18.5% and -17.3%, -20.6% and -16.3%, respectively), in BA vs. control group. In conclusion, dietary BA supplementation may improve the production performance and egg quality of laying hens, and showed the potential to maintain healthy intestinal microflora of hens.

Keywords: Nutrition, Poultry, Laying hen, Probiotic, Gut health

243 Comparative effects of probiotics, prebiotics, and synbiotics on growth performance, carcass traits, immune function, and gut morphology of turkeys

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This study investigated the effects of dietary supplementation with probiotics, prebiotics, and their combination (synbiotics) on the growth performance, nutrient digestibility, immune response, and gut functions of turkey. A total of 180, 21-day-old turkey poults were acclimated for 1 week, then allocated into 4 treatments, with 3 replicates of 15 turkeys per replication. The four dietary groups were composed of basal diet (control group); or basal diet supplemented with 1.0×10^8 CFU/g *Lactobacillus acidophilus* and *Bacillus subtilis* 1.0×10^8 CFU/g (Probiotic group); 1% inulin and 0.5% Mannan oligosaccharides mixture (Prebiotic group) or having both prebiotic and probiotic mixtures (Synbiotic group). The birds were fed for 12 weeks and the data collected were subjected to one-way ANOVA using the general linear model procedure on Minitab 21 software. Growth performance was evaluated at two phases (4–10 weeks and 10–16 weeks), with the synbiotics group demonstrating the highest body weight gain relative to the control group at the overall phase ($P < 0.05$). The probiotic, prebiotic, and synbiotic-fed turkeys had increased feed intake at the overall phase compared to the control group ($P < 0.05$). Carcass traits remained unaffected ($P > 0.05$) but immune-related organ weights (spleen, bursa of Fabricius) were significantly higher in the probiotics, prebiotics, and synbiotic groups than in the control group ($P < 0.05$). Blood analysis revealed elevated white blood cell counts and total cholesterol in the probiotics, prebiotics, and synbiotic groups relative to the control group ($P < 0.05$). Duodenal morphometrics showed no significant differences among treatment groups ($P > 0.05$), although the lactobacillus counts were significantly higher in probiotics and synbiotics-fed turkeys compared to the control group ($P < 0.05$). Crude protein digestibility was improved, with prebiotics and synbiotics supplementation, and the probiotic diet increased nitrogen retention than the control diet ($P < 0.05$). Additionally, behavioral assessments indicated increased activity levels in synbiotics-fed turkeys compared to the control group ($P <$

0.05). Overall, synbiotics emerged as an effective dietary intervention, promoting growth performance, immune function, and nutrient utilization, underscoring their potential as a strategic supplement for turkey production.

Keywords: Synbiotics, Turkey, Growth performance, Nutrient digestibility, Behaviour

244 Necrotic enteritis in chickens: Understanding immune responses to devise novel probiotic-based disease control strategy

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Necrotic enteritis (NE) caused by virulent strains of Clostridium perfringens has been at the forefront amongst the diseases that have posed serious health and economic challenges to the poultry industry, particularly the broiler sector. In an era of no or reduced use of antibiotics in poultry production, NE control has much relied on effective non-antibiotic strategies that are currently unavailable. This is largely because immunity to NE is poorly understood. Hence, the work reported here, carried out three separate studies, was aimed at understanding host immune responses against C. perfringens in broilers to develop novel probiotics that can specifically target NE prevention. First, and experimental NE reproduction model in broilers using different C. perfringens strains was established followed by qualitative and quantitative measurement of disease pathology, immune gene expression and cellular responses. Results showed that NE-affected birds had higher ($P<0.05$) gross and histopathology lesion, augmented ($P<0.05$) lymphoid expression of pro-inflammatory (IL-1b, IL-6 and IFNg) cytokines and increased ($P<0.05$) macrophage and T cells. This finding suggested that strategies capable of dampening inflammation can prevent NE effectively. Next, about 8 chicken-origin *Lactobacillus* strains were screened using an in-vitro macrophage cell-based assay for their ability to modulate cytokine response. Results showed that strains P38, P43 and C29 were able to reduce ($P<0.05$) pro-inflammatory IL-1b and IFNg and increase ($P<0.05$) anti-inflammatory IL-10 response. Finally, broiler chickens administered orally with each of the 8 strains were challenged with virulent C. perfringens using NE-reproduction model to evaluate probiotic efficacies. Results showed that birds given P38 and C29 were able to reduce ($P<0.05$) NE lesions along with an increased ($P<0.05$) mucosal expression of IL-10 and TGFb genes. Statistical methods used in results analysis included one-way parametric/non-parametric tests. Collectively, the findings showed that NE pathogenesis involves robust inflammation and probiotic strains possessing immunomodulatory properties can prevent NE in chickens.

Keywords: Necrotic enteris, Clostridium perfringens, Chickens, Probiotics, Immune response

245 Efficacy of gut microbiota modulators from yeasts on performance of broilers chickens challenged with necrotic enteritis caused by Eimeria and Clostridium infection

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Necrotic enteritis (NE) is a severe inflammation of the small intestine that is increasingly affecting poultry due to the reduction in the use of antibiotics growth promoters. NE can be artificially induced by inoculation of coccidia and *Clostridium perfringens (CP).* Gut microbiota modulators derived from yeast cell wall are among the feed additives that improve broilers performance and confer them protection against NE by regulating the intestinal microbiota and modulating immunity. The aim of this study was to evaluate the efficacy of mannano-oligosaccharides (MOS), β-glucans, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) in reducing the effects of NE caused by coccidia and CP infections in broilers. A total of 770 1-day-old male chicks (Cobb 500) were divided into 7 treatments with 10 replicates: A negative control (NC) group without challenge; a positive control (PC) group with challenge without any additive in the diet; an antibiotic group (PCAGP) with 150 g/T enramycin; a GlucanMos® group (PCG) with MOS and β-glucans at a decreasing dose; a Golf®+GlucanMos® group (PCGG) with FOS, GOS, MOS and β-glucans at a decreasing dose; and 2 competitor groups (PCC1 and PCC2) with similar products. All the birds, except those in the NC group, were challenged by oral gavage with 5,000 oocysts of *Eimeria maxima* at 15 days of age and with CP at 18, 19 and 20 days of age. Histological examination of the small intestine was performed at 25 and 42 days of age. At day 42, all the remaining birds were euthanized and productive performance, histomorphometry, and TMBES (necrotic enteritis score; M. De Gussem (2010) were measured. Data were analyzed statistically in SAS using a multivariate analysis of variance and the results are expressed as mean and pooled standard error of the mean. On day 42, the weight of the birds in the PCGG group was significantly higher than all the others, and not significantly different from the NC group ($p=0.006$). Although there weren't significant differences, the PC group had a lower survival than any of the other groups, showing the effect of the challenge ($p=0.270$). The PCAGP, PCG and PCGG groups had the highest survival. On day 42, the VH:CD ratio was significantly higher in the PCGG group than in any of the other groups ($p=0.026$). In this study, the use of gut microbiota modulators (MOS and β-glucans) combined with prebiotics (FOS and GOS) offered the highest protection to the animals, allowing them to significantly improve their performance against the PC, and even (numerically) compared to the NC and the PCAGP.

Keywords: Gut health, Disease resistance, Yeast cell wall

246 Calcium montmorillonite Calibrin®-Z binding capacity with beta lactamase in vitro

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Beta lactamase (BL) is an enzyme produced by bacteria that contains a broad spectrum of multi-resistance to antibiotics by breaking down the beta lactam rings. This is a main reason for concern for drug resistance in hospitals and why withdrawing antibiotics in animal feeds is commonly practiced. The objective of this study was to see how effective Calibrin-Z (CZ) could bind with BL in vitro. We hypothesized that there would be a dose response with CZ binding BL at higher capacities when more CZ was added. Calibrin-Z was mixed with BL at the ratio from 0:1, 100:1, 400:1, 1000:1 to 5000:1 in vitro. To confirm the accuracy of BL activity, two concentrations (1 and 5 µl /well groups) were selected in each ratio treatment. All the tested points were duplicated. The mixtures were spun down and the supernatants were used for enzyme kinetic activity tests with the kit provided (Abcam, Waltham, MA). The mechanism is that BL can transfer the fresh substrate nitrocefin to hydrolyzed nitrocefin which can be detected by UV-VIS at 490 nm. BL activity can be calculated as an increased concentration of hydrolyzed nitrocefin per min. The relationship between various CZ to BL ratios vs. BL activities were estimated. Results: the standard curve showed a linear pattern at $y = 0.0144x + 0.0465$ with $R^2 = 0.9989$. All the enzyme

activities were calculated only within the linear response ranges. BL without CZ at 1 and 5 µl/well groups showed activities at 17.725 and 48.957 mM/min respectively. They were used as the 100% activities. When the mixing ratio was at 100:1, both 43% BL activities were reduced in 1 and 5 µl/well groups. When the ratio increased to 400:1, 70 and 65% of BL activities were reduced in 1 and 5 µl/well groups respectively with an average of 67.5% reduction. When the 1000:1 ratio was tested, 98.8 and 97.9% of BL activities were reduced in 1 and 5 µl/well groups respectively with an average of 98.4% reduction. No BL activity was found in 5000:1 ratio treatment for both 1 and 5 µl/well groups. This is defined as a 100% reduction. The binding capacity of CZ on BL can be best fitted at logarithmic relationship at $y = 12.424\ln(x) - 2.6789$ with $R^2 = 0.9408$ than other regression models. Conclusion: Calibrin-Z can bind with beta lactamase at a great logarithmic relationship under this new developed analytical protocol, and Calibrin-Z can remove almost all beta lactamase at 1000:1 ratio.

Keywords: Calibrin-Z, Beta-Lactamase, Enzyme Activity, Multi-resistance, Pathogenic Bacteria

247 Precision biotic: an innovative solution for controlling *Salmonella* Infantis and improving performance in broiler chickens

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Global restrictions on the use of antimicrobial growth promoters (AGPs) have led the food animal production industry to explore effective alternatives. Research on glycan-based precision biotics (PB) has shown promising results in modulating microbiome pathways related to performance improvements as well as enhancing resilience against enteric diseases. Foodborne infections caused by non-typhoidal serovars of *Salmonella* spp. are a significant concern for public health. This study aimed to evaluate the effect of PB in the diet for controlling *S. Infantis* infection in experimentally challenged broiler chickens. A total of 150 one-day-old chickens were randomly assigned to three treatments, with five replicates of 10 birds each for 42-days period. Two control treatments included a diet without PB supplementation, with (POS) and without (NEG) bacterial challenge, and a challenged treatment with the inclusion of 900 g/ton of PB in the feed. The birds were orally inoculated via oral gavage with a field strain of *S. Infantis* at concentrations of 10^7 and 10^9 CFU/ml in weeks 1 and 5, respectively. Feed intake (FI), body weight (BW), and feed conversion ratio (FCR) were analyzed. Microbiological cultures of the spleen, liver, cecum, and bone marrow were performed, as well as *Salmonella* counts from cloacal swabs and bactericidal activity in blood. Intestinal morphometry and integrity index, and spleen and liver lesions scores were evaluated by histopathology. Relative quantification of tight junction proteins, pro- and anti-inflammatory cytokines, and mucin genes in the ileum were performed by qPCR. Statistical analysis was performed using ANOVA and Kruskal-Wallis tests ($P<0.05$). The results showed a significant reduction in *Salmonella* fecal shedding (98%) and in organ isolation levels (50%) in the PB group compared to POS. Bactericidal activity in blood was higher in NEG (87%) and PB (79%) groups compared to POS (36%). The PB group showed improvements in BW (2511g, 2430g, and 2741g), FI (3852g, 3903g, and 3807g), and FCR (1.53, 1.61, and 1.39) for NEG, POS, and PB, respectively. Significant reductions in intestinal lesion indices and spleen damage were observed in PB compared to POS group, along with improved intestinal villi morphology in the duodenum and jejunum. Moreover, the PB group showed higher expression of ZO-1, OCL, CLDL-3, and MUC2 genes, which are associated with intestinal health. These findings suggest that the PB effectively controls *S. Infantis* infection in poultry by reducing fecal shedding, enhancing bactericidal activity, improving intestinal health and boosting performance. PB as a promising strategy for managing this pathogen in poultry production.

Keywords: Precision biotics, Salmonella Infantis, Foodborne Illnesses, Intestinal Health, Performance Improvements

248 Effects of dehulled hempseed extract on proliferation and glucose utilization of chicken macrophage-like cells

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Hempseed meal has recently been approved for use in laying hens; however, the immunomodulatory effects of hemp byproducts remain largely unexplored. In vitro studies serve as in efficient first step to screen a range of doses before moving to in vivo research. The use of hemp extracts in vitro involves solvents, making it necessary to establish ethanol tolerance to separate solvent toxicity from bioactive effects. This study aimed to assess the impact of hempseed extract (HSE) and ethanol on the proliferation and glucose utilization of a chicken macrophage-like cell line (HD11 cells). Dehulled hempseed was extracted using 70% ethanol, and HD11 cells were cultured in 20% FBS, 1% glutamine, and 1% antibiotics at 41°C with 5% CO_2. At 80% confluence, cells were treated with six HSE doses (4,000 to 125 µg/mL in twofold dilutions, with corresponding ethanol concentrations of 0.7% to 0.022%) and six ethanol-only treatments at matching ethanol levels, alongside an untreated control, with five replicates per treatment. After 24 h of incubation, media were collected for glucose analysis. AlamarBlue (10 µL) was then added to each well, and cell proliferation was assessed at 48 h post-treatment by measuring absorbance at 520 nm. Proliferation values (OD) were normalized relative to the untreated control. Data was analyzed using the PROC MIXED procedure and means were separated using the PDIFF statement in SAS. Significance was set at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Compared to the untreated control, the highest four ethanol doses and the highest three HSE doses tended to reduce proliferation ($P < 0.10$), while lower doses had no significant effect. Notably, HSE at doses 1 (74 vs. 62%, $P = 0.08$) and 2 (86 vs.72%, $P = 0.07$) tended to increase proliferation relative to their ethanol-only counterparts, with dose 4 showing a significant increase (98 vs. 71%, $P < 0.01$), suggesting a potential cytoprotective effect of HSE against ethanol-induced cytotoxicity. No significant changes in glucose levels were observed at 24 h, indicating that a longer incubation period may be required to detect metabolic effects. In conclusion, selected doses of HSE demonstrated dose-dependent improvements in macrophage proliferation compared to ethanol controls, suggesting potential cytoprotective and immunomodulatory properties. Future research will focus on evaluating other hemp byproducts, including hempseed meal, under pathogen challenge conditions to elucidate their mechanisms of action and potential contributions to poultry health.

Keywords: Hempseed extract, chicken macrophages, Cell proliferation, Ethanol toxicity, Immunometabolism

249 Effect of antibiotics in combination with postbiotics and essential oils on gut microbiome maturation in broiler chickens.

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Antibiotics have been commonly used in poultry production globally to enhance growth. However, with the implementation of bans and restrictions on non-therapeutic use of antibiotics, it has become essential to explore alternative methods to manage gut health challenges in poultry. Non-therapeutic antibiotics can help to control the pathogen load in broiler chickens, but they might also disrupt the maturation process of gut microbiome. Understanding the link between antibiotics and gut microbiome fitness is crucial for developing nutritional solutions to optimize gut microbiome profile and performance. The objective of the present trial was to assess how various antibiotics, combined with postbiotics and essential oils, influence the maturation of gut microbiome in broiler chickens. In a study using a 4 x 2 factorial design, the effects of three antibiotics— bacitracin methylene disalicylate (BMD), virginiamycin (VGM), and lincomycin (LCM) — were evaluated alongside a control diet, with or without postbiotics and essential oils. A total of 480 broiler chickens were randomly divided over 96 metabolic cages, with 5 chickens per cage. The study was divided into two phases: starter (day 0-10) and grower (day 10-21). BMD and VGM were added to all feed phases at non-therapeutic levels, while LCM was administrated through water at therapeutic level from day 0 and 4. Body weight and feed intake were measured for each phase and overall trial period, and feed conversion ratio (FCR) was calculated. Cloacal samples were collected from two broilers per cage at days 7 and 21 and analyzed using Galleon^{TM} to assess gut microbiome. Performance data were analyzed using mixed model and microbiome data were analyzed with a fixed model in statistical software. Broiler chickens fed BMD showed a higher abundance of *Lactobacillus* compared to those fed control diet on d7, resulting in a lower FCR between 0-10 d. Supplementing BMD with postbiotics and essential oils further promoted *Ruminococcus* and reduced *Listeria* during this period. VGM reduced *Lactobacillus* and promoted *Lachnospiraceae* compared to the control treatment at d21, indicating a more developed microbiome. However, it also increased pathogenic bacteria such as *Salmonella* and *E. coli*. Adding postbiotics and essential oils helped mitigate the high pathogen load. Broiler chickens administrated LCM through water showed a lower abundance of *Enterococcus* on d7, but this positive impact diminished over time, probably due to the short administration time. In conclusion, antibiotics have varying impacts on the gut microbiome maturation process. Supplementing with postbiotics and essential oils could help mitigate the negative effects of antibiotics.

Keywords: broiler, antibiotic, postbiotic, essential oil, microbiome

250 Did Raised Without Antibiotics programs change field levels of Ionophore anticoccidial resistance

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There is a concern for a potential increase in antibiotic resistance due to administering in-feed antibiotics to animals. To lower the chance of resistance development, programs such as Raised Without Antibiotics (RWA) are being used. RWA includes not feeding the anticoccidial, polyether Ionophorous antibiotics. Extensive usage of the Ionophores (IONO) has led to anticoccidial resistance to the IONO. Poultry companies producing birds using the RWA program have not used the IONO for many years. The objective of the study was to determine if this elimination of IONO resulted in a reduction in field levels of IONO anticoccidial resistance. Three anticoccidial sensitivity tests (AST) were conducted. Coccidia was isolated from 3 sources: farms that never used IONO (Never), farms that continued to use IONO (Continuous), and RWA farms (no IONO usage for 5-8 years). Each AST consisted of 42 cages with 8, 12-day old chicks. The treatments were replicated in 3 randomized blocks. Statistical analysis employed the General Linear Models procedure with a probability level of $P < 0.05$ to determine differences. Treatments were: No Additive, challenged (NMI) and nonchallenged (NMU); Chemical (CHEM) Class: Zoalene 125ppm, Amprolium 125 ppm, Nicarbazine 125 ppm, Robenidene 33ppm, Coyden 125ppm, Diclazuril 1ppm, and Decoquinate 33ppm; IONO Class: Monensin 99ppm, Salinomycin 66ppm, Narasin 79ppm, and Lasalocid 72ppm. In all ASTs, birds and feed were weighed by cage on DO (12 days of age) and D8. On D2 challenged birds were orally dosed with one of the 3 isolates. Each isolate contained *E. acervulina*, *E. maxima*, *and E. tenella.* On D8 (6 days PC), all birds were coccidia lesion scored (LES). Across all 3 ASTs, NMU grew similarly (avg. FCR 1.429). NMI across all three ASTs produced a similar moderate challenge with all species (avg. FCR 2.829 with a LES 2.73). Both CHEM and IONO significantly controlled the Never coccidia challenge. The avg. IONO control (FCR 1.639 and LES 1.0) was significantly better than CHEM control (FCR 2.077 and LES 1.3). Both CHEM and IONO significantly controlled Continuous coccidia challenge. The avg. CHEM control (FCR 1.753 and LES 1.4) was significantly better than IONO control (FCR 1.953 and LES 1.9). Both CHEM and IONO significantly controlled RWA coccidia challenge. The avg. IONO control (FCR 1.664 and LES 0.9) was significantly better than CHEM control (FCR 1.992 and LES 1.3). Conclusions are that never using IONOs the coccidia is very sensitive (low resistance); continuous usage has led to some degree of resistance; and RWA may have allowed coccidia on those farms to “rest from Ionophore” allowing for more of a chance for the sensitive coccidia to populate these farms.

Keywords: Cocccidia, Eimeria, RWA, Ionophore, Anticoccidial

251 Inhibitory effect of FloraMax B11® against *Salmonella* Minnesota and *Salmonella* Infantis

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Non-typhoidal *Salmonella* is responsible for foodborne disease outbreaks, causing economic losses in the poultry sector and posing risks to public health. The emergence of antimicrobial-resistant *Salmonella* spp. isolates represents a significant challenge for the *One Health* approach. In this context, probiotics offer an alternative due to their competitive exclusion properties and metabolite production, which exert antagonistic effects against various pathogens. This study aimed to evaluate the antagonistic effect of a commercial probiotic (FloraMaxB11®) against *Salmonella* Infantis and *Salmonella* Minnesota isolates of avian origin. *Salmonella* spp. isolates(n=16) were selected, including eight *S.* Infantis (SI) and eight *S.* Minnesota (SM) isolates. The isolates were analyzed using the *Spot on the Lawn* and co-culture techniques. In the *Spot on the Lawn* assay, FloraMaxB11® was incubated in MRS broth at 37°C for 24 hours at a concentration of 10^6CFU/mL. Then, 20 µL of the inoculum was plated on MRS agar and incubated at 37°C for 24 hours. On the following day, each *Salmonella* isolate was independently inoculated onto the pre-prepared MRS agar. After 24 hours, inhibition halos were measured, with diameters >1 mm considered satisfactory. In the co-culture assay, FloraMaxB11® was incubated in MRS broth at 37°C for 24 hours, while *Salmonella* spp. isolates were cultured in BHI broth under the same conditions. The inocula were transferred to MRS and MH broth in double concentrations and mixed. The co-culture was incubated at 37°C, and bacterial growth was evaluated at 0, 6, 10, and 24 hours by plating on MacConkey and Rogosa agar. Data were analyzed using Tukey and Dunn *post-hoc* tests, with a 5% significance level. Results showed that FloraMaxB11® inhibited 100% of the *S.* Infantis and *S.* Minnesota isolates, with mean inhibition halos of 4.15 mm and 6.21 mm, respectively. In the co-culture assay, a significant reduction in *Salmonella* isolates was observed over time. For *S.* Infantis, the mean count was 8.53 Log CFU/mL at time 0 (T0) and undetectable after 10 hours (T10), significantly differing from the positive control, which presented 8.81 Log CFU/mL (T0) and 9.35 Log CFU/mL (T10). Similarly, *S.* Minnesota isolates in co-culture showed a mean of 8.57 Log CFU/mL (T0) and undetectable values at T10, whereas the control exhibited 8.84 Log CFU/mL (T0) and 9.06 Log CFU/mL (T10).These findings indicate that FloraMaxB11® demonstrates a promising antagonistic profile, with the potential to inhibit the growth of *Salmonella* Infantis and *Salmonella* Minnesota isolates.

Keywords: Enterobacteria, poultry farming, probiotics, pathogens

252 Microbial ecology of poultry leg quarters treated with a short-duration dip (15-s) of sodium acid sulfate

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The use of sodium acid sulfate (SAS) as a short-duration dip on poultry products has been reported to reduce pathogens and increase the shelf-life of subsequent products. Despite existing preliminary data, knowledge gaps exist on the effect of SAS directly on the microbial ecology of poultry products. Therefore, we sought to determine the impact of SAS alone and in combination with peracetic acid (PAA) on chicken shelf-life and microbiota. Skin-on, bone-in leg quarters (N=300) obtained from USDA-inspected, antimicrobial-free Ross 708 chicken carcasses were not treated (NT) or treated for 15-s in either tap water (TW), SAS (3%), PAA (500 ppm), or combination of SAS+PAA. Leg quarters were rested (2-min), packaged (plastic tray with film overlay), and sampled on d 0, 4, 7, 14, 21, and 28 of shelf-life (dark storage) for microbiome sequencing via rinsing in 150 mL of neutralizing BPW. Microbial DNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq. Data were analyzed in QIIME2-2024.5 for diversity and compositional changes. Time impacted the microbial diversity regardless of treatment ($P<0.05$). Additionally, there was a decrease in microbial richness, evenness, and abundance, and an increase in phylogenetic dissimilarity, over time, of SAS and SAS+PAA-treated leg quarters as compared to NT and a decrease in abundance and increase in phylogenetic dissimilarity of PAA-treated leg quarters as compared to NT ($P < 0.05$). On d 0, PAA enriched *Pseudomonas* and *Yersiniaceae* and depleted *Stenotrophomonas* and SAS enriched *Akkemansia*, a beneficial microbiota. PAA and SAS -treated quarters had depleted *Acinetobacter*, spoilage microorganism, on d 4. On d 7, PAA enriched *Acetobacter*, SAS enriched Bacillales, and SAS+PAA enriched several taxa belonging to Lactobacillales and Bacillales families. By d 28, PAA and PAA+SAS enriched *Acetobacter,* and SAS and PAA+SAS depleted Sphingobacterium. Overall, results suggest that SAS does impact the microbial ecology and may improve shelf-life by maintaining a more diverse and beneficial microbiota.

Keywords: short-duration dips, sodium acid sulfate, microbiome, shelf-life

253 SENS-D: A convergent innovation model for rapid *Salmonella* detection and supply chain intelligence

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The poultry processing sector has made notable strides in complying with USDA-FSIS *Salmonella* performance standards, resulting in lower pathogen levels in raw poultry compared to previous years. However, the incidence of Salmonellosis in humans has remained largely unchanged over the past three

decades, signaling a critical gap between regulatory compliance and actual public health outcomes. To address this persistent challenge, a more comprehensive and innovative strategy is required—one that leverages the strengths of convergence science. Convergence science represents a collaborative, multidisciplinary, and problem-driven approach that integrates knowledge, tools, and methodologies from diverse fields to solve complex societal problems. Unlike traditional or even interdisciplinary research, convergence science dissolves disciplinary boundaries, encouraging deep integration that leads to transformative solutions. This paradigm is especially well-suited to address complex food safety issues like *Salmonella* in poultry. Our convergent team of 18 scientists, spanning five universities and a broad range of disciplines—including microbiology, engineering, data science, supply chain analytics, policy and extension—was funded by the National Science Foundation (NSF) Convergence Accelerator Phases I and II. The project aimed to develop rapid, field-deployable technologies for detecting and quantifying Salmonella serovars in poultry rinsates, alongside a robust decision support system (DSS) capable of monitoring, predicting, and improving food safety outcomes across the entire poultry supply chain. We developed two advanced sensing technologies: a bioelectrical impedance sensor and a surface-enhanced Raman spectroscopy (SERS) sensor. These systems allow for rapid detection and quantification of Salmonella within 10 to 70 minutes. These sensors are integrated into a working prototype capable of collecting critical metadata for downstream analysis. We developed a cloud-based DSS that enables real-time analytics and visualization, predictive pathogen and spoilage modeling, optimization of sensor placement, and intelligent logistics planning. As part of the NSF Convergence Accelerator program, our team participated in entrepreneurial training that equipped us to transition from academic research to commercialization leading to the formation of SENS-D LLC. Our work demonstrates the power of convergence science to create real-world solutions for persistent food safety challenges. We implore poultry scientists to break down disciplinary silos and embrace convergence approaches in both research and teaching for the future of the poultry industry.

Keywords: Convergence, Salmonella, Sensors, Decision Support System, SENS-D

254 Evaluating *in vitro* models for assessing the effects of amendments on poultry litter microbial composition

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There is currently no standardized in vitro model for evaluating how amendments affect poultry litter microbial composition. This study compared three in vitro systems—conical tubes, Whirl-pack® bags, and Dacron bags—using a commercial litter amendment (IndigoLT™, AgriGro Inc.) applied at four concentrations (0%, 1.9%, 3.9%, and 4.3%) over a 7-day incubation period. A total of 336 in vitro samples and 28 poultry house controls were analyzed to assess microbial responses over time (days 1, 3, 5, and 7). Each system contained 20 g of litter, and daily 1 g subsamples were serially diluted and plated on selective media to quantify aerobic, Gram-negative, lactic acid bacteria (LAB), and Staphylococcus populations. Colony counts were log-transformed, and data were analyzed using ANOVA, Tukey's HSD, and linear mixed-effects models ($P < 0.05$). No significant differences were observed between poultry house control litter and non-amended in vitro models for aerobic, Gram-negative, or *Staphylococcus* counts, indicating that all three systems could adequately mimic in-house microbial populations for those groups. However, LAB counts were significantly lower in non-amended Whirl-pack® and Dacron bags compared to controls ($P < 0.05$), limiting their reliability for LAB analysis. By day 7, the high-concentration amendment significantly reduced aerobic and *Staphylococcus* counts across all models ($P < 0.05$). Staphylococcus counts in the high group were over 2 logs lower than in control samples and about 1 log lower than other treatment groups. LAB counts were influenced primarily by time rather than treatment, while Gram-negative counts did not differ significantly across treatments ($P > 0.05$). Overall, all three systems supported microbial growth, but conical tubes provided the most consistent and accurate representation of poultry litter microbial composition, especially for LAB. High amendment concentrations effectively reduced key bacterial populations, including Staphylococcus, suggesting potential benefits for poultry health and food safety.

Keywords: Poultry litter, Litter amendments, Litter in vitro models, Microbial characterization

255 Evaluating natural Darkling Beetle and Salmonella control products

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As poultry producers begin to work to lower Salmonella on the farm, control of the vectors of Salmonella from flock to flock or house to house becomes more important. The darkling beetle (Alphitobius sp.) has been shown to carry Salmonella in their intestines for more than 60 days. Many of the chemical insecticide controls for beetles have been used for decades so resistance has resulted in lowered levels of control. Natural products used for beetle control may also have direct antibacterial effectiveness. Two products, a combined soap and essential oil based product (beetle spray) and an essential oil only product were evaluated in vitro against *S. enteritidis*, *S. typhimurium*, and *S. infantis* and found to be effective (SCAD, 2025). The effectiveness of the two products were then evaluated in vitro using S.E. contaminated (10^9 cfu/ml) re-used broiler litter (approximately 4 kg) in sanitized buckets. Each treatment was represented by eight buckets that were then sprayed with either water, the beetle spray product, or essential oil product only. Sample (~100 grams) were taken from each bucket on days 1, 2, 7, 14, 21, and 28 post spray. S.E. enumeration was by MPN/g (Berghaus, 2009) on day 1 and 2 and prevalence was evaluated from each bucket on days 1, 2, 7, 14, 21, and 28. Fisher's exact test was used to compare litter samples between treatments. MPN's were log-transformed prior to analysis. All replicates of buckets litter were S.E. positive immediately after S.E. spray. On day 1, the water only was 100% S.E. positive, while the beetle spray and essential oil were 88%. The S.E. prevalence continued to decline until day 28 when all buckets litter were S.E. negative. The post S.E. and pre-treated MPN were 6 $\log_{10}$ cfu/g and immediately post treatment, there was a significant ($P = 0.02$) reduction by the essential oil only application (5.27 cfu/g). litter were S.E. negative. The post S.E. and pre-treated MPN were 6 $\log_{10}$ cfu/g. The mean MPN of the essential oil only application was significantly lower than that of

the water only application over the two-day follow-up period (4.34 vs 5.29 $\log_{10}$ cfu/g; P = 0.024). Future evaluations will use these products sprayed on contaminated litter that will then have broilers grown in these pens to determine if lowering the S.E. level in the litter will also lower the S.E. level in broilers.

Keywords: Salmonellla enteritidis, darkling beetles, broilers

256 Persistence and translocation of a *Salmonella*-specific bacteriophage from eggshell exterior to interior surface under hatching conditions in the absence of *Salmonella*

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Bacteriophages offer a promising strategy to reduce *Salmonella* on hatching eggs. This study assessed the persistence and movement of *Salmonella*-specific SP6 phage from the outer to the inner surface of the eggshell under hatching conditions (37°C, 60% RH) across two trials using a shared design. Eggs were sprayed with SP6 (10^9 PFU/mL), air-dried, and incubated for 24 h. At 0 and 24 h, five eggs per time point per treatment were sampled to quantify SP6 and indigenous microbiota on both outer and inner shell surfaces. Outer shell phages were recovered by LB broth rinsing, serial dilution, and plating on 0.5% LB top agar. Inner shell analysis involved surface-decontaminated, aseptically cracked eggs, with the shells and membranes macerated in LB and processed similarly. Indigenous aerobic bacteria were enumerated on TSA; microbial and phage counts were log-transformed and analyzed via ANOVA with Tukey's HSD ($P < 0.05$). In Trial 1, unwashed eggs from a poultry house resulted in a decrease in outer shell phage counts at 24 h compared to 0 h, but a significant increase on the inner shell surface ($P < 0.05$). At 24 h, inner shell phage counts were comparable to initial outer shell levels at 0h, with 92% of recovered PFUs proportional to outer shell values, indicating translocation. Indigenous microbiota increased significantly on the outer shell but remained unchanged on the inner shell. Trial 2 used clean (store-bought) and dirty (poultry house) eggs. At 0 h, phage counts were higher on dirty eggs compared to clean eggs ($P < 0.05$). By 24 h, clean eggs showed increased phage counts on both shell surfaces, while dirty eggs showed a decrease on both ($P < 0.05$). Aerobic bacteria on clean egg exteriors dropped below detection and remained stable on inner shells, while dirty eggs showed an increase externally and a significant rise internally ($P < 0.05$). Together, these findings demonstrate that SP6 phages can persist on eggshells and translocate to the inner shell surface under incubation conditions. Results highlight the influence of surface cleanliness and environmental factors on phage behavior and support the need to consider shell penetration dynamics when applying phage-based controls in hatcheries.

Keywords: Bacteriophage, Salmonella, Eggs, Hatching environment

257 Effects of a commercial triple-strain *Bacillus*-based probiotic on cecal colonization with *Salmonella enteritidis* in commercial pullets at the onset of pre-lay period

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Salmonella enteritidis (SE) is a significant challenge in laying hens, which directly impacts consumer food safety. Effective SE control is critical to preventing Salmonellosis outbreaks from consumption of eggs. A commercial triple-strain *Bacillus*-based probiotic was tested to determine its effect on colonization by *Salmonella* in the ceca of commercial layer pullets. Potential for reduction in *Salmonella* colonization was assessed by prevalence and enumeration following oral inoculation of a selected nalidixic acid resistant SE strain. Two treatments were tested, each containing 250 day-of-hatch Hyline W-36 layer pullets. Dietary treatments were: 1) No supplement (Control, CON), and 2) Probiotic (PRO, 1.6×10^6 CFU/g of finished feed). Environmental swabs were collected from each experimental group and tested to ensure freedom from SE prior to challenge. At 16 weeks of age, the SE challenge strain was inoculated orally at a dose of 3.9×10^8 CFU/bird. Pullets from each experimental group (n=24) were euthanized at 6-, 10-, 14-, and 17-days post infection (dpi). Contents from the ceca were aseptically collected for prevalence and enumeration of SE. To evaluate the effect of treatment on SE counts over time in independently sampled birds, we conducted a non-parametric two-way ANOVA using ranked LogCount data by means of ProcGLM in SAS (Statistical Analysis Software, Cary, NC). The analysis revealed a highly significant effect of time post-infection (dpi; $p < 0.0001$), indicating a strong decline in SE burden across all birds as the infection progressed. There was also a statistically significant main effect of treatment group ($p = 0.0310$), with PRO treated birds consistently showing lower SE counts compared to control birds at each time point. However, the interaction between time and treatment was not significant ($p = 0.2596$), suggesting that while the PRO treatment reduced the overall level of infection, it did not significantly alter the shape or trajectory of decline over time. These results support the conclusion that the PRO treatment effectively reduced SE load but did not fundamentally change the time-course pattern of resolution. These findings also confirm previous results obtained with the probiotic and highlight a promising strategy to complement pre-harvest food safety programs.

Keywords: Bacillus, probiotic, layer, food safety, Salmonella

258 The effect of oregano essential oil on Lactobacilli bacteria

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Antimicrobials are widely used to support poultry health either as prophylaxis or treatment for disease. However, recognition of increasing resistance means that the poultry industry needs to identify alternative strategies. Oregano essential oil (OEO) has been widely shown to have extensive antimicrobial activity against various pathogens including e. coli, salmonella and C. perfringens.Research has shown that due to the complex mode of action and composition of OEO, resistance development is unlikely. Orego-Stim (OS) is an oregano essential oil-based product developed to support the health and performance of livestock. Traditional antimicrobials are often broad-spectrum, mitigating not only pathogenic microbes, but also beneficial bacteria leading to dysbiosis and potential for opportunistic pathogens to proliferate. Whilst extensive research has been undertaken to elucidate the antimicrobial activity of OEO against pathogens, its effect on beneficial bacteria is largely unknown. To elucidate effects on beneficial bacteria a study was performed that compared the effect of OEO and OS to a traditional antimicrobial (amoxicillin (AM)) on various lactobacilli bacteria strains.

Material and Methods A minimum inhibitory concentration (MIC) assay was utilized to assess the minimum concentration of each antimicrobial substance that is required to inhibit growth of *Lactobacillus Casei, Lactobacillus rhamnosus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus delbruekil*. All bacteria were grown on MRS agar and cultured at 30-35°C in either aerobic or anaerobic conditions as appropriate for the strain. Serial dilutions of the OEO, OS and AM were prepared. and then applied individually to petri dishes streaked with each bacterial strain and incubated for 72 hours in triplicate. Growth was then observed for each dilution to determine at what dilution bacterial growth started to be inhibited. Statistical analysis was performed using IBM SPSS 26 by one-way ANOVA. Results found that for all bacteria tested both OEO and OS had significantly greater (p=<0.001) minimum inhibitory concentration than amoxicillin with MIC values ranging from 0.0977-1.563ppm for amoxicillin, 781-3130ppm for OEO and 50,000-500,000ppm for OS respectively. All MIC results for OEO and OS were considerably higher than the commercial recommended application rate into feed/water (300ppm and 3000ppm respectively). Discussion and Conclusion The results of this study provide an initial indication that OEO and Orego-Stim may not have indiscriminate antimicrobial activity when applied at the normal rate.

Keywords: Gut Health, Antimicrobial resistance, Essential oils, Oregano, Antibiotics

259 Phage cocktail delivered in water significantly reduced *Salmonella* colonisation in broiler chickens

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Bacteriophages (phages) are viruses of bacteria and can be used as antimicrobials to treat human and animal bacterial infections. We have isolated phages that target and kill poultry associated *Salmonella* serotypes. The objective of this study was to determine if a phage cocktail delivered in water can reduce *Salmonella* infection in experimentally challenged chickens. Three doses of the phage cocktail were tested and were administered in water throughout the study (0-35 days) or for the last week (28-35 days). 1760 Ross 308 male broilers were used. Chickens were divided into 10 treatments groups each with 160 chickens. The control treatment groups were: T1 no challenge, no phage; T2 no challenge, phage at dose of 10^6 PFU/day 0-35 days; T3 no challenge, phage at dose of 10^6 PFU/day on days 28-35; T4 challenged, no phage. T5, T6 and T7 were challenged and administered phage in water on days on days 28-35 at doses of 10^5, 10^6, and 10^7 PFU/day respectively. T8, T9 and T10 were challenged and administered phage in water on days on days 0-35 at doses of 10^5, 10^6, and 10^7 PFU/day respectively. Chickens in groups T4 to T10 were challenged via oral gavage on day 4 with *S.* Typhimurium strain 4/74 at a dose of 5 x 10^6 CFU/per bird. Pooled faecal samples from each pen were collected on days 8, 10, 14, 21, 27, 31, and 35 (last day of the trial); and caecal samples were collected on days 14 and 35 to determine *Salmonella* and phage abundance. Methods and materials: Xylose Lysine Deoxycholate agar was used to enumerate *Salmonella* and lysogeny broth medium was used for phage abundance counts. Statement of statistical analysis: One-way ANOVA was used to compare *Salmonella* and phage counts between groups. Performance data was analysed using JMP Pro 14. Results: Faecal samples collected on day 31 showed that regardless of phage dose, phage treatment significantly reduced *Salmonella* abundance in groups administered the phage cocktail in the last week (p<0.001). By day 35 in both faecal and caecal samples *Salmonella* was not re-isolated and not detectable (detection limit 10 CFU/g) in all groups T5, T6 and T7. In groups T8, T9 and T10, where the phage cocktail was administered throughout the study, *Salmonella* abundance was significantly lower (<0.001) at all faecal sampling points, regardless of the phage dose. By day 35 in groups administered the higher (T8) and lower (T10) phage doses *Salmonella* was not re-isolated and not detectable. We demonstrate that our phage cocktail delivered in water, one week prior to slaughter most optimally removed Salmonella infection from the flock. Our study highlights phages are a promising option for use in reducing or eliminating poultry infections.

Keywords: Salmonella, Poultry, Bacteriophage, Phage, Antimicrobials

260 Cecal microbiota profile and cecal digesta concentration of short chain fatty acids in pullets fed yeast bioactives and omega-3 fatty acid under different spacing allowance

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Gut microbiota influences performance and immunity in poultry. The study investigated the impact of supplementing yeast bioactives (YB) and co-extruded full-fat flaxseed (N-3 FA) diets fed to Lohmann LSL pullets reared under different spacing allowances (SA) from hatch to 16 weeks of age (woa). A total of 2,832-day-old pullets were reared in enriched cage system under High (HSA, 348 cm^2/bird) or Low (LSA, 284 cm^2/bird) SA and fed either control corn-soybean basal diet (C), C+3% N-3 FA, or C+0.05% YB. Birds had free access to feed and water and were sampled for ceca digesta at 4 and 16 woa for determination of cecal microbial community and diversity via 16S rRNA and the concentration of short chain fatty acid (SCFA) . The data analyses model had SA, diet, and sampling age as the fixed factors, while non-parametric correlations were applied to explore relationships between SCFA and bacterial taxa. There were no ($P>0.05$) SA and diet effects on the α-diversities indexes at any time. At 4 woa, β-diversity ($P<0.05$) revealed microbiota dissimilarity between HSA and LSA with highest *Bacteroides* abundance in LSA. Beta-diversity analysis at week 16 ($P=0.001$) showed significantly different clustering, with N-3 FA-fed LSA differed from N-3 FA-fed, YB-fed and control HSA groups (P<0.01). At both sampling points, the concentration of propionic and iso-butyric acids was higher ($P<0.05$) in LSA than HSA pullets. At 16 woa, highest and lowest n-butyrate concentrations were noted in N-3 FA-, and control-fed birds, respectively (P<0.05), regardless of SA. There was a positive correlation between *Barnesiellaceae* and n-valeric and acetic acid concentration of N-3 FA-fed birds at 4 woa. In YB-fed pullets, *Lactobacillaceae* and *Enterobacteriaceae* positively correlated with n-butyric acid. At week 16 multiple bacterial families had positive and negative correlations with SCFAs. This study's findings highlight that SA, YB, and N-3 FA modulate cecal microbiota composition, potentially influencing SCFA production.

Keywords: Gut microbiota, YB, N-3 FA, Spacing allowance, pullets

261 Omics-based studies indicate that *Bacillus velezensis* can be an alternative for *Salmonella* Heidelberg control and intestinal health improvement in broilers

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Although many studies show the benefits of probiotics for pathogen control and intestinal health improvement, there is a limited number of manuscript studies using "omic" techniques to enhance the understanding of the interaction between the intestine and probiotics. *Bacillus velezensis* (BV) is a species that has been little explored as a probiotic in birds. This study evaluated the effectiveness of BV in inhibiting *Salmonella* Heidelberg (SH), presenting potential benefits for poultry health and food safety using "omic" techniques. First, we conducted *in vitro* experiments measuring inhibition zones and analyzed the results. Additionally, we carried out an *in vivo* experiment involving 100 broiler chicks, divided into four treatment groups (Positive Control, Negative Control, Broilers challenged with SH receiving BV in feed, and non-challenged broilers receiving BV in feed). In broilers, we assessed the impact of BV on cecal microbiota, fecal metabolites, and SH inhibition in the cecum. We used metagenomic and metabolomic analyses to evaluate microbial diversity and metabolic profiles. For SH quantification, we evaluated the data using the Shapiro-Wilk test and a two-way ANOVA. For metabolomics, we first applied a filtering step, and metabolites that differed according to t-test and showed a fold change greater than 2 were selected for Pearson's correlation analysis. Due to high multicollinearity among metabolites within each age group, we then performed a principal component analysis (PCA) separately for each group. Other statistical methods, such as a multivariate analysis of variance (MANOVA), were also applied. For the metagenomic data, we assessed alpha and beta diversity. Additionally, PCA results were used in a k-nearest neighbors (k-NN) classification model. For all analyses, statistical significance was considered at $p < 0.05$. Our study found that BV effectively inhibited SH in cecal content when the broilers reached 28 days ($p<0.05$). Metabolic class analysis revealed five major classes influenced by the treatment, with probiotics enhancing beneficial metabolites such as fatty acyl glucosides (surfactant molecule), lignin and artemisinin (antioxidant molecules), taurodeoxycholic acid (bile acid), and reducing those associated with SH infection. The treatments significantly influenced microbial richness and diversity, with BV-rich diets and SH infection promoting a diverse yet distinct bacterial profile. These findings suggest that BV can enhance poultry gut health and control SH growth in the cecum, showing changes in metabolites and microbiota as a protective effect in the intestinal tract, offering a promising alternative to antibiotics.

Keywords: poultry health, antibiotic resistance, food safety

262 Probiotics treatment reduces Salmonella shedding in broiler chickens

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Salmonella is one of the most important foodborne pathogens affecting public health and food safety. In humans, Salmonella infections are typically caused by the consumption of raw or undercooked poultry products. Therefore, controlling Salmonella at the poultry farm level is crucial. With growing concerns over antibiotic-resistant bacteria, probiotics have emerged as a promising alternative for controlling foodborne pathogens in poultry. The present study investigates the efficacy of lactobacilli in reducing Salmonella shedding in broiler chickens. A total of 51 lactobacilli isolates from the intestines of healthy broiler chickens were assessed for anti-Salmonella Typhimurium activity using an agar well diffusion assay. Isolates exhibiting the strongest antagonistic effects (*L. salivarius-26*, *L. reuteri-42*, *L. salivarius-43*, *L. crispatus-44*, L. *crispatus-46*, and *L. gasseri-49*) were selected for in vivo experiments. In Experiment 1, 210 birds were randomly assigned to 14 treatment groups. On day 2 post-hatch, birds in groups 1–12 received oral treatment with one of the six selected Lactobacillus species at two different doses (10^6 and 10^7 CFU/ml). The positive control group received phosphate-buffered saline. On day 3 post-hatch, birds in all groups (except the negative control) were challenged with Salmonella Typhimurium (10^4 CFU/ml). On day 5 post-infection, cecal contents were collected from 10 birds per group, and Salmonella colonies were enumerated after serial dilution (up to 10^{-7}). In Experiment 2, birds were treated (15 birds per group) with a lactobacilli cocktail containing all six selected species at three different doses (10^6 to 10^8 CFU/ml). All procedures were performed following the protocol established in Experiment 1. Statistical significance among groups was analyzed using a one-way ANOVA followed by Tukey's HSD test. The results demonstrated that oral treatment of birds with *L. salivarius-26 and L. crispatus-44* at 10^6 CFU/ml and *L. gasseri-49* at 10^7 CFU/ml significantly reduced the number of the Salmonella Typhimurium colonies compared to the positive control group. In addition, lactobacilli cocktail supplementation at 10^7 CFU/ml significantly reduced salmonellae shedding compared to the positive control group. In conclusion, probiotic supplementation has the potential to reduce *Salmonella* shedding in broiler chickens in a dose-dependent manner, highlighting the need for further research to optimize strain selection, dosage, and application strategies for effectiveness.

Keywords: Probiotics, Salmonella, Colony Counting, Chickens

263 Not Presented

264 Effect of deoxynivalenol and an algae-clay detoxifier on intestinal morphology, and liver oxidative stress in broiler chickens

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Chronic exposure to deoxynivalenol (DON) remains a persistent concern in broiler chickens, as it can adversely affect the digestive system. These detrimental effects can occur at DON levels below the Food and Drug Administration (FDA) maximum threshold of 10 mg/kg feed. This study aimed to evaluate the impact of natural DON contamination in feed on digestive health, and liver oxidative stress in broiler chickens, while also assessing the efficacy of an algae-clay-based detoxifier in mitigating these effects. One-day-

old male chickens (n = 600; Ross 308) were divided into three treatments with ten replicates (20 birds/replicates). The chickens were fed a control diet (CON) with negligible levels of DON (0.2 mg/kg) or diets naturally contaminated with significant levels of DON (2.9 mg/kg), either supplemented (2 g/kg diet; ALG) or not with the detoxifier (DON). Samples of jejunum from one bird per pen at d14, d28, and d37 were collected and fixed for histological analysis (NanoZoomer scan). The evaluated parameters were: mucosal damage, villus height (VH), crypt depth (CP), VH:CP and villus area. A liver sample was collected and submitted to RNA isolation (SV Total RNA Isolation System), and total RNA was quantified by spectrophotometer. qPCR was performed using the MyIQ single-color real-time PCR detection system and MyiQ System Software. Data were analyzed using the efficiency-corrected DeltaDelta-Ct method. Statistical analysis performed using GenStat® software, by comparing the dietary treatment as main factor on the physiological parameters, and means compared by Tukey's test, $p<0.05$. Mucosal damage was observed in the jejunum villus ($p=0.04$) in birds exposed to DON (day 28). It was observed villus shortening on day 37 in chickens fed the DON diet. These negative effects were not observed when the DON diet was supplemented with the ALG detoxifier ($P=0.01$). On day 37, the mRNA expression of glutathione synthetase was increased in the liver of broilers fed DON diet ($p<0.01$). However, its expression was similar to the control when the birds were supplemented with the ALG detoxifier. In conclusion, dietary contamination with DON (~3 mg/kg) had a negative impact on chicken intestinal morphology and the liver redox system. When the algae-clay detoxifier was added, the harm caused by DON was no longer observed.

Keywords: Mycotoxin, Gut integrity, Macroalgae

265 Performance and ileum microbiota of broilers fed with symbiotic

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To evaluate the effects of symbiotic supplementation (Pollax® - Ekoa Life Sciences) as a replacement for the anticoccidials (ANC) nicarbazin (NC) and salinomycin (SAL) and the antibiotic growth promoter (AGP) bacitracin methylene disalicylate (BMD), an experiment was conducted with 1200 1-day-old male chicks, COBB500®. The experimental design was completely randomized (CRD), in a 3x2+1 factorial scheme: 3 inclusion levels of Pollax® (0.05%, 0.10% and 0.15%), 2 absence or presence of ANC and AGP plus the Negative Control (NC), totaling 7 treatments, 6 treatments (3x2) with 30 birds with 6 replicates and the NC with 15 birds with 8 replicates. The experimental diets were mashed based on corn and soybean meal, and the nutritional program consisted of a pre-starter diet – 0 to 12 days, growth 1 - 13 to 28 days, growth 2 – 29 to 39 days and finisher – 40 to 43 days, with feed and water provided ad libitum. The density was 10 birds/m^2. The dosages of NC, SAL and BMD were 125 ppm, 60 ppm and 55 ppm, respectively. The characteristics evaluated weekly were feed intake (FI), body weight gain (BWG), body weight (BW), feed conversion (FCR) and mortality (M), with BWG and FCR being corrected with the weight of dead birds. Normality was verified by the Shapiro-Wilk test (1%) and homogeneity of variances by the Bartlett test (1%). Analysis of variance (ANOVA) was performed in CRD. The effect of ANC and AGP and the comparison of the control with the other treatments were evaluated by the F test, while the effect of Pollax® was analyzed by polynomial regression. Statistical analysis was performed in RStudio using the ExpDes.pt package. Microbiome studies used amplicon sequencing of the 16S rRNA gene. The HiSeq next-generation sequencing platform (Illumina, San Diego, CA, USA) was used to prepare metagenomic sequencing libraries of 16S ribosomal RNA, focusing on the variable V4 region. The total number of species and the distribution of abundance of the species found in the samples were determined by alpha diversity, which was calculated using the Chao (community abundance) and Shannon and Simpson (diversity) indices. At 42 days, a quadratic effect of the Pollax® inclusion level was observed for BW (P = 0.05946), BWG (P = 0.05800), Corrected BWG (P = 0.064675) and FCR (P = 0.08953). The Shannon (4.0171 ± 0.5907) and Simpson (0.9289 ± 0.0270) indices indicated high microbiota diversity and Chao1 (715.1425 ± 165.6171) a good abundance. It is concluded that the performance, diversity and abundance of the microbiota were benefited by the use of Pollax®.

Keywords: body weight, feed conversion ratio, nutrition, prebiotic, probiotic

266 Evaluation of symbiotic for commercial laying hens: Performance and internal and external quality of eggs

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Several feed antibiotic growth promoters (AGP) have been banned and not used in feeds in certain countries due to increased concerns about the potential for antibiotic resistant strains of bacteria and residues of antibiotics in animal tissues. To evaluate the effects of symbiotic supplementation (Polaris® - Ekoa Life Sciences), an experiment was conducted with 192 Hisex White® layers, in the range of 18 and 34 weeks of age. The experimental design was completely randomized (CRD), in a 4x2 factorial scheme: 4 inclusion levels of Polaris® (0,00%, 0,05%, 0,10% and 0,15%) and 2 (absence or presence of AGP bacitracin zinc (BZ), totaling 8 treatments with 6 replicates of 4 birds. The experimental diets were mashed based on corn and soybean meal, and the nutritional program consisted of the Production 1 diet that was modified according to feed intake (95g/b/d 2850 kcal ME/kg, 18,0% CP, and 100g/b/d 2850 Kcal ME/kg, 17,6% CP) and feed and water were provided ad libitum. The BZ dosage was 15 ppm (15g/ton of feed). The cage/bird space was 1250 cm^2 and the aviary was open and equipped with fans. The evaluated characteristics were body weight and uniformity (BW), feed intake (FI), egg production (EP), egg weight (EW), egg mass (EM) and feed conversion by EM (FCR/EM), with BW being checked at 17 and 34 weeks, EW weekly, FI every two weeks and EP daily. The EggTester equipment was used to analyze the internal quality (IQ) and external quality (EQ) of the eggs at 21 and 32 weeks. Normality was verified by the Shapiro-Wilk test (1%) and homogeneity of variances by the Bartlett test (1%). After removing the outliers and confirming the model assumptions, analysis of variance (ANOVA) was performed. The effect of AGP was evaluated by the F test, while the effect of Polaris® was by polynomial regression. Statistical analysis was conducted in RStudio using the ExpDes.pt package. The BW gain was improved from 17 to 34 weeks (1,160 g to 1,408 g) and the same for weight uniformity (79,69% to 97,27%). In the range of 18 to 34 weeks, there was a quadratic effect of Polaris® levels for FCR/EM (P = 0,0378) and the FI was higher without the use of BZ (P = 0,0759). In the range of 20 to 34 weeks, there was a quadratic effect of Polaris® levels for FCR/EM (P = 0,0849) and the FI was higher without the use of BZ (P = 0,0543). There was a significant interaction (P = 0,0072) for shell thickness (ST) at 21 weeks, where the use of Polaris® 0,15% without BZ provided the best values. At 31 weeks, there was no effect of Polaris® on the IQ and EQ of the eggs, but there was an effect (P = 0,0225) of BZ on ST providing better values. It is concluded that the use of Polaris® effectively improves FCR/EM and ST, and that BZ also improves ST.

Keywords: egg mass, feed conversion ratio, Haugh unit, prebiotic, probiotic

267 Optimising the inclusion of xylooligosaccharides: effects on performance, nutrient digestibility, intestinal morphology, and litter properties in growing broiler chickens

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Xylooligosaccharides (XOS) are prebiotics that can be incorporated into broiler diets to promote gut health, potentially boosting growth performance and nutrient digestibility. The current study assessed the effects of dietary supplementation with varying levels of XOS on growth performance, nutrient digestibility, intestinal histomorphology, and litter properties in

broilers raised for up to 28 days. A total of 720 male Hubbard broiler chicks were allocated to 36 littered floor pens or replicates (experimental units) with 20 chicks per replicate and 12 replicates per treatment. Three dietary treatments in mash were fed to the birds as CON (Control without XOS), XOS1 (CON + XOS 1%), and XOS2 (CON + XOS 2%). The birds had *ad libitum* access to feed and water throughout the experimental period. Growth performance was recorded on pen-based, while nutrient digestibility was measured using the total collection method for faecal material, and litter nitrogen and moisture were analysed on day 28. Data were analyzed by one-way ANOVA to determine the effect of treatments on response variables at $P < 0.05$. Results indicated that XOS2 significantly increased body weight gain ($P = 0.014$), digestibilities of dry matter ($P < 0.001$), crude protein ($P = 0.009$), and fat ($P = 0.002$) compared to CON. No significant difference ($P > 0.05$) in ash digestibility was observed between the treatments. XOS2 increased ($P < 0.05$) ileum villus height, crypt depth, and villus-to-crypt depth ratio compared to CON. However, no differences ($P > 0.05$) in body weight gain, dry matter digestibility, crude protein digestibility, fat digestibility, ileum villus height, crypt depth, and villus-to-crypt ratio were observed between the treatments XOS1 and XOS2. In addition, XOS2 significantly reduced litter nitrogen ($P < 0.001$) and litter moisture ($P = 0.002$) compared to CON. Nevertheless, no differences in litter nitrogen and moisture were observed between XOS1 and XOS2. These findings suggest that including XOS at 1% has the same positive effects on growth performance, nutrient digestibility, ileum histomorphology, and litter properties in growing broilers as XOS at 2%, considering the cost-effectiveness of feed additives.

Keywords: Xylooligosaccharides, Growth performance, Nutrient digestibility, Intestinal health, Litter characteristics

268 Effect of a stimbiotic feed additive on cecal fermentation in broiler chickens: An *ex vivo* simulation approach

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The rationale of this work was to examine the effects of xyloglucan-oligosaccharides (XGOS) on cecal fermentation dynamics in broiler chickens using a small-scale *in vivo* trial and an innovative *ex vivo* model developed by Alimetrics Research. This model simulates the cecal environment, allowing for assessment of microbial community adaptations to dietary changes. Fresh cecal samples were collected from chickens fed a wheat-soy diet, with or without XGOS (100 g/tonne) over 21 days, fostering diet-specific microbial populations. XGOS (Aspratis GmbH) is a soluble dietary fibre, classified by AOAC 991.43, comprising oligosaccharides with a degree of polymerization (DP) of 7-9 from tamarind pulp production.Anaerobic 9-hour *ex vivo* fermentation was optimized for cecum conditions, including a controlled temperature of 38°C, redox potential, and pH. Substrates for microbial fermentation were prepared from the centrifugate of the blend of digesta supernatant from the broiler distal ileum and cecum. Fresh cecal microbial inocula from 16 individual birds (8 per diet) were used to ensure biological replicates. XGOS was added to simulation vessels with both inocula types at concentrations of 1, 2.5, and 5 mg/mL, with results compared to a negative control (NC) without XGOS. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$). The study tested the hypothesis that dietary adaptation to XGOS would alter the metabolic capabilities of cecal microbiota. Therefore, short-chain fatty acids were measured at 0 and 9 hours, with gas production measured at 3-hour intervals. Results showed that *ex vivo* fermentation with inocula from XGOS-fed birds significantly increased cecal bacterial cumulative gas ($p = 0.004$) and butyric acid ($p = 0.001$) production, while lactic acid levels decreased numerically after 9 hours of fermentation. Additionally, the inclusion of XGOS at a 5 mg/mL dose in the fermentation vessels enhanced acetic acid production with both control ($p = 0.023$) and XGOS ($p = 0.011$) inocula types compared to the respective NC, suggesting that acetogenic cecal bacteria directly utilized the prebiotic as a growth substrate. Overall, the findings revealed that XGOS enhances cecal microbial activity in broilers by promoting acetic acid production and increasing butyric acid generation, likely through lactate utilization, *i.e.*, bacterial cross-feeding. This research highlights (i) the stimbiotic effect of XGOS and (ii) the intrinsic value of the controlled *ex vivo* methodology with true biological replicates in elucidating the mode of action of feed additives, with implications for optimizing poultry nutrition and gut health.

Keywords: Cecal fermentation, Ex vivo model, Stimbiotic feed additive, Microbial metabolism, Xyloglucan-oligosaccharide

269 Standardized natural citrus extract to improve gut health and growth performance

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Maintaining optimal intestinal health is crucial to ensure the good productivity of broiler chickens. Indeed, when intestinal health is altered, nutrient digestion and absorption are most often affected. This results in bird performance decline, increased susceptibility to intestinal disease, and economic losses for the farmers. The aim of this study is to evaluate the effect of a Standardized Natural Citrus Extract (SNCE) on both broiler chickens' performance and gut health. 756 one-day-old Ross 308 males were randomly divided into 2 groups: a control group (**CTL**) fed a standard diet without supplementation and a citrus group supplemented with 250 ppm of SNCE, with 14 and 13 replicates of 28 birds each, respectively. Growth performance was recorded weekly until d 35. At the end of the supplementation phase, 20 birds per group were randomly selected for short-chain fatty acid (SCFA) analyses in the caeca and jejunal permeability was assessed by measuring transepithelial electrical resistance (TEER) of 10 jejunum samples per group. A veterinary histopathologist also blindly performed histopathological observations from ten jejunal samples from 5 replicates of each group. Statistical analysis was performed using Student's test (*t*-test) with GraphPad Prism software (Version 8.0.2). The Shapiro–Wilk normality test was previously performed to determine if data were parametric. Statistical significance was considered at $p < 0.05$ SNCE dietary supplementation improved broiler chickens' final bodyweight (2364.34 g) as well as average feed intake (3281.44 g) compared to the CTL group (2201.83 g and 3096.24 respectively). As a result, The European Efficiency index was also improved in the SNCE group (461.24 points), compared to the CTL group (426.31). Regarding gut health, the SCFA measurement showed a significantly lower concentration of iso-butyric acid (0.06 mM/g), iso-valeric acid (0.07 mM/g), and

total putrefactive SCFA (0.13 mM/g) in the SNCE group compared to the CTL group (0.08, 0.11, and 0.19 mM/g respectively). The differences in gut permeability measured as TEER value indicate that using SNCE lowered the risk of gut inflammation. This was correlated with the histopathological observation which showed that the jejunum samples from the SNCE group were characterized by scant inflammatory cellular infiltrates while the inflammation was severe in the CTL group. This study showed that feed supplementation with SNCE improved gut health parameters, which was correlated with better growth performance.

Keywords: Citrus extract, growth performance, gut health, broiler chickens

270 Effect of a novel phytogenic feed additive (Fytera Perform) on broiler uniformity

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Several experiments have been conducted with Fytera Perform (FP) under controlled conditions. In many of those studies, an increase in flock uniformity was observed. Consequently, we performed a study under simulated commercial conditions to investigate this effect. Thus, this experiment aimed to evaluate a novel phytogenic feed additive (Fytera Perform, Selko USA, Indianapolis, IN) on performance and pen uniformity within an ABF production system utilizing diets and protocols. Three treatments were administered to the birds: a control diet without phytogenics was provided through the withdrawal (WD) phase, along with two FP treatments. One FP diet was supplied until the finisher phase (FPF) at 45 days, and the other was given until the WD phase (FPWD) at 57 days. A total of 1,800 straight-run Ross 708/YPM birds were allocated to 12 replicates per treatment within six completely randomized blocks and challenged with used litter at placement and with a 20x live coccidia vaccine at 15 days of age. Data were analyzed using a one-way ANCOVA in JMP 17 (SAS, Cary, NC), with pen density as a covariate to account for early mortality in birds fed FPF. Birds fed FPF showed statistically improved Feed Conversion Ratio, adjusted for mortality (FCRM), by 2.2 points at 57 days of age compared to the Control group (p=0.048). However, the data from this group exhibited greater variability than the other two treatments (Control and FPWD), indicating a less uniform population, which suggests that other factors affected this group. A higher early mortality, leading to increased space, was confirmed as a potential reason for the variation observed. Birds fed FPWD showed a numerical improvement in FCRM by 1 point, while male birds evisceration yield improved by 0.15% and front half percentage by 0.39% compared to the Control. These improvements resulted from reducing the lower-performing birds in the population production curves, thereby increasing uniformity in this group. For example, the live weight uniformity of 57-day-old male birds fed FPWD improved by reducing its population standard deviation by 38% (p=0.04) compared to the control-fed bird population. This study enhances our understanding of the effects of FPWD on processing yields of broilers at 57 days of age and its positive impact on their uniformity. Further research is necessary to substantiate the effects of FP on performance, uniformity, and processing yields.

Keywords: Phytogenic feed additive, broilers, performance, uniformity

271 Effects of plant-derived Isoquinoline alkaloids on broiler performance and welfare parameters

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This study aims to assess the effects of a standardized blend of plant-derived isoquinoline alkaloids (IQ) in water on broiler performance and the welfare parameters blood corticosterone, and faecal secretory IgA. IQs originating from the *Papaveracaea* plant *Macleaya cordata* are known to benefit in heat stress situations and present anti-inflammatory, and immunomodulatory properties. In animal nutrition, IQs have been used as phytogenic additives (Sangrovit® product line) to improve zootechnical performance and gut health. Studies indicate that its inclusion in the diet can enhance nutrient digestibility and reduce the need for antibiotic growth promoters. A total of 576-day-old Ross 308 male broilers were used, the birds were distributed in three treatments, TC: Treatment control (no additives), T50: 50 mg Sangrovit® WS/l, and T100: 100 mg Sangrovit® WS/l (T50 and T100 applied via water from d1 until d28), with twelve replications and sixteen broilers each and weekly recording of body weight and feed consumption until slaughter (d35). Blood corticosterone and faecal secretory IgA were measured on days 28 and 35, for two broilers per pen. All normally distributed data were analyzed using one-way Analysis of Variance (ANOVA) using SAS 9.4 (SAS, 2012). Statistical significance was declared at $p \leq 0.05$, and mean differences were separated using Tukey's test (Tukey, 1991). Final body weight was significantly increased in the T50 and T100 compared to the TC group, with weights of 2297g, 2304g, and 2229g, respectively. The results for average daily gain (ADG) and feed conversion ratio (FCR) were as follows: ADG—TC: 62g, T50: 64g, and T100: 65g; FCR—TC: 1514 g/kg, T50: 1482 g/kg, and T100: 1484 g/kg. Sangrovit® WS treatments demonstrated significant differences on ADG compared to the TC group. On day 28, corticosterone levels were unchanged across treatments. By day 35, T100 and T50, both, showed significantly lower values compared to TC ($p \leq 0.001$) with 43.92ng/ml, 62.01ng/ml and 67.5 ng/ml, respectively. At 28 (T100) and 35 days (T50, T100), treatments with Sangrovit® WS resulted in statistically higher secretory IgA values compared to the TC group ($p \leq 0.001$). D28 values were at 0.87 mg/ml (TC), 0.85 mg/ml (T50), and at 1 mg/ml (T100). On day 35, the values recorded were at 1.26 mg/ml (TC), 1.54 mg/ml (T50), and 1.73 mg/ml (T100). The utilization of Sangrovit® WS improves performance, reduces blood corticosterone and increases faecal secretory IgA.

Keywords: Broiler, Corticosterone, Inflammatory reaction, Isoquinoline Alkaloids, Sanguinarine

272 Effects of Sanguinarine like alkaloids on immune response of broiler chickens

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The aim of this study is to evaluate the impact of Macleaya cordata extract on the production of inflammatory cytokines in broiler chickens vaccinated against coccidiosis. Alkaloids are a diverse class of nitrogen-containing natural products present in bacteria, fungi, animals and plants, they have attracted significant interest

recently due to their varied biological activities. This study focuses on a product derived from the extract and processed leaves of *Macleaya cordata*, a member of the *Papaveraceae* family native to China and Japan. A total of 528 slow feathering females Cobb 500 were used, the birds were distributed in 24 floor pens, 22 birds per pen. Three treatments were compared: NC: negative control (no coccidia vaccine or additive), PC: positive control (coccidia-vaccinated, no additive), and MC: a group vaccinated and fed with a diet containing 60g/ton of a product composed by Macleaya cordata extract and processed leaves. All normally distributed data were analyzed using one-way Analysis of Variance (ANOVA), statistical significance was declared at $p \leq 0.05$, and mean differences were separated using Tukey's test. TNF-alpha, an inflammatory cytokine, was measured in blood at 14, 21, and 42 days. Intestinal integrity was assessed using FITC Dextran via oral gavage and plasma recovery at the same intervals. Birds in the MC treatment exhibited FITC Dextran levels of 356.6, 385.0, and 398.7 ng/mL on days 14, 21, and 42, respectively. These levels did not significantly differ from those in the NC group and were numerically lower than those observed in the PC group, which showed 345.1, 389.9, and 492.7 ng/mL on days 14, 21, and 42, respectively. For TNF-α, MC showed values of 86.1, 101.0, and 88.3 on days 14, 21, and 42, respectively, which were statistically different from PC values on day 14 (122.5) and day 42 (102.8). These findings indicate that the Macleaya cordata extract may enhance gut health, presenting a promising alternative to antibiotics in poultry growth.

Keywords: Broiler, Coccidia Vaccine, Inflammatory reaction, Isoquinoline Alkaloids, Sanguinarine

273 Oregano-derived essential oils: Effects on egg production and eggshell quality in laying hens

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This study is investigating the efficacy of Ecodiar Powder, a natural essential oil product derived from Greek oregano, as a feed supplement to enhance egg production and eggshell quality in laying hens. The experiment utilized 30 replicates of 21 hens each, totaling 630 hens fron 40 to 60 weeks of age. Three treatment groups were established: a control (0 g/ton Ecodiar Powder), a low-dose group (150 g/ton, equivalent to 0.33 lb/ton), and a high-dose group (300 g/ton, equivalent to 0.66 lb/ton). Hens were fed a 16% crude protein, 2800 kcal/kg diet, with Ecodiar Powder incorporated by substituting corn. Feed is provided ad libitum, and hen-day egg production (HDEP), hen-housed egg production (HHEP), feed intake (FI), feed conversion ratio (FCR), egg quality parameters, and mortality data collected every 28 days. The physical egg quality parameters measured were egg weight (EW), shell color, albumen height (AH), haugh unit (HU), eggshell breaking strength (EBS), eggshell elasticity (ESE), vitelline membrane strength (VMS) and vitelline membrane elasticity (VME). The experiment was designed as a completely randomized design, with strain as a fixed effect and time as a random effect. Each housing condition and egg color type were analyzed separately using the PROC GLIMMIX procedure in SAS 9.4; significance was determined at $P \leq 0.05$, with means separated using Tukeys HSD test. Results demonstrated an improvement in HDEP and FCR for Ecodiar Powder supplemented groups compared to the control. Specifically, the high-dose group exhibited the most significant increase in total egg production ($P=0.018$) and HDEP. The mean HDEP was 94 % in the control group, 93% in the low-dose group, and 95% in the high-dose group ($P=0.002$). The FCR, measured as grams of eggs per gram of feed, was 0.522 in the control, 0.521 in the low-dose group, and 0.536 in the high-dose group ($P<0.001$), indicating that the high-dose group converted feed into eggs more efficiently. Furthermore, eggshell quality, ESE and VME were enhanced in the supplemented groups. The mean ESE was 0.27 mm in control, 0.27 mm in the high-dose group, which were lower than and 0.29 mm in the low-dose group ($P<0.001$). The mean VME was 1.66 mm in control, 1.65 mm in the low dose, which were lower than 1.73 mm in the high dose group ($p<0.001$). These findings suggest that Ecodiar Powder supplementation, particularly at 0.66 lb/ton, effectively bolsters egg production and eggshell quality in high production laying hens.

Keywords: Oregano, essential oils, laying hen

274 Olive-Soluble (OS): A sustainable added-value stream from extra-virgin Olive Oil production, rich in polyphenols, targeting the needs of modern chicken-broiler production

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Modern poultry broiler production faces several global challenges, including the ban on sub-therapeutic doses of antibiotics used as growth promoters. Furthermore, modern broiler growth imposes a significant metabolic demand (~12 times that of a human adult), with production aiming for maximum live body weight (BW), which contributes to chronic inflammation and broader metabolic syndromes (myopathies collectively referred to as «woody breast»). This creates broader animal welfare and consumer concerns. Consequently, there is a need to develop sustainable solutions to support the industry. Several botanicals have been proposed, and we tested an olive-soluble (OS) extract rich in polyphenols and other bio-actives, such as triterpenes, alkaloids, and tocopherols, derived from Greek extra-virgin olive oil production. A total of 480 one-day-old male Ross 308 chicks were randomly assigned in a completely randomized design to four treatments: (n = 8 pens/treatment; n = 15 birds/pen): 1) Negative control (NC), fed a corn-soy-based diet representing the industry's gold standard; 2) Positively-challenged control (PC), fed a wheat-rye-based diet without xylanase, which is known to induce intestinal challenges and growth retardation; 3) PC + OS at 0.035%; and 4) PC + OS at 0.05%. Standard growth parameters, including BW, feed intake (FI), and feed conversion ratio (FCR), were measured throughout the various growth phases, as well as cold carcass weights at the end of the trial (day 35). The olive-soluble nutritional value is considered minimal due to its low inclusion level (only 350 and 500 ppm). Data were statistically analysed using ANOVA, followed by a Tukey test for means comparisons. The PC group showed a ~ 8% reduction in BW compared to the NC (D 35) (2,013 g vs 1,864 g; $p<0.0001$). Both OS doses not only recovered the lost BW compared to the PC but also numerically exceeded the BW of the gold-standard NC at the end of the trial (day 35) (~2,094 g for each OS group vs. NC = 2,013 g). Meanwhile, the two OS groups significantly reduced the FCR compared to the PC, although only numerically compared to the NC (OS-0.035%; FCR = 1.800, OS-0.05%; FCR = 1.774, PC; FCR = 1.950, and NC; FCR = 1.829). Notably, the PC + OS at

0.05% improved carcass yield traits, specifically increasing red meat (~30% in drumsticks, thighs, and wings) compared to the other three groups. This, along with improved blood antioxidant parameters (glutathione and total antioxidant capacity), suggests overall enhanced metabolic health for the birds in this group, warranting further investigation.

Keywords: olive-soluble, chronic inflammation, polyphenols, modern broiler production, metabolic health

275 Cannabidiol and nano-selenium mediate intestinal barrier function by affecting mucosal microstructures in the gut of chickens infected with *C. perfringens*

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Nutritional additives such as cannabidiol (CBD) and nano-selenium (nano-Se), are viable to prevent bacterial diseases such as necrotic enteritis (NE) in chickens. The effects of both agents are manifested in supporting intestinal barrier function and levels of antibodies against pathogens. To the best of the authors' knowledge, no assessment of gastrointestinal structures has been performed to date in chickens at the early stage of C. perfringens infection when supplemented with CBD and/or nano-Se. The set of experiment was as follow: A total of 360 one-day-old male broiler chicks were purchased from a local hatchery and were reared in cages. Birds in the negative control (I, CON) and positive control (II, C. perfringens) groups received a basal diet throughout the experiment. Groups CBD + C. perfringens (III) and nano-Se + C. perfringens (IV) were challenged and supplemented (on top) with 15 g/kg Cannabis sativa extract or with 0.3 mg/kg nano-Se. Chicks from CBD + nano-Se (V) group and CBD + nano-Se + C. perfringens (VI) were fed a control grower diet supplemented with both additives, but the second group was challenged with C. perfringens. Experiment lasted 35 days, growth performance and gut ultrastructure's were evaluated, and data were compared using one-way ANOVA (Duncan procedure) considering $P<0.05$ as significant. The results shown that body weight gain (BWG) in the experiments on days 9-23 and 24-35 was not significantly different between groups, while BWG calculated for the entire experimental period was significantly lower in the C. perfringens-infected group and in the CBD-infected group compared to the negative control. Both compounds improved the indices of histomorphometry of duodenum, jejunum, and ileum. Examination of the structures and ultrastructures of the gastrointestinal tract showed that CBD + nano-Se supplementation did not manifest adverse effects on the host gut indices. There were significant correlations between markers of jejunum function and epigenetic and oxidative indicators. In conclusion, the current study provides evidence that CBD and nano-Se mediate epigenetic and oxidative DNA changes and affect intestinal development and functionality in broiler chickens at an early stage of infection with C. perfringens. Funded by the Minister of Science under the Regional Initiative of Excellence Program.

Keywords: cannabidiol, gut structures, C. perfringens, histology, broiler chickens

276 Impact on the performance of broiler breeders supplemented with cinnamaldehyde and thymol

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Cinnamaldehyde and thymol are two substances used in the poultry industry for their potential to enhance performance and reduce pathogen growth. The main objective of this study was to assess the performance of broiler breeders supplemented with a blend of cinnamaldehyde and thymol (EO). This study was conducted on a commercial farm with a total of 57,500 broiler breeders (Ross 308 AP). The birds were divided into two groups: CON (commercial diet+ sanguinarine (100g/ton)) and EO (commercial feed + essential oils). The EO group received a combination of cinnamaldehyde and thymol, supplied at a dose of 100g/ton of feed, providing 4.5g of cinnamaldehyde and 13.5g of thymol per ton of feed. At 20 weeks of age, the birds were distributed into three separate houses. Two of these houses were administered the EO treatment, while the third house received the CON treatment. The supplementation of the test diets began at 24 weeks until 55 weeks of age. Variables related to animal performance were evaluated, including weekly egg production divided into three phases (25-30 weeks, 31-39 weeks, 40-64 weeks), breeder and male breeder livability, and the incidence of dirty and broken eggs. Additionally, variables such as hatching, fertility, and embryo mortality were also assessed. The data were analyzed for normality and outliers, with bird houses defined as covariate for statistical analysis. Analysis of variance was performed using JMP Pro 16 software, with statistical significance defined as $P<0.05$ and a tendency as $P<0.10$. The Enviva EO treatment showed a tendency to increase weekly egg production during the peak production phase (31-39 weeks) at 81.97% compared to the control treatment at 80.75% ($P<0.10$). For the remaining phases, there were no statistically significant differences. Male breeder livability was significantly ($P<0.05$) higher in the EO group (99.13%) compared to the control (99.02%). No differences were observed for female breeder livability. The EO treatment resulted in a significant reduction in the percentage of dirty eggs and broken eggs, at 9.54% and 1.26% respectively ($P<0.05$), compared to the CON group at 10.53% and 1.38%. No significant differences were observed between treatments for hatching rates, fertility, and embryo mortality. In conclusion, the study found that supplementing broiler breeders with a blend of cinnamaldehyde and thymol (EO) improved some performance parameters. Specifically, the EO treatment tended to increase weekly egg production during the peak production phase and led to a significant reduction in the percentage of dirty and broken eggs.

Keywords: Breeders, Essential Oil, Performance

277 Potential significance *of Corchorus capsularis* on turkey meat oxidative stability, meat composition, and performance

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Functional foods are defined as foods that may impart a health benefit to the consumer. Traditional food ingredients have increased scientific attention due to their potential to generate functional foods and nutraceuticals. Plants have provided nourishment and other health benefits to humans and animals. Jute

is an important cash crop in Bangladesh which contains a large amount of dietary fiber, rich in vitamins (E, C, B_2, and B_9) and minerals that may contribute to preventing various diseases. A study was conducted to determine the feeding effect of *Corchorus capsularis* leaf as a functional feed ingredient on growth performance and meat quality in turkey meat. A total of one hundred twenty (120) 7-day-old turkey poults were split into three dietary treatments, with four replications having ten poults in each group. The three dietary treatments were formulated using basal feed as follows: control T_0- control feed; T_1- supplemented with 0.5% and T_2- 1% Jute leaf meal. Turkey poults were reared up to 70 days of age and fed ad libitum. The analytical measurements were performed in triplicates, and the results were presented as the average of the three analyses ± standard error mean. Statistical analysis was conducted using one-way ANOVA with the SPSS statistical package. There were no statistically significant differences in feed intake and average daily gain however body weight at 70 days was significantly different ($p \leq 0.05$) in T_2 group, 1520.09g than the control 1407.54g. The feed conversion ratio was slightly lower in the additives group (4.72, 4.61 respectively) than control (4.98). There was no significant difference in proximate component values of breast and thigh meat. The lowest ($p \leq 0.05$) thiobarbituric acid-reactive substances values of turkey breast and thigh meat were obtained in T_2 groups compared to T_0 group after the fourth week of preservation. Thigh meat cholesterol and fatty acid content were also significantly difference in T_2 additives group 16.82 mg/100g. The results of the present study elucidated that the dietary inclusion of *C. capsularis* could be the promising functional ingredient to produce value-added turkey meat in terms of enhancing oxidative stability and lowering cholesterol in meat.

Keywords: Turkey, Corchorus capsularis, Cholesterol, Lipid oxidation, Value-added

278 Not Presented

279 In-vitro inhibition of *Enterococcus* strains isolated from commercial broilers by a three-strain *Bacillus* direct-fed microbial product (DFM)

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Enterococcus spp. are commensal bacteria found in the avian gastrointestinal tract and may opportunistically infect the host, causing disease including locomotor disorders and septicemia. The incidence of such *Enterococcus* infections has been increasing recently and become a major concern among poultry producers. The objective of this study was to quantify and characterize *Enterococcus* spp. at a commercial poultry operation and determine whether a three-strain *Bacillus amyloliquefaciens* direct fed microbial (DFM; Enviva® PRO) could inhibit *in vitro* growth. All samples were collected at a vertically integrated broiler complex in the Southeastern United States. At varying ages (13-58 days of ages) samples were collected from 18 different flocks. All birds were Cobb x Cobb and were raised under the same feed programs of corn/soybean meal-based diets and similar management programs. Three birds from each house were humanely euthanized and the gastrointestinal tract (GIT) was collected. Bacteria adherent to the epithelial lining were cultured on SBM agar using a spiral plater (Interscience easySpiral). CFU/g was calculated from plate counts, and putative *Enterococcus* colonies were confirmed via 16S sequencing. Colonies of *Enterococcus* spp. associated with poultry pathology were selected for in-vitro inhibition with cell-free supernatant (CFS) from three strains included in Enviva® PRO. In total, 504 *Enterococcus* colonies were isolated and sequenced, and 9 separate species were identified. 53.97% were *Enterococcus faecalis*, followed by *E. faecium* at 15.48% and *E. durans* at 13.49%. Across all ages, total Enterococcus were between 3.64 and 6.40 log CFU/g. Age effect was observed for some *Enterococcus* spp. with *E. durans* increasing over time, and *E. faecium* decreasing over time. *E. faecalis* declined during ages 0-14 d, then increased from 14-28d. The most species diversity was observed at earlier ages. In the cell-free supernatant assay, a clear species-specific effect was observed. *E. durans* was strongly inhibited (on average 91%), *E. faecium* and *E. hirae* were moderately inhibited with 68% and 67% respectively, and *E. faecalis* was poorly inhibited with 26%. In summary, the inclusion of a multi-strain DFM to poultry diets may potentially inhibit the growth of specific pathogens endemic to field conditions.

Keywords: Enterococcus, DFM, broilers, commercial complex, in vitro

280 Difference in saponin-based formulations in maintaining broilers' zootechnical performances under experimental condition

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Saponin containing feed additives are proven as an effective tool for avian coccidiosis management. However, the efficacy of commercial products can significantly vary due to differences in saponin concentration, structure, and biological activity which are influenced by several factors including plant species, plant part, geographical origin, and extraction method. Although the saponin level is a good indicator of quality, it is insufficient, as not all saponins performs the same way on coccidiosis. Therefore, mastering the characterization and standardization of rich-in saponins plants help to maximize formulating potential. This study aims to evaluate the different saponins-rich formulations supplemented in feed on maintaining broiler performances under experimental conditions. 1020, seven-day-old Ross 308 broiler chickens were randomly divided into 6 groups (6 to 7 replicates per group): UUC (n=180): uninfected untreated control; IUC (n=210): infested untreated control; P1 (n=210); P2 (n=210); P3 (n=210): infested, supplemented saponins-rich additive formulation 1/ 2/ 3 respectively, at 250g per ton of complete from day 7 to day 42. Tested saponin-based additives were formulated from different plants and standardized to bring the similar saponin content per ton of feed. Coccidiosis challenge was performed orally at 14 days olds. 1-week post-challenge, 2 birds per replicate were randomly selected to score the intestinal lesion (ILS). Performances including live weight, feed intake, feed conversion rate (FCR) and mortality were monitored. Statistical analysis was performed using GraphPad Prism software (Version 8.0.2). Statistical significance was considered at $p < 0.05$. The experimental infestation was successful as evidenced by the significant reduction in live weight (2267g vs. 1407g) and increase in FCR (1.4 vs. 1.8) in the IUC compared to the UUC group. Performance restoration was not equal among supplements. P3 birds performed the best, showing significant higher live weight and lower FCR ((2241g; 1.42) compared to P1 (1623g; 1.5) and P2 (1641g; 1.54) respectively. Global ILS was significant improved in all groups compared to IUC but no significant difference observed among supplements. No statistical difference in mortality was found. This study demonstrated that saponin-based additives can play as a "stand-alone" solution for managing coccidiosis in broiler chickens. However, the final results vary from saponins sources, emphasizing the importance of characterization, standardization and formulation to achieve consistent outcomes. Further in-field studies should be carried to confirm these results.

Keywords: Saponins-based additive, Avian coccidiosis, cocci vaccination, Saponin plants, coccidiostat alternative

281 Effect of dietary inclusion of grape pomace on physiological and molecular marker of gut health and oxidative stress in coccidia-challenged broilers (*Gallus gallus*)

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Grape pomace (**GP**) is an agricultural byproduct rich in bioactive compounds that have proven to have strong antioxidant, antimicrobial, and immunomodulatory properties in chickens. As a part of a defense mechanism, coccidiosis initiates the innate immune response, triggering oxidative bursts and generating large amounts of free radical species that negatively affect the gut health of chickens. This study aimed to evaluate the effect of the inclusion of GP in broiler diets infected with coccidiosis on physiological and molecular markers of gut health, immune response, and oxidative status. A total of 120 male broiler chickens were randomly assigned to one of the four treatments. The four treatment groups were i) non-challenged control without any dietary feed additives (**NCC**), ii) challenged control without any dietary feed additives (**CC**), iii) CC with 0.5% dietary inclusion of concord grape pomace (**0.5GP**), and iv) CC with 0.75% dietary

inclusion of concord grape pomace (**0.75GP**). On d 14, broilers grouped in CC, 0.5GP, and 0.75GP were orally inoculated with 20× doses of commercial live coccidiosis vaccine to induce coccidiosis. Markers of gut health, intestinal lesions, tight junction protein, histomorphology, and gut permeability were measured at d 20 and d 26. The inflammatory response was measured as mRNA expression of inflammatory cytokines and CD4+ and CD8+ T cell differentiation at d 20 and d 26. mRNA expression of oxidative pathways (MAPK-Nrf2/ARE) was measured at d 20 and d 26. Data thus obtained were analyzed using one-way ANOVA. A significant level was set at $P \leq 0.05$, and a tendency at $P < 0.1$, and means were separated using Fisher's LSD. Results indicated that 0.75% inclusion of GP reduced the occurrence and severity of the intestinal lesions associated with *Eimeria* at d 20 ($P < 0.05$). The inclusion of either 0.5 or 0.75% of GP improved the villus height and reduced crypt depth in all three sections of the small intestine compared to CC during the acute and recovery phases of coccidial infection ($P < 0.05$). 0.75GP upregulated the expression of CLDN at d 20 compared to other treatments ($P = 0.037$). At d 20, 0.75GP tended to maintain the same level of IL-1β as that of NCC and downregulated the expression of IL-10 ($P < 0.1$). During the recovery phase, the CD8+ T-cell population was lowered in the 0.75GP group compared to CC and 0.5GP ($P = 0.059$). At d 20, the MAPK-Nrf2/ARE pathway was activated by the oxidative burst from a coccidial challenge; however, 0.75% GP inclusion was able to prevent it ($P < 0.05$). These findings suggest that grape pomace possesses the potential as a sustainable feed additive to improve gut health, immune response, and oxidative stability in broilers infected with coccidiosis.

Keywords: Grape pomace, coccidiosis, gut health, oxidative stress, immune response

282 Evaluation of a new combination of postbiotics and essential oils on broilers raised under various coccidiosis control programs with a mild Enterococcus challenge

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Innovative feed additives coming from combination of different molecule categories such as postbiotics and essential oils (Biostrong™ Dual (Dual)), are important for optimizing poultry production. Postbiotics have been shown to support a healthy immune system, promote beneficial bacteria, and maintain gut balance, while essential oil compounds have been shown to improve gut health by aiding nutrient absorption, thereby enhancing poultry performance. The objective of this trial was to evaluate the impact of Dual during a mild *Enterococcus* challenge under various coccidiosis control programs in broilers. A total of 2,880 day-old male Cobb 500 byproduct chicks were placed on used shavings in 4'x8' floor pens (0.80 sq ft/bird) and randomly assigned to 1 of 6 treatments (6 treatments x 12 replicates x 40 birds/replicate). The trial ran for a total of 42 days and birds were fed standard corn-soybean meal-based diets in a three-phase program (starter d 0 – 14, grower d 14 – 28, finisher d 28 – 42) that met nutrient requirements. All birds were raised in a house with a well-established, naturally occurring *Enterococcus* challenge. Treatments included chemical coccidiostat, ionophore coccidiostat, and coccidiosis vaccine, with or without Dual (0.8 lb/t). Pen and feeder weights were captured on d 0, 14, 28, and 42 for performance parameters. On d 7, 19 and 35, 1 bird/pen was randomly chosen for spleen swabs for *Enterococcus* analysis. Data were analyzed as a 2x3 factorial design coming from a combination of Dual (with or without) and coccidiosis control program (chemical, ionophore, vaccine). No interactions between Dual and coccidiosis control program were detected ($P > 0.05$) for any of the growth parameters at any timepoints. For the overall trial, Dual improved ($P < 0.004$) feed conversion ratio (FCR) and bodyweight (6.55 lb) adjusted FCR by over 2 points compared to no Dual in the diet. The Dual treatments were effective in reducing the prevalence of *Enterococcus* strains in spleen culture swabs over time with the percentage of *Enterococcus* strains in the Dual group being lower compared to the no Dual groups at d 35. The Dual treatments reduced the prevalence of both highly pathogenic and opportunistic *Enterococcus* strains in spleen swabs at d 35, indicating Dual may have a broad-spectrum impact on different types of *Enterococcus*. The trial demonstrated that Dual significantly improved FCR and bodyweight (6.55 lb) adjusted FCR, while effectively reducing the prevalence of *Enterococcus* strains in broilers. These findings are relevant for broiler producers as they show Dual as a tool to support growth performance and health, leading to more efficient and profitable broiler production.

Keywords: Enterococcus, Coccidiosis program, Postbiotics, Essential oils, Broilers

283 Precision biotics in laying hens: a key solution for reducing *Salmonella* Enteritidis excretion and improving food safety

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Due to global restrictions on the use of antimicrobial growth promoters (AGPs) in animal feed, the industry is actively seeking alternative solutions. Glycan-based precision biotics (PB) have demonstrated potential in enhancing performance and disease resilience by modulating microbiome pathways. Foodborne infections caused by non-typhoidal *Salmonella* serovars, particularly *Salmonella* Enteritidis, are a significant public health concern. Effective control methods are needed in production systems to ensure food safety. The objective of this study was to evaluate the effects of PB on controlling *S.* Enteritidis infection and excretion in commercial laying hens. A total of 45 hens, aged 28 weeks, were randomly assigned to three groups with five replicates (three birds each) over 12 weeks, all groups fed with the same basal diet. Treatments included: non-challenged hens (NEG), challenged hens (POS), and challenged hens supplemented with 900 g/ton of PB in the feed. At week 2, POS and PB groups were inoculated with 10^9 CFU/ml of a field strain of *S.* Enteritidis via oral gavage. Weekly sampling of cloacal swabs and eggs was performed to measure *Salmonella* counts, while bactericidal activity in blood was assessed at 7 days and 10 weeks post-infection. At the end of 12-week period, animals were euthanized, and samples of spleen, liver, heart, ovarian follicles, oviduct, uterus, cecum, and bone marrow were cultured. Histological lesions scores were measured, and gene expression of tight junction proteins and mucins was measured using qPCR. IgA concentration in cloacal swabs was quantified by ELISA at 7 and14 days post-inoculation and at the end of the trial. Feed intake, body weight, egg production, and feed conversion were measured weekly. Statistical analysis was performed using ANOVA and the

Kruskal-Wallis test ($P < 0.05$). The results showed a significant reduction in *Salmonella* fecal excretion (96%) and egg contamination (93%) in the PB group compared to POS. Blood bactericidal activity was significantly higher in the PB group (70.89%) than the POS (56.26%) and NEG (41.39%) groups. Histological lesion scores were significantly lower in the PB (53%) and NEG (50%) groups compared to POS (57%). The PB group showed higher expression of tight junction proteins ZO-1, MUC2, and CLDL-3. These findings demonstrate that precision biotics significantly reduce S. Enteritidis excretion and infection in laying hens, supporting their potential as a highly effective control strategy for Salmonella, thereby improving food safety. Additionally, the enhanced blood bactericidal activity and reduced histological lesions observed in the PB group further highlight its benefits in strengthening immune defence and gut health.

Keywords: Precision Biotics, Salmonella Enteritidis, Food Safety, Foodborne Illnesses, Laying Hens

284 A precision biotic enhances the growth performance of broilers fed nutritionally reduced diets

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Precision biotics (PB) are a new category of feed ingredients specifically developed for their ability to modulate the intestinal microbiome. This modulation increases the production of short-chain fatty acids (SCFAs), which in turn helps regulate intestinal metabolism and health. The aim of this study was to evaluate the effects of a PB (Symphiome®, dsm-firmenich) on the growth performance, carcass yield, and ileal digestibility of broiler chickens fed nutritionally reduced diets. A total of 552 Cobb 500 broiler chicks were allocated to a randomized block design with three treatment groups, eight replicates per treatment, and 23 birds per replicate pen. The treatments included a positive control diet (PC), a negative control diet (NC) with approximately 3% less Apparent Metabolizable Energy (AME) and 2.5% fewer digestible amino acids (dAA), and the NC supplemented with 700 g/MT of PB. The feeding program was divided into four phases: Starter (0-14d), Grower (15-28d), Finisher (29-42d), and Withdrawal (43-49d). Growth performance was evaluated for each feeding phase as well as the cumulative periods. The experiment lasted 49d. At d42, 3 birds per replicate were selected based on the average pen weight, and ileal contents were collected to assess the digestibility of dry matter (DM), nitrogen (N), and fat. The data were analyzed by ANOVA, and the means were separated by Tukey's Test (n=8; $P<0.05$). From 0-14d and 0-28d, the growth performance of the birds was negatively affected by the nutritional reduction ($P<0.01$), with no positive effect of the PB. From d28 onwards, the nutritional reduction continued to impair the performance of the birds, but supplementation with PB enhanced several parameters. From 0-42d, PB increased body weight gain (BWG; $P<0.05$) by 69g and improved the feed conversion ratio (FCR; $P<0.01$) by 3.5 points. From 42-49d, PB improved the FCR of the birds to a level similar to that of the PC group ($P=0.01$). Over the entire experimental period (0-49d), PB supplementation increased BWG by 137g and improved the FCR by 6.7 points ($P<0.01$). Additionally, the digestibility of N and DM was significantly enhanced ($P<0.01$) with PB supplementation. Supplementation with PB in nutritionally reduced diets significantly enhances the growth performance and ileal digestibility of broiler chickens. While nutritional reductions initially impair performance, PB supplementation improves BWG, FCR, and intestinal digestibility, particularly from d 28 onwards. These findings demonstrate the potential of PB to modulate the intestinal microbiome and improve overall broiler health and productivity, making it a valuable nutritional strategy for broiler production under suboptimal dietary conditions.

Keywords: broilers, microbiome, precision biotics, nutritional reduction, short-chain fatty acids

285 Effect of alternative pronutrients on liver health: a model of macroscopic evaluation and its correlation with histopathological findings

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Pronutrients derived from *S. marianum* and *C. scolymus* induce measurable changes in broiler liver tissue, enabling predictive macroscopic lesion classification correlated with histological findings and improved productive performance. A total of 168 male Ross 308 broilers were randomly assigned to control or treatment groups. Control birds received a basal diet, while the treated group received the same diet with 0.5% of a plant extract mix (15% *S. marianum*, 15% *C. scolymus*, 10% betaine HCl). Birds were raised for 35 days, and blood, organ, and performance data were collected. Birds were weighed weekly. Blood samples were taken on days 21 and 35 for biochemical analysis (cholesterol, triglycerides, proteins, glucose). Organs were weighed and liver morphometry (length, width, thickness) recorded. Color parameters (L*, a*, b*) were used for liver evaluation. Histopathology assessed lesion types and severity. A completely randomized design was applied with 7 replicates per group. Normality and homoscedasticity were tested. Depending on distribution, T-test or Mann-Whitney U test was used. Data were analyzed using Python®. PCA and Random Forest models were applied for pattern recognition and lesion classification. Biochemical changes were observed within the treatment group between days 21–35: increased total protein ($p=0.029$), globulin ($p=0.0096$), cholesterol ($p=0.0031$), and triglycerides ($p=0.0045$). At 21 days, control birds had heavier ceca ($p=0.037$). At day 35, treated birds showed stronger correlations between body weight and digestive/immune organs. Liver morphometry indicated proportional growth. Treated birds showed more intense skin and liver color. A healthy liver color scale was established. Bile duct hyperplasia was more severe in control birds ($p=0.0001$). Treated birds were heavier at day 35 (2121.6g vs 1947.7g; $p=0.022$) and had a higher productivity index ($p=0.012$). Pronutrients positively modulated liver and immune physiology, improved performance, and enabled liver lesion classification using a practical model. No adverse hepatic effects were observed. The application of the liver color scale, along with length, width, and thickness measurements, proved to be a useful tool for the rapid and objective assessment of liver status in the field, enabling the detection of subclinical alterations and showing potential for integration into broader monitoring systems alongside histological correlations.

Keywords: liver tissue, macroscopic evaluation, data analysis, histopathology, pronutrients

286 Treatment of chickens with probiotics under conditions conducive to necrotic enteritis development

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Necrotic enteritis (NE) poses a significant challenge to the global broiler industry, particularly with the increasing restrictions on using antibiotic growth promoters. Probiotics have emerged as a promising alternative for effective disease control. This study evaluated the efficacy of a lactobacilli cocktail under high stocking density, an experimental condition conducive to NE in broiler chickens. One-day-old male broiler chickens (n=352) were assigned to four treatment groups in a 2×2 factorial design to examine the effects of probiotic supplementation (with vs. without) and stocking density (low vs. high) on body weight gain, intestinal immune system, and gut microbiome of broiler chickens. Chickens were divided into two groups based on stocking density: high stocking density (30 birds/square meter) and normal stocking density (15 birds/square meter). On days 1, 7, 14, and 20 of age, birds in both stocking density groups were treated with either 10^8 colony-forming units of lactobacilli or phosphate-buffered saline. Body weight and lesion scores were recorded on days 21 and 24, respectively. Intestinal tissues were collected to analyze immunoregulatory gene expression using quantitative real-time PCR and to assess lymphocyte populations using flow cytometry. Cecal contents were collected and DNA was extracted for metagenomic analysis of the cecal microbiome using 16S rRNA gene sequencing. Statistical significance among groups was analyzed using a two-way ANOVA followed by Tukey's HSD test, with a significance level of α=0.05. Probiotic treatment improved body weight gain compared to non-treated controls ($P < 0.05$), and reduced gut lesion scoring in the birds raised under high stocking density ($P < 0.05$). Probiotic treatment increased the frequency of Bu-1^+ B cells and $CD3^+CD4^+$ T cells in the cecal tonsils ($P < 0.05$) and enhanced the relative expression of antimicrobial peptides (zonula occludens and occludin) in the ileum ($P < 0.05$). However, it decreased the expression of heat shock proteins, interleukin (IL)-18, IL-1β, and interferon (IFN)-γ ($P < 0.05$). Probiotics also enhanced alpha diversity and the abundance of *Christensenellaceae*_R-7 group, *Angelakisella*, and *Clostridia_vadinBB60* group compared to the high stocking density control group ($P < 0.05$). The findings of the present study suggest that probiotics can be supplemented in chickens to mitigate the stress caused by high stocking density and the consequent development of NE by maintaining gut microbiota homeostasis, regulating gut immune responses, and improving gut barrier integrity.

Keywords: Lactobacilli, Necrotic Enteritis, Cytokines, Lymphocytes, Gut Microbiome

287 1,25-dihydroxyvitamin D_3 supplementation modulates tight junction permeability and humoral immunity against a bacterial chondronecrosis with osteomyelitis lameness in broiler chickens

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Bacterial chondronecrosis with osteomyelitis (BCO) lameness causes substantial issues on animal well-being and production of broilers. Strategies such as early feed restrictions and feed supplementations have been tested to alleviate BCO incidences. We have published a study demonstrating the supplementation of Panbonis®, containing glycosylated $1,25(OH)_2D_3$ from *Solanum glaucophyllum,* to broilers for the first 28 days reduced BCO by approximately 50%. In this study, we investigated the impact of the G-$1,25(OH)_2D_3$ supplementation for 14, 21, and 28 days on the expression of tight junction (TJ) proteins and humoral antibodies to further understand the mode of action of the G-$1,25(OH)_2D_3$ supplementation in protecting birds from BCO. A total of 800 birds were divided into four treatment groups with four replicate pens per treatment. T1 was a negative control diet, and T2, T3, and T4 were supplemented by 1 µg/kg of G-$1,25(OH)_2D_3$ for 14, 21, and 28 days, respectively. 100 birds were placed on two wire floor pens as BCO seeders to induce BCO lameness outbreak in the research barn. Tracheal mucus, blood, and tissue samples (ileum, jejunum, and trachea) from 5 birds per treatment were collected at d12, d14, and d28 post hatch. We measured the relative mRNA expression levels of CLDN1, CLDN5, occludin (OCLDN), and zone occludin (ZO-1) of the epithelial barrier in the respiratory and gastrointestinal (GI) tracts using RT qPCR and the titers of IgA and IgM antibodies in the tracheal mucus and blood using ELISA. The mRNA expression level was analyzed by using CFX Maestro™ software (Biorad, California, USA) and *P*-value was determined by using One-Way Anova. T3 and T4 presented the highest relative mRNA expression levels of TJ proteins in GI and respiratory tracts at d28, respectively ($P < 0.05$). CLDN1, OCLN, and ZO-1 were significantly upregulated in both GI and respiratory tracts at d28 ($P < 0.05$), but limited expression of CLDN5 was observed. The results indicated a time-dependent pattern of TJ gene expression, with significant upregulation at d28, but no or limited TJ expressions at d14 and d21, respectively. ELISA tests demonstrated an increase in IgA titer intra-tracheal mucus and IgM in blood from d14 to d28. T4 produced the highest IgA and IgM levels at d28 with no statistical difference from T1 ($P > 0.05$). Overall, supplementation of G-$1,25(OH)_2D_3$ for 21 and 28 days strongly modulates tight junction permeability by upregulating CLDN1, OCLDN, and ZO-1 in GI and respiratory tracts, yet confers a modest effect on IgA and IgM production. These findings highlight the action mode of G-$1,25(OH)_2D_3$ supplementation in enhancing the gut and respiratory barrier integrity against BCO.

Keywords: Broiler, Epithelial Integrity, Vitamin D3, Lameness, Bone

288 Use of a *Quillaja saponaria* and aluminosilicate blend, Excential Sapphire Q, in vaccinated broilers during a strong enteric challenge

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Eimeria and *Clostridium perfringens* are among the most important pathogens when considering intestinal health of broiler chickens. In addition to mortality, these pathogens result in performance suppression. Phytogenic compounds have been reported to support existing enteric health programs. Excential Sapphire Q (ESQ, Orffa Additives B.V.) is a combination of *Quillaja saponaria* and activated aluminosilicate supporting broiler health. The current study included 5 treatments: an unchallenged control (fresh shavings), *Eimeria* challenge without coccidia vaccine, challenge with vaccination, ESQ in the grower and finisher diet (200 ppm) + vaccine, as well as ESQ in all phases

(200 ppm) + vaccine. All groups except the unchallenged control were placed on reused litter which contained *C. perfringens* from a previous trial. Each treatment consisted of 12 replicates of 25 male Ross broiler chickens. Vaccinated groups received a 1x dose of a commercial coccidiosis vaccine prior to placement. Challenged treatments received ~100,000 *E. acervulina*, ~50,000 *E. maxima*, and ~75,000 *E. tenella* per bird on day 22. At day 28, lesion scores were evaluated in 3 birds per replicate and blood was collected for FITC-d analysis. Excreta was collected on day 14, 21, 28, and 29 to evaluate oocyst shedding. Birds and feed were weighed on day of test 0, 14, 28, and 42 to calculate performance metrics. Performance data were subjected to an ANOVA and means were separated using LSD procedure at a 95% confidence interval.ESQ in the starter phase had a lower feed conversion than the unvaccinated challenged control on day 14 ($P < 0.05$). By day 28, the broilers were experiencing the peak intestinal disturbance from the *Eimeria* infection. During the 14-29 day interval, vaccinated groups had greater body weight gain than the unvaccinated challenged control ($P < 0.05$). Vaccinated groups had lower oocyst output, gross lesions, and serum FITC-d compared to the unvaccinated challenged control($P < 0.05$). Groups receiving ESQ maintained greater feed intake than the unvaccinated challenged control during the grower phase ($P < 0.05$), with the vaccinated control statistically intermediate to these treatments. Overall (0 to 42 days), the group which received ESQ through all phases had greater body weight gain (2.706^{b}) compared to the vaccinated control (2.625^{c}). ESQ in the grower and finisher diet was intermediate to these groups(2.670^{bc}).

Keywords: Phytogenic, Eimeria, Coccidia, Enteric, Clay

289 Characterizing the state of the gut microbiota of chicks in commercial settings

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The gut microbiota performs a number of important functions for the host. An underdeveloped gut community or host intestinal system might result in an under-optimal performing gut community. Furthermore, the state of the gut community and host intestinal system affect the potential of gut pathogens to infect the host, and the ability of probiotic strains to colonize the host and provide a beneficial effect. To understand the ecological state of the gut of newly hatched chicks, we exposed newly hatched broiler chicks to bacteria from different sources. One group was given adult hen cecum content which include a wide variety of chicken adapted bacteria. A second group was given mouse cecum content which includes diverse xeno bacteria. A third group was given both and a fourth group was an untreated control. After a week we characterized gut community composition by 16S rRNA sequencing and determined which input bacteria were able to colonize and which bacteria were pushed out by the incoming bacteria. Most of the bacterial amplicon sequence variants (ASVs) of a broad phylogeny found in the input hen sample were able to colonize newly hatched chicks in a gut segment specific manner. Specifically, the cecum community was completely transformed while the jejunum community was only moderately changed (T-test, $P<0.05$). In contrast, only 3 mouse ASVs were able to colonize newly hatched chicks, and only when chicks were treated with the mouse cecum content alone. Interestingly, treatment with hen cecum content resulted in the expulsion of less competitive Lachnospirales and Oscillospriales ASVs and in a change of the bacterial community from Enterobacterales to Burkholderials. Our results show that the intestinal tract of newly hatched chicks is mature enough to support a much more developed gut community and that the delayed development of the gut microbiota is the result of lack of exposure to adult bacteria, likely because of the practice of growing chicks in the absence of adults. Our results also show that even in the relatively empty gut of newly hatched chicks, competition is enough to inhibit most xeno derived mouse bacteria, and that exposure to hen cecum content increases competition substantially. These results also highlight the advantage of the use of host adapted bacteria as probiotics over non-adapted strains.

Keywords: Gut microbiota, colonization potential, adapted and xeno bacteria, broiler chicks, bacterial competition

290 Recent advances in mineral nutrition and trends in their supplementation for productivity, health, and sustainability

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Minerals are involved in various metabolic processes, it is clear that mineral nutrition plays a crucial role in the growth, development, and overall health of poultry. Recent advancements in poultry nutrition have focused on optimizing mineral supplementation for improved productivity, health, and disease resistance. This review aims to summarize the current trends and recent developments in the field, highlighting the importance of trace and macro minerals such as calcium, phosphorus, magnesium, zinc, manganese, iron, copper, and selenium in broilers, broiler breeders, layers, and turkey. Emphasis is placed on the balance between mineral bioavailability and interaction with other nutrients, as well as the advancements in mineral forms, including organic and inorganic minerals, which have demonstrated improved absorption and utilization. The minerals in a poultry diet, like most nutrients, must be absorbed across the gastrointestinal mucosa and enter the blood if it is to be of value to the animal. Once absorbed, these minerals are used in many ways by the animal's body. These minerals may play integral roles as cofactors of enzymes involved in controlling free radical within the body and are considered vital to antioxidants' capabilities. Moreover, new research has highlighted the role of minerals in immunomodulation and stress response, contributing to enhanced disease resistance and performance under challenging conditions. The use of precision feeding strategies and the development of more efficient mineral supplementation methods, including the use of in-feed premixes and the incorporation of minerals into functional feeds, are discussed. Challenges in mineral deficiency and toxicity, as well as the environmental impact of mineral excretion, are also addressed. Overall, continued innovation in mineral nutrition strategies is essential to meet the evolving demands of the poultry industry, ensuring sustainable production and animal welfare.

Keywords: mineral nutrition, poultry, Sustainability, bioavailability, supplementation

291 Comparing the efficacy of different organic zinc sources on growth performance of broiler chickens

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Organic minerals are those bonded to one or more carbon-containing molecules to increase their bioavailability. Most common ligands are proteinates, amino acids and small peptides. Not all organic minerals are equivalent, their quality depending on strength and stability of the bond with the ligand, but also on the type of ligand as it's defining its absorption pathway and efficiency. This study aimed to compare different organic Zn sources effectiveness on broiler chickens performance. The feeding trial was carried out at the Federal University of Viçosa, Brazil. A total of 2000 one-day-old male Cobb 500 chicks of 50 g initial body weight were weighed individually and by pen for equal weight distribution and placed into 24 pens. Chickens were assigned to 10 treatments with 10 replicates (20 birds/pen) and raised for 42 days (factorial design 5 x 2; 5 Zn sources vs 2 levels). The nutritional program consisted of 2 diets: 1–21 days and 21-42 days, formulated to meet or exceed the nutritional requirements for broilers according to Rostagno et al., 2017. Treatments differed by the mineral source and by Zn level (40 or 80 mg/kg). Four types of organic Zn were used: Zn Proteinate, Zn Glycine, Zn Methionine, Zn amino acids chelate and one inorganic form, Zn sulfate. Chickens had ad libitum access to water and feed in mash form. Performance parameters were recorded 1-21, 21-42, and 1-42 days by pen. Production efficiency index calculated as follows: PEI= (weight gain x survival) / (age of slaughter in days x FCR) x 100. Statistical analysis performed using PROC GLM of SAS software, by comparing the different Zn sources by Two-way ANOVA, Duncan's test, $p<0.10$. Broiler chickens fed Zn amino acids chelate had most efficient FCR in both 40 and 80mg/kg feed levels, optimized by -2 pts and -5 pts, respectively (Zn 80mg, $p=0.0346$). Likewise, chickens fed Zn amino acids chelate had higher weight gain and thus higher final weight at 42 days, +42g and +57g, respectively (Zn 80mg, $p=0.0936$), compared to other sources. Feed intake was similar for all Zn sources except for Zn-Methionine which increased intake (+170g) and higher weight but worse FCR at Zn 40mg/kg level. These results highlight the differences in terms of efficacy of different Zn sources (inorganic and organic) and even within organic sources, variability is observed. It emphasizes the importance of careful evaluations of growth performance and economic feasibility for decision-making in animal nutrition strategies.

Keywords: Organic minerals, Feed conversion, Weight gain

292 Estimating dietary vitamin E needs of broilers

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Two experiments were conducted to assess vitamin E responses in Cobb 500 male broilers. In Experiment 1, 540 vent sexed chicks were distributed across 45 floor pens and fed 1 of 5 dietary treatments (9 replicate pens each) from 1 to 14, 15 to 28, and 29 to 42 d of age consisting of either vitamin E or Cabanin (a vitamin E replacement compound). Treatments across phases represented: 1) 10 IU vitamin E; 2) 10 IU vitamin E with a half dose (20-40 g/ton) of Cabanin; 3) 10 IU vitamin E with a full dose (40-80 g/ton) of Cabanin; 4) 20 to 30 IU vitamin E with a half dose (20-40 g/ton) of Cabanin; and 5) 30 to 50 IU vitamin E with a full dose (40-80 g/ton) of Cabanin. Post ANOVA and t test, overall (1 to 42 d) live performance did not differ ($P > 0.05$) among treatments. Day 42 processing yields, and Pectoralis major color, pH, and drip loss did not differ among treatments ($P > 0.05$). However, the myopathy spaghetti breast was lowest in birds fed treatment 1 compared to treatment 5 ($P < 0.05$), with other treatments being intermediate. As a result of few differences between 10 and 50 IU vitamin E, a second experiment was conducted to assess broiler responsiveness up to 30 IU vitamin E. In Experiment 2, 576 vent sexed chicks were distributed across 48 floor pens and fed vitamin E doses from 1 to 18 d of age of 0 to 30 IU vitamin E in 5 IU doses (6 replicate pens per treatment). An additional treatment represented a vitamin E replacement of Cabanin (80 g/ton) and 10 IU vitamin E. No differences occurred for 1 to 18 d BW gain, feed intake, or livability. However, feed conversion corrected for the weight of mortality and culls decreased linearly ($P = 0.054$) as dietary

vitamin E increased. Regarding vitamin E replacement, birds fed 10 IU vitamin E with Cabanin compared to birds fed 10 IU vitamin E tended to be more efficient: feed conversion of 1.23 vs 1.25 (P = 0.013) and corrected feed conversion of 1.22 vs 1.24 (P = 0.160). Although few broiler responses were observed from increasing vitamin E from 10 to 50 IU (Experiment 1) or 0 to 30 IU (Experiment 2), further assessment of dietary vitamin E needs should encompass levels above 30 IU.

Keywords: Vitamin E, broiler

293 Different dietary sources and inclusion levels of copper modify microbiome profile, explaining performance differences

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Copper (Cu) supplementation above nutritional requirements has improved broiler performance, showing significant changes in microbial population. The study evaluated Cu sources and levels on performance, and microbiota abundance. Cu sources tested were Sulfate, Tribasic Cu Chloride (TBCC) and Cu Methionine-Hydroxy-Analogue Chelate (MHAC). A total of 3,240 Ross 708 male broilers were assigned to 120 floor pens with used litter across 6 dietary treatments with different sources and inclusion level: negative control (NC, no added Cu), Cu sulfate ($CuSO_4$, 125 ppm), TBCC (TBCC125, 125 ppm), MHAC (MHAC30, 30 ppm), TBCC step-down (TBCC-SD, 125 ppm d0-14; 100 ppm d15-28; 75 ppm d29-43; 0 ppm d44-56), and MHAC step-down, (MHAC-SD, 30 ppm d0-14; 24 ppm d15-28; 18 ppm d29-43; 0 ppm d44-56). Broilers were housed on used litter and administered a 5X dose of coccidiosis vaccine on d0. Ileal content of 1 bird/pen were collected on d14 and d28 (15 replicate pens/treatment) for microbial profiling via 16S rRNA sequencing. Alpha diversity was assessed using Chao1 (species richness), Shannon (richness and evenness), and Simpson (community dominance), with higher Chao1/Shannon and lower Simpson values indicating greater diversity. Beta diversity was evaluated using Bray-Curtis and Euclidean distances, with treatment differences assessed via PERMANOVA ($p<0.10$). Wilcoxon tests were used for treatment comparisons, with significance set at $p<0.10$. Differential relative abundances at the phylum, family, and genus levels were determined using ANCOMBC2, and the taxa that differed significantly based on Kruskal-Wallis tests were further evaluated for the effect size using LEfSe. For differential abundance analyses, significance was determined at $Padj<0.10$. All analyses were performed using specialist R scripts. At d14, there were no significant differences in alpha or beta diversity between MHAC30 and TBCC125 ($p<0.10$). By d28, MHAC30 tended to increase family alpha diversity (Chao1; $p<0.10$) compared to TBCC125, suggesting greater family richness, though no differences were observed at the genus level. MHAC30 also increased the abundance *Nocardiopsaceae* and *Corynebacterium.* Both abundance of *Nocardiopsaceae* and *Corynebacterium*. Both MHAC-SD and TBCC-SD benefited from step-down programs that started at d28, where the MHAC-SD substantially impacted overall community structure and differential abundance. Notably, it increased the abundance of *Candidatus* A*rthromitus* (segmented filamentous bacteria) and *Clostridiaceae* compared to the TBCC-SD. These findings suggest that in birds challenged with 5X dose of coccidiosis vaccine, the MHAC-SD promotes a microbiome composition with greater abundance of known beneficial bacteria.

Keywords: broiler, copper, microbiome, coccidiosis

294 Evaluation of a digestible calcium and phosphorous system formulated with different limestones and phytase matrix values on performance and bone mineralization

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To date, Ca is formulated in poultry diets on a total Ca (tCa) basis. Recent work has elucidated standardized ileal digestible Ca (dCa) coefficients for many commercial feed ingredients and contributions from phytase enzymes, thus providing an opportunity to formulate broiler diets on a digestible Ca basis. To evaluate the potential advantage of formulating on a dCa basis, a 2x2x2 factorial arrangement of two Ca and P systems, two limestones (LS) with different chemical and physical characteristics and two matrix values applied to the phytase was used. The two Ca and P systems tested were digestible Ca (dCa) and digestible P (dP) (Angel et al., 2022) and tCa and available P (aP) (Aviagen, 2022). The LS tested differed in characteristics mainly solubility (medium and fast solubility), contributing to different dCa coefficients when using the Ca digestibility prediction equation version 1, 12/2023 (Angel et al., 2023). The matrix values assigned to the phytase were either a static contribution of tCa or dCa and dP or aP irrespective of LS, or a predicted contribution of these nutrients based on the LS utilized. Male Ross 308 broiler chicks (n=5,280) were placed at day-old in 96 floor pens and fed treatment diets to 32 d of age Performance was measured at the end of each dietary phase, being d 10, 16, 24, and 32. Toe ash (%) was recorded on d 10 and expressed on a DM basis. Feed cost was used to calculate the cost of production ($ cents/kg broiler) using feed ingredient prices December 2024. Data were analyzed with JMP 17.0 (JMP, 2022) using the fit model platform where any main effects and interactions were determined. When the model was significant, means were separated using a protected Tukey HSD (Tukey, 1949) with significance defined at $P \leq 0.05$. Where no interactions were present, main effects were reported. During all phases, a main effect ($P < 0.05$) of feeding diets formulated on a dCa and dP basis compared to a tCa and aP for FCR was observed**.** Diets formulated using dCa and dP versus formulating using tCa and aP increased ($P<0.05$) d 32 BW (1945.21 vs. 1919.86 g, respectively). There were no interactions present for the toe ash, however there was an improvement ($P<0.05$) in toe ash when utilizing a dCa and dP system (14.82 vs 14.48 %), a medium solubilizing LS (14.87 vs 14.43 %) and a predicted matrix (14.78 vs 14.52 %) for phytase. The use of a slow-soluble LS or the dCa and dP system decreased ($P<0.01$) production cost (46.00 vs 46.50 and 45.85 vs 46.64$c/kg, respectively). The application of a dCa and dP system in feed formulation together with the consideration of LS quality and the subsequent impact on phytase efficacy shows promise to support broiler performance and skeletal integrity.

Keywords: Calcium, Broilers, Mineralization, Phytase

295 The effects of zinc source and dose in diets supplemented with a high dose of phytase on performance and mineral excretion in broilers to 37 days of age

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The inclusion of high doses of phytase enzymes has been shown to increase P digestibility and reduce undigested phytate in the small intestine. Since phytate chelates zinc, reducing its availability, high phytase can potentially also alter the broiler response to supplemental Zn. The objective of the study was to evaluate the effect of supplemental Zn from two sources on Zn excretion, and tibia Zn content in broilers when diets contained 2000 FTU phytase. Day-old Ross 308 chicks (n=5760) were allocated to 84 floor pens in a complete randomized block design, utilizing 7 treatments with 12 replicate pens per treatment. A corn-soy basal diet (T1) was formulated to meet nutrient requirements of broilers in five phases to 37d of age and with remaining treatments being T1 supplemented with 30, 60 or 90ppm Zn from either Zn-sulphate (ZnS) or Zn-amino acid (Zn-AA) complex. All diets contained phytase included at 2,000 FTU/kg feed. Data were analyzed in JMP 16.0 (JMP, 2022) using one-way ANOVA, regression analysis, and slope ratio analysis. Where appropriate, means were separated using a protected Tukey HSD test at $P < 0.05$. There were significant linear and quadratic effects of supplemental Zn from either source on broiler body weight (BW) and mortality corrected feed conversion ratio (mFCR) at 24d of age. In contrast, there was no response to supplemental ZnS compared to T1 at 30 or 37d. Broilers fed diets containing 60ppm Zn-AA produced heavier ($P < 0.05$) BW compared to those fed T1 or 60ppm ZnS on d10, 24, 30, and 37. Additionally, 60ppm Zn-AA yielded the lowest ($P < 0.05$) mFCR on d37 (1.303) compared to both T1(1.356) and 60ppm ZnS (1.336). A quadratic response ($P < 0.01$) was noted for Zn-AA, with a plateau in BW and feed conversion ratio (mFCR) occurring when broilers were supplemented with 60ppm Zn-AA on d37. Tibia Zn was increased with supplemental Zn from either source in a step-wise manner. A slope ratio analysis of tibia Zn content indicated that the relative biological availability of Zn-AA was 1.25 times that of ZnS. The excretion of Zn increased linearly ($P < 0.001$) with Zn dose from both Zn sources, with the highest Zn excretion observed at 90 ppm for ZnS (100.20 mg/kg BW) and Zn-AA (98.16 mg/kg BW). In conclusion, the response of broilers to supplemental Zn differed significantly between Zn sources. There was no benefit of supplemental ZnS on broiler performance at 30d and 37d, while supplemental Zn from Zn-AA significantly improved BW and mFCR compared to ZnS with an optimum of 60ppm.

Keywords: Broiler, Zinc, Requirement, phytase

296 Evaluation of different trace mineral sources in commercial egg production

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To evaluate the performance of laying hens fed different trace mineral sources during five different production phases, an experiment was conducted using 1,080 Lohmann LSL hens at 21 weeks of age. The birds were housed in a natural environment facility with California-type cages. The hens were distributed in a completely randomized design with four treatments, each with 13 replicates of 20 hens. Diets were formulated based on yellow corn, soybean meal, dried distillers' grains, and vegetable oil. Energy and amino acid levels were calculated according to Rostagno (2016) recommendation. The treatments were: T1: Positive control (inorganic); T2: IntelliOpt 50% (organic and hydroxy); T3: IntelliOpt 100% (organic and hydroxy); T4: IntelliBond (hydroxy). Weekly, data on laying percentage, average egg weight, feed intake per hen per day, feed conversion ratio, egg mass per hen per day, mortality, and the percentage of dirty and broken eggs were recorded. At the end of the trial, cumulative mortality rate, the number and weight of eggs per housed hen, and overall feed conversion were calculated. At weeks 31, 42, 52, 65, and 83, 120 eggs per treatment were collected to evaluate egg quality (shell resistance and internal quality measurement). Additionally, eight hens per replicate were weighed to assess body weight gain or loss and flock uniformity. At the end of the study, four fecal samples per treatment were collected and analyzed in a laboratory for calcium, phosphorus, zinc, manganese, and copper content. The data were analyzed using a completely randomized design with a factorial arrangement, where the first factor was the different treatments, and the second factor was the trial weeks. Mean comparisons were performed using Tukey's multiple comparison test ($p<0.05$). Results obtained over the 62-week trial period showed that the best egg production was observed in T2 (93.99%), the highest egg weight in T3 (60.5g), and the highest egg mass in T2 and T3 (56.8g and 56.6g, respectively). The lowest feed intake was recorded in T3 and T4 (103.2g and 103.4g, respectively), while the best feed conversion ratio was observed in T3 (1.872 kg:kg). The T3 also had the lowest percentage of broken eggs (0.52%), and T2 had the lowest percentage of dirty eggs (3.61%). Consequently, the highest number of eggs per week was recorded in T2 and T4 (6.29 and 6.30 eggs, respectively) ($p<0.001$). Furthermore, lower excretion levels of zinc (801.6 ppm), manganese (336.1 ppm), and copper (48.1 ppm) were observed in T2. It can be concluded that the addition of IntelliOpt Concept and IntelliOpt 50% in a commercial laying hen production cycle improved performance parameters compared to an inorganic trace mineral source.

Keywords: organic minerals, hydroxychloride minerals, inorganic minerals, laying hens, production parameters.

297 Utilizing a dynamic nutrient system for precision poultry nutrition and performance optimization

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Accurate feed formulation is essential for optimizing poultry production efficiency, yet traditional static nutrient systems often fail to account for ingredient variability, leading to inefficiencies in performance and economic returns. This presentation evaluates the impact of dynamic nutrient modeling and real-time data integration on improving feed formulation accuracy, cost management, and bird performance. A dynamic nutrient evaluation framework was implemented, integrating real-time ingredient analysis, predictive equations, and advanced data modeling. Unlike static approaches that rely on periodic updates, dynamic systems incorporate continuous nutrient assessments to refine estimates of digestible amino acids, metabolizable energy, and retainable phosphorus. Nutrient variability was assessed using near-infrared reflectance spectroscopy (NIR) and laboratory analysis across multiple ingredient sources. Predictive models were applied to estimate real-time nutrient values, enabling formulation adjustments. The impact of dynamic versus static systems on feed cost efficiency and key performance metrics, such as feed conversion ratio (FCR) and weight gain, was analyzed. The use of dynamic nutrient models improved ingredient characterization, reducing feed formulation costs. Additionally, real-time ingredient digestibility assessments support more informed ingredient supplier selection, ingredient quality and cost optimization strategies. The integration of real-time data analytics and dynamic nutrient modeling in poultry feed formulation significantly enhances production efficiency by addressing ingredient variability and optimizing dietary precision. Transitioning from static to adaptive nutritional strategies presents a valuable opportunity to improve economic and performance outcomes in commercial poultry operations.

Keywords: poultry nutrition, dynamic formulation, feed cost optimization, nutrient variability, precision feeding

298 Use of powder or granulated calcium carbonate in the diet of starter chicks on the growth performance and tibia characteristics

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The objective of the study was to evaluate the productive responses, measurements, dry matter and ashes content and breaking strength of the tibia and weight of the digestive organs in starter chicks fed diets added with two types of calcium (Ca), powder (Pow) or granulated (Gra), to cover the Ca recommendations. Two experiments were done with male Ross 308 chicks, from 5 to 21 days, housed in cages in pairs. In Exp. 1, there were 90 cages assigned to three diets: 1) a control diet with levels of Ca and available phosphorus (avP) based on strain recommendations and the use of powder Ca carbonate (PowCa); 2) the same diet with 50% PowCa and 50% GraCa with a particle size of 3 mm; 3) the same diet with 100% GraCa. At 12 days of age, half of the chicks received a 10x oral dose of a coccidia (cocc) vaccine. At day 21, performance variables were registered, and tibia were obtained for measurements of diaphysis and epiphysis length and thickness; dry matter (DM) and ashes content were determined. In Exp. 2, there were 60 cages assigned to two diets: 1) a control diet with Ca and avP based on strain recommendations and the use of PowCa carbonate and 2) the same diet with 100% GraCa with a particle size of 3 mm. At day 21, performance variables were registered, and tibia were obtained for measurements and breaking strength determinations. The proventriculus, gizzard, small intestine, liver and pancreas were removed and weighed. Results were subjected to ANOVA. In Exp. 2 chicks in each pen were classified as light or heavy and the effect of body size was added to the analysis. In Exp. 1, the final body weight (BW) and weight gain (WG) were lower and the feed conversion ratio (FCR) was higher in non-challenged cocc chicks receiving 100% GraCa; the rest of the treatments had similar responses (type of Ca and cocc challenge, $P < 0.05$). The DM of the tibia was higher ($P < 0.05$) in broilers receiving 100% GraCa. In Exp. 2, the final BW and WG were higher and the FCR was lower in chicks receiving GraCa ($P < 0.01$). The diameter of the diaphysis was higher in heavy chicks added with GraCa while the other treatments had similar values (type of Ca and body size, $P < 0.01$). In light chicks, the BW, the tibia weight, length, diameter of diaphysis and breaking strength, as well as the weight of the gizzard, small intestine and pancreas were lower ($P < 0.05$) compared to heavy chicks. In conclusion, the results indicate that in Exp. 1 the productivity decreased but in Exp. 2 the productivity was enhanced with GraCa; no large changes in tibia mineralization were observed with the addition of GraCa. It was not clear whether there is a benefit to using GraCa in the diet of starter chickens.

Keywords: Broiler chickens, Granulated calcium, Productivity, Tibia characteristics

299 Effects of the level of calcium of the pre-lay diet on egg production and egg quality of white laying hens from 17 to 38 weeks of age

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The objective of this research was to study the effects of feeding a pre-lay diet, containing 2.4 vs. 3.7% Ca, from 17 to 20 wk of age, followed by a commercial diet (3.7% Ca and 0.34% digestible P), on performance and egg quality traits of Lohmann LSL Classic white laying hens from 21 to 38 wk of age. A deficiency in Ca might result in poor bone mineralization whereas an excess might affect feed intake (FI) and egg weight. Consequently, inadequate levels of Ca in the pre-lay phase, might affect bone strength and shell quality for the whole egg production cycle. In practice, most of available guidelines for laying hens recommend the use of a pre-lay diet with 2.0 to 2.5% Ca, from 16 wk of age until reaching 5 to 15% egg production, a strategy that complicates the logistics of the feed supply chain and results often, in a high percentage of hens laying eggs while consuming less Ca than needed which lead to an increase in the utilization of medullar bone. The experimental design was completely randomized with two treatments and 20 replicates of one hen each per treatment. Egg production (average of 97.5% for the last wk of the experiment), egg weight, and percentage of unsaleable eggs (dirty, broken, and shell-less) were recorded daily and FI and BW were determined by phase (pre-lay from 17 to 20 wk and laying from 21 to 38 wk of age). In addition, egg quality traits (Haugh units, shell thickness, shell strength, and percentage of shell of the eggs) were evaluated in the first three eggs produced, the first three eggs collected after 7 days of lay and then, in an egg every two subsequent wk (first egg laid in each

period). Data on BW, ADG, FI, egg production, egg weight, egg mass, FCR, and egg quality traits were analyzed by ANOVA as a completely randomized design using the MIXED procedure of SAS with dietary Ca as main effect. In addition, the number of days in which the hen no laid, and the percentage of unsaleable eggs, were analysed by phase as a binomial distribution, using the GENMOD procedure of SAS. From 17 to 20 wk of age, no significant differences between treatments were detected for any of the egg production or egg quality traits studied. From 21 to 38 wk of age, FI, egg production, egg weight, egg mass, FCR, and egg quality traits were not affected by the Ca content of the diet. However, the percentage of shell-less eggs were greater (1.52 vs. 0.13%; P < 0.001) in hens fed 2.4% Ca than in hens fed 3.7% Ca. Also, BW gain tended to be lower (1.36 vs. 1.85 g/d; P = 0.077) in hens fed the low Ca diet. In conclusion, the use of a diet containing 3.7% Ca from 17 to 38 wk of age, will simplify feed management without compromising hen production or egg quality traits in the period considered.

Keywords: calcium level, egg quality, egg quality, white laying hens, Pre-lay

300 Effect of DL-methionine and Methionine hydroxy analogue on the performance and gut health of aflatoxin-exposed broilers

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The effects of DL-methionine (DLMet) and methionine hydroxy analogue (MHA) on growth performance, detoxification enzyme levels and immunity in broilers exposed to aflatoxin (AFL) were investigated until 21 days of age. A total of 540 broilers were randomly assigned to 36 pens (15 birds/pen, 6 pens/treatment) and fed one of 6 treatments: controls (T1: DLMet; T2:MHA; at recommended levels, i.e 0.56% and 0.84%), T3: T1+200 µg/kg AFL, T4: T2+200 µg/kg AFL, T5: 0.2% DLMet above the recommended level + 200 µg/kg AFL; T6: 0.2% MHA above the recommended level +200 µg/kg AFL. In all corresponding treatments, DLMet was supplemented at a 65:100 ratio of MHA. One-way ANOVA was used to analyze the data, and the means were compared by Tukey's tests. Chi-squared test was used to analyze the mortality rate. The AFL-exposure did not increase mortality, which was 4% across treatments (P=0.44). At 7 days, body weight (BW) was unaffected by AFL (P=0.30), nor at 14 days (P=0.06). However, final BW was significantly (P=0.0005) lower in AFL-exposed birds (T3 to T6, mean: 743 g) compared to T1 (804 g). AFL exposure significantly increased the Feed conversion ratio (FCR) by 6 points in T3 (1.36, P=0.03) compared to T1 (1.30). Overall, using DLMet or MHA in feed resulted in similar BW, BWG and FCR between control groups (T1vs.T2) and between AFL-exposed groups (T3 vs. T4 vs. T5 vs. T6). T cell proliferation was similar between controls and AFL-exposed groups T3 and T4. However, the 0.2% additional supplementation of DLMet and MHA (T5 and T6) in AFL contaminated diets enhanced the ability to fight infection with increased T cell proliferation (P=0.0007). AFL contaminated diets increased serum AST activity (P<0.001), indicating liver and muscle damage, but these effects were reversed by the additional 0.2% DLMet and MHA (T5 and T6) supplementation. The hepatic Cytochrome p450 enzyme CYP1A1, was significantly reduced in AFL-exposed groups (P=0.0011, -0.8 folds) but the additional 0.2% increase in methionine-source increased CYP1A1 levels (+0.3 folds). Glutathione peroxidase (GSH) levels remained stable despite the presence of aflatoxin-, however the additional 0.2% MHA (T5, P=0.018) decreased the GSH. In conclusion, using DLMet at 65:100 of MHA resulted in similar growth performance to MHA-fed animals, regardless of AFL-exposure. AFL decreased growth performance compared to T1. Numerically, DLMet treatment (T1) had the best performance results. T cell proliferation and GSH were unaffected by AFL but influenced by methionine level and source, while AST activity and CYP1A1 expression were negatively impacted by AFL, with increases in methionine restoring their levels. An increase of DLMet may be recommended in AFL exposed broilers.

Keywords: methionine, broilers, aflatoxin, performance, enzymes

301 Evaluating choline sources: Impact on broiler performance and leg health in a choline deficiency model

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Choline is a vital nutrient for broiler performance and health, functioning through various metabolic pathways. It is essential for the synthesis of acetylcholine, which supports muscle and brain function. Choline also plays a critical role in bone formation, cell membrane integrity, lipid metabolism, methyl donation, and blood clotting, wich are crucial for animal welfare. Although most feed ingredients contain some amount of choline, there are significant variations in both content and bioavailability, prompting nutritionists to supplement additional choline in broiler diets. Selecting an appropriate supplemental choline source is essential to prevent performance issues, welfare concerns, and economic losses. This trial aimed to evaluate the efficacy of choline chloride compared to biocholine on broiler performance and leg skeletal structure in a 28-day dietary choline deficiency model. A total of 1,120 one-day-old male Ross 308 AP broilers were distributed by seven treatments, 16 replicates, and 10 birds per cage. Treatments were distributed in a completely randomized design using the GLM procedure of SAS, 2009. Significance was accepted at the P≤0.05 level and mean differences were separated using the Tukey's HSD test. The treatments included T1, a control diet deficient in choline; T2 to T4, three diets with increasing levels of choline chloride 60% (CC60) at 446, 894, and 1340 mg/kg of feed; and T5 to T7, three diets formulated with biocholine (BC) at the manufacturer's recommended inclusion rates of a 1:4 ratio to CC60 (112, 224, and 335 mg/kg of feed). From days 1 to 28, choline deficiency significantly reduced feed intake (FI) and body weight gain (BWG) in broilers on the control diet compared to those receiving CC60 supplementation (P<0.01). Supplementation of additional choline chloride led to substantial improvements in performance compared to control and BC diets. In contrast, broilers fed BC diets did not exhibit similar benefits, showing FI and BWG comparable to those on the deficient diet. By 28 days of age, although not statistically significant, broilers receiving higher levels of CC60 demonstrated numerically better feed conversion ratios than those fed control and BC diets. The choline deficiency significantly increased the frequency of valgus-varus deformities in broilers fed control and BC diets (P<0.05), while only the inclusion of choline as CC60 effectively reduced the occurrence of leg deformities and improved leg conformation (P<0.05). In

conclusion, biocholine was not an effective choline source compared to choline chloride for broilers. Birds supplemented with BC presented signs of choline deficiency, including reduced growth performance and increased leg deformities.

Keywords: Choline supplementation, Growth performance, Valgus-varus deformities, Nutritional deficiency, Animal welfare

302 Effect of dietary fat sources on performance, carcass yield and intestinal histomorphometry of broiler chickens

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Fat functions as an essential macronutrient that significantly impacts health, immune responses, and production performance in broilers. The quality and composition of dietary fat are inherently variable, depending on the source. This study evaluated the effects of various dietary fat sources on growth performance, carcass yield, and intestinal morphometry in broiler chickens. In this study, a total of 1,089, non-vaccinated, male, day-old Ross 708 broiler chicks were randomly allocated to dietary treatments containing a distinct fat source: soybean oil (T1), poultry fat (T2), soap stock (T3), and palm oil (T4). Birds were provided ad libitum access to feed and water, with environmental parameters (temperature, humidity, and lighting) maintained according to standard commercial guidelines. Body weight and feed intake were recorded weekly to assess average weight gain, feed intake, and feed conversion ratio (FCR). On day 42, one bird per pen was euthanized using carbon dioxide asphyxiation for necropsy. Intestinal samples, collected in formalin, were embedded and stained using hematoxylin and eosin (H&E) staining. Intestinal segments were analyzed under light microscopy for the measurement of villi height, crypt depth, and villus-to-crypt ratio. A total of 377 chickens were processed on day 43, and breast fillet, boneless thigh, drumstick, wing, and carcass yield were determined based on the cold, without-giblets (WOG) weight. Statistical analysis was performed using the PROC ANOVA procedure in SAS, with significance set at $P \leq 0.05$. The result from this study indicates that FCR and average body weight are not significantly different among treatment groups. Additionally, no significant differences were observed among treatments for their effect on parts yield, carcass yield, and intestinal morphometry ($P > 0.05$). Mean ileal crypt depths were 106.65, 91.92, 91.44, and 103.79 µm for T1, T2, T3, and T4, respectively. Jejunal crypt depths averaged 116.37, 121.32, 126.35, and 116.57 µm for T1, T2, T3, and T4, respectively. Ileal villus heights were recorded as 614.46, 657.38, 618.26, and 674.05 µm for T1, T2, T3, and T4, respectively. Parts yields ranged from 28.29% to 28.71% for breast fillets, 9.84% to 10.07% for wings, 13.29% to 13.55% for thighs, and 12.25% to 12.44% for drumsticks, with a carcass yield ranging from 76.78% to 77.79%, among treatments ($P > 0.05$). These findings indicate that the fat sources studied could be chosen interchangeably in feed formulation based on their availability and cost changes, without adversely affecting broiler performance or economic profitability.

Keywords: Carcass yield, FCR, Intestinal Morphometry, Fat, Broiler

303 Can combined dietary strategies and photoperiod management affect meat quality and the occurrence of breast muscle myopathies?

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Breast muscle myopathies (BMMs) [white striping (WS), wooden breast (WB), and spaghetti meat (SM)] pose a significant burden to broiler production. The aim of the study was to assess the effect of combined dietary strategies and photoperiod management on the occurrence of BMMs as well as on meat quality, productivity and welfare indicators. A total of 1,908 male chicks were randomly assigned to four treatment groups according to a 2x2 factorial design with "diet" and "photoperiod" as the main factors (9 pens/treatment, 53 birds/pen). The birds were subjected to either standard photoperiod (STP) (groups A and B), as per EU industry norms, or short photoperiod (SHP) with prolonged darkness (groups C and D). While groups A and C were fed a commercial control (CON) diet, groups B and D received a dietary treatment (TRT) with phytase "superdosing", 0.06% guanidinoacetate (GAA) supplementation throughout the trial, increased amino acid density in the starter phase, and -15% lysine reduction in the grower phases. Growth performance was monitored, and FPD and BMM incidence were evaluated on about 400 birds per group. Meat quality was assessed on fillets not showing any macroscopic sign of myopathies (n=15/group). Performance and meat quality data were analyzed using two-way ANOVA, considering the effects of "diet" and "photoperiod", and their interaction. Logistic regression with Pearson's chi-squared test were used to evaluate BMM and FPD data, and risk ratios were calculated. Compared to CON, TRT diet reduced the risk for WS and WB (-26% and -19.2%, respectively; $P<0.0001$), but increased the SM risk (+37.7%, $P<0.0001$). SHP reduced the SM risk (-13.7%, $P=0.0241$) compared to STP, but worsened FPD severity (+74% risk, $P<0.0001$). As for meat quality, the TRT diet negatively impacted pHu (4% lower in TRT, 5.44 vs. 5.64 in CON; $P<0.01$) and drip loss (90% higher, 2.36 vs. 1.24% in CON, $P<0.001$), but increased marinade uptake (21% higher, 10.5 vs. 8.7% in CON,$P<0.01$). SHP increased cooking loss (23.4 vs. 21.7%, $P<0.05$) with no additional effects. Compared to CON, the TRT diet increased feed conversion ratio (1.63 vs. 1.57, $P<0.001$), reduced body weight (3,845 vs. 3,930 g, $P=0.02$), and lowered breast yield by 2.35%. Mortality was lower in SHP than STP (2.4 vs. 4.2% respectively, $P<0.05$). Overall, the TRT diet effectively reduced WS and WB occurrence, but increased SM prevalence and negatively impacted meat quality and performance. SHP lowered mortality but increased FPD severity and had limited direct effect on the BMM rate. These findings provide insights into the optimization of broiler management strategies to balance productivity, welfare indicators, incidence of BMM and overall meat quality traits.

Keywords: Breast muscle myopathies, Footpad dermatitis, Meat, Photoperiod, Diet

304 Productive energy caloric values of protein and fat in soybean meal, corn, and diets for broilers

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Productive energy (**PE**) caloric values of nutrients in soybean meal (**SBM**), corn, and diets for broilers were determined with 5 experiments (**E1** to **E5**) from 1 to 56 d. E1 to E4 determined corn

PE, were fed pelleted diets in 5 feeding phases, and their diets included SBM dilutions with cellulose or corn, and levels of oil or total digestible amino acids (**TDAA**). E5 determined PE of SBM and diets, and used 96 pens assigned to 1 of 8 blocks. Pens in each block were restricted-fed one of 12 treatment mash diets for one week. E5 treatments varied in TDAA, dCP, dFA, dST, and non-starch polysaccharides (**NSP**). Fresh birds were used in each feeding phase (E1 to E4) or block (E5). Response variables: In E1 to E5, BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were recorded. Body composition and processing weights were measured with Dual Energy X-Ray Absorptiometry; fasting heat production (**FHP**) with calorimetry chambers. PE (kcal/kg) was: PE = net energy (**NE**) gain (**NEg**) + NE maintenance (**NEm**; FHP = NEm). In E5, market value gain (**MVG**; cents/bird/d; = processing weights X prices) and gross profit gain (**GPG**; cents/bird/d; = MVG – feeding cost, cents/bird/d) were also calculated. Stats: ANOVA and Tukey test compared treatments and mixed models were fitted in JMP. Results of E1 to E4: dST, dCP, and dFA explained 72.8, 20.8, and 6.4% of corn PE. Corn PE (kcal/kg) = 136.5 +80.55 dCP +65.29 dFA +35.49 dST –13.66 age –0.454 (age –31.1548) (R^2=0.62). Results of E5: dCP and TDAA explained 92% and 88% of diet NEg, 85% and 81 % of diet PE, and 96% and 96% of SBM PE, respectively. SBM PE = [504 +60 dCP +29 dFA +31 dCP/NSP (R^2=0.58; 98% accurate)] or [462 +68 TDAA +36 dFA +37 TDAA/NSP (R^2=0.57; 98% accurate)]. Diet PE = 562 +81 TDAA +35 dFA (R^2=0.80). CP, TDAA, and dFA favored BWG (P≤0.019) and FCR (P<0.014). dCP and TDAA favored protein gain (P<0.010), but dFA provided no additional predictive ability (**APA**; P=0.858). dST provided no APA for BWG, FCR, or PAC (P=0.139). PE predicted BWG, FCR (P<0.001), and PAC (P<0.017). Models for MVG or GPG, both including PE intake (kcal/bird/d) and the PE/TDAA intake ratio, showed positive signs for PE intake (P<0.001) and negative signs for PE/TDAA (P≤0.001), implying that MVG and GPG increase when PE comes from amino acids. Overall results: PE caloric coefficients (kcals predicted per g of nutrient) were obtained. Corn: 7.5, 6.1, and 3.2 kcal per g of CP, FA, and starch, respectively. SBM: 7.9, 7.3, and 3.9 kcal per g of TDAA, dCP, and dFA, respectively. Diet: 10.5, 9.6, and 4.7 kcal per g of TDAA, dCP, and dFA, respectively. In conclusion, amino acids are the main PE driver. Protein calories are more valuable than other nutrients for meat gain, carcass value, and profit.

Keywords: Productive energy, Caloric value, Soybean meal, Broilers, Protein calories

305 Influence of the origin of the soybeans on the chemical composition and protein quality indicators of soybean meals arriving to European ports

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We studied the chemical composition and protein quality of 27 soybean meals (SBM) from Argentina (ARG), Brazil (BRA), and USA in equal number, collected at random at European feed mills in 2024. The meals were analyzed by wet chemistry for proximal components, amino acids (AA), oligosaccharides, neutral detergent fiber (NDF), non-starch polysaccharides (NSP), and minerals. Also, urease activity (UA), KOH solubility (KOH), protein dispersibility index (PDI), and trypsin inhibitor activity (TIA) of the samples were determined. The data were analyzed as a completely randomized design using ANOVA, with SBM origin as main effect. In addition, Pearson correlation (r) analyses were conducted to determine the relationships between all the variables studied. On 88% DM basis, no differences among SBM origins were detected for CP (46.1 ± 0.58%), but, Lys (6.14 vs. 5.99 vs. 6.07%; $P < 0.05$), TSAA (2.74 vs. 2.65 vs. 2.69%; $P < 0.01$), and the sum of Lys, Met, Cys, Thr, and Trp (14.1 vs. 13.9 vs. 14.0%; $P < 0.01$) were higher, per unit of protein, for the USA meals than for the BRA meals with the ARG meals being intermediate. Ether extract was higher for the ARG than for the USA meals with the BRA meals being intermediate (1.93 vs 1.22 vs. 1.76%; $P < 0.05$). Sucrose (6.75 and 6.60 vs. 5.67%; $P < 0.05$) and stachyose (4.57 and 4.75 vs. 4.13%; $P < 0.05$) contents were higher for the ARG and USA meals than for the BRA meals. Crude fiber (5.31 vs. 4.49 vs. 5.16%) and NDF (11.1 vs. 9.20 vs. 10.7%) were higher ($P < 0.05$) for the USA meals than for the ARG meals with the BRA meals being intermediate. Also, total (15.4 and 15.2 vs. 14.3%; $P < 0.01$) and insoluble (11.9 and 11.7 vs. 11.0%; $P < 0.01$) NSP contents were higher for the BRA and USA meals than for the ARG meals. The P content was higher ($P < 0.05$) for the ARG meals than for the BRA and USA meals. All protein quality indicators, except for UA that was similar for all countries, were higher for the USA meals than for the South American meals ($P < 0.05$). Independently of the origin, significant correlations were observed among the traits studied. Per unit of protein, Lys, Cys, and the sum of Lys, Met, Cys, and Thr were correlated ($P < 0.01$) with PDI (r = 0.638, 0.630, and 0.662, respectively) and KOH (r = 0.701, 0.543, and 0.643, respectively). TIA values were correlated ($P < 0.001$) with KOH (r = 0.694) and PDI (r = 0.810), but not correlation was detected with UA ($P > 0.05$). In summary, the chemical composition and protein quality indicators, except UA, of SBM vary with the country of origin of the SBM. These indicators are useful tools for assessing the nutritional value of SBM, except for UA, which does not seem to be useful in the case of well-processed meals.

Keywords: amino acid profile, carbohydrate fraction, protein quality indicators, proximal analysis, soybean meal origin

306 Influence of the use of tannins on the performance, egg quality and intestinal morphometry of layers

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Tannins are a group of polyphenolic compounds that are considered secondary metabolites found in plant vacuoles. Some tannins can have positive effects on lipid peroxidation and serum biochemistry. The effects of including hydrolyzed tannin from Portuguese chestnut (Castanha Sativa) and Quebracho condensate (Schinopsis Lorentzii) on feed of laying hen on productive performance, egg quality, serum biochemistry, antioxidant activity in yolk and liver, organ weight, and intestinal morphometry were evaluated. A completely randomized design was used, with 5 diets: Negative control (NC) (basal feed); Treatment 2 - Positive control (PC) (basal feed + virginamycin); Treatment 3 - (0.5 TanH) basal feed + 0.5 kg/ton of TanH; Treatment 4 - (1.0 TanH) basal feed + 1 kg/ton of TanH; Treatment 5 - (1.0TanC) basal feed + 1 kg/ton of TanC, 10 replicates of 6 Hy-line W36 birds aged 76 weeks. Productive performance (feed consumption, feed conversion, egg mass), egg quality, blood biochemical profile, organ weight, resistance to shell breakage, and intestinal histology were analyzed

over four 28-day cycles. The data were submitted for Tukey test using the Statistical Analysis System program and were considered significant when P≤0.05. Tannins had no significant effect (P>0.05) on performance and egg quality variables. The tannin addition had no influence on eggshell strength (shell hardness and fracturability) (P>0.05). The inclusion of 1.0TanH tannins increased the levels of cholesterol, albumin, triglycerides, and total proteins (P<0.05). Albumin and triglyceride levels decreased in the blood of birds with negative control (P<0.05). There was a decrease in the total protein content with the addition of 1.0 TanC (P<0.05). The inclusion of 1.0TanH reduced lipid peroxidation in the yolk and liver (P<0.05) and the negative control obtained the highest average concentration of malonaldehyde (MDA) in the yolk and liver tissue. There was no effect of the treatments on the height, width, and area of the villi, crypt depth and villus:crypt ratio of the birds. There was also no significant effect on the relative weights of the organs and the length of the intestine (P>0.05). Under the conditions of this experiment, we concluded that the inclusion of 1.0TanH reduces lipid peroxidation in the yolk and liver and improves the serum biochemistry of the layers without negative effects on performance and egg quality Hy-line W36 between 76-91 weeks of age.

Keywords: antibiotics, antioxidant, natural products, condensed tannin, hydrolyzed tannin

307 Chemical composition, nutritional value, and physiological effects of alkaline hydrolyzed feather meal in Japanese quail

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The nutritional, physiological, and chemical properties of alkaline hydrolyzed feather meal (AHFM) were studies. AHFM, which was chemically hydrolyzed with NaOH, was analyzed and compared to 3 commercially available feather meals. Then, a total of 250 ten-day-old Japanese quail were allocated randomly into 5 treatments receiving 0%, 2%, 4%, 6%, and 8% AHFM with 5 replications of 10 birds each cage to evaluate the performance, organ weights, and some blood parameters. Data were analyzed using SAS software, and Duncan test to separate means at 0.05 level of significance in a completely randomized design. Two birds per cage were euthanized for sampling (n=10/treatment). AHFM yielded a significantly higher in-vitro pepsin digestibility of protein (P < 0.05). 8% AHFM group showed lower feed intake for the entire period higher cumulative FCR (P < 0.05). The internal organ weight data reveals significant differences in the relative weights of the gizzard, intestine, liver, and heart (P < 0.05). The relative weights of the gizzard and intestine showed an increase with increase in AHFM level (P < 0.05). While the difference in carcass weight among the groups receiving AHFM was not statistically significant, a gradual decline in carcass weight was observed with higher levels of AHFM in the diet compared to the control group. No significant effects of AHFM inclusion were observed on blood globulin, albumin-to-globulin ratio, glucose, or hematocrit levels (P > 0.05). However, the 8% AHFM group showed significant differences in total protein, albumin, uric acid, calcium, and triglyceride concentrations compared to the control (P < 0.05). Although ileal villus height, thickness, crypt depth, and the villus height-to-crypt depth ratio increased with higher treatment levels, none of such differences were statistically significant (P > 0.05). Villus height and the villus height-to-crypt depth ratio were significantly affected by increasing levels of AHFM (P < 0.05). Additionally, varying levels of AHFM had no significant effect on any of the evaluated tibial traits (P > 0.05). Increasing levels of alkaline-hydrolyzed feather meal in the diet significantly influenced meat malondialdehyde (MDA) content (P < 0.05), in which the lowest level was recorded in the group receiving 8% AHFM. In conclusion, while AHFM demonstrated higher in-vitro digestibility and influenced some physiological and biochemical parameters, its inclusion at higher levels resulted in lower feed intake, and higher FCR, indicating that optimal inclusion levels should be carefully considered for balanced growth performance.

Keywords: Feather meal, processing, chemical composition, digestibility, Japanese quail

308 Effects of different dietary forage sources and quantitative feed restriction on carcass traits and meat quality attributes of native Bangladeshi geese

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The present study was designed to assess the efficacy of different fodder varieties named as, Spinach (*Spinacia Oleracea*), Cabbage (Brassica Oleracea var. Capitata), Oats (*Avena sativa*) fodder as a supplementation along with concentrate feed for determining carcass characteristics, meat color and texture, pH, drip loss, cooking loss, and water holding capacity (WHC) of Bangladeshi native geese. A total of 60 goslings were reared in a semi-intensive production system in the Bangladesh Livestock Research Institute, Savar. They were randomly divided into 4 groups with 3 replicates having 5 goslings in each replication. Maize soya-based diet was considered as the basal diet (2750 ME and 17% CP). At an early age, birds were given feed as 10 grams, then gradually increasing upto 200 grams, and marked as T_0- 200g concentrated feed, T_1= 160 g Basal diet+ 276 g oats fodder, T_2= 160 g Basal diet + 276 g cabbage, T_3 = 160 g Basal diet+ 276 g spinach daily. After 3 months of rearing those birds were slaughtered for meat quality analysis at 13 weeks of age. A calibrated pH meter used to measure the pH values of the breast and thigh meat 24 hours of slaughter. Meat color was determined in triplicate using a Chromo meter CR-8, (3nh, China) and texture by using a texture analyzer (Tx-700, France). The data were statistically analyzed by one-way analysis of variance (ANOVA) through the use of SPSS software (Ver. 23.0). Differences among groups were compared using the Duncan's method. The results were presented as means with their pooled standard errors. Treatment had a remarkable effect on live weight and total muscle weight, breast meat drip loss, WHC, and pH after 24 hours (p < .05). In case of meat color attributes, results showed that breast meat saturation index and thigh meat hue angle significantly varied among different groups (p<.05). Results also disclosed that, in case of beast mucle texture analysis- force minimum, fracture, force maximum, cohesiveness and gumminess values were significantly varied, on the other hand in case of thigh meat all the above values, as well as springiness and chewiness values, were also significantly varied among different treatment groups. From all the values we found, satisfactory results in all cases were found in 2 groups: that's were cabbage and oats supplemented group. We may conclude supplementation of native Bangladeshi adult geese with 160 g of concentrate feed + 276 g of

oats fodder or cabbage daily was found to be beneficial for improving their meat quality.

Keywords: Geese, Oats fodder, Meat quality, Restricted feeding, Cabbage

309 Not Presented

310 Effects of breeder genetic line and age on specific gravity and translucency

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Specific gravity (SG) is a standard method for evaluating eggshell quality; however, the combined influence of breeder age by genetic line on SG remains unclear. This study evaluated the effects of breeder age on SG and eggshell properties on two genetic lines. Eggs were collected from a Cobb breeder flock at 35 (N = 112) and 48-wk (N = 115) and from a Ross breeder flock at 35 (N=112), 48 (N=115), and 56-wk (N=103) and stored at 19 ± 1°C, for 6 days. The SG was obtained with saltwater solutions. Eggshell thickness was measured using an ultrasonic gauge. Egg width and length were measured using an electronic caliper, and eggshell translucency scores (TS) were determined with the BlueBox®. The data were analyzed using contingency and regression analyses. The average egg weights for Cobb were 61.6 and 69.9 ± 0.4 g at 35 and 48 wk, respectively. For Ross, the average egg weights were 59.4 ± 0.5, 66.7 ± 0.4, and 70.6 ± 0.4 g at the evaluated periods. Results indicated that 93.5% of eggs from 35-wk-old Cobb breeders had SG between 1.075 and 1.085 on the first d of storage; however, SG distribution changed daily ($P < 0.001$) to 86.1% between 1.075 and 1.060 by day 6. For 48-wk-old Cobb breeders, the distribution for day 1 was similar, with 95.5% of the eggs distributed between 1.075 and 1.085. However, by day 6, 99.1% of eggs were distributed between 1.060 and 1.075. At 35 weeks, 90.4% of Ross eggs had a SG between 1.075 and 1.085, and 3.2% had an SG 1.065 or 1.070 on the first d of storage. By day 6, 92.6% of the eggs had SG between 1.070 and 1.080. In eggs from 48-wk-old Ross breeders, 87.1% of the eggs had SG between 1,075 and 1,085, and 10.3% <1.075 on day 1. On day 6, all eggs had SG of 1.080 or lower, with 48.3% at 1.070 or lower. In 56-wk-old Ross breeders, 78.8% of the eggs had SG 1.080 or 1.085, and 17.7% had 1.075 SG or lower on the first d of storage. However, on day 6, all floated at 1.075 SG solution or below, with 81% floating between 1.065 and 1.060. Multivariate analysis revealed weak correlations ($P < 0.001$) between SG and shell thickness ($r = 0.30$), shell weight determined by the Harms method ($r = 0.47$), and surface area ($r = -0.26$). Translucency and SG showed a very weak correlation ($r = -0.03$, $P < 0.05$). However, 73.8% of Cobb eggs with TS1 had 1.075 to 1.085 SG, and 68.5% of eggs with TS3 had 1.070 or 1.075 SG. In contrast, 53.5% of Ross eggs with TS1 had 1.075 to 1.085 SG, and 65.8% of eggs with TS3 had 1.070 to 1.075 SG. These results indicate that SG is significantly reduced by egg storage independently of the genetic line, especially in older breeders. The SG should be evaluated only during the first day of storage. Translucency and SG measure different properties of the eggshell.

Keywords: Specific gravity, Egg storage, Breeder age, Eggshell quality, Translucency

311 Starting low and ending high with breeder males

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A low male percentage at the onset of lay is a promising strategy to reduce excessive mating, feather and skin damage in females and to maintain fertility. However, there is limited information regarding the impact of different male-to-female ratios in broiler breeder production. To address this gap, an experiment was conducted to study the effects of a low male-to-female ratio starting at 20 weeks of age (woa). The study took place at a commercial Cobb 500 breeder farm with a total of eight breeder houses. Four breeder houses implemented the standard male percentage (STN) approach, beginning at 20 woa with 8.7% and once spiking (+1.4%) at 36 woa. The other four houses followed the low male-to-female ratio (LOW) approach, starting at 20 woa with 6.9% males with adding 1.2% and 1.4% spiking males at 36 and 44 woa, respectively. Each house contained separated feeders for males and females, nest boxes, and drinking nipples following the Europe breeder house layout (central slats with litter areas on both sides). Mating behavior of males and the feather cover of females was assessed at 28, 37, and 48 woa. Incubation traits were recorded every 5 weeks. Production data was analyzed using ANOVA and mating behavior and feather cover using GLMM. The LOW houses showed a trend toward more successful matings ($P = 0.060$; 58.9% vs. 49.1%) compared to the STN houses. Additionally, more voluntary matings were observed in the LOW houses ($P = 0.036$; 22.2% vs. 12.6%) compared to the STN houses. There was an interaction effect between male-to-female ratio and age on average feather cover score. At 28 weeks of age (woa), no differences in feather cover or thigh skin damage were observed. However, at 37 and 48 woa, feather cover was better and skin damage incidence was lower in the LOW houses. No differences in reproductive performance were observed between the two groups. The findings from this on-farm study indicate that a lower male-to-female ratio can improve mating behavior and feather cover, without negatively affecting reproductive performance.

Keywords: broiler breeder, male-to-female ratio, mating behavior, feather cover, fertility

312 Dietary tryptophan supplementation to mitigate the effects of heat stress on slow growing male broilers raised on pasture

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Tryptophan (Trp) plays a role to mitigate stress consequences in poultry due to its reported antioxidative activity. Dietary Trp may be a viable tool to mitigate the effect of heat stress (HS) on chickens raised outside on pasture. This study evaluated the efficacy of supplemental Trp to mitigate the impact of heat stress on slow growing male broilers (SGB) raised on pasture to 63 d of age. A randomized complete block design with a factorial arrangement of treatment with 7 replicates and 12 birds/replicate was used. Significant differences among treatments (TRT) means were determined using Tukey's-HSD test with a 5% level of probability. The factors for this study include added dietary Trp (0% Trp or 0.26% Trp) x HS conditions (non-heat stress, NHS or HS). Chicks were placed in pens (0.914 m x 1.83 m) outside on established pasture at 21 d of age. Birds were fed diets with and without Trp supplementation and were exposed to either an acute

HS environment (33.8°C temperature for 6 hours at 28 d of age and 31.6°C for 6 hours at 57 d of age, or a control (NHS temperature regime (23.8°C for 6 hours at 28 d of age and 20°C for 6 hours at 57 d of age). The dependent variables measured were weekly body weight gain (BWG), weekly feed conversion ratio (FCR), feed intake (FI), mortality, and percent carcass weight. No significant interactions were detected for d 63 BWG and FCR therefore only main effects will be presented. No significant effect of Trp (2.35 kg and 2.41 kg; 0%Trp and 0.26% Trp, respectively) or HS on 63d BWG were detected (2.4 kg and 2.36 kg; NHS and HS, respectively). Similar results were found for the effect of Trp on FCR. However, the HS treated birds had a higher ($P < 0.05$) FCR (2.2) compared to the FCR of NHS treated birds (2.14). Additionally, FI during the d57 HS exposure was lower ($P < 0.01$) in the HS treated birds (7.20 g/bird) compared to the NHS treated birds (13.5 g/bird). Mortality and carcass weight was not affected by the TRT. In conclusion, supplemental Trp did not mitigate the effect of HS for pasture raised SGB raised to 63 d of age. However, exposing SGB to HS had a negative impact on their final FCR. Additional studies feeding higher Trp levels to SGB are planned.

Keywords: heat stress, pasture, slow growing broiler, tryptophan

313 Feed and water access during transportation positively impacts thermoregulation and stress levels in broiler chicks

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The transportation of day-old chicks from the hatchery to the barn can take up to 24 hours. Prolonged transportation under suboptimal environmental conditions may cause stress and adversely affect chick development. This study evaluated the effects of providing feed and water during transportation on body temperature, stress and growth performance in newly hatched chicks. Day-old broiler chicks (n=624) were obtained from a commercial hatchery and randomly assigned to two treatments (6 transportation baskets/treatment): access to feed and water during transportation (FWT) and no access to feed and water (CON). Group body weight and cloaca temperature (10 chicks/replicate) were measured pre- and post-transport. After transportation, feed consumption, water consumption, body weight and blood samples (2 chicks/replicate) were collected to analyze plasma glucose and corticosterone concentration. During the grow-out period, chicks from each transportation treatment were randomly allocated into three floor pens and kept for 33 days to assess growth performance. Data were analyzed using one-way ANOVA (Proc Mixed, SAS 9.4) and significance was declared at $P \leq 0.05$. Over the four hours of transportation, the average feed and water consumption of FWT chicks were 1.3±0.05 g/chick and 2.5±0.15 g/chick, respectively. FWT chicks had higher body weight (FWT: 38.8 g bird^{-1} vs. CON: 37.2 g bird^{-1}; $P \leq 0.05$) and body temperature (FWT: 39.3°C vs. CON: 38.8°C; $P \leq 0.05$) than CON chicks post-transportation. Chicks having access to feed and water during transportation had a lower ($P \leq 0.05$) plasma corticosterone level compared to the control group. Access to feed and water had minor impacts on plasma glucose (P=0.13) post-transport and body weight on day 8 of age (P=0.09). No differences (P >0.05) in body weight, body weight gain and feed conversion ratio were observed through day 33 of age. These data indicate that the provision of feed and water during transportation enhances chicks' resilience to transport stress.

Keywords: broiler chick, transportation, nutrients access, thermoregulation, stress

314 Mitigating airborne dust in cage-free laying hen facilities: Assessing ionizer performance

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This study evaluated the effectiveness of ionizers in reducing airborne particulate matter ($PM_{2.5}$ and PM_{10}) in cage-free laying hen environments. Cage-free housing systems represent about 40% of domestic egg production, and due to the hens' access to litter for dust bathing, scratching, and other activities has raised concerns about increased particulate matter (PM) levels, which can transport airborne microorganisms and endotoxins, posing respiratory risks to both hens and caretakers. The experiment was conducted in two equivalent aviary rooms (Room A and Room B) each with a nominal 2,500 laying hen capacity. Ionizers were operated on alternating weeks, Week 1: Room A-On, Room B-Off; Week 2: Room A-Off, Room B-On; Week 3: Both Off). Airborne PM concentrations were monitored using laser particle counters (Dylos DC1700) in the morning, each day for 15 minutes. Dust accumulation was measured using eight Petri dishes with paper filters placed throughout each room. Environmental parameters, including CO_2, NH_3, temperature, and relative humidity, were recorded at 1-minute intervals. Average day-7 $PM_{2.5}$ and PM_{10} concentrations after week 1 were Room A-On = 160 and 2,666 µg/m^3, respectively, and Room B-Off = 161 and 3,747 mg/m^3, respectively. Average day-7 $PM_{2.5}$ and PM_{10} concentrations after week 2 were Room A-Off = 95 and 2,395 µg/m^3, respectively, and Room B-On = 151 and 2,683 µg/m^3, respectively. The absolute average difference (±SD) between daily $PM_{2.5}$ and PM_{10} concentrations in Rooms A and B during week 3 (both off) was 19 ±7 and 1,961 ±734 µg/m^3, respectively. Average daily PM concentration reduction between ionizers on/off for week 1 was 4% ($PM_{2.5}$) and 9.1% (PM_{10}) and for week 2 was 0.6% ($PM_{2.5}$) and 19% (PM_{10}). Petri dish dust accumulation showed a 1% to 137% (Room A) and 6% to 148% (Room B) greater mass of settled dust when ionizers were off, calculated as the difference in accumulation between ionizer-off and ionizer-on conditions at each petri dish location. These findings provide insights into particulate matter mitigation strategies in cage-free systems, emphasizing the potential of ionization technology to improve indoor air quality for modern egg production facilities.

Keywords: particulate matter, environment, ventilation, air quality

315 Assessing the detrimental effects of chronic heat stress in commercial layers in cage-free system

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Heat stress (HS) due to global warming poses a significant environmental challenge to the poultry industry, affecting both the broiler and layer chickens. As the egg industry transitions from conventional-cage systems to cage-free (CF) systems, concerns

have emerged regarding reduced egg production and quality, as well as disease transmission, leading to higher production costs. This study aimed to assess the impact of chronic HS in commercial layers raised in CF system, focusing on egg production, egg quality, and blood biochemical parameters. A total of 240 commercial white layers (26 weeks old) were randomly assigned to two temperature treatments: thermoneutral (TN, 22°C) and cyclic heat stress (HS, 35°C for 12 h followed by 22°C for 12 h) for duration of 8 weeks. Each treatment had six pens (20 birds/pen) at a stocking density of 0.139 m^2/bird. Egg production and weight were recorded daily, while body weight, feed intake, and cloacal temperature were measured weekly. Egg quality parameters, and blood gases and electrolyte levels using the iSTAT blood analyzer were assessed at the end of study. Data were analyzed using linear mixed-effect model with pen as a random effect. Results showed no significant difference in the daily egg production rate between groups, however, the HS group produced significantly ($p < 0.05$) lighter egg weight. Moreover, chronic HS significantly ($p < 0.05$) reduced eggshell breaking strength, while shell thickness, Haugh unit, yolk color, and yolk index remained comparable between groups. Despite the HS chickens consumed significantly ($p < 0.05$) less feed, their feed conversion ratio (based on egg weight) improved ($p < 0.05$) compared to TN birds. However, body weight gain did not differ significantly ($p > 0.05$) between groups. Cloacal temperature was significantly higher ($p < 0.05$) under HS conditions. Additionally, chronic HS significantly ($p < 0.05$) decreased blood pH, while increasing PCO_2 (mmHg), PO_2 (mmHg), and ionized calcium (iCa, mmol/L) levels. In conclusion, chronic HS primarily affected egg weight rather than egg number, enhanced feed efficiency, weakened eggshell strength, and altered blood pH, gases, and iCa levels in commercial layers raised under CF system.

Keywords: Heat stress, Layers, Egg production, Egg quality, Blood parameters

316 Insights for designing future vaccines in poultry

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The broiler industry exclusively relies on live vaccines, which present significant challenges such as interference with USDA disease monitoring, need for extended withdrawal periods, challenges in vaccine application uniformity, cold chain maintenance, and adverse reactions. Given the disadvantages of live vaccines, killed vaccines offer a viable alternative. Though killed vaccines offer a safer alternative, killed vaccines are not applied in US broiler industry because three primary limitations hinder their use in broilers: administration methods, the need for a second dose, and performance impacts due to adjuvants. Currently, all available killed vaccines must be manually injected, which is impractical for large poultry farms. Advances in vaccine administration technology have made administration of the first dose in hatchery a feasibility. However, killed vaccines typically require a second dose 7 to 10 days after the first, and manually injecting millions of birds on farms at this age is virtually impossible in the U.S. broiler industry. As a result, killed vaccines have not been widely adopted for broilers, leaving the industry reliant almost entirely on live vaccines. Developing novel killed vaccine platforms that can deliver vaccines as a single-dose can be advantageous for the US broiler industry. Nanoparticle based vaccines can release antigen release over 10 days, which can eliminate the need for second dose. Previous research has identified that nanoparticle-based vaccine platforms induce robust mucosal and cell-mediated immune responses and are efficient in reducing food borne pathogen loads in poultry. Alum-free single dose killed vaccines can potentially improve animal welfare by minimizing animal handling and avoiding inflammation at the site of injection. This seminar will highlight how nanoparticle-based killed vaccines could redefine broiler and layer bird vaccination by addressing the shortcomings of current live and killed vaccines.

Keywords: Vaccine, Foodborne, Pathogen, Immunology

317 Lymphocyte populations in the liver and secondary lymphoid organs of White Leghorn and UCD200/206 Scleroderma/Systemic sclerosis-expressing chickens differ after vaccination with *Salmonella* Typhimurium bacterin

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Although *Salmonella* vaccination programs effectively decrease *Salmonella* load in poultry flocks, there are several cases where intramuscular (i.m.) vaccination with *Salmonella* bacterin induces severe injection site reaction with associated hemorrhagic hepatopathy in breeder flocks. The objective of this study was to assess lymphocyte populations after a primary i.m. vaccination with *Salmonella* Typhimurium (ST) bacterin in UCD 200/206 (UCD) White Leghorns (WL) that naturally express an autoimmune disorder akin to scleroderma/systemic sclerosis (SSc) in humans and in WL controls. To do this, 16-wk-old UCD and WL pullets were i.m-vaccinated with formalin-inactivated ST or sterile PBS (N=6-9 birds/line). Prior to (0d) and at 3d and 7d post-vaccination (p.v.), birds were euthanized to measure total organ weights and collect samples of liver, spleen, and cecal tonsil (CT). Lymphocyte profiles of each organ sample were assessed by immunofluorescent staining of single cell suspensions and population analysis by FACS. Cell populations were normalized as "cells/mg of tissue." Data were analyzed by three-way ANOVA to test the effect of vaccination, time, line, and their interactions ($P \leq 0.05$; 3 birds/vaccination/time/line). Relative spleen and liver weights (% body weight) were lower ($P<0.001$) in the UCD than in the WL, independent of vaccination. In the spleen, there was a three-way interaction ($P=0.02$) for $CD4^+$ T cells whereby concentrations were increased with ST vaccination in WL by 7d p.v. but were not affected in UCD. In the liver, independent of vaccination, $CD4^+$ T cell concentrations dropped ($P=0.03$) at 3d p.v. in UCD but did not change over time in WL. For $CD8\alpha^+$ T cells, concentrations in UCD spleens dropped ($P=0.02$) below those of WL at 3 and 7d p.v., independent of vaccination. In the liver, $CD8\alpha^+$ T cell- and γδ T cell-concentrations peaked at 3d p.v. in UCD and were higher ($P<0.02$) than in WL only at this timepoint, independent of vaccination. In contrast, splenic γδ T cell concentrations were not affected by time, line, or vaccination. Lymphocyte concentrations in the CT of both lines were unaffected by vaccination, but UCD birds had overall lower ($P<0.03$) $CD4^+$ T cell-, γδ T cell-, and B cell-concentrations when compared to WL. Although there were no visible lesions on the organs of WL or UCD birds following i.m. ST or PBS vaccination, peaks in cytotoxic T cells and γδ T cells in UCD livers suggest induction of T cell mediated, tissue-specific inflammation. Moreover, these results provide evidence of divergent quantitative and temporal immune responses to ST vaccination or sterile injury in the livers and spleens of birds with and without susceptibility to fibrotic disease development.

Keywords: Salmonella vaccine, Lymphocytes, Fibrosis, Pullets, Immunology

318 The effects of primary and secondary immunizations with a *Histomonas meleagridis* cathepsin protease recombinant protein in different vehicles on local and systemic immune responses in turkeys

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There are currently no drugs or vaccines available to control *Histomonas meleagridis* (HM). As a result, there is a need to explore effective vaccination strategies against HM in turkeys. This time-course study assessed the local cellular and systemic humoral immune responses to primary (naïve) and secondary (sensitized) immunizations with an HM cathepsin-protease vaccine using the growing feather (GF)-pulp bioassay in female turkey poults. Vaccine treatments consisted of HM-protein in emulsion (HM-Emul), in PBS (HM-PBS), or vehicles alone (3 birds/treatment). Sensitized poults received a primary s.c. vaccination at 1d of age (250 µL/poult). At 5-wks, both sensitized and naive poults were immunized by intradermal (i.d.) GF-pulp injections (10µL/GF; 250 µL/poult). Immunofluorescent staining and FACS was used to assess pulp leukocyte profiles in GFs collected before (0d) and at 3h, 1d, 2d, 3d, and 5d post-GF-pulp injection (p.i.). HM-protein-specific IgG in blood collected before

(0d) and at 3 and 5d p.i. were measured by ELISA. GF and blood data were analyzed by two-way ANOVA and two-way repeated-measures ANOVA, respectively, and significance was set at $P \leq 0.05$. In naïve poults, primary HM-immunization resulted in highest heterophil infiltration (% pulp cells) at 3h, with a gradual decline to 0d levels by 5d p.i. ($P \leq 0.05$). Macrophage infiltration was highest at 1d and remained elevated by 5d p.i.; macrophage levels were higher with HM-PBS than HM-Emul ($P \leq 0.05$). There was a transient peak in B cells at 1d p.i., reaching higher levels with HM-PBS than HM-Emul ($P \leq 0.05$). HM-PBS also recruited higher levels ($P \leq 0.05$) of $CD4^+$ cells than HM-Emul, with highest levels observed at 3d p.i. with both vaccines. Levels of $CD8\alpha^+$ cells had a similar time course to $CD4^+$ cells, independent of vaccine. In sensitized poults, secondary immunization resulted in maximal heterophil levels at 3h that gradually declined to baseline by 5d p.i., whereby levels with HM-PBS were higher than with HM-Emul ($P \leq 0.05$). Independent of vaccine, macrophages reached peak levels at 2d and 3d and remained elevated at 5d p.i. ($P \leq 0.05$). HM-PBS recruited more B cells, that reached a transient peak at 2d p.i. with both vaccines. Independent of vaccine, levels of $CD4^+$ cells peaked at 2d p.i. and remained above 0d levels thereafter ($P \leq 0.05$). $CD8\alpha^+$ cells were higher with HM-PBS than with HM-Emul regardless of time. In sensitized poults, HM-specific IgG was elevated at all time points, independent of vaccine. The HM-PBS vaccine formulation was more effective in stimulating tissue cellular and systemic humoral immune responses following primary and secondary immunizations and, hence, may provide a more effective defense against HM in turkeys than HM-Emul.

Keywords: Histomonas meleagridis, histomonosis, vaccine, cathepsin protease, growing feather-pulp bioassay

319 Further observation confirm the phagocytic capacity of avian erythrocytes-chickens and turkeys

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Phagocytosis was recently added to hemostasis and gas transport, established functions of the nucleated avian erythrocyte (RBC). This fundamental function of the innate immune system was demonstrated for RBCs in lame duck blood. Given the importance of phagocytosis in general, can the duck observations be extended to other poultry? The description of the problem is to show evidence for phagocytosis by chicken and turkey RBCs. Wright-Giemsa and Gram-stained blood and bone marrow films were examined by microscopy at 100x for evidence of phagocytosis. Positive cells were photographed at 100x magnification. Results: Bacteria were seen in 31/47 (66%) microscopic fields of blood from a 6wk isolater raised SPF chick (TWBC ~ 25K, H/L ratios ~0.15). Bacteria were free swimming, attached to RBC or other cells (cell associated bacteria, CAB). Some bacteria were internalized by RBCs and then degraded (phagocytosis). In some instances the internalized bacteria were seen in clear (Hb-free) cytoplasmic vacuoles. The hemogram of this chick was remarkable as a disintegrating thrombocytic embolus with intact bacteria, toxic heterophils, secretory monocytes, reactive basophils, and a Turk cell (proplasmacyte) were also present. Erythroid atypia included reticulocytes, polychromatic RBCs, and irregularly shaped types (akanonistosis). Phagocytic RBCs were both polychromatic (young) and mature RBCs. Phagocytic RBC were found in commercial and experimental turkeys at several ages. RBCs were attached by diplococci and other bacteria. A vacuole containing 3 pairs of partially degraded diplococci indicates RBCs have a capacity to phagocytose multiple bacteria. Phagocytic RBC can be differentiated from punctare and network reticulocytes; two atypical RBC forms that retain cytoplasmic ER. Microscopic examination of blood obtained from chickens and turkeys indicates their RBCs are capable of phagocytosis as has already been demonstrated in the duck. Phagocytosis, a fundamental process of innate immunity is now shown as a property of the avian RBC. Therefore, it joins the thrombocyte, the monocyte, and granulocytes, as an example of a phagocytic cell. It is not yet known if phagocytosis by avian RBCs requires possession of an active (open chromatin) nucleus.

Keywords: phagocytosis, erythrocyte, chicken, turkey, innate immunity

320 Immune profile and immunometabolic recovery of adult inbred poultry genetic lines in response to intramuscular lipopolysaccharide challenge

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Previous research has characterized genetic profiles and immune traits of highly inbred chicken genetic lines. Fayoumi lines (M5.1, M15.2) are known for their resistance to bacterial and viral infections and heat stress, while Leghorn lines (Ghs-13, Line-8) display lower disease resistance. Research is lacking in investigating the line-specific metabolic and immune responses to lipopolysaccharide (LPS). Therefore, this study investigated immune profiles and metabolic phenotypes of peripheral blood mononuclear cells (PBMC) from highly inbred genetic lines under resting and stressed metabolic states. Fifty-four adult birds from 5 highly inbred genetic lines (M5.1, M15.2, Ghs-13, Line-8, and Sp-21.1) were randomly assigned to 1 of 2 treatments, resulting in a 5 x 2 factorial of genetic line by intramuscular injection (0.9% sterile saline or 2.4 mg/kg BW LPS, *Escherichia coli* O55:B5). Body weight was recorded at baseline before injection and 24 h post-injection (hpi), and cloacal temperature at baseline, 2 hpi, and 24 hpi. Blood was collected at all timepoints for flow cytometry and metabolic analysis. Data were analyzed using the SAS 9.4 MIXED procedure with fixed effects of genetic line, LPS inoculation, and interaction followed by Tukey-Kramer adjustment, with significance at $p \leq 0.05$. Baseline immune profiles varied by line ($p \leq 0.001$). At 2 hpi, LPS did not significantly impact body weight or temperature but influenced all queried cell populations, decreased ATP production, and decreased glycolytic rates (70.2-80.5% and 68.0-70.7%, respectively; $p \leq 0.02$). Line-8, M5.1, and Sp-21.1 LPS birds had increased $CD3^+$ cells by 59.6%, 62.3%, and 51.8%, respectively, while Ghs-13 and M15.2 birds were unaffected (2 hpi; $p \leq 0.0001$). Sp-21.1 had the highest $CD3^+CD1.1^+$ T cell presence by 40.6-74.9%, while LPS decreased $CD3^+CD1.1^+$ T cells by 34.1% (2 hpi; $p \leq 0.0001$). Within $CD3^+CD4^+$ T cells at 2 hpi, M5.1, M15.2, and Line-8 controls had 14.9-66.5% higher prevalence in circulation than LPS birds, while $CD3^+CD4^+$ cells were 12.2% lower in Ghs-13 post-LPS ($p \leq 0.0001$). $CD3^+CD8\alpha^+$ populations increased by 41.1-63.2% in M5.1, M15.2, Line-8, and Sp-21.1 LPS birds but remained similar in Ghs-13 at 2 hpi ($p \leq 0.0001$). By 24 hpi, immune profiles and glycolytic rates were largely recovered from LPS exposure, while genetic line effects persisted, indicating line-specific immune responses to LPS ($p \leq 0.04$). Genetic-line specific changes in circulating immune cells may indicate inherent pathogen response capabilities. Further understanding cellular preference and metabolic switching during inflammatory challenges could provide insight into how to best support and optimize birds based on genetic line during production cycles.

Keywords: flow cytometry, highly inbred genetic lines, immunometabolism, LPS

321 Gene expression analysis of newly identified chicken 1α-hydroxylase (CYP27B1) in the lymphoid system

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Vitamin D synthesis requires two enzymatic steps to form 25-OH Vitamin D in the liver and subsequently 1,25-OH_2 Vitamin D (1,25D3) in the kidney. The last step is catalyzed by the enzyme 1α-hydroxylase (CYP27B1) which has been termed a missing gene in birds. We recently reported on the identification of the chicken CYP27B1 gene locus and its coding sequence. Using this sequence information, we established a qPCR assay to gain new inside into the tissue expression pattern. As expected, highest expression was observed in kidneys and largely restricted to the proximal tubular epithelium as shown by RNAScope analysis. Thus, chicken CYP27B1 shows identical features to its mammalian counterpart. Evolutionarily, the first functions of 1,25D3 were related to detoxification and the energy metabolism in the innate immune system and current research is focused on its role in vertebrate immune regulation in health and disease. Using the newly acquired sequence information, we next investigated vitamin D receptor (VDR) and CYP27B1 expression in immune organs of the chicken. High expression of CYP27B1 and VDR was found in the Bursa of Fabricius and the cecal tonsils, both being B-cell rich compartments. Dissociation of bursal tissue revealed that CYP27B1 was low in B-cells but high in the bursal stroma, thus indicating a regulatory function of 1,25D3 in B-cell development. Using RNAScope, we attempted to identify the CYP27B1 expressing cells but due to the technical incompatibility of RNAScope with antibody staining we could not unequivocally identify the source of 1,25D3 production as of now. However, macrophages are known as an important source of 1,25D3 in mammals. We therefore used the chicken macrophage cell line HD11 and primary splenic macrophages to further investigate CYP27B1 expression and regulation. Low expression levels were found in resting macrophages while LPS stimulation with 100 ng/ml of LPS significantly upregulated CYP27B1 expression within 6 hours, while VDR expression remained unchanged. Finally, we activated splenic B- and T-cells with CD40L and T-cell receptor crosslinking, respectively. While CYP27B1 expression remained largely unchanged over 24 hours, VDR expression was upregulated in T-cells but not B-cells within 6 hours. These first results show that 1,25D3 is involved in the avian immune system. Enzymatic dissociation of bursal tissue combined with flow cytometric cell sorting should enable exact identification of 1,25D3 producing cells in the bursa of Fabricius. Functional studies related to bursal B-cell development will help to better understand the interaction of 1,25D3 and avian immunity.

Keywords: 1, 25(OH)2D3, avian immunity, lymphocytes, macrophages, vitamin D receptor

322 Nature versus nurture: can genetic selection overcome environmental differences to achieve consistent results between experiments?

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It is the overarching goal of research to gain insight by extrapolation; to apply results more broadly and characterize phenomena beyond a specific experiment. Lines of White Leghorn chickens have been under continuous divergent selection for high (HAS) and low (LAS) antibody response to injection with sheep erythrocytes (SRBC) for 50 generations. These lines are a model for understanding the genetics of immunology, however their utilization begs the question of result consistency when the chickens are raised in different environments. Serendipitously, two separate experiments, one in Blacksburg, VA, in November 2023 and the other in Morgantown, West Virginia in August 2024 were conducted using members of HAS and LAS generation 50. Both experiments included injection with SRBC, with samples collected five days post injection. There were no other treatments, though the VA flock were two days older on day of injection than the WV flock. Bursas, the avian immune organ responsible for B-cell production, necessary for antibody response, were collected for gene expression analysis in both experiments. RNA from 24 bursas; 6 chickens from each line and flock, were sequenced at the NC State University Genomic Sciences Lab on the Illumina NovaSeq 6000, in one flow cell. Differential gene expression analysis was subsequently performed using CLC Genomics Workbench (Qiagen, Hilden Germany) with FDRp $\leq$0.05 considered significant. There were 909 differentially expressed genes (DEG) identified between WV HAS vs. LAS and 910 DEG between VA HAS vs. LAS. Of those, 488 DEG were common between the two flocks, with 480 having directionally consistent expression including a majority of MHC and related immune genes. Comparison pathway analysis of the HAS vs LAS DEG unique to either flock indicates high similarity of functional networks, suggesting that while the individual DEG may differ, the resulting physiological responses are consistent between flocks. There were only 41 DEG in HAS and 54 in LAS between flocks, which with over 11K expressed genes sequenced may be negligible, however pathway analysis suggests a more inflammatory state in the WV flock. These data support general consistency of bursal gene expression of HAS vs. LAS under different environmental conditions and increases confidence if comparing results from multiple unrelated experiments. However, the magnitude of DEG varied such that direct comparisons between experiments are not recommended. Future research may include exploration of gene expression of other organs from these experiments to further validate the results.

Keywords: genetic selection, immune response, HAS/LAS, RNASeq, immunogenetics

323 A study of the behavioural output differences in turkeys vs broilers response to photoperiod

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Management and welfare research focusing on turkeys is generally much more difficult compared to broiler research. Bird size, strength, curious nature, and costs mean turkey research is much less common, and as a result, many management decisions for use in turkey flocks are based from research conducted on broilers. But should that be? Is a turkey really just a very large broiler? This presentation will focus on similarities and differences in behavioural output for broilers and turkeys exposed to different photoperiods/scotoperiods. Understanding these differences or similarities could answer the question of the acceptability of this practice.

Keywords: Turkeys, Broilers, Photoperiods, Behaviour

324 Differences in feeder usage and feeding behaviors by broiler chicks with supplemental and standard feeders

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Supplemental feeders are used with standard feeders to improve feed access for broiler chicks in early life. This study aimed to evaluate differences in feeder usage and feeding-related behaviors between supplemental feeders and standard feeders. On the day of hatch, YP x Ross 708 male broiler chicks (n =360) were randomly placed into 12 floor pens (30 birds/pen). Each pen had a C2 feeder, and a supplemental feeder (feed tray (FT) or turbo grow (TG)). Treatments were FT, C2 in pen with FT (C2FT), TG, and C2 in pen with TG (C2TG). Behavior frequency was measured on days 1 and 4 for each feeder separately via 5-minute sampling every 30 minutes. Behaviors were eating whilst outside the feeder, eating from the edge of the feeder, eating whilst inside the feeder, entering-exiting the feeder, attempting to enter the feeder, moving inside the feeder, litter pecking within the feeder, feed scratching, feed selection, jostling at the feeder, and displacing other birds from the feeder. To assess the behavioral differences between standard feeders and supplemental feeders a one-way ANOVA (PROC GLIMMIX) was performed with pen as a random effect and means separation via Tukey-Kramers. Total eating was higher with supplemental feeders than the standard feeders ($P<0.01$), with total eating highest for FT (11.63), then TG (7.78), followed by C2TG (3.67) and lowest in C2FT (3.24) ($P<0.01$). Chicks ate most from outside TG (7.19), then C2TG (2.66), followed by C2FT (2.03) and least from FT (1.34) ($P<0.01$). While more chicks ate inside FT (10.18), then in C2FT (1.10) and C2TG (0.91), and least in TG (0.15) ($P<0.01$). More chicks ate from the edge of the feeder in TG ($P<0.01$; 0.31). Chicks were most successful at entering-exiting the feeder with FT (7.15), then C2FT (0.53) and C2TG (0.45) and least with TG (0.30) ($P<0.01$). FT also had the lowest unsuccessful attempts to enter the feeder ($P<0.01$; 0.06). Movement inside the feeder occurred mostly in FT (3.65), then in C2FT (0.42) and C2TG (0.39), and least in TG (0.05) ($P<0.01$). Litter pecking, feed scratching, and feed selection were highest in FT ($P<0.01$; 0.13, 0.29, 0.52, respectively). Jostling at the feeder was highest with TG (0.13) whereas displacement occurred more with both TG (0.09) and FT (0.04) ($P<0.01$). These results indicate that chicks prefer supplemental feeders to standard feeders for feeding-related behaviors. TG encouraged birds to eat from outside the feeder, resulting in higher jostling, whilst FT allowed birds to eat from inside and explore within the feeder. Overall, the study suggests that the use of supplemental feeders changes the behavioral expression of broiler chicks, with more frequent feeding with supplemental feeders than the standard feeders.

Keywords: feeder management, behavioral expression, turbo grow, feed tray, bird activity

325 Behavioral strategies of broilers during early life thermal conditioning and subsequent heat stress

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Thermal conditioning (TC) during a broiler's first week can increase heat stress tolerance. This study was designed to examine if it affects heat stress mitigation behaviors. Cobb 500 broilers were housed in six environmentally controlled chambers (2 pens/chamber, 30 birds/pen). On day (d) 4, three chambers underwent TC at 37.8°C for 24 hours, while the control (CON) stayed at 28°C. On day 5, after TC, birds were moved so that CON and TC chicks were housed in the same chambers (different pens) for the remainder of the experiment. From d28-34, all birds experienced eight hours of cyclic heat stress (HS) daily. Video analysis occurred on d4, d26 (PRE), d28 (BEG), d31 (MID), and d34 (END) with scan sampling every 15s for 30mins in the AM and PM for one pen/chamber (CON and TC: n=3 chambers). Behaviors were mutually exclusive, with heat stress behaviors (wing spreading, panting, drinking) scored over walking, standing, sitting and lateral sitting. Linear mixed-effects models in R were used. PRE-HS was excluded from panting analysis (not observed). During TC, more CON broilers walked (CON=15.7%, TC=8.0%; $p=0.002$) and stood (CON=21.1%, TC=6.0%; $p<0.001$), whereas TC exhibited more wing spreading (CON=2.31%, TC=25.9%; $p<0.001$) and drinking (CON=2.31%, TC=25.9%; $p<0.001$). Sitting did not differ (CON=50.9%, TC=43.5%; $p=0.13$). More broilers (CON and TC combined) were observed walking PRE (3.14%) compared to MID (2.73%), BEG (1.25%), and END (1.03%) of HS ($p<0.001$). Standing followed a similar pattern, PRE=14.36% with fewer at BEG (9.64%), MID (4.85%) and END (5.18%; $p<0.001$). Panting increased with HS, peaking at END (80.9%), followed by BEG (70.7%) and MID (64.3%; $p<0.001$). Wing spreading was greatest at MID (5.40%), compared to PRE (0.91%), BEG (0.26%) and END (0.18%; $p<0.001$). Drinking peaked at MID (5.81%), slightly higher than PRE (5.22%) and BEG (5.37%), but decreased at END (3.90%; $p<0.001$). Sitting was the predominant PRE (70.39%), but was rarely observed without HS behaviors during BEG (7.31%), MID (2.55%), and END (2.02%; $p<0.001$). Lateral sitting similarly decreased from PRE (2.89%) to BEG (0.51%), MID (0.25%), and END (0.10%; $p<0.001$). During HS, walking tended to be more common in CON (2.01%) than TC (1.85%) broilers ($p=0.07$). No differences were found in standing (CON=8.75%, TC=8.27%; $p=0.85$), panting (CON=70.6%, TC=73.3%; $p=0.66$), wing spreading

(CON=1.07%, TC=1.06%; p=0.85), drinking (CON=5.35%, TC=4.8%; p=0.55), sitting (CON=9.05%, TC=6.93%, p=0.47) or lateral sitting (CON=0.72%, TC=0.66%; p=0.53) during HS. While early life TC had little effect on behaviors during subsequent cyclic HS, responses varied across HS days, indicating that broilers may adjust thermoregulatory strategies over time.

Keywords: Broiler, Behavior, Thermal conditioning, Heat stress

326 Dietary olive oil influences the profile of metabolites crossing the blood-brain barrier in broiler chicks

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Dietary fat types modulate the dorsal raphe nucleus (**DRN**) and hypothalamic (**HYP**) serotonergic regions of the brain to influence birds' behavior. Identifying metabolites that cross the blood-brain barrier (**BBB**) in response to dietary changes could support the formulation of diets that promote positive behavior. This study aimed to unravel how monounsaturated fatty acids in the form of olive oil, influence the composition of metabolites crossing the BBB into serotonergic rich DRN and HYP in broiler chicks. Day-old (Ross 708; n = 160) broiler chicks were assigned to two dietary treatments consisting of a corn-soybean meal (**SBM**) basal, into which poultry fat (**CON**), and olive oil (**OLIV**) were incorporated at 3% level with 5 replicate pens/16 chicks each. On d 20, plasma, and brain segments (DRN and HYP) were aseptically collected and subjected to HPLC-MS metabolomics profiling. This was followed by multivariate analysis and principal component analysis (PCA) to discern distinct metabolite profiles. HMDB and KEGG databases were used to annotate differentially expressed metabolites and pathway enrichment analysis. A total of 15,084 metabolites were differentially expressed, of which 617 metabolites ($P < 0.05$) were annotated. Among the metabolites that crossed BBB, a serotonergic enhancer such as S-succinylcysteine known to amplify serotonergic signaling pathways linked to calmness and reduced aggression was upregulated in both plasma and DRN. Moreover, among stress modulators metabolites, huperzine A upregulated in plasma and HYP is associated with enhanced nicotinic acetylcholine receptor signaling, which regulates stress responses and appetite regulation. Whereas gamma-glutamylleucine, a metabolite that modulates GABAergic signaling by reducing stress-induced hyperactivity; and epigallocatechin which improves stress resilience by normalizing the hypothalamic-pituitary-adrenal axis activity were upregulated in plasma and HYP but downregulated in DRN. Glutamate metabolism pathway, a primary energy source for intestinal cells that influence hypothalamic appetite regulation was enriched in plasma and HYP but not significantly in DRN. Enriched pathway of aspartate metabolism known to influence neurotransmission by impacting mood and behavior was altered in plasma and DRN; and phenylacetate metabolism (precursors to neurotransmitters of serotonin and dopamine) was enriched in plasma but not significantly in DRN. Overall, dietary olive oil influenced metabolites that crossed BBB into DRN and HYP, capable of inducing behavioral changes such as reduced aggression, improved cognitive functions and stress response in broiler chicks.

Keywords: Olive Oil, Dorsal raphe nucleus, Blood-brain barrier, Hypothalamic, Broiler chicks

PSA Industry Research Forum: A conversation with our stakeholders

327 Broiler Industry: Research needs and opportunities
Ashley Peterson*[1] [1]*National Chicken Council, Washington, District of Columbia, United States*

No abstract required.

328 Turkey Industry: Research needs and opportunities
Lindy Chiaia*[1] [1]*National Turkey Federation, Washington, District of Columbia, United States*

No abstract required.

329 Egg Industry: Research needs and opportunities
Oscar Garrison*[1] [1]*United Egg Producers, Johns Creek, Georgia, United States*

No abstract required.

330 USPOULTRY Update: Partnership and funding opportunities
Denise Heard*[1] [1]*USPOULTRY, Tucker, Georgia, United States*

No abstract required.

331 Moving forward with dietary amino acid research in broilers

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Increased soybean acreage and processing for oil in the U.S. has resulted in a decrease in soybean meal price that has not been seen since 2020. Competitively priced soybean meal allows for U.S. integrators to increase amino acid density. Other geographical regions that limit dietary soybean meal inclusion due to tight supply and above average prices, however, favor diets that allow for CP reductions. Research designed to evaluate bird responsiveness to amino acid density should be implemented with occurrence for the following reasons: 1) to quantify the bird's maximum response to dietary Lys, and 2) to quantify the bird's genetic potential for growth responses vs carcass yields. Once the former are established, the level of dietary Lys is set based upon feed price and the price of poultry parts (e.g., bone-less skin-less breasts). Regarding reduced CP, researchers around the world continue to research reduced CP in broiler diets shedding light on essential and non-essential amino acid minimum needs, as well as dietary energy. Nutritionists feeding birds for optimal amino acid density may include 3 to 4 feed grade amino acids, whereas nutritionists feeding birds reduced in CP may incorporate 4 to 7 feed grade amino acids. In the U.S., the commercial acceptance of L-Val occurred much faster than L-Thr, and it is hypothesized that the acceptance of L-Arg, L-Trp, L-Ile, and L-His HCl will be commercially accepted quicker than that of L-Val. In order to achieve the former, dietary nutrient minimums for His, Phe + Tyr, Pro, and some of the non-essential amino acids must be established. The former points will be addressed during this presentation.

Keywords: Amino acid, lysine, valine, histidine, broiler

332 Evaluating the effects of increasing digestible arginine to lysine ratio levels on the growth performance of Ross 708 x YPM male broilers during the finisher and withdrawal phases

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The objective of the following experiments was to determine the optimal digestible arginine (dArg) to digestible lysine (dLys) ratio of Ross 708 x Yield Plus Male (YPM) broiler males from d22-32 and d33-42 of age based on growth performance. Experiment 1 evaluated six different dArg:dLys ratios (90%, 98%, 106%, 114%, 122%, 130%) fed during the finisher phase from d22-32 of age. Experiment 2 evaluated the same six dArg:dLys ratios during the withdrawal phase from d33-42 of age. Each experiment utilized 2,256 male broilers that were randomly allotted to the dietary treatments in a randomized complete block design with 47 birds/pen and 8 replicate pens/treatment. All birds were provided a common starter (d0-10) and grower (d11-21) diet that was formulated to meet the nutritional recommendations of Ross 708 x YPM male broilers from d0-21 of age. The birds in experiment 2 were fed a common finisher diet from d22-32 of age before receiving the experimental diets at d33 of age. Birds and feed were weighed on D21, 32, and 42 to calculate feed intake (FI), BW gain (BWG), and mortality corrected FCR (FCRc). The data was analyzed using a one-way ANOVA mixed models procedure of JMP Pro 17 and polynomial contrasts were utilized to evaluate the linear and quadratic effects of increasing dArg:dLys ratios. In experiment 1, BWG from d22-32 of age was not different (P=0.147) between dArg:dLys ratio treatments, however FCRc decreased (1.483, 1.490, 1.462, 1.461, 1.445, and 1.451) linearly (P<0.01) and quadratically (P<0.01) as dArg:dLys ratios increased. In experiment 2, BWG from d33-42 of age increased (1.295, 1.262, 1.296, 1.331, 1.339, and 1.249 kg) linearly (P<0.01) as dArg:dLys ratios increased. As dArg:dLys ratios increased, FCRc from d33-42 of age decreased (1.674, 1.663, 1.625, 1.593, 1.579 and 1.643) linearly (P<0.01) and quadratically (P=0.022). These data indicate that increasing dArg:dLys up to 122% in the finisher phase improves FCRc from d22-32 of age and improves BWG and FCRc during the withdrawal (d33-42) phase.

Keywords: Amino Acids, Arginine, Lysine, Broiler, Ross 708

333 Effects of variable digestible arginine to digestible lysine ratios on the growth performance of male Ross 708 x YPM broiler chickens through 42 days of age

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The objective of the following study was to determine the impact of variable digestible arginine (dArg) to digestible lysine (dLys) ratios on the growth performance of Ross 708 x Yield Plus Male (YPM) broilers through a 42-day production period. An experiment was conducted utilizing 4,800 male broiler chicks allotted to 12 treatments in a 2 x 6 factorial arrangement of dArg:dLys (90%, 98%, 106%, 114%, 122%, 130%) and phase of application (finisher or withdrawal). In total there were 8 replications with 50 birds/pen and 4 feeding phases. All birds were fed common starter (d0-10) and grower (d11-21) diets, formulated to meet Aviagen's nutritional recommendations for the Ross 708 broiler. The experimental period began for the first application phase (48 pens) during the finisher phase (d22-32) while the other application phase was fed a common finisher diet. All birds were fed the experimental diets during the withdrawal phase (d33-42). Birds and feed were weighed on D21, 32, and 42 to calculate feed intake (FI), BW gain (BWG), and mortality corrected FCR (FCRc). The 12 treatments were arranged in a randomized complete block design and data were analyzed with a mixed model using one-way ANOVA procedure of JMP Pro 17. Means were separated using a Student's T-test post-hoc analysis and $P \leq 0.05$ was considered significant. The main effect of dArg:dLys indicates that birds fed 106%, 114% or 122% dArg:dLys had the highest d33-42 BWG, while birds fed 130% had the lowest. All other treatments were intermediate for d33-42 BWG ($P < 0.001$). Interactive effects show that birds fed 106%, 114% or 122% from d22 or 114% or 122% from d33 had the highest d33-42 BWG, while birds provided 130% from d22 or d33 or 90% from d22 had the lowest ($P = 0.050$). The main effect of the phase of application indicates that there was no difference in d33-42 BWG regardless of when the titration began ($P = 0.999$). The main effect of dArg:dLys indicate that birds fed 122% dArg:dLys improved d33-42 FCRc by 10.3 points compared to those fed 90% ($P < 0.001$). Interactive effects indicate birds provided 106%, 114% or 122% from d22 or 122% from d33 had the lowest d33-42 FCRc, while birds provided 90% for only the withdrawal phase had the highest

d33-42 FCRc ($P = 0.037$). The main effect of phase of application indicates that birds fed the titration from d22 reduced d33-42 FCRc by 3.0-points when compared to those fed the titration only from d33-42 ($P < 0.001$). These data indicate that increasing dArg:dLys up to 122% provides a performance improvement regardless of when you provide the increased level.

Keywords: Amino acids, Arginine, Broiler, Ross 708 x YPM, Performance

334 Dietary arginine silicate inositol complex (Nitrogisine®) supplementation supports egg quality and bone metabolism during later laying phases in hens

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Dietary interventions to support late cycle laying hens are one method to maintain or improve egg production, hen health, and shell quality through the end of the production cycle. Arginine silicate inositol (ASI) was investigated as a nutritional intervention to enhance performance, egg characteristics, and bone quality in late-laying hens. Lohmann LSL-Lite hens were housed in conventional cages with 7 birds per cage and 10 replicate cages per treatment. Each cage was randomly assigned to one of five dietary treatments: control diet, or the control diet plus 0.025%, 0.05%, 0.10%, or 0.15% ASI. Baseline body weight, whole body dual energy x-ray absorptiometry (DXA), tibia DXA, bone breaking strength, egg quality, and serum metabolites was taken at week 65 and again at study conclusion at week 81. Egg production was recorded daily and egg quality was assessed weekly. Body weight and feed intake were measured every four weeks along with serum and total fecal collection. Data were analyzed with a one-way ANOVA in SAS 9.4, with significance set at $\alpha \leq 0.05$. No significant difference in body weight change, feed conversion, or egg production was observed with the supplementation of ASI ($P > 0.05$). ADFI significantly increased for hens fed 0.025% and 0.05% ASI compared to control ($P < 0.05$). Eggshell strength was significantly improved for hens supplemented with 0.05% ASI at weeks 71 and 73 ($P < 0.05$). Dietary ASI increased the bone mineral content (BMC) and fat mass compared to control at 81 weeks ($P < 0.05$). Calcium excretion was significantly lower for 0.10% and 0.15% ASI at week 69 ($P < 0.05$), while phosphorus excretion was not significantly different between treatments ($P > 0.05$). There was a linear decrease in iron, manganese, and zinc excretion with increasing supplementation of ASI at 81 weeks ($P < 0.05$). Overall, a dose-dependent response to supplemental ASI supported egg quality and bone metabolism likely through improved mineral retention and calcium bioavailability.

Keywords: bone quality, dual energy x-ray absorptiometry, egg quality, laying hen , laying performance

335 The response of broiler performance to chestnut tannins is dependent on the dietary amino acid density

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In modern broiler production, various nutritional strategies can be implemented to meet their amino acid (AA) requirements: standard levels of digestible AA to meet the requirements for optimal performance versus higher levels of digestible AA to meet the requirements for optimal breast meat deposition. In addition to optimal nutrient supply, feed additives can support better performance of broilers and improve feed efficiency but unfortunately, feed additives are often validated in only one single nutritional context. The goal of this study was therefore to test the interaction between nutrition (dietary AA density) and the addition of a chestnut tannins - KingBrown® (KB). KB is used to support broiler performance under coccidiosis challenge but can also bind to feed proteins and endogenous enzymes and reduce the AA availability if provided at a high dosage. The research question is therefore: does the dietary AA density have an impact on the performance response of broilers to KB? In total, 1,056 one-day-old Cobb 500 male broilers were subjected to 6 dietary treatments, with 8 replicates of 22 broilers per pen from 0 to 35 days. Treatment HAA was the control treatment with a high AA provision (1.30, 1.20 and 1.10 % dLys in starter, grower and finisher, respectively) and LAA was the treatment HAA with a reduced AA provision (-0.10 % dLys for all phases). HAA + KB0.5, HAA + KB1, LAA + KB0.5 and LAA + KB1 were respectively treatments HAA supplemented with 0.5 or 1kg/T of KB and treatments LAA supplemented with 0.5 or 1kg/T. Reducing dietary AA density decreased the overall growth performance (-50g of body weight at 35d, $P<0.05$) and increased FCR (+ 0.03 pts at 35d, $P<0.05$), and the effects were already visible from period 0-7d. There was a clear interaction between the level of supplementation of KB and AA density on growth performance in periods 0-7d and 21-35d on FCR ($p < 0.05$). HAA + KB1 increased FCR by 0.03 pts in both periods compared to the HAA treatment while there was no negative effect of HAA + KB0.5 and there was no negative effects of treatments LAA + KB0.5 and LAA + KB1 when compared to treatment LAA. This interaction indicates that different levels of chestnut tannins should be fed to broilers, depending on the dietary AA density. As this study was performed under optimal sanitary conditions, further research should be conducted to study those responses under more challenging conditions and to better understand the mode of action.

Keywords: Amino acid density, Chestnut tannins, Essential oils

336 Effect of methionine source and levels on nitrogen utilization in growing broilers

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Efforts to reduce nitrogen (**N**) excretion in animal production have steadily increased. Methionine (**Met**), the first limiting amino acid in broiler diets, must be optimized to maximize performance and lower N excretions. Evidence suggest that dietary Met sources can affect the N retention of broilers. This study evaluates the effects of Met source and level on the performance and dietary N retention of growing broilers. One hundred twenty day-old male Ross 308 chicks were raised in floor pens on a commercial starter diet until 13 d of age. Ninety six birds were then selected and placed in individual metabolic cages from 13 to 23 d of age and assigned to one of the four diets ($n = 24$) with either DL-methionine (**DL-Met**) or hydroxy methionine (**OH-Met**) as the supplemental Met source to supply digestible total sulfur amino acid (**TSAA**) at suboptimal (65% TSAA:lysine) and optimal (75% TSAA:lysine) levels. Both Met sources were used on an iso-molecular basis. The N retention assay was done by total excreta collection from 20 to 23 d. Results were analyzed using a two-way ANOVA with Met source and level as the main factors along with their interaction. For the N retention parameters, dry matter intake was used as a co-factor. Post-hoc means comparison was done by Tukey's Test. The zootechnical performance parameters during 13 to 23 d were not

impacted by Met source ($P \geq 0.62$), level ($P \geq 0.59$), or their interaction ($P \geq 0.43$). There were no significant differences between source, level, and their interaction for dry matter and total N intake ($P \geq 0.48$). However, a significant interaction of Met source and level was observed showing that feeding broilers with OH-Met at optimal TSAA level allowed a significant reduction ($P < 0.01$) in total N output and the highest ($P < 0.01$) N retention compared to other treatments. This N retention was mainly explained by the significant reduction ($P < 0.01$) in uric acid excretion without changes in fecal N excretion ($P > 0.10$), demonstrating the metabolic origin of the N saving. These findings confirm the equivalence of DL-Met and OH-Met for broiler performance. However, results challenge conventional dietary recommendations method, suggesting that dietary Met needs may be overestimated when based solely on performance. In conclusion, optimizing Met supply is crucial not only from a growth standpoint but also from an environmental perspective. Furthermore, our results showed that compared to DL-Met, OH-Met has a greater potential to reduce metabolic N excretion and optimize N utilization and reduce metabolic excretion.

Keywords: broiler, metabolism, methionine, nitrogen retention, uric acid

337 Effects of methionine sources on performance and productivity in broiler breeders

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Previous studies suggest a bioavailability value (RBV) of 65% for methionine hydroxy analogue (MHA) relative to DL-Methionine (DLM), which can be shown when replacing MHA with DLM at a 100:65 ratio (weight basis) while maintaining performance in different species. The objective of this study was to evaluate the RBV of MHA in broiler breeders. A total of 824 Fast Feather Broiler Breeder Hens (Cobb 500™) and 80 Roosters (Cobb Male™) were randomly allocated to two treatments containing either MHA calcium salt (**MHA-Ca**, 84% purity) or DLM (at 65% of MHA-Ca, weight basis, **65DLM**, 99% purity) with 8 replicates of 50-51 birds, from 28-48 wks of age. A breeder 1 diet was formulated to meet or exceed all nutrients according to the breeder guideline and used throughout the trial. The male to female ratio was 1:10, and birds were fed a common breeder diet for a 4-wk acclimation period (24-28 wks). Each pen was then individually weighed to ensure no differences in average body weight or flock uniformity were present before the beginning of the trial. Feed was provided at 7 am daily and amounts were calculated based on average bodyweight, body weight gain, and weekly egg production to determine daily caloric requirements at the start of each wk. Birds had free access to water over the 9-hr period following feeding. Light and temperature followed the breed guideline. Egg production was evaluated weekly, performance and egg quality (8 eggs/pen) every 4 wks, and feathering (25 hens/pen) and fertility (16 eggs/pen) at the end of the study. One-way ANOVA was used and significances were considered at $P<0.05$ (JMP 17.0). No differences were seen for body weight, flock uniformity or mortality at any time point ($P>0.10$), and these results followed closely the breed performance objectives. Egg production and egg mass were similar between the treatments ($P>0.40$), however, they were slightly below Cobb objectives. Egg weight, Haugh units, and vitelline membrane strength were not different between the treatments ($P>0.10$), however, shell strength tended to be higher in the MHA-Ca-fed group at 32, 40, 44, and 48 wks ($0.05<P<0.10$). Fertility, total hatch, and hatch of fertile were similar between treatments ($P>0.10$) at 48 wks. 65DLM significantly reduced the severity of feathering loss in the back/rump area ($P=0.03$), while MHA-Ca improved feathering in the legs ($P<0.05$) at 48 wks. Overall, no significant differences between methionine sources were observed in the majority of measured parameters during the 20-wk production period, despite replacing 100 units of MHA-Ca with 65 units of DLM. Therefore, these results suggest that the RBV of 65% for MHA relative to DLM is also applicable for broiler breeders.

Keywords: Nutrition, Amino Acids, Broiler Breeder, Methionine, Performance

338 Fasting heat production and metabolic responses of L-citrulline-fed broiler chickens subjected to acute heat stress conditions

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L-citrulline is a non-essential amino acid with thermoregulatory, anti-inflammatory, and immuno-modulatory functions in poultry. Oral and dietary provision of L-citrulline have proven beneficial in mitigating the adverse impacts of heat stress in chickens. To understand the mechanisms behind L-citrulline-induced thermotolerance, this study investigated the efficacy of dietary L-citrulline supplementation on the fasting heat production and metabolic responses of broiler chickens under acute heat stress. In this study, 384-day-old broiler chickens were fed a basal diet (CON group) or basal diet supplemented with 1% L-citrulline (LCT group). At 18 days old, 32 broilers were moved into the respiratory chambers for 3-d acclimatization, and arranged using a 2 by 2 factorial design of two diets (CON vs. LCT group) and two temperatures (24 ^{o}C (TNZ) vs. 35 ^{o}C (HS)) into 4 treatments of 8 replicates each. Birds were fasted for 24h, then the HS group was exposed to 35 ^{o}C, 65 %RH for 5 hours. One chicken from each replicate was selected for blood and tissue collection. Data was analyzed using the T-test to compare main effects or two-way ANOVA procedure on the statistical analysis software. The results showed that there were no significant interactions of diet by temperature on the body temperature, metabolic responses and thermosensory genes that were detected ($P > 0.05$). However, the LCT-fed broilers had lowered body weights and average daily gains relative to the CON group ($P < 0.05$). The oxygen consumption, carbon dioxide expiration, and total heat production were also elevated in LCT-fed broilers compared to the CON group. LCT upregulated the mRNA expression of TRPV1, TRPV3, and TRPM8 in the hypothalamus and TRPA-1 in the skin of broilers compared to CON groups ($P \leq 0.05$). Following HS exposure, the rectal, surface, and core body temperatures were elevated in HS-broilers compared to the TNZ-housed broilers ($P < 0.05$). The oxygen consumption, total heat production, and fasting heat production of $BW^{0.70/d}$ were significantly higher in the HS group than in the TNZ broilers ($P < 0.05$). Furthermore, the mRNA expression of skin TRPV3 was downregulated, whereas, both the skin and hypothalamic expression of TRPM8 tended to increase with HS exposure. Altogether, this study revealed that acute heat

stress increased the basal metabolic rates and heat generation in broilers, which was not alleviated with dietary L-citrulline supplementation. Moreover, it was evident that both factors may function independently during thermoregulation by influencing the expression of both hot and cold-sensitive ion channels in thermosensitive organs.

Keywords: Broilers, Heat production, Metabolic rate, L-citrulline, Thermoregulation

339 Precision biotics: an effective strategy to enhance performance and egg quality in commercial laying hens raised in a cage-free system

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This study aimed to evaluate the effects of supplementation with glycan-based precision biotics combined with a commercial symbiotic on the productive performance and egg quality of commercial laying hens raised in a cage-free system. The experiment involved 1,120 Hy-Line Brown hens (54 weeks of age), assigned to two treatments: a commercial symbiotic (probiotic + mannan-oligosaccharides + beta-glucans) and a commercial symbiotic + glycan-based precision biotic. A completely randomized design was used, with eight replications and 70 hens per unit. The birds were fed mash diets based on corn and soybean meal, with the respective treatments included. Environmental conditions and nutritional management were standardized according to breed-specific recommendations. Performance variables (laying rate, feed intake, egg weight and mass, and feed conversion rate) and egg quality parameters (Haugh unit, shell strength, shell thickness, and yolk index) were assessed between 58 and 90 weeks of age. Data were analyzed using ANOVA with repeated measures over time ($p < 0.05$). Supplementation with precision biotics significantly increased laying rate (83.97% vs. 79.68%; $p < 0.01$) and egg mass per hen per day (53.14 g vs. 50.07 g; $p < 0.01$), indicating improved productivity in older hens. Although feed intake was higher in the supplemented group with precision biotics (125.14 g vs. 118.30 g; $p = 0.01$), feed conversion remained unchanged between treatments (2.37 vs. 2.36; $p > 0.05$), suggesting that the increased feed intake did not negatively affect feed efficiency. No significant differences were found in average egg weight (64.34 g vs. 64.19 g; $p = 0.35$), indicating that the increase in productivity did not compromise egg size. Regarding egg quality, supplementation did not adversely affect the evaluated parameters. The Haugh unit was similar between treatments (83.19 vs. 83.08; $p = 0.97$), and no significant differences were observed in shell strength (4.02 kgf vs. 3.97 kgf; $p = 0.61$), shell thickness (0.38 mm for both groups; $p = 0.34$), or yolk index (0.43 for both treatments; $p = 0.13$). Incorporating glycan-based precision biotics into the diets of cage-free commercial laying hens significantly improved productivity, particularly in older hens, without adversely affecting feed conversion or egg quality. This approach holds significant potential for optimizing performance in long-cycle commercial systems.

Keywords: Cage-free systems, egg quality, laying hens, performance, precision biotics

340 A Silymarin-based formulation promotes broiler performance: Antioxidant mechanisms as key drivers supported by integrated field and mechanistic insights

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The study assessed broiler response to a silymarin-based formulation (**SBF**) and developed a mechanistic model to describe the antioxidant mechanisms associated with observed response. Twelve broiler houses (15,000 birds each) were assigned to a control feeding program (T1) or fed 35 ppm of phytogenic extract (SBF; from Liverin-M, 7% silymarin; reformulated to 28%, Liverin-NFC; CKM SAC, Peru) from 21 to 35 days (T2). Performance (feed intake, **FI**; BW gain, **BWG**; fed conversion ratio, **FCR**), energy gain (**EGA**), net energy (**NE**) for gain (**NEg**), fasting heat production (**FHP**), NE for maintenance (**NEm**), and total NE (**NEt**; calculated as productive energy, **PE**; PE = NEg + NEm) were assessed in 22-28, 29-35, and 22-35 d periods, and European Broiler Index (**EBI**) in 29-35 d. BW, processing weights (carcass, **CAR**; breast fillet, **BRE**; tenderloins, **TEN**; wings, **WIN**; leg quarters, **LQU**; total white meat, **TWM**, TWM = BRE+TEN) and bird market value (**MKV**, $/bird) were estimated at 42 d. A mechanistic model of the associated antioxidant effects was developed and complemented with literature data. A flexible P-value criterion was used, and ANOVA was run on JMP Pro 16. SBF showed no effect on FI (P=0.760) but improved BW (35 d, P=0.053), BWG (29-35 d, P=0.060; 22-35 d, P=0.085), FCR (29-35 d, P=0.024; 22-35 d, P=0.058), and EBI (P=0.037), increased body energy (35 d, 3,612 vs. 3,481 kcal/bird, P=0.053), EGA (29-35 d, 164.4 vs. 149.7 kcal/bird/d, P=0.054), NEg (29-35 d, 962 vs. 897 kcal/kg, P=0.033; 22-35 d, 1,021 vs. 974 kcal/kg, P=0.068), with no notable change in NEm (kcal/kg; P>0.69), which translated into extra NEt kcals (29-35 d, +53 kcal/kg; 22-35 d, +52 kcal/kg). SBF reduced FHP (22-35 d, 109.2 vs. 110.4 kcal/kgBW/d, P=0.052), reducing the FHP/EGA ratio in 7.9% (29-35 d), which implies a more efficient use of dietary energy for gain. SBF increased CAR, BRE, TEN, WIN, LQU, TWM (P≤0.034), and MKV (+0.129 $/bird; P=0.033). The mechanistic model suggests that SBF reduces maintenance expenditure, FCR, and FHP, supports immune activity, and influences microbiota, while it increases BWG, NEg, and NEt, lowering feeding costs and increasing MKV. It suggests that response does not necessarily depend on pathological/toxic challenges but that the free radical-scavenging activity of SBF, along with its effect favoring bird's inner antioxidant systems, support the high metabolic activity of modern lines, that the benefit of antioxidant effects of SBF is more relevant the faster the broilers grow, and that the combination of flavonolignans and their bioavailability are crucial due to likely synergistic effects. In conclusion, the SBF promoted performance, improved energy efficiency, and increased meat production and broiler market value.

Keywords: Silymarin-based formulation, Antioxidant, Broiler, Performance, Productive energy

341 Liver health dynamics, amino acid metabolism, and growth kinetics of commercial broilers under a hepatic challenge and fed a Silymarin-based formulation

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The study aimed to determine the effect of a silymarin-based formulation (**SBF**) in broilers under a natural hepatic challenge on liver health, its influence on protein anabolism, and the growth curve, and to develop a mechanistic model to explain the action

mechanisms associated with such effects. Houses in a broiler complex (n=12, total 180,000 birds) with hepatic challenge background were randomly allocated to a control diet or to a treatment group (fed 35 ppm of phytogenic extract, SBF; from Liverin-M, 7% silymarin; reformulated to 28%, Liverin-NFC; CKM SAC, Peru) from 21 to 35 days. Bird mortality (%), liver lesion prevalence (**LLP**, %), and intensity (**LLI**, 0-3 score) were assessed between 29 and 35 d. BW gain (**BWG**), protein accretion (**PAC**, g/bird/d), and PAC efficiency (**PACe**, %) were determined from 22 to 28 d, 29 to 35 d, and 22 to 35 d. Gompertz curves were modeled for each house, and the maximum asymptote obtained was set as a common value to re-fit them all. Then, growth rate (**GR**) and inflection point (**IP**) were obtained, and the age at a commercial BW (2.7 kg, **CA**) was inversely predicted for each house. A mechanistic model explaining the observed response and the relationship between liver function, protein anabolism, and growth rate was developed and enriched with literature data. A relaxed P-value criterion was considered. ANOVA to compare the treatments and modeling approaches were run in JMP. SBF reduced LLP, LLI ($P<0.001$), and mortality ($P=0.007$). It improved PAC ($P=0.058$) and PACe ($P=0.028$), increased the Gompertz curve GR ($P=0.081$), and reduced CA (–1.4 d, $P=0.043$). The mechanistic model indicates that SBF reduces liver lesions, mixed muscle protein degradation rate, amino acid catabolism, maintenance amino acid expenditure, and mortality while increasing essential and non-essential amino acid pools, mixed muscle protein synthesis rate, PAC, PACe, BWG, and leanness, reducing the age at commercial BW and favoring economics. It suggests that these responses do not necessarily depend on pathological hepatic challenges but support the demanding anabolic activity of modern broiler lines and that the specific combination of flavonolignans and their availability have a major influence on broiler response. In conclusion, SBF reduced subclinical liver damage and mortality while improving protein accretion and growth, and reduced the age to reach a commercial BW, supporting economics.

Keywords: Silymarin-based formulation, Amino acid metabolism, Protein accretion, Growth curve, Broilers

342 Effects of Plantago ovata seed supplementation on performance, egg quality, and immune response in laying Japanese quail

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The effects of Plantago ovata seed (PO) supplementation were evaluated on performance, egg quality, organ weight, and immune response in laying Japanese quail in a 6-week experiment. A total of 120 75-day-old quails were randomly distributed into 6 treatments with 4 replicates of 5 birds per cage. Birds were fed a standard basal diet according to NRC (1994) recommendations, with treatments consisting of a control diet (0 g/kg PO) or diets supplemented with PO at 10, 20, 30, 40, or 50 g/kg, along with ad libitum access to feed and water. A one-way ANOVA analysis was performed on data with 0.05 significance level. Feed intake data revealed a significant impact of supplementing PO, showing a linear increase in week 2 and a decrease in week 6 ($p < 0.05$), while overall feed intake was not affected significantly ($p > 0.05$). No significant effects were observed in egg production and egg mass among treatments ($p > 0.05$). However, a significant increase in egg weight of the birds receiving 20 g/kg PO was observed compared to control group ($p < 0.05$). Regarding external egg quality measurements, a decline in albumen height and a linear increase in eggshell weight percentage was observed with increasing the levels of PO ($p < 0.05$). A significantly higher shell strength was seen in the group receiving 30 g/kg PO, and the control, 10, and 30 g/kg groups showed the thickest shells ($p < 0.05$). The influence of supplementing PO on intestinal bacterial populations was also significant, showing a linear increase in total aerobic bacteria, coliforms, and lactic acid bacteria as well as both linear and quadratic trends in spore-forming bacteria ($p < 0.05$). No significant differences were seen in moisture content of excreta, even though the 10 and 20 g/kg levels of PO yielded lower moisture level in excreta than 30-50 g/kg PO supplemented groups. FCR did not show any significant effects of the treatments ($p > 0.05$). Regarding immune response, a significant increase was revealed in skin thickness to PHA injection in male birds, in which the groups receiving 20 and 30 g/kg PO showed the highest response ($p < 0.05$). The weight of gizzard and the length of duodenum increased with increasing the level of PO ($p < 0.05$). Likewise, the length of jejunum + ileum increased significantly in those receiving 30 and 50 g/kg PO ($p < 0.05$). These results suggest that dietary supplementation of PO at moderate levels (20–30 g/kg) can enhance egg weight, eggshell quality, and immune response without negatively affecting overall performance in laying Japanese quail.

Keywords: Plantago ovata seed, egg quality, immune response, intestinal microbiota, Japanese quail

343 Meta-analysis of a protected organic acid blend effect on growth performance of broiler chickens

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Studies have shown that dietary organic acid supplementation can improve growth performance and gut health of broiler chickens although its effect lacks consistency. Meta-analysis is a statistical tool which can be used in poultry nutrition to increase statistical power and provide more accurate estimates by combining data across studies. A meta-analysis was conducted to evaluate the effect of a protected organic acid blend (POAB, AVIMATRIX® Feed Solution, benzoic acid, calcium formate and fumaric acid blend) on growth performance of broilers. The meta-analysis included 8 trials, and each trial consisted of 2 dietary treatments: Negative Control (NC) without organic acid supplementation and POAB supplemented at 0.5 or 0.532 kg/ton diet. Body weight, mortality adjusted FCR, and feed intake were determined at various weigh days and data were grouped into 0-14 d, 0-21 d, 0-28 d, and 0-42 d phases. Treatments from each trial had between 8 to 25 replicate pens/treatment and 8 to 40 birds/replicate pen, which added up to 257, 185, 111, and 134 observations for 0-14 d, 0-21 d, 0-28 d, and 0-42 d respectively. All trials had a dietary challenge such as wheat without xylanase, canola, rye, and poultry byproduct meal, and/or a pathogenic (*Eimeria* or *C. perfringens)* challenge, with 5 trials having both dietary and pathogenic challenges Performance data were analyzed using a mixed model where the effect of POAB level was a fixed effect, trial was a random effect, continuous day variable was used as a covariate. Pen was the experimental unit. Least square means were compared using Student's t procedure and a P-value of equal or less than 0.05 was considered statistically significant. Dietary supplementation of POAB at 0.5 or 0.532 kg/ton significantly and consistently increased body weight and improved FCR ($P<0.05$)

without altering feed intake (P>0.05) of broilers on days 14, 21, 28, and 42. For the overall 0-42 d period, the improvement was 3.5 points (95% confidence interval [2.1, 4.8]) for FCR (1.663 vs 1.628 for NC and POAB) and 62 g (95% confidence interval [32, 93] for body weight (2.299 vs 2.361 kg for NC and POAB) (P<0.05). In conclusion, the meta-analysis demonstrated that the POAB can be an effective tool in improving growth and production efficiency under various dietary and pathogenic challenging conditions in broiler chicken production.

Keywords: organic acid, benzoic acid, broiler, growth performance, meta-analysis

344 Evaluation of guanidinoacetic acid supplementation on broiler performance at different dietary energy levels and feeding phases

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Guanidinoacetic acid (GAA) is the metabolic precursor of creatine, which plays an important role in energy metabolism, particularly in muscle cells, and consequently supports muscle growth. GAA supplementation has been shown to improve feed efficiency and energy utilization in broilers. This study aims to evaluate the response of broilers to GAA supplementation in diets with gradually reduced apparent metabolizable energy (AMEn, N-corrected) during different feeding phases. Three independent experiments (Expt) were carried out with 3600 one-day (d)-old male Ross 308 broilers in each Expt, with 18 groups/Expt, 5 pens/group and 40 birds/pen. In Expt 1, 2 and 3, birds were randomly assigned to the groups on d 1, 10 and 24, and the starter, grower and finisher phase were investigated respectively. In Exp 2 and 3 birds were fed with basal diet without GAA supplementation until the start of the experiment. Basal diets were formulated with 3100, 3200, 3300 kcal/kg in starter (d 1-10), grower (d 10-24) and finisher phase (d 24-42) respectively, and those experimental diets were formulated to reduce AMEn up to 400 kcal/kg in 50 kcal/kg steps and supplemented with GAA (0 or 600 g/t feed). Daily weight gain (DWG), daily feed intake (DFI), feed conversion ratio (FCR, mortality adjusted), energy (EER) and protein efficiency ratio (PER) were measured. Data was analyzed using two-way ANOVA of SAS (ver. 9.4), with multiple linear and piecewise linear regressions. Significance was considered at P<0.05. DFI (R^2=0.76-0.97, P<0.01), FCR (R^2=0.91-0.97, P<0.01) and EER (R^2=0.40-0.84, P<0.01) increased linearly, whilst PER decreased linearly (R^2=0.91-0.98, P<0.01), with the dietary AMEn reduction in all phases. Birds with GAA supplementation had significantly (P<0.05) better FCR, EER and PER during grower (Expt 2) and finisher (Expt 3) phases than the unsupplemented birds. DWG was decreased and DFI was increased significantly (P<0.05) by the reduction of AMEn in all phases. The dietary AMEn levels for optimal DWG were lower when GAA was added in diets (2918, 2996 and 3084 vs. 2975, 3075 and 3175 kcal/kg for the starter, grower and finisher phases, respectively). In conclusion, dietary AMEn reduction reduces the growth performance and PER, but increases the EER of broilers, while GAA supplementation improves EER, PER and FCR of birds independently of dietary energy level and feeding phase. The results indicate that GAA supplementation allows for dietary energy reduction and the magnitude of the energy sparing effect increases from early to late feeding phases.

Keywords: Nutrition, Poultry, Performance, Guanidinoacetic acid, Energy sparing

345 Isoacids in broiler diets: effects on performance

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A study evaluated the use of a blend of isoacids (isobutyrate e 2-methyl butyrate - 2:1) in broiler diets from 1 to 35 days of age. A total of 896 mixed Cobb broilers were used, distributed in a completely randomized design in a 2x4 factorial scheme: 2 dietary densities - low (LD) and high (HD), and 4 inclusion levels of isoacid (ISA) - 0; 0.25; 0.50 and 1.00%, for a total of 8 treatments with 8 replicates of 14 birds each (7 males + 7 females). The diets were mashed based on corn and soybean meal with phytase and xylanase added. HD diets were formulated with 3% more metabolizable energy and 9% more amino acids, related to DigLys, than the LD diets. Data on feed consumption, body weight, weight gain, and feed conversion were evaluated. The data were analyzed by ANOVA, and if significant, the Tukey test was used (P<0.05). There was no significant interaction between the factors studied. Birds fed the high-density diet with 1% ISA supplementation had a body weight of 2.489 g (P<0.001) at 35 days and a weight gain of 2.248 g (P<0.001) between 1 and 35 days, higher than the other treatments. In conclusion, results showed the potential of using isoacids in HD diets at 1% inclusion, as there was an improvement in broiler performance. Future studies will help to better understand the role of isoacids as modulators of gut health and immune response, contributing to more efficient and sustainable diets in poultry production.

Keywords: feed conversion, metabolizable energy, nutrient density, short chain branched carboxylic acids, Weight gain

346 Effect of organic acids and mannan oligosaccharides on production parameters and egg quality of laying hens

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Actual demand of antibiotic-free products has prompted producers to reduce or eliminate the use of antibiotics growth promoters (AGP) in laying hen feed and use alternative additives. The objective of this study was to evaluate the effect of an additive (Uniwall MOS 25®) composed of a synergistic combination of organic acids (Acetic acid, Formic acid, Ammonium formate and Ammonium propionate), with prebiotics (mannan oligosaccharides and beta-glucans) for the replacement of conventional AGP in diets of Babcock Brown laying hens since 21 to 41 weeks of life on performance and egg quality. A total of 156 birds housed in 52 cages were randomly distributed and subjected to different diets in four treatments, with 13 replicates per treatment and 3 birds per replicate as follows: Negative Control (NC); Positive Control (PC) - 50 ppm Zinc Bacitracin; T3: 1.0 Kg/ton (Uniwall MOS 25®); T4: 2.0 Kg/ton (Uniwall MOS 25®). Data was analyzed using one-way ANOVA in a randomized complete design. Mean separation was performed using Tukey's test. The results obtained in both periods evaluated (21-31 and 32-41 weeks) showed that supplementation with different levels of Uniwall MOS 25® significantly increased daily egg production

and daily egg mass (P<0.05), evidencing a similar behavior to the PC. Furthermore, feed conversion was better in treatments supplemented with Uniwall MOS 25® and the PC. Hens fed the NC diet had a lower laying percentage and egg mass production than hens fed diets supplemented with prebiotics and organic acids and with the AGP. At both stages (21-31 and 32-41 weeks), egg weight increased significantly (P <0.05) with the treatments supplemented with Uniwall MOS 25® and the PC compared to the NC, showing an improving behavior in egg weight values 32-41 with respect to the first period evaluated (21-31 weeks). The highest values were presented in birds on the experimental diet with AGP, followed by T4 (Uniwall MOS 25® 2 kg/Ton) and then T3 (Uniwall MOS 25® 1 kg/Ton) throughout the trial. PC showed the highest shell strength in both phases of the experiment (p<0.05). Albumen height did not show significant differences in the first phase, but differences did appear in the second stage (P=0.0487), indicating that supplementation may influence internal egg quality over time. It can be concluded that the inclusion of organic acids and MOS in the diet of laying hens has a similar positive effect to that observed with conventional AGP (Zinc Bacitracin), optimizing production parameters and egg quality, making it an effective alternative to antibiotic growth promoters.

Keywords: organic acids, prebiotics, performance, laying hen, egg quality

347 Not Presented

348 Fermented feed modulates growth performance and intestinal health in broiler chickens challenged with Necrotic enteritis

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This study aimed to evaluate the effects of dietary fermented feed (FF) supplementation on growth performance, intestinal health, permeability, and body composition in broiler chickens challenged with necrotic enteritis (NE). 288 0-day-old Cobb 500 male broiler chickens were used in a completely randomized design with 4 treatment groups with 6 replicates of 12 chickens per battery cage: negative control (NC; basal diet), positive control (PC; basal diet + NE challenge), 5% FF + NE challenge (F5), and 15% FF + NE challenge (F15). Fermented feed, prepared by solid-state fermentation of corn and soybean meal, was utilized. NE challenge was induced by *Eimeria maxima* (day 14) and *Clostridium perfringens* (days 18-19). Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), lesion scores, intestinal permeability, jejunal *Clostridium perfringens* counts, and body composition (DEXA, day 21) were measured. Data were analyzed by one-way ANOVA, with Tukey's HSD test employed for post-hoc comparisons. Statistical significance was determined at $p < 0.05$. At 7 and 14 days, F15 supplementation increased BWG and improved FCR compared to NC and PC. From 14-21 days, F15 maintained greater BWG than NC and PC. Overall, F15 supplementation improved BWG and FCR over 21 days. NE challenge increased lesion scores in PC, F5, and F15 compared to NC. Intestinal permeability was increased in PC and F5 compared to NC. F15 supplementation reduced intestinal permeability compared to PC. Jejunal *Clostridium perfringens* counts were increased in PC, F5, and F15 compared to NC; F5 had lower counts than PC. F5 and F15 supplementation increased body fat percentage compared to NC and PC. No differences were observed in bone mineral density or bone mineral content. Dietary supplementation with 15% FF improved growth performance and intestinal barrier function under NE challenge, as evidenced by improved BWG, FCR, and reduced intestinal permeability. While FF supplementation did not mitigate intestinal lesions, these findings suggest that FF may have potential as a feed additive to enhance broiler performance under NE challenge. Further research is needed to explore the effects of FF on nutrient digestibility and intestinal nutrient transport.

Keywords: fermented feed, necrotic enteritis, broiler, growth performance, intestinal health

349 Effects of commercial mycotoxin deactivator on performance, gut and organ morphometry, and liver color in broiler chickens fed diets contaminated with Fumonisin and Deoxynivalenol

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Mycotoxins are frequently detected in poultry feed. According to the DSM-Firmenich World Mycotoxin Survey, 28,388 samples from 95 countries were analyzed in 2024. Deoxynivalenol (DON) and fumonisins (FUM) were among the most prevalent, with global contamination rates of 63% and 61%, respectively, posing significant risks to poultry health. The objective of this study was to evaluate the effects of including a commercial mycotoxin deactivator (Mycofix® Select 5.0) on performance, gut morphometry, organ morphometry and liver color in broiler chickens fed diets contaminated with DON and FUM. A total of 336 one-day-old male Ross 308 chickens were randomly allocated to 28 pens (12 birds per pen) in a 2 X 2 factorial arrangement: with or without an average of 10,790 ppb FUM and 6,025 ppb DON, and with or without 2 kg/t of Mycofix® Select 5.0. Diets were formulated to meet breeder nutritional recommendations using corn, soybean meal and DDGS in mash form. Birds were reared to 28 days of age. Variables analyzed included weekly body weight (BW), feed intake (FI), adjusted feed conversion ratio (Adj. FCR), mortality, intestinal morphology (villus length and width, villus number, villus-to-crypt depth ratio, absorption area), organ morphometry (liver, proventriculus, gizzard, thymus, spleen and intestine), and liver color at 22 days. Significant differences ($P < 0.05$) in performance were observed due to FUM and DON. Their inclusion resulted in lower BW and higher Adj. FCR. Chickens fed FUM and DON without the addition of Mycofix® Select 5.0 had the lowest body weight. Duodenal morphometry showed significant differences ($P < 0.05$) for both main factors. The addition of the deactivator resulted in greater villus length, thickness, villus-to-crypt depth ratio and surface area, whereas the inclusion of FUM and DON addition reduced villus length, villus number, villus-to-crypt depth ratio, and surface area. Organ morphometry differed significantly due to the inclusion of Mycofix® Select 5.0, with higher absolute liver, proventriculus, and thymus weights, and greater relative thymus weight. Liver color at day 22 was affected by FUM and DON, which increased a* and b* values. These results demonstrate that dietary inclusion of Mycofix® Select 5.0 in mycotoxin-contaminated diets improved broiler performance, intestinal morphometry, and organ development, mitigating the negative effects of FUM and DON.

Keywords: mycotoxins, fumonisins, deoxynivalenol, broiler chickens, deactivator

350 Effect and prediction of carbohydrase enzyme supplementation on growth performance of broilers

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A meta-analysis investigated the performance of exogenous enzyme (Rovabio Advance; Adisseo), across 27 trials with 84 diets. The dataset included 2,774 individual data entries covering various strains over 35-to-42-day trial periods. A random-effects model was used for data analysis. The primary objectives were to evaluate the average response of the enzyme on broiler performance, identify the key factors influencing its effectiveness, and explore potential strategies for optimizing feed formulation. Studied parameters were feed intake (FI), feed conversion ratio (FCR) and average daily gain (ADG). Enzyme significantly improved performance of broilers. Average (min/max) were 0.83 (-4.0/4.6) and -1.3 (-6.8/2.3) for ADG and FCR, respectively. The analysis demonstrated that diet composition was the most significant factor affecting enzyme response, explaining approximately 79% of the observed variability. Principal component analysis suggests that the source and level of raw materials and nutrients can explain 21 and 59% of the variability, respectively. Predictive equations were developed for FI, ADG and FCR prediction, based on metabolizable energy, available phosphorus and digestible amino acids, with respective determination coefficients being 0.93, 0.90 and 0.90 for FI ADG and FCR, respectively. The enzyme effect was predicted taking into account soluble arabinoxylan content with determination coefficients of 0.93, 0.95 and 0.93, Taking the soluble arabinoxylan content into account, the predicted enzyme effect on the coefficients on FI, ADG and FCR was 0.93, 0.95 and 0.93, respectively. The findings underscore the value of customizing feed formulations based on specific nutrient compositions and substrate content, enabling more effective utilization of enzymes in poultry nutrition. The results also suggest the need for further research to optimize enzyme application in diets with varying nutrient densities, substrate content and for other feed additives like phytase and protease. Additionally, the potential of using soluble arabinoxylan (AX) content to better predict enzyme response was discussed, along with the need for more precise calibration techniques, such as NIRS, for evaluating dietary AX levels.

Keywords: xylanase, broiler, performance, meta analysis

351 Efficacy of phytase supplementation on growth performance, protein efficiency ratio and pre-cecal phosphorus digestibility in broilers

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The objective of this 35-d study was to investigate the impact of increasing doses of a novel biosynthetic bacterial 6-phytase on growth performance, protein efficiency ratio and pre-cecal phosphorus digestibility (pcPd) in broilers fed corn-soybean meal-based diets that were low in available P (avP) and calcium (Ca) from 1 to 35 days of age. Male Ross 308 chicks (n = 1,200) were randomly allocated to one of five experimental dietary treatments with 12 replicate pens per treatment. The experimental dietary treatments consisted of a positive control (PC) diet with calculated non-phytate P (NPP) and Ca levels of 0.45% and 0.90%, and 0.33% and 0.66% for the starter and grower diets, respectively, negative control (NC) diet similarly reduced by 0.22% points in avP and calcium (Ca) of 0.22% points compared to the PC diet, and the NC diet supplemented with phytase (Phy) at 250, 500 or 1000 phytase units (FTU)/kg diet. Body weight (BW) and feed intake were measured at 21 and 35 days of age, and average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated. In addition, ileal digesta samples were collected on day 21 for pcPd determination. Compared with the PC, the NC group had reduced ($P < 0.001$) final BW (-25.8%), ADG (-26.2%), ADFI (-24.1%), PER (-3.2%) and pcPd (-8.6% points) and increased ($P < 0.001$) FCR (+2.9%). Increasing phytase dose linearly and quadratically improved ($P < 0.001$) the performance, PER and pcdP. In comparison with the NC group, the highest dose of phytase improved ($P < 0.001$) the final BW (+40.9%), ADG (+41.9%), ADFI (+36.6%), PER (+3.7%), pcPd (+21.6% points) and FCR (-3.8%). These findings highlighted that supplementing a biosynthetic bacterial 6-phytase can be an effective strategy at improving broiler performance, PER and P digestibility when fed diets low in avP and Ca.

Keywords: phytase, performance, phosphorus

352 Evaluation of the effects of bile acids and emulsifier on the digestibility of crude protein and ether extract in broilers

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The present study aimed to evaluate the effect of bile acids (BAs), specifically cholic and deoxycholic acids, and an emulsifier, composed of soy lecithin, glyceryl ricinoleate and mono and diglycerides of fatty acids, on the digestibility of ether extract and crude protein in broilers. The study was conducted at the Poultry Technology Research Center/Federal University of Lavras, in Lavras, Minas Gerais, Brazil. A total of 240 one-day-old male Cobb 500 chicks were randomly assigned to five treatments with eight replicates of six birds each, totaling 40 metabolic cages. The treatments consisted of T1 – basal diet (BD), T2 – BD + 500 g/ton of emulsifier, T3 – BD + 100 g/ton of BAs, T4 - BD + 150 g/ton of BAs, and T5 - BD + 200 g/ton of BAs. The diets were formulated according to the nutritional levels recommended by the lineage manual for the initial (1-21 days of age) and final (22-39 days of age) phases. The diets were based on corn and soybean meal and were offered in the form of bran. Both BAs and emulsifier were added as a replacement for the inert material (kaolin). Total excreta collection was performed in the last three days of both the initial phase (21 days of age) and the final phase (39 days of age), to evaluate the digestibility of ether extract and crude protein. The results were statistically analyzed by the SNK test at a 5% probability level, using the SISVAR program (2016). Regarding crude protein digestibility in the initial phase, only T5 showed better results when compared to T1 ($P<0.05$). However, no differences were observed in crude protein digestibility between T5 and the other treatments (T2, T3 and T4) ($P>0.05$). In the final phase, T3, T4 and T5 showed better crude protein digestibilities ($P<0.05$) when compared to T1. However, there was no difference in relation to T2 ($P>0,05$). Additionally, regarding the digestibility of ether extract in the initial phase, T4 and T5 presented better results ($P<0.05$) when compared to T1, but there was no difference ($P>0.05$) in relation to T2 and T3. In the final

phase, all treatments (T2, T3, T4 and T5) presented greater digestibility of ether extract in relation to the basal diet (T1) ($P<0.05$). Therefore, it can be concluded that the inclusion of bile acids in broilers diet provided an improvement in the digestibility of crude protein and ether extract in both phases of rearing, while the inclusion of emulsifier increased the digestibility of ether extract only in the final phase. This result allows for better performance of broilers, as well as reduced production costs when using bile acids in the diet.

Keywords: Cholic acid, Deoxycholic acid, Emulsifier, Crude protein, Ether extract

353 Gastrointestinal functioning in poultry in health and disease: roles of neuropeptides, hormones and immune modulators

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Optimal functioning of gastro-intestinal tract in poultry is essential to the efficient production of their meat and eggs. The gut in birds follows the general vertebrate pattern with a mouth, an esophagus, stomach, small intestine and associated liver and pancreas and a large intestine with ceca. Differences in modern birds include the absence of teeth, the presence of a distensible expansion of esophagus, the crop, and division of the stomach into the glandular proventriculus and muscular gizzard, The present review will focus on recent studies and, in particular, from our laboratories on the foregut, namely the crop, proventriculus and duodenum. These organs contain epithelial and muscle layers with a glandular mucosa present in proventriculus and duodenum. The crop is a site for the storage of feed with the gradual release of feed between times of eating or feeding. One of the mechanisms for this is contraction of the crop which is induced by the neuropeptide, ghrelin. The crop is a site for bacterial fermentation together with breakdown of phytate and other nutrients by exogenous enzymes present as a component of feed. Furthermore, pathogens can not only be associated with the crop epithelium but also impact food safety. Pathological effects of Salmonella on the crop are mitigated by antibodies to toll-like receptor 4 added to the feed. Not only does ghrelin influence contractility of the crop, it and its receptor are also synthesized by crop tissues based on *in vitro* studies. It is noted that ghrelin is also produced by proventriculus and duodenal tissue as is the opioid neuropeptide, Met-enkephalin. There is a dearth of information on the roles of neuropeptides, growth factors and immune modulators on gut functioning and development/regeneration. An example of a recent study demonstrated that insulin-like growth factor 1 (IGF-1) increased cell division in duodenal explants. While this is arguably to be expected, what was unexpected was that Met-enkephalin also stimulated duodenal cell division and synergizes with IGF-1 to markedly increase cell division. What is also not known are effects of nutrients and other feed components of the release of neuropeptides, growth factors and immune modulators. What are not known is whether other neuropeptides and gastro-intestinal hormones influence cell division or immune responses. A case is advanced that there needs to be shifts in the understanding of the physiology of the gastro-intestinal tract.

Keywords: neuropeptide, ghrelin, Met-enkephalin, crop, Salmonella

354 In silico design, optimization and evaluation of a multi-epitope vaccine targeting Clostridium perfringens collagen adhesin protein (CNA)

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Necrotic enteritis (NE) is a complex enteric infectious disease caused by pathogenic *Clostridium perfringens*, leading to significant economic losses estimated at approximately USD 6 billion annually in the global poultry industry. The rising incidence of NE has been linked to the voluntary reduction or withdrawal of antibiotic growth promoters in animal feed. Consequently, the development of effective NE-specific vaccines has become a critical priority for the poultry industry. *C. perfringens* poses a substantial threat to both human and animal health, with collagen adhesion protein (CNA) identified as a key virulence factor in NE pathogenesis among poultry. Targeting CNA as a primary vaccination antigen offers a promising strategy for mitigating *C. perfringens*-induced infections. In this study, we designed and optimized a multi-epitope vaccine against CNA, incorporating $CD4^+$ helper T (Th) lymphocyte, $CD8^+$ cytotoxic T lymphocyte (CTL), and B-cell epitopes. Using bioinformatics tools and computational algorithms (IEDB MHC-II binding server, ABCpred server, Vaxijen v2.0, AllerTop v2.0, etc.), we identified epitopes optimized for enhanced immunogenicity and antigenicity while minimizing allergenicity. A 115-amino-acid peptide, encompassing the most immunogenic epitopes, was selected as the vaccine candidate. This multi-epitope vaccine demonstrated strong potential for inducing immunity against pathogenic C. perfringens and NE, as evidenced by the stimulation of IgG and IgM antibody responses, plasma cell activation, Th memory cell formation, and CTL responses—comparable to those elicited by the full-length CNA protein (647-amino-acid peptide). Additionally, this shortened vaccine candidate exhibited higher stability and solubility, further supporting its potential as an effective NE vaccine.

Keywords: Clostridium perfringens, necrotic enteritis, collagen adhesion protein (CNA), multi-epitope vaccines (MEV), immunoinformatics

355 Phytogenic supplementation modulates the ileal and cecal microbiota supporting gut health during a necrotic enteritis challenge

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Necrotic enteritis (**NE**), caused by *Clostridium perfringens* (**CP**), is a critical poultry disease associated with severe economic losses to the industry. The effects of phytogenic blends additive on the ileal and cecal microbiota of broiler chickens was assessed during a 42-day (**d**) NE model study. Day-old Ross 708 chicks (n=450) were allocated to one of three groups: 1) negative control (**NC**; corn/soybean diet), 2) NC + Avilamycin/Amprolium (**PC**), and 3) NC+ Alterna® (**ALT**). All birds were orally inoculated with 2,000 sporulated oocysts of *Eimeria maxima* and 1×10^8 CFU of CP on d 14 and d 19, respectively. On d 14, d 21, and d 42, ileal and cecal mucosal scrapings were collected (n=6/group), microbial DNA was extracted, and the V3-V4 regions of the 16S rRNA gene were sequenced and analyzed using QIIME2. Results were considered statistically significant at $P \leq 0.05$. The alpha diversity index (Chao1) was significantly higher in ALT compared to NC at d 14 and d 42 in the ileum and ceca, suggesting greater microbial

richness. At d 21, PC exhibited a higher Shannon index in the ceca compared to NC and ALT. During peak infection at d 21, NC had a higher abundance of harmful microbiota such as *Enterococcus cecorum* and *Desulfovibrio* whereas ALT showed a greater abundance of beneficial microbiota such as *Prevotellaceae* spp., *Akkermansia* spp., *Candidatus Arthromitus*, *Blautia* spp. By d 42, ALT group had a differential abundance of beneficial microbiota such as *Blautia* spp., *Clostridia vadinBB60*, and *Ruminococcus* spp. These are involved in gut barrier integrity, immune modulation, and short chain fatty acids (**SCFAs**) synthesis. BUGBase analysis showed ALT having a reduction in potentially pathogenic phenotypes in the ceca at d 42. Additionally, stress tolerant microbiota were significantly lower in ALT compared to NC at d 14 and d 21 but significantly increased by d 42, suggesting microbiota adaptation following recovery. PICRUSt2 analysis showed ALT having a significantly greater expression of functional genes involved in menaquinol-8 biosynthesis and formaldehyde assimilation at d 14, while at d 21 had a significantly enhanced methyl ketone biosynthesis. At d 42, ALT group had a greater abundance of functional genes biosynthesis of antioxidant related compounds such as methyl ketone, mycothiol, adenosylcobalamin, and ergothioneine pathways compared to NC and PC in both ileum and ceca. Therefore, phytogenic supplementation supported intestinal health in broilers during a NE challenge by promoting the growth of beneficial ileal and cecal microbiota involved in SCFAs production, gut barrier integrity, immune modulation, and oxidative stress protection, and reduced pathogenic and increased stress-tolerant phenotypes.

Keywords: Necrotic enteritis, Clostridium perfringens, phytogenic, microbiota, short chain fatty acids

356 Prembion: A breakthrough precision biotic for poultry gut health and stressor defense

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Prembion is an innovative biotic feed ingredient derived from spent barley, developed to support animal health and performance under stress. To evaluate its efficacy, a high-challenge trial was conducted targeting key pathogens: *Clostridium perfringens*, *E. Coli*, *E. acervuline*, *E. maxima*, *E. tenella* (Coccidia) and *Salmonella* (*Typhimurium* and *Heidelberg*), with a mortality threshold set above 6%. The trial involved 624 mixed-sex Cobb 500 broilers per treatment group, with 12 replicates of 52 birds each (50:50 male-to-female ratio), over a 42-day period. Prembion was tested at four inclusion levels: 25 ppm, 50 ppm, 75 ppm, and 150 ppm. All birds received a Coccidiosis vaccine at the hatchery. Performance was assessed at days 10, 18, 32, and 42, measuring: BWG FCR Mortality was tracked daily, and pathogen enumeration was done at day 42. Under severe challenge conditions, Prembion significantly improved bird performance: FCR improved from 1.94 in the positive control group to 1.87 at 75 ppm ($p<0.05$) and 1.86 at 150 ppm ($p<0.05$)—gains of 7 and 8 points, respectively. BWG increased across all effective doses. EPI scores (European Production Index) recovered by 56% at 75 ppm ($p<0.05$) and 77% at 150 ppm ($p<0.05$), compared to the non-challenged group. Pathogen Reduction At 75 ppm on day 42, Prembion achieved: Two log10 units reduction of *E. coli* Two log10 units reduction of *C. perfringens* One log10 units reduction of *Salmonella* One log10 units reduction in total Coccidia counts as well as Coccidia counts in the duodenum, ileum, and ceca These results are consistent with immune modulation observed in vitro, particularly the upregulation of beta-defensin antimicrobial peptides. Beta-defensins are antimicrobial peptides that play a key role in innate immunity. They recruit immune cells like macrophages, dendritic cells, and T cells to infection sites, enhancing immune response. They also modulate cytokine and chemokine production, promoting a pro-inflammatory environment that helps clear pathogens. Activation occurs when pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), detect pathogens. This triggers signaling pathways that induce beta-defensin expression. Inflammatory cytokines like IL-1β, TNF-α, and IL-6 also stimulate their production during infection. Prembion demonstrates strong potential as a biotic feed solution in high-stress, pathogen-rich environments. It not only improves performance metrics like FCR and BWG but also significantly reduces gastrointestinal pathogen loads. The immune-enhancing action—driven by defensin activation—positions Prembion as a robust tool for maintaining animal health.

Keywords: gut health, innate immunity, various stressors, sustainbility, animal health

357 Develop long-term avian airway organoids for avian influenza virus research

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Avian influenza virus (AIV) poses a significant threat to both poultry and humans. The tracheal epithelium serves as the first line of defense against AIV, and understanding host-pathogen interactions, specifically in epithelial cells, is crucial for combating AIV infections. However, a long-term avian primary tracheal cell model is currently unavailable. The recent development of mammalian 3D airway organoids for human COVID-19 studies represents a significant advancement in respiratory research, providing a convenient and physiologically relevant model for studying respiratory diseases. We hypothesized that avian tracheal organoids could also be established to investigate host-pathogen interactions for poultry. The objectives of this research were to: 1) develop avian tracheal organoids; 2) cryopreserve and passage them for long-term use; and 3) characterize the different cell populations within the tracheal organoids by using western blot, immunofluorescence, qRT-PCR, and RNA-sequencing. Statistical analyses were performed using one-way ANOVA with JMP14. Tracheae from 90-week-old layers (n=10) were cleaned with PBS and incubated with collagenase. Isolated chicken tracheal epithelial cells were embedded in Corning Matrigel and cultured with homemade tracheal organoid media. The growth and morphological changes of avian tracheal organoids were monitored under a Nikon Ti microscope. Every seven to ten days, avian 3D tracheal organoids were passaged by using a 1:5 dilution. Avian tracheal organoids were frozen using Gibco™ Recovery™ Cell Culture Freezing Medium. A time course study of avian tracheal organoids showed significant growth from days 1 to 5 in the organoid expansion media from passage 0 ($P<0.05$), with an increase in both organoid size and diameter observed each day. Cryopreserved tracheal organoids could be regrown and propagated after one year. Western blots confirmed the presence of airway basal stem cells using the basal cell marker TP63, with increased expression at passage 7 compared to passage 3. Western blotting and qRT-PCR were used to confirm the expression of cell markers for four distinct cell types: mucin-producing goblet cells,

basal cells, ciliated cells, and club cells in the tracheal epithelium. We are currently analyzing the gene profiles of tracheal organoids from early to late passages. We have successfully established 3D avian tracheal organoids that can be cryopreserved and passaged for long-term use, providing a valuable model for investigating host-pathogen interactions associated with AIV. This model holds promise for elucidating the molecular mechanisms underlying AIV infection and may facilitate the development of novel therapeutic strategies to combat avian influenza.

Keywords: Airway Organoids, Avian, Host-pathogen, Model System, Avian influenza virus

358 Comparative evaluation of probiotic potential of *Lactobacillus* isolates from healthy and colibacillosis-affected broilers against avian pathogenic *Escherichia coli* challenge

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Avian pathogenic *Escherichia coli* (APEC) disrupts the delicate balance of gut microbiota impairing growth and nutrient assimilation in poultry. Despite a significant reduction in *Lactobacillus* (L) populations, certain microbial strains persist alongside *APEC* under disease conditions, indicating potential resilience or competitive adaptation. We hypothesize that these microbial strains may exhibit enhanced resilience, making them viable probiotic candidates. This study evaluates the probiotic potential of *Lactobacillus* (*L*) isolates from healthy and diseased broilers and assesses their efficacy in mitigating APEC infection. A total of 120 *L* isolates were collected from healthy and colibacillosis diseased broiler flocks and subjected to in-vitro probiotic characterization, safety profiling and molecular identification. Based on results 4 promising strains were selected for in-vivo trials: *L. casei*-NK1 and *L. paracasei* NK3 from healthy broilers, and *L. plantarum* NK2 and *L. rhamnosus* NK4 from diseased broilers. A 35-day feeding trial was conducted on one-day-old broiler chicks, randomly distributed across seven treatment groups: a negative control group (NC, no treatment), an APEC group, a commercial probiotic-fed group (CProb), and four *L*-fed groups (LNK1-LNK4). The APEC, CProb, and LNK1-LNK4 groups were infected with *E. coli O78* strain at day 11 of age. Body weight gain (BWG) and bacterial shedding was monitored weekly. Logistic regression was used to estimate the probability of probiotic selection, while one-way ANOVA was applied to evaluate the treatment effects on BWG and bacterial load. Results showed that *Lactobacillus* isolates from diseased broilers exhibited significantly higher auto-aggregation ($P < .001$, OR = 1.066) and competitive exclusion ($P = .036$, OR = 1.180) compared to those from healthy broilers and diseased isolates were 2.8 times more likely to be selected as probiotics ($P = .098$, OR = 2.8). The BWG of LNK1 and LNK2 groups were comparable to the CProb probiotic group, and all groups significantly outperformed the APEC group ($P < .05$). *E. coli* shedding was significantly reduced ($P < .05$) in the LNK1-LNK3 groups to levels comparable to the CProb group. These findings suggest that *L* isolates from diseased broilers are more resilient in terms of colonization and competitive exclusion abilities, with antimicrobial potential similar to isolates from healthy broilers. Future research plans on metagenomic analysis of gut microbial diversity and immunological parameter evaluation.

Keywords: Probiotics, Poultry health, APEC, Lactobacillus

359 *Salmonella* Heidelberg-induced dysbiosis in broiler chickens: A microbiota disruption perspective

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Salmonella Heidelberg (SH) colonizes the intestinal tract of chickens, multiplies in macrophages, and disseminates via the bloodstream or lymphatic system. Understanding its impact on the intestinal ecosystem provides valuable insights into microbial interactions and supports control strategies. This study investigates SH-induced infection and dysbiosis in broilers, focusing on microbiota response and susceptibility. A total of 100 one-day-old male chicks were distributed into four pens and challenged with SH. At 3 and 15 days of age, all birds received an SH strain via gavage. Cecal contents were collected from two birds per pen at 7 (D10) and 18 (D21) days post-infection (dpi) for bacterial DNA isolation. The V3-V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq, and sequences were processed via Sapiens's bioinformatics pipeline. To assess SH-induced dysbiosis, microbiota from challenged birds were compared to a reference database containing microbiome samples from d21 broilers, along with their health and performance data. Microbial composition was analyzed at the genus level. LEfSe (Linear Discriminant Analysis Effect Size) identified 23 bacterial biomarkers (LDA >2.0). In the healthy group, 21 biomarkers were detected, with 12 classified as positive (P) and 9 as negative (N), corresponding to a relative abundance of 26.2% (P) and 12.4% (N). In the SH-challenged group at 9 dpi, only 11 biomarkers were identified (6P, 5N), with a sharp reduction in relative abundance (0.9% P, 4.3% N). By 18 dpi, partial microbiota recovery was observed, as biomarkers increased to 18 (10P, 8N), with relative abundances of 3.0% (P) and 13.4% (N). However, the positive-to-negative biomarker ratio (Healthy: 2.12:1 | Sal9d: 0.21:1 | Sal18d: 0.23:1) highlights severe dysbiosis in SH-challenged birds, with persistent microbial imbalance. Additionally, a sigmoid machine learning model classified the degree of dysbiosis between 0 and 1, where higher values indicate greater dysbiosis. The results of this model were: Healthy: 0.65, Sal9d: 0.98, and Sal18d: 1.0, confirming the severity and persistence of dysbiosis in the SH-challenged birds. Our findings reveal that S. Heidelberg infection induces significant and prolonged dysbiosis in broilers, characterized by a drastic reduction in beneficial microbial biomarkers and an altered positive-to-negative biomarker ratio. While partial microbiota recovery was observed by 18 dpi, the persistent imbalance suggests long-term consequences for intestinal health. These insights emphasize the need for targeted strategies to mitigate Salmonella-induced dysbiosis, enhancing gut resilience and poultry performance.

Keywords: Health challenge, Microbial biomarkers, 16S rRNA sequencing, Gut health

360 Impact of selected pharmacological and nutritional interventions on *Histomonas meleagridis* transmission in turkey poults

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Histomonosis, also known as blackhead disease, is a parasitic disease caused by the protozoan *Histomonas meleagridis*. This disease primarily affects turkeys, leading to 70-100% mortality. Nowadays, there are no commercial treatments or vaccines available. Therefore, this study aimed to evaluate different pharmacological and nutritional strategies to mitigate the presentation of enterohepatic lesions and transmission of *H. meleagridis*. Two independent experiments were conducted using a horizontal transmission model previously established by our laboratory. Each experiment used 7 replicates of 6-10 female turkey poults per group. In experiment 1, treatment groups included a 1) Positive control, 2) Halquinol 0.05%, 3) Iodoquinol 0.1%, 4) Nitazoxanide 0.05%, 5) Rye 20%, and 6) Rye 40%. In the second experiment, the treatments were 1) Positive control, 2) Berberine 0.075%, 3) Decoquinate 0.004%, 4) Acriflavine 0.05%, 5) Quercetin 0.1%, and 6) Rye 40%. Treatment administration started on day 7 until the end of the study. On day 10 of age, 50% of the poults per replicate were intracloacally challenged with 100,000 histomonads/0.5 mL, and wax paper was placed in the battery cages to promote transmission of the protozoa. 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 in the disease model (seeders and contacts). 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 significant improvement in performance parameters, lesion scores, or percentage of transmission was observed in the pharmacological groups, berberine or quercetin, compared to the positive control ($P>0.05$). Interestingly, turkey poults fed diets containing rye showed a reduction in horizontal transmission of around 45% and lower cecal lesion scores (0.42 vs. 1.76) in comparison to the positive control in both experiments ($P<0.05$). In conclusion, the pharmacological and phytogenic compounds evaluated in the current set of experiments did not reduce transmission of *H. meleagridis*. Nevertheless, the consumption of rye appears to mitigate horizontal transmission, which could be related to the induction of intestinal inflammation or cecal microbiome changes. Further research is currently being conducted evaluating other alternative chemical compounds, feed additives, and nutritional inclusion levels.

Keywords: Histomonas meleagridis, transmission, lesions, performance, turkeys

361 Not Presented

362 Thrombocyte behavior in Mareks challenged, vaccinated, and control chickens of ADOL Lines 6.3 and 7.2

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Background: The avian thrombocyte (Th) the most numerous white blood cell, is normally found at ~ 30,000/µL. Statement of the problem: As hemostasis and phagocytic abilities of Th are known, a description of how the Th interacts with other immune cells is the objective. Basic Th morphology of the ancestral stocks of lines 6.3 and 7.2, experimental chickens important to Marek's disease research, is in the Atlas of Avian Hematology. When Th transition to reactive types specific (alpha and dense) granules multiply and the cytoplasm becomes vacuolated. The present observations emphasize Th cellular behavior; how they interact with each other and with the cells of the other series. This was studied in blood sampled from Mareks infected/vaccinated and control birds of both lines 6.3 and 7.2. The observations, extend basic Th morphology to ploidy (nuclear size), Th-Th and Th-RBC interactions, and Th-leukocyte (WBC) aggregations. Method: Light microscopy of Wright-Giemsa-stained blood using a100x (Olympus CX-41) oil objective with Infinity Image Capture-6.5. Samples were from chicks aged between 19-49 d. Controls, Marek's challenged, CVI988 vaccinated, and MD challenged/vaccinated are the treatments. Results: solitary Th were differentiated into types based on granules as "typical," containing α (alpha) and dense granules, and "secretory" with both granules and vacuoles (Golgi). Some Th form an anuclear version, a "thromboplastid" reminiscent of RBC erythroplastids, by nuclear expulsion. The sizes of Th aggregates are taken to be proportional to the amount of stress, like the H/L ratio. Th aggregate with RBCs in the form of "toroids" [Th-RBC-Th] indicate adhesive surface modifications. Th were phagocytosed by monocytes indicating their numbers may be reduced by autophagocytosis (thrombocytopenia). Conclusions: There is sufficient treatment variation among these blood films to illustrate multiple Th cell behaviors, an often-overlooked component of the "blood picture." The present observations show how including behavior adds power to hematological analyses usually based on tallies as are the SDC and WBC. The benefit occurs at little additional cost. The implication is clear; excluding behavior obscures a true blood picture.

Keywords: thrombocyte, behavior, blood, analysis, cytology

363 Avian reovirus clusters isolated in broilers with suggestive clinical symptoms in Brazil in 2024

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The Avian Reovirus (ARV) is of significant relevance in poultry production, as it is the primary causative agent of avian viral arthritis and tenosynovitis. This study aimed to investigate the occurrence of ARV in broilers exhibiting clinical symptoms and analyze the relationship between the identified cluster, age, location, and its distribution across Brazil. A total of 960 broiler flocks, presenting clinical signs suggestive of ARV (leg problems and increased passage rate), from various Brazilian states were analyzed. Isolation was performed on pooled samples of the small intestine (duodenum and pancreas regions), heart, or joints, with each pool consisting of samples from five birds. The samples were inoculated into 6-day-old SPF (Specific Pathogen-Free) embryonated eggs and passaged in chicken embryo kidney. After isolation, RT-qPCR was conducted to confirm the presence of the viral kidney Sequencing and phylogenetic analysis were performed by amplifying the sigma C gene and sequencing the nucleotides. Descriptive statistics and Pearson correlation analysis ($p < 0.05$) were applied using GraphPad Prism 10.2. A total of 390 ARV isolates were obtained from 360 flocks, and 190 isolates were sequenced. Cluster 4 was the most prevalent (41.05%), followed by clusters 2 (20.53%), 6 (14.74%), 5 (14.21%), 1 (11.58%), and 3 (8.95%). All isolates originated from flocks younger than 45 days, with no association between the identified cluster and the age

of the birds isolated (< 45 days). The highest ARV isolation rate was observed in the intestine (58.61%), followed by tendons (48.01%) and heart (20.20%). In the intestine, the distribution of clusters mirrored the overall results, but a lower isolation rate of cluster 6 (6.67%) was observed in tendons, and a lower isolation of cluster 5 (2.63%) was seen in the heart. Cluster 5 was present in 15% of tendon samples. The distribution of different clusters across regions showed variation; in the Northeast, clusters 2 and 6 were absent, with the highest isolation of clusters 1 and 3 (both 33.33%). In the Southeast and South, all clusters were identified, with clusters 4 and 2 being more prevalent, and cluster 5 occurring at 12.5% and 11.11%, respectively. In the Central-West, cluster 3 was not identified, and clusters 4 (40.74%) and 1 (29.63%) were the most frequent. This study highlights the widespread presence of various ARV clusters in Brazil, including clusters associated with milder lesions (such as cluster 4) and those linked to more severe lesions (clusters 5 and 6).

Keywords: Epidemiological analysis, Biosafety, Reovirus, Cluster, Chicken

364 Separating chicken's head and leg in thermal images via object detection and machine learning models to predict avian influenza and Newcastle Disease

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The body temperature of animals is closely related to their metabolism and vital activities, which can indicate their physiological status and health. This temperature changes proportionally when animals experience disease, specific physiological changes, or environmental changes. Early and accurate diagnosis of poultry diseases through non-invasive methods is of great importance for the poultry industry. The objective of this research was to separate chicken's head and leg in thermal images via object detection model and use machine learning model to predict avian influenza and Newcastle disease. A total of 80 Ross 308 broiler chickens were infected with the virus. The virulence type was velogenic strains for Newcastle disease and H9N2 for avian influenza. The detection model was You Only Look Once (YOLO)v8 and the classification model was Support Vector Machine. Thermal images were evaluated in three different modes, including original images, background-removed images, and images with chickens' head and leg extracted by the YOLO-v8 model. Thermal images were captured from 8 to 64 h after disease infections with 8 h intervals. The results showed that the diagnosis accuracy in three visual groups was 75.89%, 83.93% and 92.48%, respectively, for avian influenza and 83.04%, 91.52% and 94.20%, respectively for Newcastle disease. The algorithm's detection accuracy of detecting Newcastle disease was higher than the influenza. In addition, the model can identify the two diseases within 8 h of disease infection with over 90% accuracy. The results also show that the share of texture-related features was more significant than other features. Focusing on the head and leg areas led to increased accuracy of diagnosis, which allows for a more accurate diagnosis in the early stages.

Keywords: Thermography, Image analysis, Machine learning, Influenza disease, Newcastle disease

365 Effect of organic trace minerals vs. zinc sulfate on performance, carcass yield, and tibia zinc content of broilers raised in floor pens

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Zinc is an essential trace mineral for developing and maintaining key physiological functions in poultry. Inorganic and organic zinc sources are commonly used in poultry diets. This study evaluated different organic zinc sources compared to zinc sulfate in broilers. Day-old chicks were randomly assigned to nine treatments: negative control (NC) group with no supplemental zinc and eight groups with 10 or 60 ppm added zinc from either zinc sulfate (ZnSO4), zinc glycinate (ZnGly), zinc amino acid-hydrate (ZnAAH), or zinc methionine hydroxy analogue (ZnMHA). Zinc sources were supplemented based on their mineral concentration, excluding the ligand's nutritional contribution. All diets were corn-SBM based and supplemented with 500 FTU/kg of phytase. Each treatment was replicated in 8 floor pens with 16 birds per pen. On Day 21, two birds per pen were randomly selected and euthanized, and the right tibiae were collected for Zn analyses. On Day 43, hot carcass yield was recorded as the percent of live weight of six randomly selected birds per pen. Data were analyzed using a two-way ANOVA to evaluate the zinc source and level main effects and their interaction with room and block effects as random. In addition, pre-planned contrasts were conducted to compare treatments to NC. Overall (D0-42), zinc source affected body weight gain (BWG) and feed intake (FI) ($P<0.05$), feed conversion ratio (FCR) ($P<0.1$), and hot carcass yield ($P<0.1$). BWG significantly increased by 3 and 2.6% for birds fed ZnSO4 and ZnGly, respectively, compared to ZnMHA (3,456 g, $P<0.05$). ZnMHA had the lowest overall feed intake than other sources ($P<0.05$, 4,906 Vs. 5,048 g). ZnGly had the lowest FCR across sources and significantly reduced overall FCR by 3.6 points compared to NC (1.413 Vs. 1.449, respectively, $P<0.05$). FCR was reduced by 4 and 3 points for birds fed ZnGly and ZnMHA, respectively, compared to ZnAAH ($P<0.05$). Hot carcass yield was significantly increased by 0.62% in ZnGly supplemented birds compared to NC ($P<0.05$; 76.06 Vs. 75.44%, respectively). In addition, ZnSO4 ($P<0.1$) and ZnMHA ($P<0.1$) improved carcass yield by 0.54 and 0.52%, respectively, compared to NC. Overall feed intake was affected by source-by-level interaction which was mainly driven by reduced FI for ZnMHA treatments. On D21, Tibia Zn content was significantly affected by zinc level but not source with 60 ppm treatments increasing tibia Zn compared to 10 ppm ($P<0.05$, 433 Vs. 408 ppm, respectively). In this study, zinc glycinate and zinc sulfate were the most efficient zinc sources based on overall BWG, FI, FCR, and hot carcass yield. Future studies will evaluate zinc source and phytase interaction and investigate the effect of supplemental zinc on carcass yield.

Keywords: Broiler Performance, Zinc Sulfate, Zinc Glycinate, Zinc Amino Acid Hydrate, Zinc Methionine Hydroxy analogue

366 Evaluation of digestible calcium requirements of Ross 308 broilers by meta-analysis, with a specific focus on the interaction between phosphorus and calcium response

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Recent research on digestibility of calcium (Ca) allows poultry nutritionists to formulate on a digestible Ca (dCa) basis. This study aims at determining the dCa requirements (for maximizing growth, feed efficiency and bone mineralization) for broiler chickens using a meta-analysis approach. A database was constructed, comprising 317 observations from 67 experiments reported in 24 scientific articles. The experiments had to test at least three levels of dietary Ca with a constant available P level within each experiment. All the published diets were recalculated on the same formulation matrix system. The meta-analysis revealed that to maximize average daily gain, the dCa requirements were 0.49%, 0.43%, and 0.38% for the starter, grower, and finisher phases, respectively based on a quadratic response with an available P of 0.52, 0.42 and 0.36. To maximize tibia ash percentage, the requirements were 0.57%, 0.53%, and 0.47% for the same phases and same level of available P. While the results for the grower and finisher phases are relatively consistent, the starter phase results are less clear, maybe due to the complex interaction between Ca and P. Indeed, high levels of dCa combined with low available P levels negatively impacted growth performance due to the complexation of Ca and P, resulting in P more unavailable to the animal. Further research on health parameters such as gizzard pH and litter moisture are required to fully understand the benefits of dCa on health and welfare of broilers.

Keywords: Calcium, Phosphorus, Digestibility, Meta-analysis

367 Comparison of defatted and non-defatted methods for determining bone ash, calcium, and phosphorus in broiler tibias

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This study aims to compare two methods for determining bone ash, calcium (Ca), and phosphorus (P) content in broiler bones, focusing on the impact of defatting versus a non-defatting approach. Bone composition, particularly the ash, calcium, and phosphorus content, is a key factor in understanding poultry nutrition and skeletal health. Various methods for determining these components lead to variability in results across studies. Traditional techniques typically involve defatting bones using solvents such as petroleum ether, which pose safety risks due to their flammability and toxicity. An alternative approach involves boiling the bones, which may provide a safer, simpler process. However, the impact of defatting versus boiling on the accuracy of ash, Ca, and P measurements is not well understood. Thirty right and left tibias from the same broiler were collected from a commercial slaughterhouse. The bones were cleaned, and tissues manually removed. The right tibias were processed using the conventional method, and the left tibias were processed using the alternative method. The conventional method involved drying bones at 105°C overnight, defatting in Soxhlet extractors with

petroleum ether for 12 hours, drying at 105°C for 24 hours, weighing, and then ash was obtained in a muffle at 600°C for 8 hours. The alternative method involved boiling the bones for 4 hours, drying at 105°C overnight, further drying for 7 days at 105°C in a drying oven, weighing, and ash was obtained in a muffle at 600°C for 8 hours. Both methods were used to determine bone ash percentage, and Ca and P deposition. Statistical analysis was performed using a mean test (Tukey) and Pearson's correlation (SAS software). No significant differences ($p > 0.05$) were found between the methods for ash and P content. A 1.05% reduction in Ca levels was observed in the alternative method compared to the conventional method ($p = 0.0209$). The correlation between methods for ash and P was high but not statistically significant ($p < 0.05$). However, the correlation for calcium was significant ($r = 0.79$, $p = 0.0497$). This study indicates that both defatted and non-defatted methods can be used to determine ash and P content in broiler bones. However, for calcium analysis, the conventional method provides greater accuracy, as the alternative method may underestimate calcium levels.

Keywords: broilers, bone quality, bone ash, calcium content, skeletal health

368 Calcium:phosphorus ratio and calcium levels: impacts on performance and bone health in broiler chickens

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The aim of this study was to evaluate the impact of two calcium to available phosphorus (Ca:P) ratios, with or without a reduction in total calcium levels in the diet, on the productive performance and bone health of broiler chickens. A total of 1,200 Ross broilers were distributed in a completely randomized design with four treatments, 10 replications, and 30 birds per experimental unit. The treatments consisted of two Ca:P ratios (1.65:1 and 2:1) and two total calcium levels: a positive control (PC, no reduction) and a negative control (NC, with a 0.18% reduction). The experimental diets, based on corn and soybean meal, were supplemented with phytase (1,000 FTU/kg of feed), providing a 0.15% increase in total calcium and available phosphorus. At 42 days, performance parameters (weight gain, feed intake, mortality, and feed conversion ratio [FCR] corrected for mortality) and bone quality indicators (plasma calcium and phosphorus levels, bone strength, and tibia mineral content) were evaluated. Data were analyzed using two-way ANOVA (GLM, SAS), with mean comparisons performed using Tukey's test ($P < 0.05$). No significant effects of Ca:P ratios or calcium levels on feed intake and weight gain were observed. However, FCR was influenced by the interaction between Ca:P ratios and calcium levels, with increased FCR in the NC diet at the 1.65:1 ratio (1.580) compared to the PC diet (1.482), suggesting reduced feed efficiency with lower calcium. At the 2:1 ratio, no significant difference was found between NC (1.505) and PC (1.492), indicating that a higher Ca:P ratio may mitigate the negative effects of calcium restriction. Plasma calcium concentration was higher at the 2:1 ratio (10.9 mg/dL vs. 10.4 mg/dL), regardless of diet, while plasma phosphorus levels were not significantly affected. Bone strength was higher at the 2:1 ratio, but calcium reduction compromised bone quality in both ratios, particularly at 1.65:1 (40.8 vs. 24.5 kgf). Bone mineralization was affected, with greater calcium content in the tibia at the 2:1 ratio (18.0% vs. 17.6%) and in the PC diet (18.3% vs. 17.3%). Tibia phosphorus was higher in the PC diet (9.00% vs. 8.73%). These findings indicate that the Ca:P ratio significantly influences broiler performance and bone health. The 2:1 ratio proved more effective in maintaining FCR and bone strength, mitigating the adverse effects of calcium restriction. Adjusting the Ca:P ratio in diet formulation is essential to optimize both productivity and bone health, particularly in diets with lower calcium levels.

Keywords: broiler performance, bone quality, calcium to phosphorus ratio, poultry nutrition, calcium content

369 Evaluation of a quick and easy to use application of 25-hydroxyvitamin D_3 on vitamin D status, growth performance and bone characteristics of broiler chickens

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In animal production, optimal vitamin D levels in feed are crucial for bone health, immunity, and muscle development. However, stressful situations like vaccination, heat stress, and transport can harm health and performance. Therefore, quick, easy, and preventive complementary solutions are needed for feed manufacturing during these critical moments. In the current study, the application of 25-hydroxyvitamin D3 (25OHD3, Hy-D®, dsm-firmenich, Switzerland) in both feed and in water using effervescent tablets were compared to the classical vitamin D3 feed supplementation at the same (4000) bioequivalent IU of vitamin D in broiler chickens. A total of 270 one-day-old Ross 308 male chicks were raised in floor pens and assigned to three treatments (15 birds × 6 replicates): T1, Control 4000IU/kg vitamin D3 in feed; T2, 69µg of 25OHD3/ kg of feed; T3, 34.5 µg 25OHD3/L of water. The water dosage was half that of the feed, assuming animals drink twice as much as they eat, and in both cases, 25OHD3 was supplemented on top of 1240 IU of vitamin D3 via feed. Whole blood was collected at days 9, 21 and 35 and analyzed for 25OHD3 concentration. Zootechnical parameters (weight gain, WG; and feed conversion ratio, FCR) including water (WI) and feed (FI) intake were evaluated at day 21, 35 and the overall period. Tibia were collected from 2 birds per pen to evaluate bone characteristics. Data were analyzed using one-way ANOVA with Tukey's HSD ($P < 0.05$). On average, individual birds ate 3671 g feed and drank 7386 ml of water confirming the estimation (2:1). No statistical differences were detected on WI nor FI between treatments at any of the periods evaluated indicating that similar levels of bioequivalent vitamin D IU were consumed. However, plasmatic 25OHD3 concentration was higher ($P<0.001$) in T2 (54.6; 47.7 and 42.5 ng/ml) and T3 (45.7; 42.1 and 32.7 ng/ml) compared to T1 (22.5; 22.8 and 19.3 ng/ml) at every of the time points evaluated, showing a better vitamin D status. Moreover, T2 showed better ($P<0.001$) WG and FCR compared to T1 and T3 at day 21, but it tended ($P=0.1$) to be similar to T3 in FCR (T2 and T3 = 1.36 vs T1= 1.39) at the overall period. No statistical differences were detected for bone mineralization at d21 and d35, probably because the animals did not receive any kind of challenge. To conclude, administering 25OHD3 via drinking water using effervescent tablets seems a promising rapid and effective solution to improve the vitamin D status of the flock, and consequently to protect and ensure flock performance during critical moments of the production cycle.

Keywords: 25-hydroxivitamin D3, effervescent tablets, drinking water, vitamin D status, performance

370 Impact of different levels of vitamin d_3 in the diet on serum levels of 25-hydroxy-vitamin d_3 in long-term laying hens

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This study aimed to evaluate the effects of two levels of vitamin D3 in the diet on plasma 25-hydroxycholecalciferol [25(OH)D3] concentrations in laying hens in long-cycle production systems. A total of 320 Lohmann LSL LITE NA laying hens (one day old) were randomly assigned to two dietary treatments in a completely randomized design. Each treatment included 10 replicates, with 16 birds per experimental unit. The treatments consisted of two dietary vitamin D3 levels added to the feed: 2,700 and 5,760 IU/kg. Environmental conditions and nutritional management were standardized according to breed-specific recommendations. Blood samples were collected from one bird per experimental unit at ages 18, 30, 54, 78, and 102 weeks to quantify plasma 25(OH)D3 levels. Data were analyzed using repeated measures ANOVA over time, with means compared using the F-test at a significance level of 5% ($P \leq 0.05$), utilizing SAS® software (SAS® Studio) and the Proc MIXED procedure. The results demonstrated a significant interaction between 25-hydroxy-vitamin D3 (25(OH)D3) levels and hen age ($P = 0.0005$), with variations in plasma vitamin D3 levels depending on age and dietary vitamin D3 levels (2,700 and 5,760 IU/kg) ($P < 0.0001$). At 18 weeks, serum 25(OH)D3 levels were 34.6 ng/mL (2,700 IU/kg) and 63.8 ng/mL (5,760 IU/kg), with a significant difference between treatments. Plasma 25(OH)D3 concentrations progressively declined with age, reaching 19.0 ng/mL (2,700 IU/kg) and 35.7 ng/mL (5,760 IU/kg) at 102 weeks. The overall average serum 25(OH)D3 levels were 20.7 ng/mL (2,700 IU/kg) and 52.1 ng/mL (5,760 IU/kg). These findings emphasize the importance of maintaining adequate vitamin D3 supplementation throughout the laying cycle to ensure skeletal health and eggshell quality, especially in long-cycle production systems. The observed decline in 25(OH)D3 levels over time suggests potential challenges in bone mineralization and eggshell integrity, which could adversely impact productivity. Further research is needed to better understand the physiological mechanisms of vitamin D3 metabolism in aging hens and its effects on egg production and quality. This knowledge is crucial for optimizing the performance and longevity of commercial laying flocks, thereby enhancing both egg production efficiency and bird health over time.

Keywords: laying hens, plasma concentrations, poultry nutrition, 25-hydroxycholecalciferol, vitamin D3

371 1,25-Dihydroxyvitamin glycosides from plant origin (*Solanum glaucophyllum*) in diets for 76-week-old laying hens

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This experiment aimed to evaluate the effect of supplementing 1,25-dihydroxycholecalciferol glycosides (G-1,25$(OH)_2D^3$) in layer diets from hatch to 76 weeks of age on eggshell morphology and strength. A total of 900 one-day-old Hisex Brown hens (36.23 ± 0.16 g) were assigned to two dietary treatments: a control group (CON, 0 µg kg^{-1}) and a supplemented group (SG, 1 µg kg^{-1} G-1,25$(OH)_2D^3$). The birds were raised in rearing cages, and at 17 weeks of age, 160 hens were transferred to laying cages and assigned to their respective treatments. Hens were distributed across 10 replicates, with each experimental unit (EU) consisting of 8 birds. The study followed a completely randomized design. Throughout the experiment, diets were corn-soybean-based, with vitamin D levels meeting nutritional recommendations for each phase and not exceeding 4,000 IU kg^{-1} of feed. At 76 weeks of age, four eggs per EU were collected and analyzed for shell-breaking strength and thickness. Additionally, one eggshell per EU was dried and examined using scanning electron microscopy (SEM) for morphological analysis. Data were assessed for normality and homogeneity before variance analysis. When significant effects ($P < 0.05$) were detected, means were compared using the F-test. Hens fed the SG diet produced eggs with greater shell strength, 3.91 to 3.47 kgf ($P = 0.0028$). Morphological analysis revealed an increase in the height of the mammillary ($P = 0.049$), palisade ($P = 0.035$), and inner layers ($P = 0.0545$), resulting in thicker shells in SG-fed hens ($P = 0.0002$). However, no significant differences ($P = 0.3179$) were observed in the crystals present in the shell. Under these experimental conditions, G-1,25$(OH)_2D^3$ supplementation from hatch to 76 weeks improved eggshell thickness and strength, enhancing shell quality.

Keywords: Shell egg, Morphology, Quality, Strength

372 Calcium requirements in growing Japanese quail: Growth, bone health, and immune function

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Calcium (Ca) is an essential mineral for optimal growth, bone development, and physiological functions in Japanese quail. This study aimed to determine the optimal dietary calcium requirements for quail at different growth stages by evaluating its effects on performance, bone health, carcass traits, and immune responses. Two separate feeding trials were conducted: one from 11 to 24 days post-hatch and another from 21 to 35 days post-hatch. Diets with varying Ca levels (0.45–1.05%) were tested in a completely randomized design. In both trials, body weight gain (BW gain) and feed conversion ratio (FCR) exhibited significant quadratic responses ($P < 0.05$) to increasing dietary Ca. Optimal Ca levels for BW gain and FCR were estimated at 0.75–0.84% and 0.74–0.83%, respectively, in older quails, while younger quails showed similar estimates at 0.788% and 0.800%. Bone parameters, including tibia ash, tibia Ca, and bone breaking strength (BBS), responded strongly to dietary Ca, with optimal levels ranging from 0.67 to 0.87%. Carcass analysis revealed that thigh meat yield (TMY) was significantly influenced by dietary Ca ($P = 0.012$), whereas breast meat yield (BMY) remained unaffected. Serum biochemical parameters, including calcium, phosphorus, and alkaline phosphatase (ALP), showed no significant differences among treatments. Immune responses, assessed by total immunoglobulins (Ig-Total) and IgY, suggested a higher Ca requirement (~0.852%) than that needed for growth and bone development. These findings indicate that while dietary Ca levels of approximately 0.80% optimize growth and feed efficiency, higher levels may be necessary to fully support bone mineralization and immune function in Japanese quail. This study

provides practical recommendations for formulating calcium-sufficient diets across different growth phases in quail production.

Keywords: Calcium, Japanese Quail, Bone Health, Growth Performance, Immune Response

373 Interaction effect of feeding mycotoxin-contaminated corn fines diet and heat stress on liver cytokines and jejunal nutrient transporter gene expression in broilers

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Heat stress and mycotoxin-contaminated feed significantly impair broiler chicken growth performance, resulting in poor feed efficiency and compromised overall health. Hence, the objective of this study was to evaluate the effect of thermoneutral (TN: 29.4 ºC) vs. cyclic heat stress (HS: 34 ºC, 6 h/d from d 9-21); and mycotoxin-contaminated corn fines (CF) on cytokines and nutrient transporter gene expression in the liver and jejunum. The regular corn in the basal diet (0% CF) was replaced with 0, 33, 66, or 100% CF to produce the CF-containing diets. The analyzed concentration of mycotoxins in CF was deoxynivalenol-9.2 ppm, aflatoxin-6.0 ppb, total fumonisins-31.7 ppm, and zearalenone-2.1 ppm. A total of 384, 21-d-old male Cobb broilers were distributed into eight treatments in a 4 x 2 factorial arrangement consisting of 4 CF-based diets with or without HS (n=8). All birds were raised under thermoneutral temperature (32º C) for the first 9 d prior to exposure to HS. Liver samples and mid-jejunal mucosal scrapings were collected 12 d post-HS. Data were analyzed using the Proc GLM procedure in SAS software. Significant diet x temperature interactions were observed ($p < 0.05$) for liver IL-1β, IL-10, and IL-6 mRNA expression. Birds in the 0% CF-HS group had increased ($p < 0.05$) IL-1β expression than the birds in the 0% CF-TN treatment. Birds on the 100% CF-TN treatment had higher ($p < 0.05$) IL-1β mRNA expression in the liver compared to 0% of the CF-TN control group. Expression of liver IL-10 gene increased ($p < 0.05$) in the 66% CF-HS treatment group, compared to the 0% CF-TN control group. Similarly, in birds fed diets containing 100% CF in the HS group, the liver IL-6 gene expression significantly ($p < 0.05$) increased compared to the 0% of CF-TN control group. There were no significant interaction effects on liver HSP70 mRNA expression. However, among HS treatment groups, 66% and 100% CF groups had increased HSP70 mRNA expression by 2.5 fold ($p < 0.05$). There was a significant diet x temperature effect on jejunal NaPi-IIb mRNA expression, where 0% CF-HS group had increased ($p <0.05$) NaPi-IIb expression, and HS did not alter NaPi-IIb expression among CF groups. There were significant interaction effects on jejunal Claudin-1 and SGLT-I mRNA expression ($p < 0.05$). Among the TN treatment groups, 66% CF-TN groups had increased claudin-1 mRNA expression compared to the 0% CF-TN control group. Among HS treatment groups, the expression of SGLT-1 was decreased ($p < 0.05$) in the 66% CF-HS group compared to 0% CF-HS group. In conclusion, the mycotoxin-contaminated diet altered liver cytokine expression and jejunal nutrient transporter gene expression when the birds were undergoing heat stress.

Keywords: broiler, corn fines, cytokines expression, jejunum, mycotoxin

374 Characterization of the oxidative balance function of intestinal tissue during coccidiosis infection in chickens through a novel transcriptomics data analysis

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This study characterized the oxidative balance function of chicken intestinal tissue with coccidiosis through a novel method for pathway activation mapping using mRNA data. Two publicly available datasets were reanalysed. In Experiment 1 (Bremner et al., 2021), 512 jejunum samples were collected from Leghorn-type chickens assigned to either a non-challenged control, or a *E. maxima* challenged treatment at 21 d, and sampled at 2, 4, 6 and 8 days post-infection (DPI). In Experiment 2 (Ghareeb et al., 2024), 20 ileum samples were collected from broiler-type chickens assigned to either a non-challenged control, or a *E. maxima* challenged treatment at 14 d of age, with samples collected at 6 DPI. In both experiments, RNA was extracted, and mRNA was sequenced using the TrueSeq RNA library preparation kit and the Illumina platform (150 PE, poly-A enriched). Data were quality control checked and aligned to the *Gallus gallus* reference genome (GRCg7b, Ensembl) using Kallisto. Gene expression data were normalised and tested for differentially expressed genes versus non-challenged controls, followed by a novel topology-based Quantitative Pathway Activation method (Biofractal, Portugal) to quantify the activation or inhibition of pathways related to oxidative stress, anti-oxidant systems, and anti-oxidant nutrients, using a curated pathway catalogue for animal nutrition and health research. A significance threshold of adjP<0.05 was used for pathway activation. As expected, coccidiosis challenge resulted in oxidative stress in jejunal mucosa, indicated by activation of oxidative stress induced senescence and Keap1-Nrf2 signalling versus non-challenged chickens in both experiments. Anti-oxidant enzymatic systems, and glutathione metabolism were progressively inhibited by coccidiosis infection, reaching a lower limit at 6 DPI. The metabolism of vitamin E, vitamin A, cysteine and homocysteine catabolism, and the pentose phosphate pathway were also heavily inhibited in the coccidiosis challenged groups, along with mitochondrial biogenesis and uncoupling. There was a clear shift in the metabolism of the jejunal tissue with the activation of glycolysis and pyruvate metabolism, and the inhibition of fatty acid beta-oxidation compared to the non-challenged control. Effects on oxidative balance and nutrient metabolism were remarkably similar between both experiments. This data reanalysis expands the reported changes on glutathione metabolism and ROS production in macrophages during coccidiosis, using a common pathway activation method and a focused pathways database in both experiments to identify mechanisms and potential nutritional strategies that mitigate intestinal oxidative damage and preserve functional capacity.

Keywords: oxidative stress, nutrient metabolism, coccidiosis, transcriptomics

375 Energy density of diets formulated with productive energy: Linear and quadratic effects on improved broiler performance, processing yield, and economics

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This study assessed the relationships between energy density of diets formulated with productive energy (**PE**) and broiler performance, body composition, processing yield, and economics. 45 floor pens were allocated to each of 5 independent feeding

phases (fresh birds each). In each phase, 3 treatments were assessed, each with 6 replicates: Diets formulated with apparent metabolizable energy (**AMEn**, T1) or with 83% (T2) or 92% (T3) of PE recommended values. BW, BW gain (**BWG**), feed intake (FI), feed conversion ratio (**FCR**), and mortality (**MO**) were determined. BWG was fitted to Gompertz 3P curve 1st derivative, and cumulative BW (**cBW**) and BWG (**cBWG**) were calculated. Cumulative FI (**cFI**) was obtained based on FCR and cBWG, and cumulative European Broiler Index (**EBI**) was calculated. Body composition and processing weights (carcass, **CAR**; breast fillet, **BRE**; tenderloins, **TEN**; wings, **WIN**; leg quarters, **LQU**) were determined with Dual-Energy X-Ray Absorptiometry across the study. Nine additional pens were fed the treatment diets continuously from 1 to 56 days, and 50 birds from each treatment were processed at the end of the study to determine CAR, LQU, WIN, and breast meat + wing (**BPW**) yields, along with meat quality (white stripping, **WS**; woody breast, **WB**). Feeding costs were expressed per bird (**FCB**), per kg BW (**FCW**), and per kg carcass (**FCC**). Market value (**MKV**, $/bird) was determined based on the weight of each processing part and its market price, gross profit (**GPR**, $/bird) as MKV – FCB, and ROI as GPR/FCB. All data were adjusted to processing weights, and response at 100% PE requirement (pseudo-T4) was estimated by regressing response against PE density (T2, T3). Monte Carlo and bootstrapping in JMP were used to obtain T4 data. ANOVA and Student's t-test, correlation among PE density and responses, and linear and quadratic regressions for response based on PE density were run. Increasing PE density produced linear positive effects on cFCR, EBI, CAR, LQU, WIN, FCC, and MKV ($P<0.05$) and quadratic effect on feed ROI ($P<0.05$). 92% PE (T3) showed lower feeding costs and higher gross profit and ROI than AMEn birds ($P<0.05$). Estimated response at 100% PE indicated better cBW, cBWG, cFCR, EBI, CAR, LQU, FCC, MKV ($P<0.001$), GPR ($P=0.002$), and ROI ($P<0.001$) than T1. A 1 million broiler complex fed with 92% PE diets would produce weekly a net balance of additional 101,000 kg carcass, $71K in total feeding cost savings, extra $207K in total cut-up parts market value, and additional $258K in gross profit. Improved BW, FCR, and EBI were determined at densities between 92% PE and 100% PE compared to AMEn. In conclusion, PE shows potential to improve production efficiency and economics of broiler production compared to AMEn.

Keywords: Productive energy, Energy density, Processing yield, Broilers, Economics

376 Higher amino acid intakes associated with diets formulated with productive energy boost protein accretion, weight gain, and processing yield in broilers

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The study assessed the effect of the continuously increased digestible amino acid intake driven by diets formulated with productive energy (**PE**) on broiler performance and body composition. It developed a mechanistic model to explain the response. 90 floor pens were assigned to 5 feeding phases and treated as independent experiments using fresh birds in each phase. Starter, Grower, and Finisher 1, 2, and 3 pens had 30, 23, 18, 16, and 16 birds, respectively. Each phase included 3 treatments: T1, diets formulated with apparent metabolizable energy (**AMEn**), and T2 and T3 formulated with PE (83 and 92% of PE requirement, respectively). Feed intake (**FI**), BW, BW gain (**BWG**), feed intake (**FI**), feed conversion ratio (**FCR**), and body composition and processing weights (using Dual-Energy X-Ray Absorptiometry) were determined by phase. The gains of BW, processing weights (carcass, **CAR**; breast fillet, **BRE**; tenderloins, **TEN**; wings, **WIN**; leg quarters, **LQU**) and body chemical fractions (protein, **PR**; fat, **FA**; lean mass, **LM**) were fitted to Gompertz 3P First Derivative (**GFD**) and the calculated cumulative effects (**C-EFF**) of feeding the treatment diets continuously across phases (**cFI**, **cBW**, **cCAR**, **cBRE**, **cTEN**, **cWIN**, **cLQU**, **cPR**, **cFA**, **cLM**, respectively) were obtained by applying GFD's obtained coefficients to the basic Gompertz 3P equation. Cumulative BWG (**cBWG**) was calculated with cBW, and cumulative FI (**cFI**) with FCR and cBWG. 9 additional pens were fed the treatment diets continuously 1-56 days, 148 birds were processed at 56 d to determine processing weights, and actual cumulative effects (**A-EFF**) were obtained by fitting the data to Gompertz 3P model. Response to 100% PE requirement (T4) was estimated by the linear regression of T2 and T3. Bootstrapping and Monte Carlo techniques were applied in JMP Pro 16 to obtain T4 data. ANOVA and Student's t-test were applied. C-EFF and A-EFF data were compared. Increasing PE (T2, T3, and T4) produced higher effects under the actual continuous feeding scenario (A-EFF) compared with their corresponding calculated effect (C-EFF) on BW ($P=0.01$), carcass yield, body protein, and body lean mass ($P<0.05$) at 56 d, suggesting an improved protein synthesis rate the higher the diet PE. C-EFF of T4 on BW indicated +67 g at 56 d ($P=0.119$) compared with its A-EFF of +144 g ($P<0.001$) at the same age. The mechanistic model indicated that the higher amino acid intake driven by productive-energy-formulated diets induced a higher mixed muscle fractional synthesis rate, increasing protein accretion and BWG. In conclusion, feeding continuously PE-formulated diets improves protein accretion, BW, and carcass yield. PE diets show potential to improve broiler response and production efficiency.

Keywords: Productive energy, Amino acid intake, Processing yield, Broilers, Economics

377 Cluster-based analysis of DDGS nutrient and antinutrient variability for improved precision

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Dried Distillers Grains with Solubles (**DDGS**), recognized for their variable nutrient composition, present a challenge for precision nutrition. This study aimed to characterize the variability of nutrients and antinutrients in DDGS samples clustered by proximate composition (88% DM-basis), and to evaluate the implications of using average cluster composition or overall averages on feed formulation. Data (N = 6,896) was obtained from a commercial (ABVista) database of user-submitted near-infrared spectroscopy (**NIRS**) readings, from 2019 to 2024, and analyzed using JMP 18. Before clustering, distributions were fitted to each DDGS nutrient and antinutrient available, and the data was best represented by variants of normal mixtures. Clusterization of sample reading into 3 groups (C1, C2, C3) using the Normal Mixtures platform. A one-way ANOVA was used to determine differences between clusters, with $P \leq 0.05$ considered significant, and Tukey's HSD was used to separate means. Starter, grower, and finisher diets, meeting or exceeding the breeder recommendations (Aviagen, 2022), were formulated using the average nutrient composition of all samples, the lowest total non-starch

polysaccharides (**NSP**) cluster , and the highest total NSP cluster. To focus solely on DDGS nutrient and antinutrient variability, the DDGS inclusion was fixed at 10%, and average corn and soybean values were used. The C1 (12.7% of samples) had higher ($P \leq 0.001$) total NSP (24.04%), insoluble NSP (21.32%), crude fat (7.55%), and crude fiber (8.81%), as well as lignin (3.80%). Additionally, C1 had the highest ($P \leq 0.001$) phytic P (0.32%) and Starch (5.41%), but similarly high values were observed for C2 (0.32% and 5.39%, respectively). For C2 (45.4% of samples), higher ($P \leq 0.001$) crude protein (**CP**; 31.24%), neutral detergent fiber (**NDF**; 31.97%), and moderate crude fiber (7.76%) were observed. The C3 (41.98% of samples) exhibited a more balanced nutrient composition, with intermediate levels of protein (29.55%) and fat (7.30%). However, C3 also had the lowest ($P \leq 0.001$) lignin (3.21%) and phytic P (0.26%) contents. Similar values for total NSP were observed for C2 (23.28%) and C3 (23.27%), as well as insoluble tNSP (20.61 and 20.67%, respectively). Formulations with high and low total NSP clusters, using only average corn and soybean meal values, resulted in a small increase in total NSP, ranging from 9.69 to 10.34%. Additional higher insoluble NSP (+0.06%) and lignin (+0.04%) were observed when formulating with the high total NSP Cluster (C1). Identifying the variability in the nutrient and antinutrient characteristics of ingredients, even with small dietary inclusions such as DDGS, can help nutritionists improve precision nutrition.

Keywords: DDGS, nutrient, antinutrient, non-starch polysaccharides, feed formulation

378 Evaluation of two energy systems currently available for broiler: Performance and economic responses

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This study evaluates the performance and economic outcomes of Ross 308 broilers from 0 to 35 days of age, consuming a three-phase feeding program (starter: 0–10 days, grower: 11–28 days, and finisher: 28–35 days). The trial followed a 3x3x3 factorial scheme, considering three energy systems—Metabolizable Energy Poultry (MEpoultry), Net Energy (NE), and Effective Energy (EE), three dietary energy levels (recommended energy, and reductions of 75 and 150 kcal/kg), and three growth phases. Each treatment included eight replicates, with 19 broilers per replicate. Diets were formulated according to the assigned energy system and level, with NE estimated as NE (MJ/kg) = 0.815 × MEpoultry – 0.26 × Protein + 0.20 × Lipid - 0.24 × NDF, and EE estimated as EE (MJ/kg) = MEpoultry – 0.038 × FOM - 0.0467 × dProtein + 0.036 × dLipid, where Protein, Lipid, NDF, FOM, dProtein, and dLipid represent crude protein, ether extract, neutral detergent fiber, fecal organic matter, digestible protein, and digestible lipid, respectively (all in g/kg). Feed prices ranged from €0.403 to €0.337/kg for MEpoultry-based diets, €0.394 to €0.324/kg for NE-based diets, and €0.403 to €0.330/kg for EE-based diets. Feeding cost was calculated as feed intake (kg) × feed price (€/kg), revenue as live weight (kg) × €1.28/kg, and margin over feeding cost (MOFc) as revenue – feeding cost. A three-factor repeated measures design was used. NE-based diets at the recommended energy level led to higher body weight gain (BWG) during the grower (P = 0.017) and finisher phases (P = 0.001) compared to MEpoultry. EE-based diets did not result in significant differences in BWG or feed intake (FI) compared to MEpoultry across all phases (P > 0.05). However, feed conversion ratio (FCR) was 0.02 points lower (P < 0.05) for broilers fed an EE-formulated diet at the recommended energy level but 0.02 points higher (P < 0.05) at the 75 kcal/kg reduction. NE-based diets at the recommended energy level reduced feeding cost by €0.019 per bird at 28 days (P = 0.018) and 35 days (P = 0.001), leading to higher MOFc over the full production cycle (P = 0.045). However, at reduced energy levels (75 and 150 kcal/kg), NE-based diets did not provide economic advantages over MEpoultry (P > 0.05). EE-based diets tended to reduce feeding costs (P = 0.053) but did not significantly improve revenue or MOFc (P > 0.05). These findings suggest that both NE and EE can be reliably used in broiler feed formulations, given their minimal impact on performance. Although some economic benefits were observed with the NE system, further research is needed to provide strong evidence supporting a clear economic advantage of switching from a metabolizable energy system to a net energy system.

Keywords: net energy, effective energy, energy utilization, metabolizable energy, digestibility

379 Effect of slaughter age on carcass characteristics, meat quality and texture profile of turkey meat

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Slaughter age has a direct impact on meat quality attributes, supplier economic benefits and consumer choice. Investigating a better slaughter age for turkeys will require a thorough understanding of the relationship between meat characteristics and ages. This study evaluates the impact of slaughter age on carcass characteristics, meat quality attributes and texture profile of meat at 8,12,16, and 25 weeks old turkeys. A total 40 turkey toms (10 birds from each age group) were selected for slaughter at their respective marketing age. Turkeys were reared in floor pans and provided with corn-soya diet: ME 2950 kcal/kg and CP 16%. Proper vaccination schedule and bio-security were followed. A 10-h fasting period was applied prior to slaughter and only water was provided during this period. Furthermore, the body weight of the birds were determined before slaughter. Then edible organ weight and carcass weight were weighed following slaughter. According to standard method, carcasses were cut into parts. Weight was taken for all carcass parts. Meat color (L*, a*, b*) values were determined using Color Reader CR8 and meat pH were determined 24 hours of slaughter using pH meter (Hach, USA). Drip loss, cooking loss and water holding capacity were also determined according to standard method. Texture profile analysis (TPA) was done using Texture Analyzer (Lamy Rheology, France). One-way ANOVA was applied in data analysis and means were contrasted at $P < 0.05$. Live weight and carcass weight increased significantly with increasing age ($P < 0.0001$), from 1219.75 g and 713.17 g at 8 weeks to 4836.22 g and 3114.22 g at 25 weeks, respectively. Dressing percentage improved with increasing age (P=0.016). Thigh and breast weights increased ($P < 0.0001$) with advancing slaughter age. The older birds had higher DL% (7.232% at 25 weeks compared to 1.562% at 8 weeks) and lower WHC% (73.498% at 25 weeks compared to 82.739% at 8 weeks), indicating lower moisture retention in the older birds. Meat color values were significantly different ($P < 0.001$), with higher redness

(a*) in younger birds. TPA found significant ($P < 0.0001$) differences in fracture force, chewiness, and gumminess, indicating firmer meat of older birds. However, 16 weeks old turkeys performed better in meat quality attributes than others. Therefore, slaughter age has marked influence on carcass yield, meat quality and texture properties. Whilst older turkeys give more meat, this is most likely to be tougher with greater moisture loss, influencing processing and consumer acceptability.

Keywords: slaughter age, carcass characteristics, meat quality, texture profile analysis (TPA), turkey meat

380 Metformin as a potential therapeutic for improving ovarian function in broiler breeder hens

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Broiler breeder hens, the parents of commercial broiler chickens, often exhibit poor reproductive efficiency due to decades of intense genetic selection for growth-related traits in broiler progeny. When given unrestricted feed access, they overconsume, resulting in metabolic dysfunction, excessive fat deposition and ovarian dysfunction. To mitigate these issues, the industry standard is feed restriction, which is often imprecise, hard to manage and raises welfare concerns. Despite feed restriction, ovarian dysfunction persists, characterized by the excessive recruitment of immature follicles and a disrupted preovulatory hierarchy. The underlying causes remain poorly understood, highlighting the need for alternative solutions. Notably, ovarian dysfunction in broiler breeder hens resembles polycystic ovary syndrome (PCOS) in women, a condition linked to metabolic and reproductive disorders. Metformin, a widely used PCOS treatment, may offer therapeutic benefits for improving reproductive health in these hens. Our studies aimed to evaluate metformin's effects on ovarian function in broiler breeder hens. We hypothesized that metformin would enhance reproductive outcomes by correcting ovarian dysfunction. The first study examined metformin's effects on follicular cells isolated from broiler breeder hen ovaries. In vitro metformin treatment of granulosa cells from prehierarchical and preovulatory follicles significantly decreased progesterone secretion, associated with inhibited ERK2 and Akt signaling and reduced expression of steroidogenic genes. These findings suggest metformin may influence follicular recruitment and maturation. The second study assessed metformin's long-term effects in vivo. Dietary supplementation at 75 mg/kg body weight for 40 weeks improved ovarian function by normalizing follicular hierarchy and reproductive hormone levels. These improvements correlated with lower overall body and abdominal fat pad weight, increased and prolonged egg production and sustained fertility and hatchability. Additionally, transcriptomic analysis of liver tissue from this study revealed increased expression of genes related to estrogen-stimulated yolk precursor synthesis, insulin-mediated lipogenesis and AMPK-regulated glucose homeostasis. These findings suggest metformin supports prolonged egg production in aging hens by sustaining yolk precursor and fatty acid synthesis, typically diminished with age. Overall, our findings suggest that dietary metformin supplementation could be a cost-effective feed additive to enhance egg production and reproductive efficiency in broiler breeder hens.

Keywords: Broiler Breeder, Metformin, Ovary, Liver, Egg production

381 Early defense and immune shifts during symptom progression in the vagina of turkey hens infected with low pathogenicity influenza A virus

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In turkey breeder hens, the reproductive tract represents a potential entry route for low-pathogenicity avian influenza viruses (LPAIV), particularly through artificial insemination. The vagina, as the site of insemination, serves as the first line of defense, while the adjacent uterovaginal junction (UVJ) plays a key role in sperm storage and fertility. The aim of this study was to characterize gene expression differences in the vagina during early LPAIV infection by comparing transcriptomes before and after the onset of clinical symptoms. Additionally, we compared vaginal transcriptomes with those of the UVJ to assess whether the vagina has a more pronounced immune response. Vaginal tissue samples were collected from presymptomatic and symptomatic LPAIV-infected turkey breeder hens (n = 4 per group, 37 weeks of age at 7 weeks of lay). Samples from the UVJ were also collected. Total RNA was extracted, libraries were constructed with the TruSeq Stranded mRNA kit, and sequencing was performed on an Illumina NovaSeq 6000. Gene expression analysis was conducted using DESeq2, and genes with an adjusted p-value (FDR < 0.05) and a fold-change ≥ 1.5 were considered significantly differentially expressed. RNA sequencing identified 14,337 genes expressed in the vagina, with 663 genes differentially expressed between presymptomatic and symptomatic groups, and 14,226 genes expressed in the UVJ, with 5,090 significantly differentially expressed. When comparing gene expression between the vagina and UVJ, 871 differentially expressed genes were identified in presymptomatic birds and 3,050 in symptomatic birds. Gene Set Enrichment Analysis (GSEA) revealed upregulation of pathways related to the ribosome and oxidative phosphorylation in the vagina of presymptomatic birds, with a shift toward the calcium signaling pathway in symptomatic birds. The vagina showed significant upregulation of genes associated with skin barrier establishment and natural killer (NK) cell-mediated immunity compared to the UVJ. In symptomatic birds, Gene Ontology (GO) analysis suggested a transition toward adaptive immune responses. The upregulation of the ribosome and oxidative phosphorylation pathways indicates increased protein synthesis and energy production as the infection progresses. The calcium signaling pathway enrichment in symptomatic birds suggests a role in immune regulation and inflammation. The stronger early immune activation observed in the vagina likely serves as a protective mechanism, whereas the UVJ may be more susceptible to viral replication. These findings suggest that enhancing vaginal immune responses may limit viral infection, reduce spread, and mitigate reproductive impacts in infected flocks.

Keywords: Avian influenza, Gene expression, Immune response, Reproductive tract, Turkey

382 The effect of genetic selection on gene expression in the developing neuroendocrine system of broiler chickens

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Broiler selective breeding has improved growth rates, muscle mass, and feed efficiency. However, these genetic modifications also impact the neuroendocrine systems which regulate growth and other physiological processes. Embryonic development represents one-third of broiler growth to market weight. To understand the

impact of genetic selection on neuroendocrine regulation of growth during embryonic development, we examined mRNA expression for growth-related genes in the hypothalamus and anterior pituitary of two chicken breeds: the modern commercial Ross 708 broiler and the Athens Canadian Random Bred (ACRB), the oldest established control strain for meat-type chickens. Hypothalami and pituitary glands were dissected from four males and four females of each breed at embryonic days 10, 12, 14, 16, and 18 (n=4 for each combination of breed, age, and gender). Levels of mRNA for genes involved in growth, metabolism, body composition, and reproduction were quantified using reverse transcription-quantitative PCR normalized to PGK1 and GAPDH mRNA levels in the pituitary and hypothalamus, respectively. Significant effects and interactions across breed, age, and gender on mRNA levels were determined using mixed model ANOVA (SAS, Cary, NC). In the adrenocorticotropic axis, hypothalamic corticotropin-releasing hormone (*CRH*), pituitary CRH receptors 1 and 2 (*CRH-R1* and *CRH-R2*), and pituitary pro-opiomelanocortin (*POMC*) mRNA levels were affected by age, *CRH* was affected by gender, and *CRH-R1* was affected by the interaction effect of breed, age, and gender ($P<0.05$). In the thyrotropic axis, pituitary thyroid-stimulating hormone β-subunit (*TSH-β*) mRNA levels were affected by breed, age, and the interaction of breed, age, and gender ($P<0.05$). Hypothalamic thyrotropin-releasing hormone (*TRH*) mRNA level was affected only by age ($P<0.05$). In the somatotropic axis, hypothalamic somatostatin (*SST*), pituitary growth hormone-releasing hormone receptor (*GHRH-R*), and pituitary adenylate cyclase-activating polypeptide receptor 1 (*PACAP-R1*) were affected by breed and age ($P<0.05$). In the gonadotropic axis, hypothalamic gonadotropin-releasing hormone 1 (*GnRH1*) and gonadotropin-inhibiting hormone (*GnIH*) were affected by breed, age, and gender ($P<0.05$). The findings indicate that genetic selection of modern broilers has affected neuroendocrine regulation of the somatotropic, adrenocorticotropic, thyrotropic, and gonadotropic axes during embryonic development. The differences identified may account for a portion of the improvements in growth traits of modern broilers.

Keywords: broiler, gene expression, genetic selection, hypothalamus, pituitary

383 Neurochemical metabolic pathways display region and age-specific expression patterns in the chicken intestinal tract

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Little is known regarding the distribution and expression of proteins involved in the synthesis, transport, and degradation of neurochemicals throughout the chicken intestinal tract. Therefore, this study aimed to identify and quantify proteins involved in the production, transport, and catabolism of neurochemicals in the chicken ileum, cecum, and colon at 14 and 28 days of age. Male broiler chickens were raised in floor pens under age-appropriate conditions. Ileum, cecum, and proximal colon samples were collected at 14 and 28 days of age. A series of commercially available primary antibodies were first tested and validated for use in chickens using intestinal and positive control tissues, including chicken brain regions, prior to the experiment which sought to characterize tryptophan hydroxylase-1 (TPH-1), monoamine oxidases (MAO-A and MAO-B), tyrosine hydroxylase, norepinephrine transporter (SLC6A2), and serotonin transporter, which were evaluated in all three intestinal segments. The samples were diluted and analyzed using the Jess automated capillary immunoassay with the Separation module, incorporating a fluorescent standard, as per the manufacturer's instructions. Protein normalization was performed following manufacturer guidelines. All data were analyzed using ANOVA followed by Tukey's post-hoc comparison. Statistical significance was set at $P<0.05$. Significantly higher levels of MAO-A and MAO-B ($P < 0.05$) were detected in the ileum compared to the cecum and colon in 14-day-old chickens. However, no significant difference in MAO-A levels ($P > 0.05$) was observed between the ileum and cecum in 28-day-old chickens. Similarly, no significant differences ($P > 0.05$) in MAO-A or MAO-B levels were observed between the cecum and colon segments at both 14 and 28 days of age. Tyrosine hydroxylase levels were significantly higher ($P < 0.05$) in the cecum and colon segments compared to the ileum at both 14 and 28 days of age. Conversely, no significant differences ($P > 0.05$) were observed among intestinal segments for SLC6A2 levels in 14-day-old broilers. The detection and quantification of neurochemical metabolic pathways in the ileum, cecum, and colon tissues provide a biogeographical map for poultry researchers that can be used to target stress-related neurochemical responses throughout the chicken intestinal tract. Further research is needed examining the translatability of these findings to other poultry species would provide valuable insights and broaden the applicability of this work.

Keywords: Neurochemical, Chicken, monoamine oxidases, automated capillary immunoassay, Intestine

384 Effect of early-life thermal conditioning on mRNA expression in tissues during heat stress in broiler chickens

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Heat stress (HS) negatively affects the health, productivity, and welfare of broilers, particularly those nearing market age. This study investigated the impact of early-life thermal conditioning (TC) on expression of genes associated with stress responses, thermoregulation, and metabolism in broilers exposed to HS. Male Ross 708 chicks were used and subjected to TC at 37.8°C or non-thermal conditioning (NTC) at 33°C for 24 hours on day 3 after hatch. Six replicate pens were used for each treatment, and pen was used as the replicate in statistical analyses. On day 31, birds from both groups (6 pens each) were exposed to either HS (35°C) or non-heat stress (NHS; 22°C) conditions for 4 hours, after which tissue samples were collected for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. A t-test for independent samples was used to compare the means of the NTC and TC groups under NHS and HS conditions, with statistical significance set at $P < 0.05$ (n=6). The results showed that early-life TC notably affected gene expression in the hypothalamus and pituitary, which are involved in stress adaptation under HS. Specifically, TC birds showed increased neuropeptide Y expression in the hypothalamus, likely enhancing stress adaptation and thermoregulation. TC also increased heat shock protein 90 mRNA levels in thigh muscle and reduced heat shock protein 70 mRNA in breast muscle, suggesting tissue-specific adaptations. Moreover, TC birds showed higher melanocortin 2 receptor expression in heart tissue under HS, indicating better cardiovascular resilience. Changes in gene expression in other tissues, like the adrenal gland, thyroid, liver, and adipose tissue, were minimal, pointing to selective effects of TC. These findings highlight that early-life TC can prime specific tissues to better cope

with HS, potentially benefiting poultry farming in hot climates. This study emphasizes the potential role of TC in improving broiler health and productivity under HS conditions.

Keywords: broiler, gene expression, heat stress, physiological effects, thermal conditioning

385 Responses to insulin-like growth factor and growth hormone may be influenced through thyroid hormone-driven changes to levels of binding proteins and receptors in avian muscle cells

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The robust accretion of muscle tissue is one of the most economically important aspects of broiler production. Growth and metabolic efficiency of muscle are regulated in part through the thyroid hormone (TH) triiodothyronine (T_3), which binds to nuclear TH receptors (THRs) to alter expression of target genes. Putative TH response elements are present in the promoters of several genes within the somatotropic axis that may affect muscle growth, including some insulin-like growth factor (IGF) binding proteins (IGFBPs), and prior work from our lab demonstrated that thyroid hormones may influence their expression. The objective of this study was to determine how biologically relevant levels of T_3 affect expression of receptors, binding proteins, and hormones in the somatotropic axis. To this end, quail muscle clone 7 (QM7) cells, a myoblast cell line in which differentiation and myogenesis can be induced, were used to assess the ability of T_3 to affect these targets. Differentiated and undifferentiated QM7 cells were treated with either vehicle or 0.04 ng, 0.2 ng, or 1 ng/mL of T_3 for 0.5, 6, or 24 hours (n=6). Total RNA was isolated, followed by reverse transcription-quantitative PCR. Data were analyzed via 2-way ANOVA followed by Fisher's LSD test. A 1-way ANOVA was used to compare vehicle-treated cells between undifferentiated and differentiated groups (n=6). In vehicle treated cells, IGFBPs 2, 5, and 7, as well as IGF1 receptor (*IGF1R*), deiodinase 3 (*DIO3*), and THR alpha (*THRA*), had higher expression in differentiated cells, while *IGFBP3* and growth hormone receptor (*GHR*) were higher in undifferentiated cells ($P \leq 0.05$). In T_3-treated groups, *DIO3* increased in a dose- and time-dependent manner, regardless of differentiation state ($P \leq 0.05$). Additionally, THR-beta (*THRB*) increased with dose regardless of treatment time in undifferentiated cells, while in differentiated cells it increased with both dose and treatment time ($P \leq 0.05$). Within the somatotropic axis, *IGF1* was not detected, and *IGF2* did not exhibit changes in response to T_3. In contrast, *IGFBP5* increased with T_3 concentration and duration in both undifferentiated and differentiated cells ($P \leq 0.05$). Both *GHR* and *IGFBP4* also increased in a dose-dependent manner, though only in differentiated cells for the latter ($P \leq 0.05$). Herein, biologically relevant levels of T_3 altered expression of binding proteins and receptors for IGFs and GH, respectively, while no changes in *IGF2* were observed, suggesting T_3 exerts influence over this axis without impacting local hormone production. As both undifferentiated and differentiated cells responded similarly, the influence of T_3 on somatotropic signaling within muscle may be independent of differentiation status.

Keywords: Myogenesis, triiodothyronine, insulin-like growth factor binding proteins, quail muscle clone 7 cells, differentiation state

386 Protein co-expression network analysis to investigate biological mechanisms associated with fillet weight in broilers affected by myopathies

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This study aimed at providing biological insights into the growth-related myopathies (white striping–WS; wooden breast–WB; spaghetti meat–SM) affecting broilers selected for rapid growth and high breast-yield by exploring the proteome profiles of affected and not-affected (Normal, N) *Pectoralis major* muscles. Twelve fillets were collected and weighed at a commercial processing plant (3/group; N and severely affected by WS, WB, or SM), and classified based on their macroscopic features. One gram from each sample was used for proteomic analysis through TMT-based mass spectrometry at the Harvard Medical School (Boston, MA, USA). After testing for normality (Shapiro-Wilk Test), fillet weights were analyzed by one-way ANOVA considering the 4 groups as the main effect (R v4.3.2). Proteomic and phenotypic data were used to perform a co-expression network analysis (WGCNA R Package; v1.72-5) and biological interpretations of co-expressed proteins were performed through the DAVID Functional Classification Tool. Gene Ontology (GO) terms with FDR<0.05 were considered statistically significant using *Homo sapiens* as the background organism. Fillet weights of severe WB, SM, and WS cases were higher than N ($P<0.05$). While WB fillets were higher than WS ($P<0.05$), no differences between SM and the other two myopathies were found. From TMT-based mass spectrometry, 4,199 proteins were quantified and mapped to the *Gallus gallus* Uniprot database. Since only proteins quantified by at least 2 peptides were considered for downstream analysis, the normalized relative abundance of 3,277 proteins was used to construct the co-expression networks. WGCNA identified 10 color-coded groups (i.e., modules) of co-expressed proteins, among which the turquoise ($r=+0.82$; $P<0.01$) and brown ($r=-0.87$; $P<0.01$) were the most significantly related with fillet weight. For the turquoise module, biological processes involved in the regulation of protein neddylation ($P<0.01$), cellular oxidant detoxification ($P<0.01$), cardiac myofibril assembly ($P<0.01$), and collagen fibril organization ($P<0.01$) were found. Respiratory chain complex III ($P<0.01$), mitochondrial membrane organization ($P<0.01$), endoplasmic reticulum lumen, and collagen-containing extracellular matrix ($P<0.01$) were among the most significant terms related to the brown module. To conclude, WGCNA applied to proteomic data allowed a biological interpretation of co-expressed protein networks significantly associated with fillet weight variability in broiler breast fillets affected by growth-related myopathies.

Keywords: Growth-related abnormalities, Pectoralis major muscles, Proteomics, WGCNA, Functional analysis

387 Comparative analysis of two dietary saturated fat types On metabolite profiles crossing the blood brain barrier of poultry chicks

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Dietary fat types play a pivotal role in modulating brain function, particularly through their influence on central serotonergic

signaling pathways. These serotonergic regions: hypothalamus (HYP) and dorsal raphe nucleus (DRN) play crucial functions in regulating various processes such as energy homeostasis, serotonergic system and growth regulation. Coconut oil (COCO), rich in medium-chain fatty acids, is rapidly absorbed without re-esterification and exhibits antibacterial, antioxidant, and anti-inflammatory properties, making it a valuable poultry feed additive. This study was conducted to investigate the influence of saturated animal fat (poultry fat) and plant fat (Coconut oil) on the composition of metabolites crossing the BBB to the serotonergic rich sections of the brain. Day-old broilers (Ross 708 male, n = 160) were distributed into two dietary treatments with five replicates of 16 birds each. The experimental treatments were; corn-soybean meal (SBM) base diet supplemented with 3% two forms of dietary fat type; poultry fat (CON), and coconut oil (COCO). Body weight gain (BWG), Feed intake (FI), and feed conversion ratio (FCR) were recorded over the 3-week period and data was subjected to T-test. Higher ($P<0.05$) BWG (0.8446 kg/bird) was recorded for COCO compared to CON (0.8132 kg/bird). Untargeted metabolomic analysis by high-performance liquid chromatography (HPLC-MS) was used to evaluate metabolite profiles in DRN, HYP, and plasma. To identify unique metabolites, univariate and principal component analysis (PCA) was employed, while ANOVA was used to identify metabolites that were significantly differential (P<0.05) in plasma, DRN, and HYP. The metabolites were then annotated using the KEGG and HMDB databases. A total of 5,193 metabolites was generated, 519 were annotated with 49 differential ($P<0.05$) metabolites while 5 metabolites crossed the blood-brain barrier (BBB). Succinate semialdehyde, a critical metabolic intermediate that influences brain function, energy metabolism, and neurotransmitter regulation was upregulated in Plasma but downregulated in DRN. Pleiocarpamine which possesses anticholinergic properties was upregulated in both plasma and DRN while both 2-Phenylethyl 3-O-beta-D-glucopyranoside and methyl 4-oxy-2-hydroxy-3,6-dimethylbenzoate were upregulated in Plasma. Two metabolic pathways (Butanoate metabolism and Alanine, aspartate and glutamate metabolism) associated with serotonin synthesis and Asp/Glu regulate stress-related neurotransmission were enriched. The study concluded that dietary inclusion of COCO appeared to improve the functional integrity of the chicken brain and facilitate critical processes such as neurotransmission, energy production, and neuroprotection.

Keywords: Coconut oil, Hypothalamus, Dorsal raphe nucleus, metabolomics, metabolites

388 Body composition at onset of lay - Effects of growth trajectory and photostimulation on relative body fat in laying hen pullets

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Recent research in caged laying hens showed that pullets will enter lay in the absence of photostimulation (**PS**) once they reach a certain body weight (**BW**). We hypothesize that the onset of lay depends on body composition, specifically, a body fat threshold. In cage-free systems, hens consume more feed than caged hens, but their increased activity may redirect nutrient deposition from fat to lean tissue. This study investigated the relationship between body composition, growth trajectory, and age at PS in cage-free pullets. Aviary-reared Lohmann LSL-lite pullets (N = 600) were placed in 24 furnished floor pens (2 perches, 2 platforms) across four rooms at 12 weeks of age (**woa**). From 14 to 21 woa, three pens per room were feed-restricted (**FR**), and three fed *ad libitum* (**AL**), leading to a 10% difference in BW by 20 woa. Two rooms received PS when the AL pullets reached the pre-determined BW target at 18 woa (**PS18**), and the remaining two when FR pullets reached the BW target at 20 woa (**PS20**). Consequently, PS and BW thresholds were either synchronous (at 18 or 20 woa) or asynchronous (under or over BW target). At 14, 16, 18, 20, 21, 22, and 26 woa, one pullet per pen (n = 6/ treatment group) was euthanized, ovaries were weighed (relative weight: **%Ov**), and ovaries and heads were removed for further processing. The relative carcass body fat (mean of 2 scans; **%BF**) was assessed using dual-energy X-ray absorptiometry. A linear mixed model was fitted in R, with feeding, age at PS, age, and their interactions as fixed effects and random effects accounted for the split-plot design and the statistical unit of pen. A Type 3 Wald Chi-square test ($\mathbf{X^2}$) was used to determine significant predictors. Pearson correlations were run between %BF and %Ov. The interaction of all three main effects influenced %BF (X^2= 15.56, df= 7, P= 0.028). Overall, %BF increased with age, from an average (**μ±SD**) of 7.4±1.43% at 14 woa to 17.9±2.27% at 26 woa. Pullets from AL groups reached a first peak at 19 woa (AL: μ= 14.4±1.39%; FR: μ= 10.1±1.39%). The feeding treatment difference diminished by 21 woa (AL: μ= 15.3±3.99%; FR: μ= 12.5±3.59%). All four groups increased their %BF slowly with age, but only the AL group with synchronized PS increased %BF between 22 and 26 woa (AL_PS18: μ= 21.6±2.66%; AL_PS20: μ= 16.1±1.67%; FR_PS18: μ= 15.9±2.48%; FR_PS20: μ= 17.8±2.25%). There was a moderate positive correlation between %BF and %Ov (cor= 0.58; 0.482-0.669, P<0.0001). These findings show an association between onset of lay and relative body fat as well as a connection between the timing of ovarian maturation and fat accumulation. Here, pullets entering lay averaged 13.4±1.46% fat, approximately 5% less than the %BF reported in caged pullets.

Keywords: Laying hen, Pullets, DEXA, body composition, onset of lay

389 Keel bone ossification and position change during puberty – An exploratory study

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Laying hens have been selected for high egg production, prolonged peak performance, extended laying cycles, and early onset of lay. Previous research showed that onset of lay is induced by photostimulation (**PS**) or by meeting a metabolic threshold. The keel bone (**KB**) is not fully ossified at onset of lay, and its position changes during puberty, ventrally tilting away from the pubic bones. It is unknown how the KB's ventral tilt and ossification respond to changes in age at first egg (**AFE**) or any potential impact KB damage prevalence. This study explored the effects of growth trajectory and age at PS on KB ossification and position. Six hundred LSL-lite pullets were placed in floor pens furnished with elevated platforms and perches. Two rooms were PS at 18 weeks of age (**woa**; **PS18**) and two rooms at 20 woa (**PS20**). Three

pens per room were fed *ad libitum* (**AL**), and three were feed-restricted from 14 to 21 woa (**FR**), with a 10% bodyweight difference by 20 woa (n = 24 pens). A subset of four birds per pen were repeatedly radiographed at 14, 16, 18, 19, 21, 22, and 26 woa. The following KB variables were collected via the Fiji application of ImageJ: length, mid-shaft width, cranial width, ossified dorsal length, and visual overlap of caudal tip and legs (yes/no). Dual-energy X-ray absorptiometry validated the visual distinction between bone tissue and cartilage on the dorsal side of the KB, as observed in the X-rays, with bone density measurements confirming bone tissue above the visual threshold and none beyond it. Ossification progression was calculated as the percentage of ossified dorsal length relative to the total dorsal length at 26 woa. Mixed-effect models were fitted in R, where feeding, age at PS, age, and their interactions were fixed effects and pen was the statistical unit. KB size parameters were analysed for 26 woa only. KB ossification was age-dependent (X^2= 873.53, df= 6, P< 0.0001), showing a steep progression from 14 woa (mean ossification (**μ**) = 62.64±0.341%) to 18 woa (μ= 90.01±0.457%) after which it slowed. Most KBs were fully ossified by 26 woa (μ= 99.27±0.155%). KB position change depended on the feeding treatment (AL before FR; X^2= 13.78, df= 1, P= 0.0002), and a tendency of an age at PS effect (PS18 before PS20; X^2= 3.32, df= 1, P= 0.069). KBs from PS18 x AL pullets were the first to tilt ventrally, and those from PS20 x FR group were the last. KB size variables were unaffected by treatment. These findings suggest that the KB ossification followed a pre-programmed process, while the KB's position was associated with AFE. Further research should validate these findings across different genetic strains and assess bone quality and damage prevalence in relation to AFE and growth trajectory.

Keywords: Laying hens, Pullets, Keel bone, onset of lay

390 Not Presented

391 Sustainable Poultry Industry 4.0: The next level technological approach for the poultry processing sector

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Industry 4.0, also known as the Fourth Industrial Revolution, will transform the poultry processing sector through advanced technologies such as Internet of Things (IoT) devices, artificial intelligence (AI), machine learning (ML), robotics and cloud computing. These technologies enable smarter, more connected operations, leading to greater efficiency, precision, and responsiveness in poultry processing. Despite the sector's technological advancements, the vertically integrated poultry industry continues to struggle with disconnected data systems that limit rapid, informed decision-making. To overcome this, the Sustainable Poultry Industry 4.0 (SPI 4.0) framework introduces interconnected technologies and platforms designed to enable real-time, data-driven decisions. SPI 4.0 aims to optimize productivity, resource management, and food safety while fostering a highly skilled, efficient, and economically competitive poultry workforce. The SPI 4.0 framework actively engages industry stakeholders to address real-world challenges related to production efficiency, quality, and food safety. Innovative rapid-sensing technologies are at the core of SPI 4.0, including microwave sensors for assessing meat quality in live broilers, multi-modal meat quality sorting, Raman spectroscopy for farm-to-fork pathogen detection, and hyperspectral imaging for biofilm identification. These tools generate valuable data from key nodes connected to the cloud and powered by AI/ML to generate real-time analytics through intuitive dashboards. Artificial Intelligence for Quality Assurance (AI-Q) is part of SPI 4.0 which integrates diverse, high-velocity data streams specifically for quality assurance to generate rapid insights creating actionable insights to improve productivity, reduce waste, and ensure safety. SPI 4.0 is being built and will be validated at Auburn University (AU) and industry partner sites to ensure practicality, economic feasibility, and scalability. Knowledge transfer through stakeholder training and commercialization efforts will empower the workforce with modern tools, creating higher-paying, technology-driven roles and increasing retention in rural and urban operations alike. Industry 4.0 offers the poultry sector a transformative path forward—one that promises smarter systems, stronger safety, sustainable growth, and a resilient, future-ready workforce.

Keywords: Industry 4.0, Artificial Intelligence, Cloud Computing, Poultry Processing, Data

392 Determination of brown egg fertility by multi-directional visible/near-infrared spectroscopy and machine learning

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Poultry products provide affordable proteins to meet daily nutrient requirements for human. In aligned with the growing human population, the poultry industry has been intensified and mechanized over the past decades. Hatchery is a key component in the modern integrated poultry industry by providing chicks for commercial production. Detection of egg fertility during the first few days of incubation allow hatcheries to exclude infertile or problematic eggs in a timely manner, reducing incubator space, utility costs, and contamination from exploder eggs. The objective of this research was to investigate brown egg fertility detection via multi-directional point visible/near-infrared (VIS/NIR) spectroscopy and machine learning. A total of 130 brown eggs were measured with the point VIS/NIR spectroscopy from days 0 to 5 of incubation. Spectral images of the eggs were collected from three directions (X, Y, and Z axes) and in the spectral range of 190-1100 nm. Four preprocessing methods (e.g., Savitzky-Golay, Gaussian, Multiplicative Scatter Correction, and Standard Normal Variet) were separately or jointly applied to filter unwanted data and noises in spectrums. Then feature selection was performed using Principal Component Analysis and T-test. Finally, the Support Vector Machine classifier was used to determine brown egg fertility based on the filtered data and extracted features. The results indicate that the best precisions (%) of determining egg fertility on days 0, 1, 2, 3, 4 and 5 of incubation were 90.3, 90.9, 91.3, 91.9, 92.3, and 94.3, respectively. The proposed framework is a useful tool to identify egg fertility in early stages of incubation and support efficient and sustainable poultry production.

Keywords: Data Fusion, Candling, Precision Agriculture, Artificial Intelligence

393 Comparative impact of electrical and controlled atmosphere stunning on chicken carcass microbiota: A 16S rRNA sequencing analysis

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While electrical stunning is commonly used in poultry processing facilities in the United States, little has been explored of controlled atmosphere stunning in terms of carcass microbiology. The objective of this study was to investigate the bacterial populations across different points in poultry processing and compare two stunning methods using microbiota analyses. For this study, a total of 112 samples (64 whole carcass rinses, 16 parts rinses, 16 ceca, 8 scalding, and 8 chilling water) were collected from different points (post-stun, post-pick, evisceration, pre-chill, post-chill, and after-debone) within the processing facility, and 12 litter samples were collected from 12 different broiler house pens. Cell pellets were prepared by centrifuging 150 mL of carcass rinses using 50 mL conical tubes and washed with phosphate buffer saline. DNA were extracted using Qiagen Power Fecal® Pro DNA Kit. The microbiome was investigated by 16S rRNA next-generation amplicon sequencing and analyzed using QIIME2 pipeline. The relative abundance results showed higher prevalence of Proteobacteria after evisceration at the phylum level while the prevalence of *Pseudomonas* and *Anoxybacillus* were higher after the chilling step and in scalding water, respectively, at genus level. The alpha diversity results showed significantly higher diversity ($P<0.05$, Kruskal Wallis test and $P<0.05$, Dunn Post hoc test) in ceca, chill water, and whole carcass rinses after stunning as compared to litter in broiler house and scalding water in the processing facility. For Beta diversity, as indicated by Bray-Curtis distances, there were significant distinctions ($P<0.05$, PERMANOVA) of bacterial populations across litter, ceca,

scalding, chilling water, and carcass rinses after stunning, post-pick, prechill, post-chill, and after-debone. However, the overall effect of stunning types was not significantly varied (P>0.05, Kruskal Wallis test). When the stunning types were compared across six different points, the diversity was significantly varied (P<0.05, Kruskal Wallis test) for pre-chill and after stunning whole carcass rinses. The overall Beta diversity was also significantly varied (P<0.05, PERMANOVA) between stunning types. Moreover, across six different points, as indicated by Bray-Curtis distances, there was significant variation (P<0.05, PERMANOVA) of bacterial population between stunning types across prechill and post-chill points. The results suggest that the environmental factors, processing steps, stunning types, and sample sources may influence the microbial diversity of raw poultry products in poultry processing facilities.

Keywords: poultry processing, stunning types, 16S rRNA sequencing

394 Effects of controlled atmosphere stunning and packaging on the quality and spoilage of raw breast fillets stored under refrigeration

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Consumer demand for humane slaughter has led to the development and adoption of controlled atmosphere stunning (CAS) methods. Prior to its industry-wide adoption, CAS should be thoroughly investigated for its effects on meat quality under various conditions of storage. Research was conducted to determine the effects of CAS compared to traditional electric stunning method on the quality of breast fillets in air and vacuum packs during refrigerated storage. Commercially raised broilers (5 weeks 4.5-5 lbs live wt) flock was split and stunned either through CAS or electric method, processed and deboned at a commercial poultry processing facility. Butterfly breast fillets were transported to Auburn University and stored overnight at 4C. The next day, breast fillets from each stunning method were packaged (3 fillets/pack) with under vacuum or air and the packages were stored at 4C and 10 packs were sampled every third day until spoilage (7 log CFU/mL). During each sampling day, samples were analyzed for meat quality (drip loss, color, bioelectrical impedance, cook loss and texture) and microbial spoilage (aerobic, anaerobic and lactic acid bacteria counts). The entire experiment was repeated as three separate trials. Data was analyzed using one-way ANOVA using Tukey HSD at p<0.05. There were no significant differences (p>0.05) in the quality and microbiological parameters of the meat on Day 0. Drip loss was higher (p<0.05) among the vacuum-packed samples compared to the air packs. There were no significant differences in cook loss till day 12 while there were significant differences in texture(force) starting day 9 of storage, however, there were no clear patterns/trends. The study indicates that CAS stunning does not have major effects on meat quality, however, vacuum packaging may affect water holding capacity.

Keywords: meat quality, gas-stunning, electric-stunning, packaging

395 Impact of egg handling and storage temperature on egg quality of shell eggs after extended storage of 27 weeks

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Eggs are an important international agricultural commodity. However, how eggs are handling and stored may differ around the world. In the US, under federal regulations, eggs are washed and stored at refrigerated temperatures. These practices can be an obstacle if the eggs are destined for export markets. The focus of this study was to evaluate the impact of egg handling and storage temperature on egg quality during a 27-week storage period. Conventional commercial white shell eggs were obtained and collected under four different egg handling treatments: 1) unwashed 2) washed (USDA-Agricultural Marketing Service (AMS), 3) washed and oiled (food-grade mineral oil), and 4) 21 days refrigerated storage (AMS) then washed. The eggs were then transported to the U S National Poultry Research Center in Athens, GA. Three cases of eggs (n=1,080, each case represented a replicate (n=3)) of each egg handling treatment were stored in either refrigeration (4°C) or room temperature (22°C). At week 0, 30 eggs are randomly selected from each treatment/replicate combination and grouped according to storage temperature to determine egg weight each week. At Week 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, 22, and 27, ten eggs were randomly selected from each replicate of egg handling and storage temperature combinations and assessed for various physical egg quality parameters. Statistical analysis was performed using the Generalized Additive Mixed Models to the time series data to capture nonlinear trends over time. The models had appropriate error distributions for each response variable, and fixed effects for handling method, storage temperature, replicate, and their interactions. Means were compared of each treatment combination within each week using the Tukey HSD procedure. The greatest decreases in egg weight were observed in eggs at 22°C for unwashed, washed, and 21-day stored then washed treatments (P <.0001). Oiled eggs stored at 22°C had comparable egg weight loss to eggs stored at 4°C. Storage temperature had a profound impact on the Haugh units. After one week of storage at 22°C (P <.0001), eggs that were washed, 21-day stored then wash, and unwashed had dropped to USDA-AMS Grade B eggs. Oiled eggs stored at 22°C were able to maintain Grade A through 4 weeks of storage (P <.0001). Haugh unit measurements for 22°C stored eggs were only achievable until 8 weeks of storage. After 27 weeks of 4°C storage, washed and oiled and unwashed eggs were able to maintain Grade A Haugh unit scores (P <0.04). 21-day held then washed eggs and washed eggs at 4°C (P <0.04) retained Grade A Haugh unit scores through 18 weeks. The results show the importance of storage temperature on preserving egg quality.

Keywords: eggs, refrigeration, egg quality, storage

396 Pea protein coating: A strategy to preserve internal egg quality and extend shell life

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The internal quality of eggs is affected by various factors, which become more evident over time during storage. Strategies like coatings have proven effective in extending egg shelf life, with mineral oil being commonly used for this purpose. However, its non-renewable origin raises environmental concerns and limits its application. In this context, protein-based coatings, such as those

formulated with pea protein (PP), have emerged as sustainable and promising alternatives. This study aimed to evaluate the internal quality of eggs stored for 28 days under controlled conditions, coated with PP solutions. A total of 204 red eggs, weighing 55–60 g, were distributed in a completely randomized design with a 4 × 4 factorial arrangement. The factors included four coatings (uncoated as control; coatings with 4%, 8%, and 12% pea protein, referred to as PP4, PP8, and PP12) and storage periods (7, 14, 21, and 28 days), along with a control on day zero. Coating solutions were prepared using concentrated pea protein (Ingredion, SP, Brazil) and glycerol as a plasticizer at a 2:1 ratio (protein:glycerol). Fresh eggs were analyzed on day zero for initial quality, while stored eggs were kept at room temperature (25° ± 2°) and evaluated weekly for weight loss, yolk index (YI), albumen pH (ApH), and yolk pH (YpH). The experiment consisted of 17 treatments, with 12 repetitions each, considering the egg as the experimental unit. Data underwent ANOVA ($\alpha = 0.05$) using the GLIMMIX procedure in SAS software, followed by Tukey's test for mean separation and regression analysis of coating levels on quality parameters. Linear effects of coating were observed for weight loss ($p<0.01$). After 28 days, uncoated eggs lost 7.66% of their initial weight, while PP12-coated eggs lost only 4.16%. Linear effects were also found for YI ($p<0.01$). Fresh eggs had a YI of 0.38 ± 0.03, while, after 28 days, uncoated eggs had a YI of 0.21 and PP12-coated eggs had a YI of 0.29. YpH and ApH showed linear responses to coating type, with lower pH in PP12-coated eggs and higher pH in control eggs. The reduction in dense albumen viscosity is attributed to amino acid chain hydrolysis, which releases water that evaporates or migrates to the yolk, leading to weight loss and reduced YI. Changes in pH result from CO2 loss and modifications in the bicarbonate buffer system, increasing pH over storage. Pea-based coatings were effective in minimizing internal quality losses in eggs during storage, especially at higher concentrations.

Keywords: Protein coating, Storage, Weight loss, Yolk index, Egg quality

397 Cracking the data: Exploring differences in statistical approaches to yolk score analysis

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Consumers continue to advocate for eggs with darker yolks, under the assumption that a darker color equals a healthier hen. When comparing the effects of feed additives on yolk color, yolk scores are the standard tool used throughout the poultry industry. Yolk scores are traditionally assessed on a 1 – 15 scale to measure color intensity based on visual comparisons with standardized color fan guides, then statistically evaluated using ordinal regression, non-parametric tests or ANOVAs, depending on how the yolk score variable is treated. However, there is no agreed upon standard methodology for analyzing yolk score data and disregard for the ordinal nature of the scale. Utilizing a previously collected dataset comparing dietary corn sources (Orange Corn: OC vs. Yellow Corn: YC), we investigated the differences in outcomes based on the statistical methodology employed to analyze yolk scores. The average yolk score of OC was 10.40 ± 0.98 (range: 8-13) and YC was 8.67 ± 1.18 (range: 6 -12). We hypothesized that analyzing data as ordinal, accounting for skewness and a non-normal distribution, would lead to a more accurate analysis than popular ANOVA techniques. Using an ANOVA, we found that OC yolk scores were higher than YC scores ($P < 0.001$). However, we found that the data violated normality and homogeny of variance assumptions necessary to run an ANOVA. When analyzed with a Brunner-Munzel test, we found that there was a similar group difference ($P < 0.001$). The relative effect size indicated that the probability of a randomly chosen YC yolk being darker than an OC yolk was 0.138 (95% CI [0.111, 0.165]), suggesting a strong effect of dietary supplementation. Comparatively, an ordinal logistic regression revealed that diet predicted yolk color scores, ($\chi^2[5] = 375$, $P < 0.001$). Hens fed the OC diet were more likely to have darker yolk color scores compared to the YC group (OR = 22.10, 95% CI [2.73, 3.48]). We investigated the effects of removing unused levels of the scale during analysis and found that keeping those levels led to less accurate odds ratios. Despite all tests observing the same treatment effect, yolk scores should be analyzed ordinally, transforming and zeroing them based on the lowest recorded yolk score or non-parametrically to provide the most accurate results.

Keywords: yolk score, data analysis, ordinal data, assumptions, score data

398 Egg quality of brown layer hens of two ages raised in a floor system in aviaries

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This study aimed to evaluate translucency as an eggshell quality parameter compared to the traditional measures. Translucency is a non-invasive methodology with an LED light source in a dark environment and can be adapted to egg classification automatic systems. A total of 90 eggs from Hy-Line Brown® commercial laying hens raised on the floor from 2 ages, 53 and 73 weeks, and subjected to 3 refrigeration storage periods (10°C) for 1, 5, and 30 days. The assay was conducted in a completely randomized design, 2 × 3 factorial scheme with 6treatments and 15 replicates. The experiment was conducted at the Experimental Aviary of EVZ/UFG. The eggs were obtained from commercial layer farms, identified, weighed using a digital scale (0.01 g). The eggs were then placed in trays in the BlueBox® equipment, photographed, digitized using the Procam® application, and assigned visual scores (1 to 4). Specific gravity was assessed using density solutions from 1.065 to 1.100, in 0.005 gaps. Additional external quality analyses included shell strength (kgf), percentage, and thickness (mm); internal quality parameters analyzed were albumen height, yolk color, Haugh unit, and shape index, assessed using the Nabel EggTester DET 6000®. Egghellswere washed, dried at 55°C, weighed, and the shell percentage was calculated based on egg weight. Data analysis was performed using the R software, and means were compared using Tukey's test ($p<0.05$). Egg weight showed higher values ($P=0.0240$) for eggs from 73-weeks hens, with no effect of storage. Translucency scores increased at 5 and 30 days of storage ($p<0.001$) but no differences between ages were found. Specific gravity decreased over storing time ($P<0.001$) and was higher in 53-weeks compared to 73-weeks hens ($P=0.05$). No differences were observed for shell color and eggshell thickness. Shape index increased at 73-weeks hens compared to 53-weeks ($P=0.03$). Eggs from 53-weeks hens had

greater shell strength values (P=0.003) than 73-weeks hens after 30 days of storage. Shell percentage increased with storage time (P=0.001), showing a rising trend from 1 to 5 and 30 days and the values were increased for 53-weeks hens compared to 73-weeks hens. About internal quality parameters, albumen height (P=0.03), Haugh unit (0.05) and yolk color (p=0.008) were reduced at 30 days of storing time and remained unaffected by hen age. In conclusion, the eggshell and internal quality of brown hens raised in cage-free systems deteriorates with storage time and hen aging.

Keywords: Shell, Storage, Translucency

ELECTRONIC POSTER PRESENTATIONS

Student Competition: Animal Well-Being and Behavior

399P Monitoring the utilization of ramps by laying hens in cage-free environment with deep learning

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Cage-free (CF) housing is generally considered better for laying hen welfare than conventional cage (CC) housing, contributing to a major industry shift in the USA. However, mislaid eggs and keel bone fractures are major challenges in the current CF housing system. Previous studies have shown that adding ramps in aviaries during the laying period can help minimize keel bone fractures. In addition, ramps may facilitate the use of nesting boxes by hens, thereby reducing floor eggs. Therefore, the objectives of our study were to develop a deep learning method for monitoring ramp utilization and potential impact on nesting behaviors in CF facilities. We proposed using two object detection models - YOLOv5u and YOLOv11, each model with five variants, *n* (nano), *s* (small), *m* (medium), large (*l*), and extra-large (*x*), to compare their performance in detecting ramp use. The ramp was made of wooden planks and featured three ladder steps, each spaced 0.15 m apart, resulting in a total length of 0.45 m. The experiment had two treatments: nest boxes with ramp access and nest boxes without ramp access. Each treatment was replicated three times among three separate rooms. The ramp was positioned at a 30-degree angle to the nest box, and ramp use was video recorded at 15 frames per second (fps) for 24 hours a day throughout a study period. About 600 Lohmann LSL Lite were raised from day 1 to 500 in three identical research rooms. Hens were raised following the Lohmann LSL Lite management guide. A total of 5,000 images were randomly selected from converted images from recorded videos at the rate of 15 fps and used for training (70%), validation (20%), and testing (10%). The performance of the YOLOv5u and YOLOv11 models was evaluated using precision, recall, mean average precision (mAP), and F1-score. A one-way ANOVA was conducted to compare the models' performance at a significance level of $P < 0.05$ for ramp use. Results indicated no significant difference in performance metrics between YOLOv5u and YOLOv11. However, the YOLOv5*x*u and YOLOv11*m* variants performed the best when compared among the five variants of each model. Their performance was similar, YOLOv5*x*u achieved an 85% precision, 75% recall, 80% mAP@0.50, 52% mAP@0.50-0.95, and a 75% F1 score, while the YOLOv11*m* variant achieved an 84% precision, 76% recall, 79% mAP@0.50, 51% mAP@0.50-0.95, and 74% F1 score. Our findings provide a baseline for automatic ramp use detection. Additionally, results suggest that the latest YOLOv11 model does not always outperform earlier versions, such as YOLOv5u. Future studies are warranted to investigate the effect of ramp use on nesting behavior and floor eggs.

Keywords: Cage-free housing, layer behavior, ramp, object detection, machine vision

Student Competition: Extension and Instruction

400P Leveraging AI-driven sentiment analysis and natural language processing to inform extension efforts in the poultry industry

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This study aimed to analyze sentiments and classify artificial intelligence (AI)-generated content in social media posts related to the poultry industry, utilizing natural language processing (NLP) techniques to inform targeted extension efforts. Social media platforms, such as X (formerly Twitter) and Reddit, increasingly shape public perception of poultry-related issues, including bird flu and biosecurity. As unverified information spread rapidly on these platforms, it can negatively impact the poultry industry. To address this challenge, there is a need for robust approaches that not only identify public sentiment but also determine whether social media posts are generated by humans or AI, which can help in developing more effective communication strategies. A total of 3,576 tweets and 2,305 Reddit posts were collected via API-based web scraping during January 2025, using 32 poultry-related keywords. Two models—AI-generated content classification and sentiment analysis—were developed using DistilBERT from Hugging Face, leveraging NLP capabilities to enhance model accuracy. The models were fine-tuned on large, labeled datasets, including approximately 1.6 million entries for AI detection and sentiment analysis. Additional training data were sourced from Kaggle and 600 Wikipedia articles on poultry, agriculture, and animal diseases. Performance was evaluated using validation accuracy, precision, and loss. The AI classification model achieved 96% accuracy and precision, with loss decreasing from 0.32 to 0.13, indicating improved model performance as it better aligned with actual outcomes. The sentiment analysis model achieved 92% accuracy and precision, with a reduction in loss from 0.34 to 0.31. Among Reddit posts, 49.4% were negative, 42.2% positive, and 8.4% neutral, while 66.5% were human-generated and 33.5% AI-generated. Among tweets, 61.6% were negative, 22.1% positive, and 16.4% neutral, with 66.8% human-generated and 38.3% AI-generated. High confidence scores (0.81–1.0) dominated both sentiment and AI-human classifications, indicating strong model reliability. These findings underscore the prevalence of negative sentiments and the growing presence of AI-generated content in poultry-related discussions. By integrating these insights into extension materials, stakeholders can enhance public awareness and support science-based decision-making in the poultry industry.

Keywords: artificial intelligence, extension education, natural language processing, sentiment analysis, social media

Student Competition: Genetics and Molecular Biology

401P Elucidate missing CHIR genes on Line-P chickens in previous transcriptomic study using long-read sequencing

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CHIRs (Cluster Homolog of Immunoglobulin-like Receptors) are key immune modulators in chickens. Bioinformatic analysis

revealed over 100 activatory, inhibitory, and bi-functional CHIR genes on chromosome 31 of the broiler (bGalGal1.matbroiler.GRCg7b) and layer (bGalGal1.pat.whiteleghornlayer.GRCg7w) genomes. However, short-read alignments showed reduced mapping on chromosome 31 for Line P, which is more susceptible to Marek's Disease Virus compared to Line N. To investigate, whole-genome sequencing (using PacBio long reads) were performed on two samples each from Lines P and N. Over 9 million raw reads per sample with an average of 8.3 kb read length were sequenced. Reads <5000 bp were filtered with FiltLong (v0.2.1), assemblies were generated via HiCanu (v2.0) and aligned to the references using minimap2 (v2.2-r1209). An average of 193094.75 contigs with an average contig length of 206 kb were generated by HiCanu. The minimap2 output indicated that although Line P had more overall aligned reads, Line N had more alignments (proper, partial, and mismatched) on chromosome 31 in both genome builds. Line P exhibited higher MAPQ scores (81.68 –90.97%) versus Line N (38.64 – 40.3%), largely due to secondary alignments in repeat regions; both lines maintained >99% overall and >96.73% primary mapping. Mean read depths were higher on the layer build (Line N: 2.59, Line P: 2.04). For structural variant (SV) analysis, Sniffles2 (v2.0.2) initially under-called variants, so SVIM (v2.0.0) was used, identifying break-ends, deletions, internal and tandem duplications, insertions, and inversions. Line N showed more deletions/insertions, while Line P had more break-ends. Only Line P showed unique internal duplications, which is completely absent in Line N. After normalization to the number of reads, a higher number of structural variants per million reads were observed on chromosome 31 in Line N than Line P. In summary, Line N's genome may contain more repetitive or structurally rearranged regions, whereas Line P's genome is structurally closer to the reference, yielding more unique alignments. Additional analysis reveals that the differences in mapping quality and structural variant profiles not only emphasize the CHIR gene family's complexity, but also suggest implications for immune response variability in chickens. The increased secondary alignments and unique genomic features in Line N versus more stable pattern in Line P offer a compelling avenue for further research. These findings may inform future breeding and disease resistance strategies, ultimately contributing to enhanced poultry health management.

Keywords: Bioinformatics, CHIR genes, Long Read Sequencing, Chicken

402P Novel computed tomography (CT) – derived traits in purebred turkeys (Meleagris gallopavo)

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As demand for affordable poultry meat has increased, turkey lines have been more intensively selected for faster growth and higher body weight. This selection strategy has been accompanied by adverse health and welfare challenges, such as bone abnormalities and lameness. Incorporating leg health traits in breeding programs could benefit bird health, production sustainability, and profitability. Leg health has traditionally been assessed using subjective walking scores (WS), which are labor-intensive, time-consuming, and can be inconsistent. Non-invasive techniques such as computed tomography (CT) scanning have been successfully used to estimate body weight (BW, kg) and breast meat yield (BMY, %) in live birds and could be used to identify and characterize new leg health traits. This study focused on defining and evaluating a new trait, tibiotarsus cortical fraction (CF), with the goal of estimating genetic parameters for CF and examining phenotypic and genotypic correlations with BW, BMY, and WS. CF is defined as the percentage of dense bone pixels (cortical bone) in relation to total pixels comprising the tibiotarsus, making it a potential indicator of cortical bone and tibiotarsus strength in live birds. The dataset consisted of scans of 4,682 toms from line A and 5,304 toms from line B, scanned between 2022 and 2024. At 20 weeks of age, BW and WS (scored from 1 = clear abnormalities to 6 = no abnormalities) were recorded, and CT scans were performed to estimate BMY and CF. A subset of 15 randomly selected birds were further analyzed. A total of 75 image slices, with five slices from each of the 15 birds, were manually annotated using Labelme software to mark the area of tibiotarsus bone in both legs. Panoptic segmentation models were trained on 64 of these slices and further refined using Detectron2 in Python v3.11. The model was then tested on the remaining 11 slices, yielding preliminary mean CF values of 52.16% for the right leg and 52.24% for the left leg, with confidence levels of 83% and 76%, respectively. Preliminary analyses showed that line B birds were, on average, 4.5 kg heavier and had a 1.2% higher BMY than line A, while both lines had an average WS of 2.0. Next steps involve characterizing CF for all scanned individuals, analysing CF, BW, BMY, and WS in a multivariate model using ASReml v4.2, and estimating genetic parameters of these traits. This work provides an initial report on incorporating novel leg health indicators to breed for increasingly sustainable turkey populations.

Keywords: artificial intelligence, leg health, meat poultry

403P Effects of *in ovo* docosahexaenoic acid supplementation on liver and ileum gene expression profiles in broiler chicken embryo

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Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid that contributes to growth, oxidative stability, immunity, and metabolism in poultry. This study aimed to investigate the impact of *in ovo* DHA supplementation on gene expression in the livers and ileum of broiler chicken embryos. A total of 480 Ross 308 broiler fertilized eggs were injected with 98% DHA solution into the yolk sac on embryonic day (ED) 11 and divided into three groups: non-injected control (CON), 50 µl DHA (DHA50), and 100 µl DHA (DHA100). Liver and ileum samples were collected from all groups on ED19. Total RNA was extracted using TRIzol, and cDNA was synthesized for quantitative PCR analysis. Gene expression was normalized with the housekeeping gene GAPDH. Data was checked for normality, log-transformed as necessary, and analyzed using One-way ANOVA with Tukey HSD test in JMP® Pro 17. Spearman's test was performed for correlation analysis,

with significance set at $p \leq 0.05$. The results showed no significant differences in the liver for the different gene expressions (*Nrf2, SOD1, SOD2, FBP1, IGF1, IGF1R, PC, PCKC*). There were no significant differences in the ileum for the immune-related genes (*AVBD4, CD3, CD56, ChB6, IL-1β, IL-4, IL-6, IL-10*), antioxidant genes (*Nrf2, SOD2*), and tight junction genes *(JAM2, TJP, OCLN, CLDN, Cadherin*). However, the DHA100 group exhibited significantly higher expression for *ZO1* in the ileum compared to the control group ($p = 0.0322$). There were positive correlations between *Nrf2* and *IL-6* ($r^2 = 0.66$, $p = 0.0202$), *IL-10* ($r^2 = 0.66$, $p = 0.0185$), *IL-1β* ($r^2 = 0.63$, $p = 0.0283$), *SOD1* ($r^2 = 0.90$, $p < 0.0001$), and *SOD2* ($r^2 = 0.77$, $p = 0.0034$) in ileum. Similarly, a significant correlation was found between *SOD1* and *IL-10* ($r^2 = 0.62$, $p = 0.0307$). In conclusion, *in ovo* supplementation of 100 µl DHA may enhance intestinal barrier function, while antioxidant and immune-related genes appear to be interconnected in response to oxidative stress in broiler chicken embryos.

Keywords: Docosahexaenoic acid, in ovo, gene expression, gut barrier, broiler chicken

Student Competition: Management and Production

404P The effect of blue or white LED lighting, compared to darkness, during simulated chick transport on the welfare and productivity of broilers

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The effects of implementing monochromatic LED lighting in chick transport vehicles have not been investigated. Shorter wavelengths (blue/green) can reduce fear and stress in broilers during rearing. This study was a 2x3 factorial design to examine if monochromatic light could reduce stress during simulated chick transport and improve productivity and welfare during rearing. As-hatched Ross 308 chicks (n=5,670) were placed in commercial transport crates and loaded onto vibration platforms (to simulate road transport conditions) for a duration (**D**) of 4 or 7h. Lighting treatments (**L**) were blue (peak = 456nm), white, or dark. Prior to simulated transport, chicks were put into crates at commercial stocking density and weighed on a crate basis. Immediately following transport at 0d, body weight (**BW**), gastrointestinal tract weight and length, yolk-sac weight, and blood gases were recorded (n=30 chicks/trt). For the first week of life (0-7d) BW, feed consumption (**FC**), and fear (via novel object test (**NOT**)) were assessed daily. At 14d and 35d BW and FC were recorded again. BW and FC values were used to calculate gain:feed (**G:F**). NOT was repeated at 16, 23, and 30d. Blood was collected at 8 and 32d to assess stress using the heterophil:lymphocyte (**H:L**) ratio. Tonic immobility, a measure of fear, was tested at 1, 4, and 31d. All data were analyzed using SAS 9.4. A two-way factorial ANOVA was used to determine if treatments impacted the above parameters (significance declared when $P \leq 0.05$). Tukey's test was used for mean separation. L had no effect on BW, FC, gastrointestinal tract weight or length, yolk sac weight, or blood gases. However, D had an effect as 4h chicks had increased BW ($P = <0.01$) and BW gain from 0-14d ($P = 0.01$). Additionally, FC was higher from 0-35d ($P = 0.01$). There were no L or LxD effects on G:F. However, D had an effect, with the 4h treatment having lower G:F from 0-35d ($P = 0.01$). An interaction existed for infectious mortality, with the 4h white treatment having the lowest mortality while the 7h treatment had the highest ($P = 0.03$). There was no impact of main effects on mortality. Birds in the 4h treatment had longer ileums ($P = 0.05$), lighter ceca ($P = 0.01$), reduced partial pressure of carbon dioxide ($P = 0.03$), sodium ($P = 0.01$) and increased plasma glucose ($P = 0.01$). L had no effect on H:L ratio; however, H:L ratios were increased in the 4h group at 32d ($P = 0.04$). L and D had no effect on NOT or tonic immobility. Overall, D appeared to influence performance whereas L did not. No treatments influenced stress or fear. Therefore, these data do not support changing from the current industry practice of transporting chicks from hatchery to rearing barn under dark conditions.

Keywords: broiler chick, wavelength, stress, fear, performance

405P Characterizing Non-Infectious Brooder Mortality: Age of Onset and Patterns in Commercial Turkey Flocks

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Mortality in the brooder can have flock long effects on the overall performance; however, ranges of common causes of non-infectious mortality are not well documented. Therefore, the objective of this project was to assess average age of onset and associated mortality percent of common non-infectious mortality with the intent to identify commercial standards. To complete this objective, 3 flocks of 3,072 male poults per flock from single hatchery were placed across 32 pens at commercial stocking densities throughout the year. Flocks were reared using commercial standards. Feed and water were provided *ad libitum.* Daily mortality and mortality cause were determined and recorded from 0-35d for each flock. Mortality were categorized as either: flip/tip over poults (TF), starve out poults (STO), spontaneous cardiomyopathy (RH), enteric disease (ED), leg issues (e.g. injured leg, slipped tendon), infections (INF), cannibalism (CAN), dehydration (DH), pendulous crop (PC), yolk rupture (YR), unknown lesions (NVL), and other (e.g. unable to reach water, accidental death). Data was analyzed in R for age of onset using the lmer package and frequency of onset using the chi.squared package. Age and morality cause were fit as fixed effects with flock fit as a random effect. Pairwise comparisons were determined using the emm package in R. Onset of morality occurred at discrete ages of the poults depending on mortality cause ($P < 0.001$). Mortality causes clustered into early morality, late mortality, and those that fell outside of these categories. Early mortality was associated with TF, STO, and YR ($P < 0.05$) with the average onset for TF at d5.3, STO at d4.8, and YR at d2.2. Late morality was associated with ED, leg issue, NVL, and RH ($P < 0.05$) with the average onset for ED at d17.2, leg issues at d15.2, NVL at d19.8, and RH at d20.3. INF, CAN, DH, and other mortality were more generally spread out during the brooder phase with the average onset for INF at d23.0, for CAN at d28.6, for DH at d7.5 and other at d17.0. Average mortality of the 3 flocks were 3.8%, 2.8%, and 3.9%. The frequency of mortality causes was different by mortality category ($P < 0.01$). Of the mortality causes, SO, RH and DH had the highest morality percent with 16.6%, 14.1%, and 11.9%, respectively. DC, CAN, and OTHER ranged between 10.0% - 10.7% and all other mortality percentages less than 6.6%. In conclusion, this research will allow growers assess their brooder flocks for abnormal mortalities and begin to identify ways to address these issues. However, additional work is needed to expand this data set to include more flocks and grower data.

Keywords: poult, non-infectious, brooder, mortality

406P Growth performance and heat stress in broiler birds reared in an environmentally controlled house and on pasture
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Pasture-raised broiler production is gaining popularity due to the consumers' increasing preference for meat perceived as more natural, nutritionally superior, environmentally friendly and ethically produced. However, the studies on difference between nutrient requirements, growth performance and feed efficiency of pasture raised broilers are limited. This study investigated the growth performance, and the expression of heat stress gene (HSP90) of broilers raised in environment-controlled houses and on a pasture. A total of 180 broiler birds (Cobb 500 males) were initially reared from d 0 to 21 on a standard starter diet in an environmentally controlled housing. Then, 100 birds (555.65 ± 25 g) were randomly selected and distributed into two groups: controlled housing environment (T1) (5 replicates, 10 birds/pen) and pasture (T2) (5 replicates, 10 birds/pen unit) and reared from d 22 to 35 on a grower diet. The average temperature, wind speed, and humidity were 63 F, 4 MPH, and 83% at 5 am;76 F, 6 MPH, and 55% at 11 am; 79 F, 6 MPH, and 50% at 5 pm; 69 F, 4 MPH, and 67% at 11 pm respectively in T2, throughout the grow-out period. The body weight was recorded on d 28 and d 35 and a t-test was performed for the group differences at $P \leq 0.05$. The breast muscle and intestinal mucosa were collected on d 35 for the analysis of heat stress gene expression (HSP90). In overall performance, the T1 group exhibited higher BWG (P=0.076), lower FCR (P=0.001), and lower FI (P=0.038) compared to the birds of pasture. However, there was no difference between heat stress gene expression (HSP90) between treatment groups. In conclusion, birds reared in controlled housing environment showed better BWG by 20.6% and lower FCR by 0.63 points compared to the pasture raised broilers when on a same nutrient density diet.

Keywords: pasture-raised broiler, cage broiler, heat stress, HSP90

Student Competition: Physiology and Reproduction

407P Egg yolk metabolites as potential indicators of quality
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Egg quality is crucial for hatchability, and although several factors can interfere with this quality, the influence of yolk compounds is poorly studied. This study aimed to evaluate the influence of yolk compounds on shell quality. Hatchable eggs from 5 flocks of Lohmann LSL Lite light breeders, aged 52 to 64 weeks, were used. A total of 99 eggs underwent the specific gravity test based on Archimedes principle. Eggs were classified into low (between 1064 and 1069g/L) and high specific gravity groups (between 1079 and 1090g/L), with 44 eggs in the low and 55 eggs in the high specific gravity groups. Metabolite extraction was followed by analysis using high-performance liquid chromatography coupled with a high-resolution mass spectrometer with electrospray ionization. After data alignment, normalization, and filtering, multivariate analysis was performed using Hotelling's T2 test. Afterward, we performed the PCA (Principal Component Analysis) and logistic regression analysis. The results revealed that Melatonin Glucuronide was more abundant in eggs with low specific gravity, with a mean intensity of 14.96 in eggs with low specific gravity and 10.68 in eggs with high specific gravity ($p < 0.05$). Dihydroxytetradecanoic Acid was more abundant in eggs with high specific gravity with a mean intensity of 15.42 in eggs with low specific gravity and 18,9 in eggs with high specific gravity ($p < 0.05$). Although we observed a difference in the mean abundance of these metabolites in eggs with high or low specific gravity, the PCA analysis did not reveal a clear separation between the groups. Furthermore, the logistic regression showed a low area under the curve (AUC), suggesting that Melatonin Glucuronide and Dihydroxytetradecanoic Acid alone are not sufficient to discriminate the groups. This is likely due to the influence of other variables on the results of specific gravity. Melatonin Glucuronide, a metabolite formed during melatonin metabolism, is present in the yolk and it is a powerful antioxidant. Studies suggest that melatonin supplementation in laying hens increases bone strength, although it reduces shell thickness. Therefore, the increased Melatonin Glucuronide in yolks of eggs with low specific gravity might suggest it affects calcium allocation, prioritizing bone health over thicker shells. Dihydroxytetradecanoic Acid, a long-chain fatty acid produced by beneficial bacteria in the gastrointestinal tract, has anti-inflammatory and antibiotic properties. Its higher presence in eggs with high specific gravity may suggest an association between egg quality and microbiota composition. This study indicates that Melatonin Glucuronide and Dihydroxytetradecanoic Acid in the yolk may influence egg shell quality.

Keywords: egg quality, metabolites, metabolomics, Melatonin Glucuronide, Dihydroxytetradecanoic Acid

408P Analysis of microbial diversity and metabolic pathways in the chorioallantoic membrane and intestine during the embryonic development
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The chorioallantoic membrane (**CAM**) in chickens is a vascularized structure that plays a crucial role in the growth and development of chick embryos by facilitating gas exchange, nutrient transport, and waste disposal. Understanding microbial diversity and the immune status of the CAM can offer valuable insights into the intestinal microbiome and post-hatch growth performances. This study aimed to 1) investigate the microbial diversity and metabolic pathways in the CAM and the whole intestine and 2) examine the effects of embryonic thermal manipulation (**TM**) on microbial diversity and metabolic pathways within the CAM and the intestine. Fertile Cobb 500 eggs (n = 600) were obtained from a local hatchery and were incubated at a standard temperature of 37.5°C with 55% relative humidity (**RH**) until embryonic day (**ED**) 11. On ED 12, eggs were allocated into two groups: 1) Control (n = 236) maintained at 37.5°C and 55% RH for 24 h/d until hatch day (ED 21), and 2) TM group (n = 238) subjected to 38.5°C and 55% RH for 12 h/d from ED 12 to ED 18,

followed by standard temperature from ED 19 to ED 21 in two incubators with automatic temperature control, 55% RH, and egg rotation (every 2 h). DNA was extracted from CAM and whole intestinal samples from ED 15 and ED 18, followed by the sequencing of the 16S rRNA gene. CLC Genomics Workbench 25.0.1 was utilized for bioinformatics analysis of microbiomes. Two novel bacterial genera, *Phenylobacterium* (detoxification) and *Limnobacter* (gaseous exchange), were identified in CAM. Three novel bacterial genera, *Phyllobacterium* (involved in nutrient cycling), Flavobacterium (promoting growth and mitigating stress), and Rubelimicrobium (influencing biochemical pathways), were identified in the intestine. *Stenotrophomonas*, *Achromonobacter*, *Hypomicrobium*, *Sphingobium*, and *Lactobacillus* bacterial genera overlapped in CAM and intestines. TM significantly increased CAM and intestines' microbial diversity (alpha and beta diversity) and metabolic microbial pathways. In conclusion, we present new evidence demonstrating that there is limited vertical transmission of microbiomes through the CAM to the intestine, along with a characterization of the CAM microbiome. The findings indicate that TM has a significant impact on microbial diversity and the metabolic pathways associated with both the CAM and intestinal microbiomes.

Keywords: Bacterial abundance, Gut microbiome, Incubation, Microbiome profiling

409P RNA sequencing-based analysis of fertility-related markers in the oviduct of broiler breeder hens

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The reproductive performance of broiler breeders is crucial in supplying fertile eggs for day-old broiler chicks. Hatchability rates in broiler breeders have recently declined compared to previous years. Hatchability is influenced by several factors, including the quality of the eggs, the fertilization potentials of ova and sperm, and the age of the hens. This study aimed to identify fertility-related genes and pathways in the uterus and magnum of the broiler breeder hens' oviduct. The uterus and magnum tissues were collected from two age groups (n=30/group) of broiler breeder hens: the peak production (35 weeks of age, referred to as "young") and the declined production phase (50 weeks of age, referred to as "aged"). The time of ovulation was predicted based on the presence of an egg in the oviduct. Total RNA was isolated from the uterus (10 to 16 hours post-ovulation) and the magnum (2 to 3 hours post-ovulation), and then subjected to RNA sequencing (n=6 per group). Additionally, selected genes from the RNA sequencing were validated using real-time qPCR. The data analysis, using the DESeq-2 package in RStudio, revealed 8 fertility-related differentially expressed genes (DEGs) in the uterus (Young vs. Aged) and 8 DEGs in the magnum (Young vs. Aged). The upregulation of the *DMRTB1* (doublesex- and mab-3-related transcription factor B1) in young hens suggests its role in chemotaxis and the nurturing of post-stored sperm in the uterus. Furthermore, *PKD2* (polycystic kidney disease) has been linked to azoospermia in mammals, and its higher expression in aged uterine tissue may contribute to lowered sperm motility. Similarly, the upregulation of *CATSPER4* (cation channel sperm associated 4) in the magnum was identified in avian species for the first time, and it is suggested to have a role in sperm motility and fertilization in breeder hens. The *HERC4* (a member of the ligase sub-family of proteins with HECT and RCC1-like domains) was also upregulated in magnum, which may relate to the structural alignment of sperm for improved motility. The results suggest that the *CATSPER4* and *HERC4* in the magnum and *DMRTB1* and *PKD2* in the uterus can serve as fertility markers to select broiler breeder hens with higher fecundity.

Keywords: Azoospermia, CATSPER4, magnum, uterus, sperm nursing

410P Age-related changes in the transcriptomic profile related to ovarian follicle recruitment in commercial laying hens

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Egg production efficiency in poultry relies on the number of ovarian follicles selected for ovulation or atresia, as well as the oviduct's ability to transform the follicle into a calcified eggshell. Therefore, developing a well-organized follicular hierarchy is essential for improving egg production efficiency. Hens experience a gradual decline in reproductive efficiency as they age, similar to mammals. However, the molecular mechanisms responsible for the decrease in small white follicles with age remain poorly understood. This study aimed to identify genes and biological pathways regulating the recruitment of small white follicles across different production cycles. Hy-line W-36 laying hens were obtained from a commercial poultry farm in three different age groups (n = 15/group): 37 weeks (peak laying phase), 67 weeks (intermediate laying phase), and 87 weeks (late laying phase). Small white follicles (n = 15/group) were collected for transcriptomic analysis. Total RNA was isolated from small white follicles (n = 5/group) and analyzed using RNA sequencing and qPCR. The RNA-seq data analysis was performed to identify differentially expressed genes (DEGs), with a log2 fold change ≥ 2 for upregulated genes and ≤ -2 for downregulated genes (p-value < 0.05). This analysis revealed 118 DEGs in 67W vs. 37W and 180 DEGs in 87W vs. 37W. Key downregulated genes in 67W vs. 37W include *ATP6V1C1* and *ATP6V0E1* (involved in receptor-mediated endocytosis), COX7C (regulates mitochondrial function and oxidative stress), and *CIT* (involved in cell division and proliferation). In contrast, *ITPR2 (*which facilitates cellular senescence by enhancing calcium transport to mitochondria) was upregulated in the 67W age group. Key downregulated genes in 87W vs. 37W are *BCL9* (a co-activator in the Wnt/β-catenin signaling pathway), *CLEC16A* (involved in autophagy and mitophagy), and *MTOR* (involved in energy homeostasis, cell growth, proliferation, and mitochondrial function). Inhibitory pathways related to follicular recruitment and aging include estrogen receptor signaling, synaptogenesis signaling, and serotonin receptor signaling, particularly between 87W vs. 37W. Mitochondrial dysfunction was observed in both groups, and the CLEAR signaling pathway was activated in both groups. This study reveals that mitochondrial dysfunction, hormone signaling pathways, and apoptosis significantly influence the reduction of ovarian follicular recruitment and egg production in aging hens.

This knowledge helps develop strategies to improve egg production efficiency in older hens.

Keywords: follicles, follicular hierarchy, recruitment, RNA sequencing, differentially expressed genes

Student Competition: Immunology, Health and Disease: Feed Additives

411P Impact of low-quality soybean meal and live coccidiosis vaccination on the cecal microbiome composition of broiler chickens

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Soybean meal (SBM) accounts for the second most abundant feed ingredient in poultry feed and derives from a variety of sources with varying quality levels. SBM quality has the potential to impact the gut microbiome due to the altered nutrient levels and bioavailability of nutrients. The microbiome is well established to be beneficial in maintaining homeostasis and contributing to the overall health in broiler chickens. The objective of the present study was to compare the effects of dietary SBM quality and live coccidiosis vaccine cycling on the broiler chicken cecal microbiome. At day of hatch (DOH), n=480 chicks (n=12 pens/group, 20 chicks per pen) were randomly assigned to either: Group 1) high-quality (HQ) SBM formulated to meet or exceed requirements or Group 2) low-quality (LQ) SBM designed to induce inflammation. The LQ SBM was produced using a 1:1:1 mixture of uncooked SBM, normal SBM, and over-processed SBM (autoclaved ~125C for 60 minutes). Chicks were orally gavaged with a 1X dose (0.25mL/chick) of a commercial live coccidiosis vaccine containing *E. acervulina*, *E. maxima,* and *E. tenella* oocysts at DOH. On d15, 28 and 42, n=8 chickens from each treatment were culled to obtain cecal content samples for microbiome evaluation. Genomic DNA was extracted and 16S rRNA V4 regions were amplified for sequencing. Alpha diversity was discerned at amplicon sequence variant (ASV) level by leveraging observed richness, Shannon diversity, Pielou's evenness, and Faith's phylogenetic diversity (Faith's PD). For alpha diversity, normality was assessed using the Shapiro-Wilk test and homogeneity of variance using Levene's test. For beta diversity, reads were normalized using cumulative sum scaling (CSS) prior to all downstream analyses. Beta diversity was assessed at ASV level using the Bray-Curtis dissimilarity, Weighted UniFrac, and Unweighted UniFrac distance metrics. To evaluate community-level differences attributed to treatment, time, and treatment-time interaction, PERMANOVA was performed with 9,999 permutations, followed by beta dispersion analysis to assess homogeneity of variances. There was a significant increase in alpha diversity within each treatment group between d15 and d42 according to Faith's PD (P=0.00) and Observed Richness. Consistent with alpha diversity, time had the greatest effect on compositional dissimilarity (Bray-Curtis and Unweighted UniFrac P=0.0004), with treatment also demonstrating a significant effect (Bray-Curtis, P=0.009). Interestingly, *Clostridiaceae* was enriched in the HQ SBM group at d15 compared to the LQ SBM group. Further investigation is needed to evaluate the relationship between SBM quality and the intestinal microbiome in broiler chickens.

Keywords: Soybean Meal, Microbiome, Gut Health, Broiler, Chicken

412P Feeding dietary black currant powder to turkey hen poults

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Producing a healthy poultry flock can be challenging today, making immune stimulation and modulation increasingly necessary in the effort against stress, pathogens and diseases. Several studies using alternative ingredients such as plant derivatives have shown variable results and efficiency. Black currant berries are being explored in poultry nutrition due to their potential health benefits such as improving immune function, reducing oxidative stress, enhancing egg production and supporting overall health. The objective of this study was to determine the effect of feeding black currant powder (BCP) at different dietary inclusion levels to turkey hen poults. LW turkey hens (432) were reared to 5 wk in 72 battery cages (6 birds/cage, 6 blocks, 12 cages/block). Diets contained increasing levels of BCP (0, 0.25, 0.5, 1, 2, and 4%). Data collection included production parameters: individual BW at placement and weekly to 5 wk and feed consumption by cage of birds. At 5 wk, one bird per cage was euthanized to collect liver and jejunum samples for gene expression and histology analysis. Feed and fecal samples were also collected for apparent metabolizable energy corrected for nitrogen (AMEn). FCR was calculated by pen. Statistical analysis was by JMP 18 and data were analyzed using the linear and quadratic regression, a significant difference was considered with a p value of $\leq$ 0.05. BW increased linearly due to BCP at 1 (p=0.01), 2 (p=0.001), 3 (p=0.03), 4 (p=0.02) and 5 (p=0.04) wk indicating that body weight increased as BCP inclusion rates increased. Similarly, FI increased at at 1 (p=0.004), 2 (p=0.01), 3 (0.02), 4 (p=0.02) and 5 (p=0.009) wk due to increased BCP inclusion. FCR was not different due to treatment. No linear or quadratic relationships were found due to BCP inclusion for gene expression (CAT, LITAF, HSP70, SOD, or GPX). There was no significant difference found for the histology in the jejunum. Dietary AMEn decreased (p=0.006) with increased inclusion of BCP. Overall, feeding black currant powder to turkeys reared under optimal conditions resulted in performance differences. However, measured gene expressions were not affected. Further research feeding black currant powder to turkeys reared under less-than-optimal conditions is warranted.

Keywords: antioxidant, blackcurrant, turkeys, performance, AMEn

413P Evaluating the impact of spraying an Enterococcus faecium probiotic at hatch and supplementing feed with a Triple-Strain Bacillus-based additive on BCO Lameness incidence in broiler chickens

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Bacterial chondronecrosis with osteomyelitis (BCO) lameness is a major animal health and welfare issue in the broiler industry worldwide. It is estimated that 1–2% of birds are condemned at market age due to BCO lameness, resulting in tens of millions of dollars in annual losses. This study aimed to evaluate the efficacy of a probiotic program incorporating an Enterococcus faecium-

based probiotic (E. faecium 669 applied as a gel spray to day-old chicks at 2 × 10^9 CFU/bird) and a triple-strain Bacillus-based feed supplement (B. subtilis 597, B. subtilis 600, and B. amyloliquefaciens 516, administered at 500 g/t of feed from day 1 to day 56), either individually or in combination, for reducing BCO lameness in broiler chickens. The study utilized two wire-flooring pens (T1) as the source of infection, simulating aerosol transmission of bacteria typical in commercial poultry houses. A negative control group (T2) received only standard feed, while the remaining birds were assigned to three treatment groups: T3 (six pens receiving the E. faecium-based probiotic as a spray to day-old chicks), T4 (six pens receiving the triple-strain Bacillus-based product in the feed continuously from day 1 to day 56), and T5 (six pens receiving both probiotic treatments). A total of 1,560 mixed-sex, fast-growing broiler chicks (Cobb 500) were randomly distributed across treatments. Cumulative lameness incidence was monitored from day 21 to day 56 through daily evaluations and necropsies. Data were analyzed using ANOVA with Tukey's HSD ($p < 0.05$). The positive control (PC) group exhibited the highest cumulative lameness incidence, exceeding 70% by the end of the trial, confirming the effectiveness of the lameness induction model. The negative control (NC) group had the second-highest lameness incidence (~50% from day 40 to day 56), highlighting the role of environmental transmission in disease progression. By day 56, probiotic supplementation significantly reduced cumulative lameness incidence compared to the NC group. Specifically, lameness incidence was reduced by 35.4% in T3, 36.7% in T4, and 47.6% in T5 ($p < 0.05$). Notably, the greatest reduction was observed in T5, suggesting a potential synergistic effect when E. faecium and the triple-strain Bacillus probiotic were administered together. These findings demonstrate the effectiveness of targeted probiotic supplementation in mitigating BCO lameness and highlight a promising strategy for improving skeletal health in broiler chickens.

Keywords: Lameness, Broilers, BCO, Probiotic, Bone

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

414P A machine vision system for detection and classification of poultry manure and disease evaluation

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The poultry industry is essential for the global supply of animal-based protein, but disease outbreaks threaten the productivity, animal health, welfare, and food safety. The traditional disease detection methods rely on manual observation of signs of diseases, which is time consuming and restricting the availability of veterinarian due to farm biosecurity management, and thus an automated monitoring system is required for that task. Previous studies applying deep learning to classify disease prevalence using fecal images have primarily focused on whole-image analysis, which may reduce classification accuracy due to background noise and irrelevant features. To address this limitation, we developed a two-stage deep learning approach that combines object detection and classification for fecal-based disease monitoring. We used an open-source polymerase chain reaction-annotated dataset consisting of 6,812 fecal images from commercial poultry farms consisting of broilers, cross-bred, and indigenous chickens. The dataset was categorized into four classes: Healthy (2,057 images), Coccidiosis (2,103 images), Salmonellosis (2,276 images), and Newcastle disease (376 images). Five different YOLO11 object detection models – YOLO11n, YOLO11s, YOLO11m, YOLO11x, and YOLO111 – were trained to locate feces on various surfaces with a dataset split of 70:15:15 for training, testing and validation, respectively. EfficientNet-b0 and EfficientNet-b1 deep learning architectures were trained on augmented datasets for disease classification. Performance metrics – precision, recall, and mean average precision (mAP) – were calculated for the developed detection models. For the classification model, accuracy wsas used as a performance metric. Out of five YOLO11 models, YOLO11n had the highest mAP of 87% for all disease classes and was selected as the best detection model for the integrated system. Out of two EfficientNet models, EfficientNet-b0 had the highest overall accuracy of 99.12%, hence was selected as a classifier for the system. To improve usability, a Streamlit-based web application was developed and deployed, allowing users to upload images for real-time analysis using fecal images. The results demonstrate that deep learning can be applied to monitor poultry disease automatically based on fecal characteristics, which provides a potential solution for early disease detection in poultry farms. Future works include integrating such monitoring systems into surveillance platforms and real-time visual monitoring systems, to enhance disease monitoring and prevention strategies.

Keywords: disease detection, fecal characteristics, machine vision, poultry production, precision farming

415P Plasma proteomic profiling reveals distinct signatures between resistant and susceptible chickens infected with highly pathogenic avian influenza virus

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Avian influenza (AI) is a viral disease with economic consequences for the poultry industry. While most chickens infected with highly pathogenic AI viruses (HPAIV) develop a lethal disease in a few days, a percentage of birds can survive the infection, illustrating a complex process that involves intricate interactions between the virus and the host's immune response. Here, we aim to investigate plasma proteomic signatures early after the challenge associated with the outcome of H7N1 HPAIV infection. Chickens were inoculated with 10^5 mean egg lethal infectious doses (ELD_{50}) of H7N1 HPAIV via intra-nasal route. Plasma samples were collected and analyzed from non-infected controls (n=9) before challenge, and at 8 (n=18), 24 (n=18), and 48 (n=15) hours post-inoculation (hpi) from inoculated birds. HPAIV-susceptible chickens were classified based on mortality, clinical signs, viral shedding, histopathological lesions, and immunohistochemistry results. Birds that did not meet any of these criteria were categorized as resistant. A total of 21 sample pools were generated by combining two or three plasma samples from each group (HPAIV-resistant, HPAIV-susceptible, and controls), with three pools analyzed at each timepoint. Samples were processed by LC-MS, and the resulting peptides were analyzed

using a library-free strategy with DIA-NN. No significant differences between resistant and susceptible birds were found at 8 and 24 hpi. Most differentially expressed proteins were identified in susceptible chickens compared to controls at 48 hpi, coinciding with the onset of clinical signs. Enrichment analysis of downregulated proteins in susceptible chickens compared to controls and resistant birds revealed pathways associated with apoptosis and the acute phase response, while upregulated proteins were linked to focal adhesion and ECM-receptor interaction. Furthermore, at 48 hpi, proteins such as ARF4, EIF4A2, and HNRNPAB were upregulated in resistant birds but downregulated in susceptible birds compared to the control group. ARF4, associated with endocytosis processes, may play a role in modulating viral entry, while HNRNPAN, involved in gene expression regulation, could contribute to a more robust antiviral response. EIF4A2, a component of the eukaryotic translation initiation complex, may enhance the efficiency of protein synthesis, potentially supporting the production of antiviral molecules. These findings suggest that resistance to HPAIV may be mediated by enhanced regulation of endocytosis, gene expression, and protein synthesis, which could collectively limit viral replication. This highlights the importance of host genetic factors in determining the clinical outcome after HPAIV infection in chickens.

Keywords: proteomics, Avian influenza, chicken, Resistance, clinical outcome

416P Phytogenic supplementation modulates performance and pathological responses of turkey poults to histomoniasis induced via lateral transmission of *Histomonas meleagridis* in battery cages

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Histomoniasis, caused by *Histomonas meleagridis*, is a protozoal poultry disease that severely damages the ceca and liver resulting in high mortality and major economic losses especially in the turkey industry. A 35-day (**d**) experiment was conducted to evaluate the effects of a phytogenic water supplement on performance and pathological variables of turkey poults during histomoniasis when raised in battery cages. Day-old female poults (n=84; 42 birds/group with 6 replicate cages each) were assigned to two treatment groups: 1) challenge group with tap water (**CTRL**) and 2) CTRL with phytogenic water supplement at 200 ppm (**PHYT**). On d 14, three poults/cage were individually wing-tagged and directly inoculated (**DI**) intracloacally with ca. 150,000 histomonads. The remaining birds were designated as non-directly inoculated (**NDI**) via lateral transmission of the parasites. Birds and feed were weighed on a weekly basis to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**), and mortality was recorded throughout the experimental period. On d 28, histomoniasis lesions in the ceca and liver were scored (0 to 4 scale) from 2 poults/cage (1 DI and 1 NDI) of the CTRL and PHYT birds. On d 35, all the remaining birds were scored for histomoniasis lesions in the ceca and liver. Performance and mortality data were analyzed using *t*-test and lesion scores data using Chi-squared test (JMP Pro 18) with significance at $P \leq 0.05$. The PHYT group showed greater ADG during the pre-challenge period (d 0–14) and reduced FCR during d 0–14 and d 28–35 compared to the CTRL group. On d 28, the PHYT group had a lower percentage of DI poults with severe lesions (score 4) in the ceca (67%) and liver (50%) compared to the CTRL group (ceca: 100%, liver: 80%). Among NDI poults, 17% in the PHYT group had mild cecal lesions (score 1) with no liver lesions, whereas 17% of NDI poults in the CTRL group exhibited severe lesions (score 4) in both ceca and liver. On d 35, the CTRL DI group had greater lesion severity with 100% of poults exhibiting severe cecal lesions (score 4) and moderate liver lesions (score 2). In contrast, in the PHYT group, only 29% of DI poults exhibited moderate cecal lesions (score 2), and none of the DI birds exhibited liver lesions (score 0). During d 14–35, PHYT poults had lower mortality rate (12.3%) compared to the CTRL group (29.4%). The phytogenic water supplementation alleviated pathological effects of histomoniasis by reducing lesions, mortality rate, and improved performance by reducing FCR during the challenge period. Therefore, this phytogenic blend demonstrated protective effects in turkey poults under this lateral transmission model of *H. meleagridis* challenge.

Keywords: histomoniasis, turkey, phytogenic, Histomonas meleagridis, lateral transmission

417P Transcriptional changes in mucosal and lymphoid immune tissue of broiler chickens during experimental necrotic enteritis

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In the current era of growing poultry without antibiotics, Necrotic enteritis (NE) in broiler flocks is negatively impacting the bird health and economy. NE is caused by Clostridium perfringens which are spore-forming, toxin-producing anaerobic bacteria. Several predisposing factors, including Coccidia infection and high-protein diet, have been implicated in disease pathogenesis. NE control via non-antibiotic means is becoming increasingly challenging due to a poor understanding of immune responses against C. perfringens during NE. Therefore, the objective of this study included reproducing NE in broiler chickens using two experimental models to evaluate host immune responses in intestinal mucosal as well as lymphoid tissues during NE in broiler chickens. The two NE predisposing models, namely the 'dietary' (high protein and wheat-based) and 'dietary + Coccidia' (a mixture of E. maxima, E. tenella and E. acervulina)' were used to evaluate mucosal (duodenum and jejunum) and lymphoid (cecal tonsil 'CT' and bursa of Fabricius 'Bursa') immune gene expression during NE. Additionally, we used two virulent strains (netB+tpeL+) of C. perfringens, Str. CP44 and Str. CP64, to reproduce NE to determine if there were any strain-specific virulence differences. The experimental groups included, 1) Uninfected, 2) Cocci-only 3) CP64-only, 4) CP44-only, 5) Cocci+CP64, and 6) Cocci+CP44. Results were analyzed using a one-way ANOVA (parametric/non-parametric) and showed that Coccidia-predisposition followed by CP44 or CP64 infection induced increased ($P<0.05$) expression of IL-1β, IL-6, IL-13 or FoxP3 genes in the cecal tonsils compared to the Coccidia-alone group. Additionally, the Coccidia+CP44 infected group had higher ($P<0.05$) IFNγ transcription in the duodenum and jejunum tissues. Furthermore, birds receiving dietary predisposition+CP44 (but no coccidia) also had elevated ($P<0.05$) expression of the IL-6 gene when compared to the negative (uninfected) control group. Collectively, our findings showed that coccidia-predisposition to NE results in an increased gene expression of pro-inflammatory cytokines (IL-1β/IL-6/IFNγ)

in both mucosal and lymphoid tissues, as well as increased immunoregulatory transcription factors such as FoxP3.

Keywords: Necrotic Enteritis, Broiler Chickens, Clostridium perfringens, Immune Response, Experimental infection model

418P Phytogenic blend positively influence the ileal and cecal microbiota during a necrotic enteritis challenge

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Necrotic enteritis (**NE**), caused by *Clostridium perfringens* (**CP**), is a major enteric poultry disease resulting in severe economic losses to the industry. A 42-day (**d**) NE model study investigated the effects of phytogenic blends additive on the ileal and cecal microbiota of broiler chickens. Day-old Ross 708 chicks (n=450) were allocated to one of three groups: 1) negative control (**NC**; corn/soybean diet), 2) NC + Avilamycin/Amprolium (**PC**), and 3) NC+ Alterna® and Synbiotec® (**SYNB**). On d 14 and d 19, all birds were orally inoculated with 2,000 sporulated oocysts of *Eimeria maxima* and 1×10^8 CFU of CP, respectively. On d 14, d 21, and d 42, ileal and cecal mucosal scrapings were collected (n=6/group), microbial DNA was extracted, and the V3-V4 regions of the 16S rRNA gene were sequenced and analyzed using QIIME2. For functional and microbial phenotype prediction analyses, PICRUSt2 and BUGBase pipelines were used. Results were considered statistically significant at $P \leq 0.05$. Compared to NC, SYNB increased a-diversity indices (Shannon, Simpson, and Chao1) at d 21 and d 42 in the ileum, indicating enhanced microbial richness. At d 21, SYNB increased the abundance of beneficial microbiota (families Lachnospiraceae, Ruminococcaceae, Bifidobacteriaceae, and Butyricicoccaceae), which support short chain fatty acids (**SCFAs**) production and gut homeostasis, with Akkermansiaceae and Prevotellaceae dominating in the ileum by d 42, which support mucosal integrity and carbohydrate metabolism. In the ceca, SYNB increased the abundance of Akkermansiaceae and Prevotellaceae at d 21 shifting to Christensenellaceae and Oscillospiraceae by d 42; these taxa are associated with gut barrier function and improved energy utilization. Stress-tolerant phenotypes were significantly lower in SYNB compared to NC at d 14 and d 21 that significantly increased by d 42. At d 14, PICRUSt2 analysis showed significantly greater abundance of functional genes involved in menaquinol biosynthesis, formaldehyde assimilation, and chondroitin sulfate degradation in SYNB birds compared to NC and PC in both ileum and ceca. At d 21 and d 42, SYNB group showed a significantly greater abundance of functional genes involved in methyl ketone biosynthesis and glycogen degradation and biosynthesis of antioxidant related compounds including adenosylcobalamin, ergothioneine, and mycothiol, respectively. This suggests that supplementation of phytogenic supported the proliferation of microbial taxa involved in SCFAs production, gut barrier integrity, and antioxidant biosynthesis, and modulated stress-tolerant phenotypes, supporting intestinal health in broilers during a NE challenge.

Keywords: necrotic enteritis, Clostridium perfringens, phytogenic, oxidative stress, microbiota

419P Assessment of neutral buffered formalin as a fixative to preserve *Histomonas meleagridis* cell morphology

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To investigate morphological stage conversion of parasitic protozoa, a fixative solution is normally used to preserve tissue samples containing the parasite obtained from different sections of the gastrointestinal tract or the excreta. To our knowledge, *Histomonas meleagridis* stage conversion has been minimally investigated so the effects of fixative on morphology need to be evaluated. The purpose of this study was to assess the durability of *H. meleagridis* cells exposed to 10% neutral buffered formalin (NBF) or 0.9% NaCl over time (d0, d3, d7, d17, and d27) to determine the resistance of the cell membrane during long periods of preservation. For this experiment, 75mL of confluent histomonas culture (6.04 $\log_{10}$ cells/mL) was concentrated at 200x*g* for 10 minutes. The supernatant was discarded, and the cells were resuspended in five replicates of 5mL containing either sterile 0.9% NaCl (treatment 1, T1) or 10% NBF (treatment 2, T2) in a 15mL conical centrifuge tube and held at room temperature for 27 days. At the previously indicated time-points, 100ul of culture was removed from each replicate tube, combined 1:1 with trypan blue, and enumerated with a Bright-Line hemocytometer at 20x magnification. Morphology was evaluated by visual examination with an inverted microscope and photographs were taken with an Accu-Scope Excelis camera for side-by-side comparison. Data was analyzed by One-way ANOVA to detect significant differences ($P\leq0.05$) and means were further separated by Tukey Honestly Significant Difference (HSD) where appropriate. While results indicated that there were significant differences across time both within and between treatment groups, $\log_{10}$ cells/mL remained consistent from d0-d27 in both the 0.09% NaCl (5.97±0.01; 5.80±0.04) and 10% NBF (5.94±0.02; 6.10±0.01). In T1, histomonads became shrunken and spherical while morphology remained unchanged in the 10% NBF-treated cells for the duration of the experiment. Further research is underway to validate that 10% NBF is capable of preserving *H. meleagridis* morphology in tissue and excreta samples obtained from infected turkeys.

Keywords: Histomonas meleagridis, stage conversion, turkey, formalin, fixative

420P A preliminary evaluation demonstrating the resilience of Histomonas meleagridis when exposed to acidic conditions in vitro

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Histomonas meleagridis transmission in turkey flocks is not fully understood and survival of the protozoan outside of a host is thought to be limited. In a preliminary *in vitro* study, we observed that histomonads were capable of surviving at room temperature in Modified Dwyer's media and horse serum (MDM) with rice starch for up to 6d. This study evaluated the ability of histomonads to withstand exposure to hydrochloric acid (HCl) simulating the acidic conditions in the gastrointestinal tract of turkeys. For the assay, *H. meleagridis* PHL strain passage 4 (P4) was grown to confluence ($<1x10^6$/mL) in MDM with rice starch in a 75cm^2 tissue culture flask. At confluence, the culture was split into

five groups: 1) P5 non-treated control, 2) P5 10m HCl exposure, 3) P5 40m HCl exposure, 4) P5 2h HCl exposure, and 5) P5 24h HCl exposure. For each group, 15mL of confluent culture was centrifuged and the pellet was resuspended in 15mL of HCl (pH 1.6) for the respective duration and incubated at 37°C. After incubation, cultures were centrifuged, and the pellet was resuspended in fresh MDM and rice starch (25cm^2 flask) followed by incubation at 40C for up to 25d. Histomonad concentration (Log10/mL) was determined using a hemocytometer before and directly after exposure to HCl acid and monitored for growth and morphology using an inverted microscope. Four days post-HCl exposure, P6 passages were made to evaluate viability. Three days post passage, P6 10m and P6 24h were supplemented with 1mL of *E. coli* (6.70 Log10 CFU/mL) and *K. pneumoniae* (5.48 Log10 CFU/mL) to determine if additional bacteria would impact histomonad growth. ANOVA was used to determine significant differences at $P<0.05$. Means were further separated using Tukey's HSD test where appropriate. Viability was subjectively monitored and assessed based on the level of confluence in the flask. Trophozoite and amoeboid morphology were observed in the stock culture prior to exposure to acidic conditions. Small, spherical, "cyst-like" structures were observed in the cultures exposed to HCl, but not in the non-treated control. Histomonad concentration (Log10 cells/mL) was significantly (P=0.0035) lower in P5 40m, P5 2h, and P5 24h (5.55, 5.45, 5.07) compared to the P5 non-treated control (5.95) and P5 10m (5.30). Viable histomonads were recovered from all groups post-HCl exposure. The addition of gram-negative bacteria in P6 appeared to increase growth rate of the histomonads post-HCl exposure, with both flasks reaching confluence within 3d. This could suggest that histomonads could survive outside a host or vector and that fecal-oral transmission may play a more critical role than previously thought. Ongoing research evaluating oral infection is underway.

Keywords: Histomonas meleagridis, hydrochloric acid, protozoa, assay, histomonosis

Student Competition: Immunology, Health and Disease: Immunology

421P In vitro evaluation of the effects of a *Saccharomyces cerevisiae*-based postbiotic on the growth of three *Salmonella enterica* serotypes

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Postbiotics are the preparation of inanimate microorganisms and/or microbial fermentation components that are beneficial for the host, and they can be effective in reducing the colonization of bacteria, including *Salmonella enterica*. *Salmonella* is an important zoonotic pathogen responsible for foodborne cases of salmonellosis worldwide. This study evaluated the antimicrobial effect of a commercial *Saccharomyces cerevisiae*-based postbiotic against *Salmonella* serotypes Enteritidis (SE), Reading (SR), and Typhimurium (ST). To obtain a uniform culture, a single colony of each serotype was cultured in buffered peptone water (BPW) overnight. Each serotype was assigned to five treatment groups: *Salmonella* with no treatment (negative control; NC), *Salmonella* treated with gentamicin at 152 µg/mL (positive control; PC), and three postbiotic treatment groups. The postbiotic was administered at three inclusion levels: 1.6 µL/mL (Group 1 [G1]; 1× of the manufacturer's recommended dosage), 3.2 µL/mL (G2; 2× inclusion), and 6.4 µL/mL (G3; 4× inclusion). Each group consisted of three replicates and was incubated at 37°C for 24 hours. Ten-fold serial dilutions were prepared down to 10e8, and aliquots of 10e5 through 10e8 were spread-plated on xylose lysine deoxycholate (XLD) agar. XLD Plates were incubated overnight at 37°C, and typical black colonies were counted to determine the colony-forming units (CFU)/mL of sample. The data were Log_{10}-transformed and analyzed using one-way ANOVA, with significance ($P \leq 0.05$) between groups determined by Tukey's post-hoc test. The results demonstrated a dose-dependent inhibition, with all postbiotic treatments showing numerical reductions in bacterial counts across all three serotypes. In the SE samples, a significant reduction was observed in G1 and G3 compared to NC**.** The mean Log_{10} CFU/mL for the NC was 10.28, while the G1 and G3 reduced SE counts to 9.46 ($P = 0.031$) and 9.40 Log_{10} CFU/mL, respectively ($P = 0.020$). G2 also showed a numerical reduction to 9.92, though it was not significantly different ($P = 0.776$). For the SR samples, there was a significant reduction in the G3 compared to NC, with a Log_{10} CFU/mL reduction from 10.27 to 9.39 ($P = 0.017$). Interestingly, bacterial counts in ST samples were significantly lower in G1 compared to the NC group, reduced from 10.39 to 9.58 Log_{10} CFU/mL ($P = 0.043$). These findings show that this *Saccharomyces cerevisiae*-derived postbiotic can significantly reduce *Salmonella* counts in vitro, which presents a potential for mitigating *Salmonella* loads and contamination in birds. Subsequently, bird trials will be conducted to evaluate its efficacy in vivo.

Keywords: postbiotic, Saccharomyces cerevisiae, Salmonella Reading, Salmonella Typhimurium, Salmonella Enteritidis

422P Primary vaccination with herpesvirus of turkey (HVT) vaccine stimulated different lymphocyte infiltration profiles in Light-brown Leghorns and scleroderma/systemic sclerosis-expressing UCD200/206 White Leghorns

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It is common practice to vaccinate chickens with live herpesvirus of turkey (HVT) vaccine to prevent infection with Marek's disease virus, a lymphoma-causing alphaherpesvirus. The UCD 200/206 is an established Leghorn line that spontaneously develops scleroderma/systemic sclerosis (SSc), akin to an autoimmune disorder in humans. The objective of this study was to compare the local cellular- and systemic humoral-primary immune responses of Light-brown Leghorn (LBL) controls and SSc UCD 200/206 (UCD) chickens following intradermal (i.d.) vaccination with a live HVT vaccine. To do this, 9-wk-old LBL and UCD pullets (N=6 pullets/line) received a primary HVT vaccination by i.d. injection into the growing feather (GF) pulps (10 µL/GF; 16 GFs/bird). Injected GFs were collected before (0) and at 0.25, 1, 2, 3, 5, 7, and 10d post-injection (p.i.) to assess local leukocyte profiles by immunofluorescent staining of pulp cell suspensions and leukocyte population analysis by flow cytometry. Peripheral blood was collected before (0) and at 3, 5, 7, 10, 14, 21, and 28d p.i. to measure HVT-specific IgM and IgG by ELISA. Data were analyzed by two-way ANOVA to test the effect of genetic line, time, and their interaction ($P \leq 0.05$; 6 pullets/line/timepoint). After primary i.d. HVT vaccination, GF-pulps of LBL and UCD pullets had similar increases ($P<0.001$) in heterophil-, macrophage-, and total lymphocyte-levels (% pulp cells) from 0 to 2d p.i. At 3d p.i., LBL pullets had greater total lymphocyte levels in GF-pulps than UCD pullets. At 5, 7, and 10d p.i., lymphocytes in GF-pulps decreased in LBL to levels similar to those in UCD and remained

elevated (P=0.02) through 10d p.i. in both lines. In GF-pulps of LBL pullets, $CD8^+$ T cells, $CD4^+$ T cells, and B cells reached maximal levels on 3d p.i. while their levels in UCD pullets were highest at 1d p.i., 2d p.i., and 5d p.i., respectively. When assessing circulating levels of HVT-specific antibodies, IgM levels in LBL and UCD pullets (P<0.08) had a transient peak at 10d p.i., and both pullet groups exhibited a gradual increase in IgG levels (P<0.001) from 0 to 28d p.i. Overall, the SSc UCD 200/206 pullets exhibited a lower ability to recruit lymphocytes to the site of HVT vaccination than LBL pullets, especially at 3d p.i. Even so, both UCD and LBL pullets exhibited similar HVT-specific antibody response profiles with IgM-to-IgG isotype switch, indicating a T-dependent primary humoral immune response. Future research will investigate how these genetic lines respond to other viruses, and investment in these studies may reveal alterations in their immune function capabilities that are associated with scleroderma/systemic sclerosis susceptibility and development.

Keywords: immunology, cell-mediated immunity, antibodies, herpesvirus of turkey vaccine, chicken

423P Beta-carotene and lipid metabolism as mediators of probiotic-induced immunometabolism modulation

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Probiotics have been shown to modulate immune function, but understanding how is unclear. Our laboratory has demonstrated increased production of immunoglobulins, as well as enhanced ATP levels in circulating immune cells with probiotic supplementation (*Lactobacillus acidophilus, Lacticaseibacillus* (formerly *Lactobacillus*) *casei*, *Bifidobacterium bifidium*, *Enterococcus faecium*). *In vitro* experiments using a T-lymphocyte-like line (CU205) treated with serum from probiotic-fed chickens also increased ATP. To narrow down the potential microbiome-immune communication factors driving this response, lipid components were removed from the serum before treating cells, which resulted in a reduction in ATP levels in the probiotic group. This suggests that lipids are critical for the probiotic-driven ATP enhancement. To test this, chickens were fed a standard grower diet either with 0.3% inclusion of probiotic or without. Serum and ileum samples were collected from both groups at day 14 and day 21 of age for global metabolomics and analyzed with Significance Analysis of Microarrays and Metabolites. The serum metabolome contained 16 more abundant lipid-associated metabolites that were also positively correlated to CU205 ATP production, 4 of which were related to the mevalonate pathway. Additionally, RNA sequencing and Ingenuity Pathway Analysis of differentially expressed genes in CU205 cells treated with probiotic serum revealed downregulation of 4 sub-pathways of the mevalonate pathway. Based on this observation, we hypothesize that metabolites that suppress the mevalonate pathway enhance ATP production in immune cells. To test this hypothesis we are systematically testing each differentially abundant metabolite for its ability to regulate ATP production in lymphocytes. This study specifically focused on beta-carotene, a metabolite that was more abundant in the probiotic serum and positively correlated with ATP production in Cu205s. Interestingly, beta-carotene is less abundant in the ileum, which may indicate greater absorption from the gastrointestinal tract into circulation. Additionally, our laboratory's previous work has shown that this probiotic increases IgA production in response to challenge, and previous literature suggests supplementing beta-carotene enhances IgA production in chickens. Collectively these studies will demonstrate to what extent beta-carotene can regulate ATP production in immune cells and how this contributes to the overall immune response.

Keywords: Microbiome, Probiotics, Immunometabolism, Lipids, Beta-carotene

Animal Well-Being and Behavior

424P Quantitative gait properties associated with subjective gait scores in male turkeys reared with access to environmental enrichment

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For commercial turkey toms, heavier body weight is associated with a higher prevalence of mobility issues. While economically beneficial, heavier body weight can negatively impact the skeletal structures of turkeys, potentially leading to conditions such as valgus/varus, crooked toes or shaky legs, and ultimately lameness. Reliable and simple gait assessment tools are crucial for testing the effectiveness of intervention strategies to reduce lameness. In this study, we compared objective gait parameters with gait scores (GS) generated from a subjective assessment technique among turkeys exposed to various environmental enrichments (EE). At day of hatch, 432 male Nicholas Select turkeys were randomly distributed among 24 pens (n=18 birds/pen). Each pen was assigned one of five EE treatments or a control (CO) with no enrichment, resulting in 4 pens/treatment. The EE included a platform (PL), pecking block (PB), ground-robot for induced locomotion (RO), straw bale (SB), or tunnel (TU). At 18 weeks of age, gait scoring was conducted on toms and 2 birds from each pen, one with a score of '0' (no gait issues) and one with a score of '1' (mild gait impairment), were selected for objective gait assessment using a pressure sensing walkway (Strideway, Tekscan™). After habituation, gait parameters were recorded and analyzed using custom software. Gait parameters, including cadence (steps/min), gait time (s), gait distance (cm), gait velocity (cm/s), step length (cm), peak pressure (kPa), and maximum force (g) were calculated for each bird. A One-Way ANOVA (JMP 18) was performed to analyze the data, with GS and EE as the main effects, and pen as a random effect. A p-value of $p<0.05$ was considered statistically significant, while a p-value of $p<0.10$ was considered a trend. The average body weight (15.10±0.64 kg) was not different between EE or GS groups. Environmental enrichments did not influence any of the gait parameters observed. Cadence, gait time, and gait velocity were affected by gait scores. Toms with a GS of '1' had a longer gait time (p=0.06) than those with a GS of '0'. On the other hand, toms with a GS of '0' had greater cadence (p=0.07) and gait velocity (p=0.09) than those with a GS of '1'. These results showed that birds with mild gait impairment walked slower and took fewer steps than birds without impairment. Overall, these results indicate that enrichments had minimal impact on gait and confirm that some gait parameters can be distinguished clearly using subjective scoring.

Keywords: Turkey, Enrichment, Welfare, Gait, Bone Health

425P Don't ruffle their feathers: Isolation in the light and dark has differential effects on vocalizations but minimal effects on brain serotonin activity in neonatal layers or pullets

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The isolation fear test measures the latency and duration of vocalizations as an indirect indicator of welfare. However, limited to no research has been done to evaluate the effects of isolation on a chicken's neurochemistry. The purpose of this study is to understand how isolation impacts central dopamine (DA) and serotonin (5-HT) activity since both are associated with an animal's affective state. To achieve this goal, hens were isolated for 90 minutes in an anechoic chamber outside of their homerooms. This timeframe ensures any potential changes in neurochemistry can be observed. In this abstract we present the two youngest ages; two adult ages are presented in an oral presentation. 36 W-36 chicks (12 days of age) and 36 pullets (12 weeks of age) were used during photophase with three treatment groups per age (12 birds/group/age). One treatment was isolated for 90 minutes in the chamber with a light on (Light), while the second was isolated with no light (Dark). Controls (Con) were collected directly from their home pens. Vocalizations were audio recorded and Adobe Audition was used to obtain latency to and duration of vocalizations for the Light and Dark groups. Birds were euthanized (intravenous pentobarbital (FatalPlus 390 mg/kg)) after 90 minutes and brains were collected. Brains were micro-dissected into the caudal (CM) and rostral mesencephalon (RM), diencephalon (DI), and medial (MT) and lateral telencephalon (LT). DA, 5-HT, and their respective metabolites were measured using mass spectrometry. The ratios of metabolites to neurotransmitters were calculated as an indicator of synaptic activity. Brain data were analyzed with an ANOVA followed by Fishers PSD post hoc test, and vocalizations with a Mann-Whitney U Test. Chicks placed in isolation with a light had a shorter duration to ($p=0.002$) and frequency of ($p=0.0039$) vocalizing compared to birds in the dark, as the Dark birds did not vocalize at all. In chicks there was a slight increase in 5-HT turnover in the DI ($p = 0.0866$) and the LT ($p = 0.0560$), suggesting a negative affective state. There were no significant differences found in DA and 5-HT levels or activity in pullets. These data combined with previous literature suggest that traditional methods of measuring stress should be used with caution in immature layers.

Keywords: Neurotransmitters, stress, layers, isolation

426P Effects of provisioning a moonlight regimen on layer fear and stress susceptibility

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Poultry are known to be sensitive to light spectrum, intensity, flicker, and photoperiod, which can influence behavioral and physiological responses related to fear and stress. This study aimed to evaluate the effects of a "moonlight" lighting regimen on fear and stress susceptibility in laying hens. Hens were reared from 18 to 48 weeks of age under one of two lighting treatments: a control group (CON) exposed to standard 3000K white LED light, and a treatment group (PSL-Moon) exposed to a poultry-specific spectrum consisting of 3000K white LED with added 630 nm red light during the day and dim monochromatic blue light at night ("moonlight"). Fear and stress responses were assessed at 48 weeks of age. Stress susceptibility was evaluated through physical asymmetry (ASYM) in three bilateral traits ($n = 120$), while behavioral fear was measured using tonic immobility (TI; $n = 120$) and inversion (INV; $n = 120$) tests. Data were analyzed using a general linear model (GLM), with significance set at $P < 0.05$. At 18 weeks, there were no significant differences between treatments in ASYM (1.7), TI, or INV responses ($P > 0.05$; SE = 0.08mm). At 48 weeks, ASYM remained similar between groups (1.8; $P > 0.05$; SE = 0.12mm), indicating no effect of lighting on stress susceptibility. However, hens in the PSL-Moon group exhibited significantly shorter times to righting (142 ± 18 sec) and first head movement (67 ± 9 sec) during TI compared to the control group (211 ± 22 sec; 115 ± 17 sec; $P < 0.05$), suggesting reduced fearfulness. No significant differences were observed in INV responses between treatments, including total wing flaps (32.0 ± 1.7), flap duration (7.4 ± 0.3 sec), or flap intensity (4.2 ± 0.1 flaps/sec; $P > 0.05$). These findings indicate that while the PSL-Moon regimen did not affect physical indicators of stress susceptibility, it may reduce fearfulness in laying hens as evidenced by tonic immobility responses. However, overall reactivity or flightiness—as measured by inversion—was not significantly affected. The PSL-Moon lighting program may offer potential welfare benefits, though further research is warranted.

Keywords: Layer, Lighting, Behavior, Stress, Welfare

Extension and Instruction

427P Assessing evolving challenges and educational needs in commercial and backyard poultry management

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The poultry industry is undergoing significant shifts in production practices, automation adoption, and regulatory challenges. This study aimed to assess current trends and challenges among commercial broiler growers and backyard poultry keepers to guide future extension efforts. The last comprehensive poultry industry surveys were conducted in 2017 (commercial broilers) and 2011 (backyard poultry) in the Delmarva area, emphasizing the need for updated data. Increasing production costs, disease risks, and regulatory compliance challenges require improved education and outreach to support poultry growers in making informed management decisions. University of Maryland Poultry Extension developed and distributed surveys using the Qualtrics platform for both commercial and small flock growers in Maryland, Delaware and the Eastern shore of Virgina. Invitations were sent via the UMD Poultry Extension and Delmarva Chicken Association listservs, with data collection occurring between January 13 and March 2, 2025. A total of 51 commercial broiler growers and 59 backyard poultry keepers participated. The survey covered flock management, production practices, challenges, and educational needs. Among commercial growers, 20% now manage flocks exceeding 200,000 birds per cycle, compared to 7.1% in 2017, reflecting ongoing trends toward efficiency and scale. Key concerns included rising input costs 42.9%, bird health 28.6%, and

government regulations 14.3%. While automation adoption is increasing, cost remains a major barrier 76.2%. Extension training has improved biosecurity 25.6% and flock performance 22.6%, with demand for future programs focused on health management 63.6% and new technologies 18.2%. Among backyard poultry keepers, chickens remain the most popular species, while species diversity has expanded, with notable increases in duck and turkey ownership. The primary purpose of keeping flocks is to produce eggs 45.9%, local egg sales 17.3% and meat for family consumption 12%. Notably, interest in meat sales has risen significantly, from 2.4% in 2011 to 12% in 2025. Key challenges faced by these keepers include predator control 40.5%, feed costs 28.6%, and access to research-based education 11.9%. Additionally, more than half of the respondents already sell or expressed interest in selling eggs and meat, yet 57% lacked awareness of meat sales regulations. The survey highlights the evolving needs of commercial and backyard poultry growers. Rising production costs, regulatory knowledge gaps, and disease prevention remain top concerns. Extension programs should prioritize health management, financial planning, and regulatory compliance education to ensure sustainable poultry production.

Keywords: extension education, flock management, needs assessment, production trend, regulatory compliance

Genetics and Molecular Biology

428P Mitochondrial calcium overload is associated with the woody breast myopathy in broilers

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The woody breast myopathy negatively impacts poultry production, welfare, and sustainability. Until the etiology is fully elucidated, however, any preventative or mitigating strategies are likely only partially effective. Previously, we showed increased levels of calcium in WB-affected tissues and in a hypoxic myoblast model, which suggests that mitochondrial calcium overload is likely a leading cause for the mitochondrial dysfunction seen in these conditions. Therefore, the objective of this study was to examine the role of calcuim overload in the mitocohndria of woody breast muscle. Calcium concentration was measured in mitochondria isolated from breast muscle from normal and severe WB (n=4 per group). Primary myoblasts were isolated from breast muscle of E18 embryos and cultured on collagen-coated culture dishes in a humidified atmosphere at 37°C. Once differentiation to myotubes occurred, two separate *in vitro* experiments were conducted. First, cells were challenged with extracellular calcium ($CaCl_2$, 10mM) for 24h to mimic the increased levels seen in WB, with control cells maintained in basal calcium concentrations (1.8mM). Second, hypoxia was induced by placing the cultures into a gas-tight modular hypoxic chamber (1% O_2/5% CO_2/94% N_2) for 24h. The control cells were maintained at normoxic conditions (5% CO_2/95% O_2). In both experiments, pre-treatment with MCU-i4 (5uM) was used to block calcium import to the mitochondria. Caspase3 activity was measured by a commercially available kit as a marker of apoptosis (n=3 per treatment), and mitochondrial respiration was measured via Seahorse XF flux analyzer (n=3-4 per treatment). Data were analyzed by Student's t-test, one-way ANOVA, or 2-way ANOVA, as appropriate. Individual means were compared by Tukey's multiple range test using Graph Pad Prism software, with significance set at $P<0.05$. Calcium concentration was greater in mitochondria of WB as compared to normal breast muscle ($P=0.06$). Caspase 3 activity in myotubes was increased both by 10mM calcium ($P<0.05$) and 24h hypoxia ($P<0.05$) and returned to control levels by pre-treatment with MCU-i4. Additionally, MCU-i4 was able to mitigate the negative effects of calcium overload on basal respiration and proton leak in myotubes. However, inhibition of mitochondrial calcium import had no effect on the reductions in ATP production and maximal respiration seen with hypoxia. These results provide initial evidence for the role of elevated mitochondrial calcium in the WB myopathy.

Keywords: woody breast, broiler, mitochondrial calcium, hypoxia, bioenergetics

429P Genetic and histopathological characterization of spaghetti meat in broiler chickens

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Spaghetti Meat (SM) is an emerging myopathy in broiler chickens, characterized by the separation of muscle fiber bundles, leading to significant economic losses. This study aimed to characterize the histopathological features of SM, identify associated single nucleotide polymorphisms (SNPs), and analyze their functional significance. SNPs were identified using GATK (v4.0) from RNA-seq data obtained from University of Guelph repository. Reads were quality-filtered (Phred score ≥20), aligned to the chicken reference genome (GCA_016699485.2) using STAR, and processed with GATK pipelines. Variants were called using HaplotypeCaller and filtered based on QD≥4.0, FS≤50.0, MQ≤45.0, and MAF>0.05. SNP allelic imbalance was assessed using an in-house Perl script, with significant thresholds of ≥2.0 (amplification) or ≤0.5 (loss of heterozygosity). The Chi-square test was applied to determine SNP enrichment in affected vs. non-affected birds at $q<0.05$. Histological analysis of 120 Bouin's-fixed breast fillets from antibiotic-free heavy birds (4.1 kg) and organic light birds (2.7 kg) classified samples as normal, moderate SM, or severe SM based on a 0–3 scale for myodegeneration, perivascular inflammation, and fibrosis/lipidosis. Histological scores of SM severity were analyzed using Kruskal-Wallis tests with Dunn's correction for multiple comparisons. Genome-wide SNP analysis identified 630 SNPs affecting 443 unique genes, with Chromosome 2 most impacted. Notable genes included *ACSF3*, *ADAM8*, *ANKS6*, *ARSG*, and *ATP10D*. Functional enrichment analysis highlighted pathways related to cytoskeletal organization, immune responses, and actin filament severing. Severely affected SM fillets exhibited significantly higher histological scores than the normal group in both heavy and light birds ($p < 0.001$ for both). Moderately affected fillets also had significantly higher scores compared to normal fillets in both heavy ($p = 0.005$) and light ($p < 0.001$) birds. However, no statistically significant differences were observed between heavy and light birds within each severity category. The characterized histological panel is currently being used to validate the association of SNPs with the phenotypes. This study provides novel insights into SM's genetic and histological basis, suggesting cytoskeletal remodeling and immune regulation in disease pathology. These findings may inform breeding strategies to mitigate the prevalence of this myopathy in commercial broiler production.

Keywords: broiler chickens, genetic analysis, histopathology, single nucleotide polymorphisms, myopathy

430P Not Presented

431P Heritability and genetic correlation of meat production traits in Mexican Creole chickens

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Unlike other countries involved in the characterization and use of their local genetic resources, in Mexico only the issue of characterization has been addressed, and few studies have focused on the estimation of genetic parameters to allow the implementation of breeding programs. The objective of this study was to estimate genetic parameters associated with meat production: heritabilities of cumulative feed conversion (CFC_{49-84}) and residual feed intake (RFI_{49-84}) from 49 to 84 days of age and the genetic correlation between these traits in Mexican Creole chickens. Data from 755 Creole chickens obtained in the third generation of random matings were used to estimate the values of these traits. A descriptive analysis of the data was performed using the UNIVARITE procedure of SAS. The birds used were identified by sex and individually weighed weekly from birth to 84 days of age. The animal model used to calculate heritability considered the additive genetic effect of the animal as the only random effect. The pedigree was integrated with information from 1052 animals. Variance components were estimated by restricted maximum likelihood methods with the REMLF90 and AIREMLF90 programs. The estimation standard errors were obtained using AIREMLF90. The CFC_{49-84} ranged from 2.21 to 8.34 g/g and the RFI_{49-84} from -1.17 to 1.22 g/g. Heritabilities of 0.132 ± 0.002 and 0.285 ± 0.001 were calculated for the CFC_{49-84} and RFI_{49-84} traits, respectively. Bivariate analyses were performed to calculate the genetic correlation between these traits. The genetic correlation between CFC_{49-84} and RFI_{49-84} was 0.371. It is concluded that the traits CFC_{49-84} and RFI_{49-84} in the Mexican Creole chickens population studied are heritable and the genetic association between them is favorable. So, it would be feasible to implement a program for the improvement of these traits through selection methods.

Keywords: Genetic parameters, heritability, genetic selection, meat production, Mexican Creole chickens

432P Heritability and genetic correlation of egg production traits in Mexican Creole hens

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Mexican Creole hens produce eggs considered to be of high quality; however, unlike in developed countries, they have not been characterized in terms of diversity and genetic improvement. The objective of the present study was to estimate the heritabilities of eggs number (EN_{21-30}) and egg mass (EM_{21-30}) in Mexican Creole hens, from 21 to 30 weeks of age and the genetic correlation between these traits. A total of 295 hens from the third generation of random matings were used. Management of the birds began at 20 weeks of age and egg production was recorded for 10 weeks (from week 21 to 30). Egg mass per bird was calculated by summing the egg weight each day. Descriptive analysis of the data was performed using the UNIVARITE procedure of SAS. The pedigree was integrated with information from 1052 animals. Variance components were estimated by restricted maximum likelihood methods with the REMLF90 and AIREMLF90 programs. The standard errors of the estimations were obtained with AIREMLF90. The animal model used to calculate heritability considered the additive genetic effect of the animal as the only random effect. The estimated EN_{21-30} and EM_{21-30} means were 30.44 ± 17.57, ranging from 0.00 to 64.00 eggs and 1.35 ± 0.78, varied from 0.00 to 2.87 kg, respectively. Heritabilities of 0.208 ± 0.031 for EN_{21-30} and 0.168 ± 0.006 for EM_{21-30} were calculated. Bivariate analyses were performed to calculate the genetic correlation between these traits. A favorable genetic correlation of 0.916 was recorded between EN_{21-30} and EM_{21-30}. It is concluded that the EN_{21-30} and EM_{21-30} traits are heritable in the Mexican Creole hens population. Therefore, it would be feasible to implement a genetic improvement program.

Keywords: Genetic parameters, heritability, genetic selection, egg production, Mexican Creole hens

Management and Production

433P Enteric neurochemical responses to heat stress in Pekin ducks are sex- and diet-dependent

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Heat stress is a major challenge in waterfowl production. Enteric monoamine neurochemicals mediate many effects of stress on poultry physiology. Little is known in ducks, however, regarding the impact of heat stress on the intestinal neuroendocrine system. As an orange corn diet in poultry was previously shown to benefit welfare and performance, we sought to determine the impact of an orange versus a yellow corn diet on duck intestinal monoaminergic responses to heat stress. Male (n=8 drakes/group) and female (n=12 hens/group) Pekin ducks were randomly assigned to either yellow (YC) or orange (OC) corn diet across 4 rooms, with each diet fed for 3 weeks before and during 3 weeks of cyclic heat stress (HS) exposure (35°C for 10h, reduced to 29°C for 14h/day). Ceca tissue and luminal content were collected before the start of HS and immediately following the cessation of 3 weeks of HS. All samples were analyzed for monoamine neurochemicals using ultra high performance liquid chromatography with electrochemical detection. The ROUT method (Q= 1%) was used to identify

outliers. Data were analyzed (with outliers removed) by two-way ANOVA with Tukey post hoc test. Differences were considered significant at $p<0.05$. Norepinephrine was increased ($p<0.05$) in YC hen but not drake cecal luminal content following heat stress. Cecal tissue norepinephrine was not affected ($p>0.05$) by HS. Dopamine concentrations were elevated ($p<0.05$) in hen but not drake OC cecal content compared to YC before the start of HS. Cecal tissue concentrations of dopamine were not affected ($p>0.05$) by HS. Serotonin (5-HT) was increased ($p<0.05$) in drake OC cecal tissue following HS, but not in the YC group ($p>0.05$). Serotonin in cecal content was not affected by HS ($p>0.05$). 5-hydroxyindoleacetic acid (5-HIAA) was decreased in drake YC and OC cecal contents following HS but not impacted ($p>0.05$) in hen cecal content. The 5-HIAA/5-HT ratio decreased ($p<0.05$) in drake OC cecal content but not in the YC group. HS decreased drake cecal tissue 5-HIAA/5-HT ratio but did not reach statistical significance ($p>0.05$). Heat stress caused monoamine neurochemical responses in the duck ceca that were dependent on orange or yellow corn diet. These findings warrant the study of feed components as a tailorable strategy to potentially control stress at the gut-level in ducks. As neurochemicals in the gut play critical roles in inflammation, foodborne pathogen colonization, and other production-relevant aspects in poultry, our findings highlight the importance of intestinal stress responses in the duck.

Keywords: Duck, Heat stress, Gut, Neurochemical, Catecholamine

434P Does exposure to blue light from 0-15 or 0-17 weeks of age affect production and health of egg-laying pullets reared to 17 weeks of age?

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Few studies have examined the effect of light color on poultry while adjusting light intensity for bird vision. The aim of this study was to determine the impact of 3 light color treatments (L) standardized to 15 gallilux: white light to 17 wk (W17), blue light (peak at 455 nm) to 17 wk (B17), or blue light to 15 wk followed by 2 wk of white light (B15) on the production and health of 2 Lohmann strains (S) (Brown-Lite (LB) and LSL-Lite (LW)) during the rearing phase. The experiment followed a 3x2 factorial arrangement (with B15 and B17 combined prior to 15 wk) in a randomized complete block design (trial as block, n=2). Pullets (n=900/S) were reared in light-tight rooms (2 rooms/L) with 6 perchery-style floor pens per room (3 pens/S/room). Pullets were fed commercial diets with *ad libitum* access to feed and water. Pullets were weighed at 0, 4, 8, 12, and 17 wk (pen basis). At 17 wk, a subsample of pullets (86 birds/S/L) were individually weighed to assess flock uniformity. Feed consumed and wasted (kg/pullet/day) was calculated from 0-4, 4-8, 8-12, and 12-17wk (pen basis). Pullets were monitored daily, and any dead or cull birds were sent to an independent laboratory for necropsy. Age to first egg was recorded on a per cage basis in the hen phase (6 cages/S/L, n=36). Data were analyzed using Proc Mixed (SAS 9.4) with room as replicate for L and pen for S. Tukey's range test was used to separate means with differences significant when $P \leq 0.05$. At 4 wk, pullets reared under blue light were heavier than those reared under W17 ($P=0.01$), but no differences existed at other ages. At 0 and 4 wk, LW pullets were heavier than LB ($P<0.01$), but were lighter at 8, 12, and 17 wk ($P<0.01$). For uniformity, an interaction between LxS was noted, where the coefficient of variation was higher in the LB pullets reared under B17 compared to the LW reared under W17 and B17 ($P=0.04$), with other treatments demonstrating intermediate levels of variability. Feed disappearance was greater under W17 than under blue light from 4-8 wk ($P<0.01$). From 0-4 wk, LW pullets had increased feed disappearance compared to LB ($P<0.01$). From 8-12 and 12-17 wk, LB pullets consumed more feed than their LW counterparts ($P<0.01$). Mortality and cull levels were very low, but more LB pullets had causes listed as unknown, compared to LW ($P<0.01$). Age to first egg was not affected by L, but LB pullets reached sexual maturity earlier than LW ($P<0.01$). In conclusion, these results provide further evidence of S differences between LW and LB pullets. Blue light increased bodyweight gain and decreased feed disappearance in early life compared to white light, with no effects of light at later ages.

Keywords: light color, pullets, health, growth, uniformity

435P Sustainable pest control: How three pests' insect of poultry farm reacts to a commercial blend of essential oils?

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Insects, including Tenebrio species and flies, are widespread in the poultry farms, causing sanitary and economic issues. They can deteriorate insulation or introduce pathogens. Essential oils are used as an alternative to chemical treatment and mitigate insecticide resistance risk. Despite their use in farms, their mode of action on the main poultry pests is still unclear. This study investigates the ability of insects to detect volatile compounds from a commercial essential oil blend, Nor Mite®, and assesses their behavioural response, specifically in *Tenebrio molitor*. To assess the insects' capacity to detect the product's volatile compounds, an electroantennography (EAG) was used. This method detects the insect's antenna's electrical response while in contact with a volatile molecule. Pure air is used as a negative control, and a mix of selected chemical compounds is used as a positive one. Due to their economic and sanitary importance in the poultry industry, three species were chosen: *Alphitobius diaperinus*, *Tenebrio molitor*, and *Sarcophaga spp.*. For each species, five individuals have been used. To assess behavioural responses to those volatile compounds, *T. molitor* larvae were used as a model system. 540 *Tenebrio molitor* larvae were chosen and put in 27 boxes containing only two filter papers. One of those papers was impregnated with Nor Mite at 200 ppm, while the other was impregnated with an aqueous solution. EAG results were analysed through a paired Mann-Whitney test associated with a Kruskal-Wallis test. The number of larvae per paper 2 and 4 hours after the deposit was recorded and analyzed through a paired student test. All statistics were done on R. The EAG showed a significant difference in the antenna response to Nor Mite compared to the negative control (p-value <0.05). No difference was found between Nor Mite and positive control at the EAG, which confirms that those insects perceive and react to Nor Mite volatile compounds. The *Tenebrio molitor* larvae showed a significant preference for control papers compared to Nor Mite-impregnated ones (p-value <0.05), inducing the volatile compounds of Nor Mite to have a repulsive effect on this species at least. These findings highlight the repellent effect of Nor Mite on *Tenebrio molitor*. On the other hand, volatile compounds of Nor-mite detected by the insect' antenna. Suggesting that the repellent effect could passe through the nervous system of the insect.

Keywords: Poultry pests, Electroantennography (EAG), Essential oils, Insect repellency

436P Play that Funky Music Pekin Ducks Part 1: Effects of auditory enrichment on Pekin Duck welfare

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In order to improve the welfare of Pekin ducks in production, our study aimed to evaluate the impact of auditory enrichment on body condition and fear responses in Pekin ducks. Research on the effects of auditory enrichment on the health of a flock and welfare in poultry is limited, with even fewer specifically evaluating Pekin ducks. We tested the hypothesis that auditory enrichment would improve duck welfare by producing a calming environment. A total of 400 day-old Pekin ducks from a commercial hatchery were randomly sorted into 4 pens in 3 rooms (12 pens total) at Purdue University, adhering to the industry standards for stocking density (30 ducks/pen). This study lasted about 2 1/2 months and age range ended around 8-10 weeks. Rooms were assigned three auditory enrichment treatments and studied: No audio (control; CON), Mozart music (MOZ), and pond sounds (POND). The enrichment of audio started on day 7, playing cyclically (one hour on, one hour off) from lights on (0300h) to lights off (2100h) at a volume of 65-75 dB. Weekly assessments included a novel object test (NOT) to assess fear response and body condition scoring of 10 ducks/pen which observed the eyes, nostrils, feather quality, feather cleaniness and foot & toe pads. All sections were measured on a scale of 0 (clear), 1 (neutral/slight damage), 2 (very poor/damaged) Body condition scores were analyzed using the PROC LOGISTIC procedure (SAS v9.4) and Firth's penalized likelihood method was used to address quasi-complete separation. NOT was analyzed by 2-way ANOVA with repeated measures using PROC MIXED (SAS v9.4) and Tukey's test for post-hoc analyses. A $p \leq 0.05$ was considered significant. The study was repeated, resulting in experiment 1 and experiment 2. For experiment 1, ducks in the POND group were more idle/non-reacting than ducks in the CON group ($p=0.0085$) reacted or made a fear response. For experiment 2, POND ducks approached more than MOZ ducks ($p=0.0277$) and CON ducks ($p=0.0238$) and CON ducks were more fearful than POND ($p<0.0001$) and MOZ ducks ($p<0.0001$). MOZ ducks had worse feather cleanliness scores, worse foot pad scores, and worse feather quality scores than POND and CON ducks. The presence of the auditory enrichment showed different outcomes in the experiments—suggest slight effects to reduced welfare, possibly related to the dynamics of MOZ music. Further research is needed to fully understand how auditory enrichment affects Pekin duck body condition and behavioral responses.

Keywords: Production, Pekin Ducks, Auditory, Enrichment

437P Play that Funky Music Pekin Duck Part 2: Effects of auditory enrichment on production variables

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In order to improve the production outputs of Pekin ducks, weaimed to evaluate the impact of auditory enrichment on bodyweight, productivity, and growth in Pekin ducks. Research on the effects of auditory enrichment on the production of a flock is limited, with even fewer specifically analyzing Pekin ducks. A total of 400-day old Pekin ducks from a commercial hatchery were randomly sorted into 4 pens in 3 rooms (12 pens total) at Purdue University, adhering to the industry standards for stocking density (~30 ducks/pen). Rooms were assigned three auditory enrichment treatments and studied: No audio (control; CON), Mozart music (MOZ), and pond sounds (POND). The enrichment of audio started on day 7, playing cyclically (one hour on, one hour off) from lights on (0300h) to lights off (2100h) at a volume of 65-75 dB. Weekly assessments included a weekly monitoring of feed intake, body weight, food conversion rate (10 ducks/pen) and biweekly necropsies (2 ducks/pen) to assess organ growth. Production metrics were collected weekly. On weeks 2, 4, and 6, 2 ducks/pen were euthanized via pentobarbital for necropsy, body weight, and organ weights (spleen, bursa of Fabricius, liver, and uropygial gland were recorded). Data were analyzed by 2-way ANOVA with repeated measures using PROC MIXED (SAS v9.4) and Tukey's test for post-hoc analyses. A $P \leq 0.05$ considered significant. For experiment 1, no significant differences were observed among groups for weekly body weights, dissection measures, or FCR. For experiment 2, ducks in the MOZ group weighed less than ducks in the POND ($p=0.0010$) and CON groups ($p=0.0109$). The second experiment suggest that MOZreduces welfare whereas this was not the case in the first experiment. Further research is needed to fully understand how auditory enrichment affects duck production.

Keywords: Production, Pekin Ducks, Auditory, Enrichment

438P Understanding varying level of hydrogen peroxide residuals in poultry drinking water in early broiler growth performance

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Hydrogen peroxide (H_2O_2) is used as a poultry drinking water sanitizer, however, the product use does create the varying levels of residuals in water and its impact on broiler performance have not been studied yet. This study investigated the effects of various concentrations of a peroxide residual created by hydrogen peroxide-based product (3% hydrogen peroxide product) in drinking water on the early broiler growth performance and amino acids (AA) digestibility. In brief, a total of 100 Cobb500 male birds were reared on a standard experimental diet from day(d) (0-13). The birds were divided into five treatment groups with 5 replications (4 birds/replication). Four stock solutions were initially created at ratios of H_2O_2 product to water for T1 (2:32), T2 (4:32), T3 (8:32), and T4 (16:32); and 1 mL of each stock solution was mixed with 100 mL of drinking water to create the drinking water solution. The municipal water (with 0 ppm free chlorine) served as control. The H_2O_2 concentrations in drinking water in T1, T2, T3 and T4 were >50, >50, >100, and >100 ppm at starting and <30, <50, <50, and >100 ppm post 24th hour of mixing, respectively. Birds were weighed on d 0, d 7, and d 13, and their water intake (WI) was recorded. Data was analyzed using one-way ANOVA with Tukey multiple range test for group differences ($P \leq 0.05$). Treatments groups- T2 -T4, showed a higher BWG and a lower FCR compared to the control group. Additionally, some of the essential (isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine) and non-essential (alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, and tyrosine) amino acid digestibility were improved with these

treatment groups. The study demonstrated that tested levels of H_2O_2 residuals did not harm the growth performance or nutrient digestion of broilers, rather benefited it.

Keywords: poultry drinking water sanitizer, hydrogen peroxide, growth performance, amino acid digestibility

439P Early non-destructive assessment of broiler egg fertility by SBSE/GC×GC-MS analysis

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The current national broiler hatchability rate is 85%, leaving 15% of incubator space underutilized. Removing infertile or non-viable eggs before incubation could reduce costs and increase incubator space utilization. To date, technologies for in-ovo detection of fertile eggs have proven elusive or costly. Among promising non-destructive methods, collecting and analyzing the volatile organic compounds (VOCs) emitted from hatching eggs using gas chromatography–mass spectrometry (GC-MS) has demonstrated its potential on Day 0. Our previous work has detected and identified VOCs emitted from broiler hatching eggs using stir bar sorptive extraction (SBSE) devices (Twister®) coupled with GC-MS and statistical analysis. An ensemble of Machine Learning (ML) techniques were used to classify eggs as "Fertile" and "Infertile". The objective of this continued research is to: i) further identify and characterize the VOCs emitted from broiler hatching eggs with increased control of variables; ii) continue to investigate VOCs that are higher in fertile eggs in order to encode their effects on embryo fertility, sex and viability. A total of 98 broiler hatching eggs were collected and stored at 18.3°C for 5 days prior to incubation. The eggs were randomly divided into two groups, control and vacuum. For control eggs, VOCs were collected by magnetically attaching a Twister® to the inside of a lid and sealing the egg inside a glass jar for 1 hour. For vacuum eggs, VOCs were collected by attaching a Twister to a vacuum suction cup on an egg for 30 seconds. VOC sampling was conducted on Days 0, 7 and 14 of incubation. Breakout analysis on Day 14 confirmed fertility status. VOCs were desorbed from the Twisters® using a thermal desorption unit (TDU) coupled to a cooled injection system (CIS) and analyzed using two-dimensional gas chromatography-mass spectrometry (GC×GC/TOFMS) in a non-targeted approach. LECO® ChromaTOF® software was used for data acquisition and analysis in conjunction with the NIST 2017 mass spectral database for compound identification. Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were performed for each VOC collection day to separate eggs into "Fertile" and "Infertile" categories. "Leave one out" technique was employed due to low number of infertile eggs resulting in F1 cross-validation score of 0.93. VOCs that contribute the most to class placement were identified. 30 second vacuum assisted collection method scores at least as many uniquely identifiable VOCs with better or similar signal strength as the one-hour collection method. These data suggest that the compound, Hexanal, is a key VOC in differentiating infertile/unfertilized and fertile eggs.

Keywords: Broiler Egg, Fertility, Non-Destructive, Volatile Organic Compound, SBSE/GCxGC-TOFMS

Physiology and Reproduction

440P Parental orange corn diet alleviated glucocorticoid response but did not alleviate the impaired growth or heightened fear caused by parental heat stress exposure in F1 Pekin ducks

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The effect of heat stress (HS) is not only immediate but can be transferred across generations with a lasting impact on the offspring. This study examined how parental HS affects offspring (F1) and whether a high-carotenoid orange corn (OC) diet fed to parents can mitigate these effects on the F1. Adult breeder Pekin ducks were fed either an OC or yellow corn (YC) diet for 3 weeks before and during 3 weeks of HS exposure, while a control group (CON) received a commercial YC diet under thermoneutral conditions. Eggs collected during the final 3 days of HS were incubated, and the resulting hatchlings (n = 56 per group: OCF1, YCF1, CONF1) were raised as meat ducks for 5 weeks. Weekly assessments of feed intake of all ducks, body weight (n = 20 ducks /treatment), and body condition scores (n = 20 ducks/treatment) were conducted. At week 3, ducks (n=8/injection type/treatment) received either an adrenocorticotropic hormone (ACTH/cosyntropin, 0.0625 mg/kg) or saline intramuscular injection, with blood samples taken at 0, 1, 2, 3, and 4 hours to measure serum glucocorticoid (GC) levels and heterophil to lymphocyte ratios (HLR). Data were analyzed using ANOVA, Kruskal-Wallis tests, or odds ratios as appropriate, with significance defined as $p \leq 0.05$. Serum corticosterone was higher ($p < 0.0001$) in the ACTH-injected groups compared to the saline groups at 1 hour with a sustained increase in YC-ACTH at 2 hours compared to the OC and CON groups. Baseline serum cortisol was lower ($p < 0.01$) in the CONF1 compared to OCF1 and YCF1 while cortisol level was lower ($p < 0.05$) in the CONF1 compared to OCF1 and YCF1 at 1 hour of injection. At 4 hours, cortisol level remained higher ($p < 0.05$) in the OCF1 and YCF1 compared to the CONF1. HLR was higher ($p < 0.001$) in ACTH injected groups compared to the saline groups at 2 and 3 hours relative to injection. Weekly body weight was higher ($p < 0.05$) in CONF1 compared to OCF1 and YCF1 at week 2, 3, 4, and 5 although there was no difference in hatch body weight. Feed intake was higher ($p < 0.05$) in the CONF1 compared to OCF1 at week 2. In conclusion, parental exposure to HS resulted in a prolonged GC response, elevated baseline GC levels, reduced body weight, and heightened fear responses in offspring. However, F1 offspring from heat-stressed parents fed OC diet showed a more rapid return to baseline for circulating corticosterone levels compared to the F1 from fed YC diet. These observations suggest a potential mitigating effect of a high carotenoid diet on stress-related physiological responses.

Keywords: Parental effect, orange corn, carotenoid, heat stress, poultry

441P Orange corn diet altered serotonin and dopamine activity in the brain while heat stress altered total antioxidant capacity in a sex dependent manner in Pekin ducks

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Carotenoids are known to have antioxidant properties. Antioxidants play an important role in the reduction of oxidative stress and are an important factor affecting heat tolerance which in turn influences welfare of poultry. We hypothesized that high carotenoid orange corn (OC) diet would alter total antioxidants capacity (TAC) and brain neurotransmitters (NT) associated with affective state in adult Pekin ducks exposed to heat stress (HS). We randomly allocated 28 hens and 7 drakes per pen to OC or yellow corn (YC) diets across 8 pens, with each diet fed for 3 weeks before and during 3 weeks of cyclic HS exposure (35°C for 10 hours, reduced to 29°C for 14 hours/day). Blood samples (n = 8/sex/treatment/timepoint) were collected before and weekly during HS. Brain samples (n = 12 hens and 8 drakes/treatment/timepoint) were collected before and at the end of HS. NT (n = 6/sex/treatment/timepoint) was assessed in 4 brain areas including diencephalon (DI), lateral telencephalon (LT), mesencephalon (ME), and medial telencephalon (MT). Data were analyzed with 2- or 3-way ANOVA, with significance set at $p \leq 0.05$ and trends at $p < 0.1$. HS decreased ($p < 0.001$) brain TAC in hens but not drakes. The effect of diet on brain or serum TAC was not significant for either drake or hen whereas HS increased ($p < 0.001$) serum TAC in both drakes and hens compared to thermoneutral groups. Hens fed OC diet showed a lower ($p < 0.05$) dopamine level before and during HS compared to the YC hens in the LT and MT. HS decreased ($p < 0.05$) DA levels in the DI of OC drakes but increased dopamine in the LT of YC hens compared to the baseline. Dopamine turnover was lower ($p = 0.055$) in the DI of OC drake compared to YC drake but higher in the LT of OC hen compared to YC hen during HS. HS decreased dopamine turnover in OC hens compared to baseline. Higher ($p < 0.05$) dopamine turnover in the drakes compared to the hens. HS decreased ($p = 0.059$) DI serotonin level in OC drakes compared to baseline. There was a sex difference ($p < 0.05$) with higher serotonin level in the hens compared to drakes. Serotonin turnover was lower ($p < 0.05$) in the ME of OC hen compared to YC hen during HS. Norepinephrine was higher in the MT of OC hens compared to YC hens before ($p = 0.06$) and during HS ($p < 0.05$). Hens showed a higher ($p < 0.05$) norepinephrine level compared to drakes before and during HS. In summary, HS altered TAC and NT in a sex dependent manner. OC diet exerted a sex dependent effect on serotonin and dopamine activities in the brain. Sex differences in NT activity may help explain the sex differences in gross effects of HS on adult ducks.

Keywords: Carotenoid, Orange corn, Antioxidant, Heat stress, Ducks

442P Vaginal swab lipid profiles of high and low fertility broiler breeder hens

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Low fertility negatively affects viable progeny numbers and economic potential of a broiler breeder hen. Successful fertilization depends on healthy sperm reaching the recently ovulated ovum. After insemination, sperm are stored in specialized storage tubules within the uterovaginal junction, where sperm are protected from eggs passing through the oviduct, immune response, and harmful metabolic waste products. Fertility may depend on the receptiveness of the vaginal tissues to sperm, which may be linked to the lipid profile of the vaginal microenvironment. Vaginal swabs could provide a non-invasive screening method for female fertility predictions if hen fertility biomarkers are established. Cobb broiler breeder hens were artificially inseminated weekly, with fertility and hatchability tracked over 30-40 weeks of age (woa). Vaginal swabs from four hens with low fertility (LF) and four hens with high fertility (HF) were extracted using the Bligh and Dryer method and subjected to multiple reaction monitoring (MRM) lipid profiling. Cotton swabs were inserted approximately 2 inches into the vagina at 35 woa. Vaginal swabs were collected immediately prior to artificial insemination, to minimize the male effect, and snap frozen. LF and HF total abundance within each lipid class were compared with a 1-way ANOVA using the PROC mixed model in SAS. Relative ion intensities of individual lipid species were compared across LF and HF using Student's t-test, $p<0.05$, in Metaboanalyst 6.0, with differentially abundant lipid species submitted as a target list into Lipid Ontology (LION) web ontology enrichment tool. Metaboanalyst biomarker analysis using classical univariate receiver operating characteristic curve analysis was also used with an area under the curve (AUC) cutoff of 0.8. Total abundance of the diacylglycerol 16:0 lipid class was more abundant in HF, while phosphatidylcholine and phosphatidylethanolamine total abundance was higher in LF ($p<0.05$). A total of 76 lipid species were significantly different between LF and HF, all of which had an AUC >0.8. Lipid species more abundant in HF were associated with LION-terms: endoplasmic reticulum, membrane component, headgroup with positive charge/zwitter-ion, glycerophosphocholines, diacylglycerophosphoethanolamines, and very high lateral diffusion ($p<0.05$, -log(FDR q)>1.3). Future studies will compare the lipid profiles captured by vaginal scrape with those detected through vaginal swab to confirm the efficiency of swabbing as a non-invasive method. Additionally, our preliminary findings on fertility-based vaginal lipid profiles will be assessed in a larger number of broiler breeder hens as well as at additional timepoints throughout the production cycle.

Keywords: Broiler Breeder, Uterovaginal Junction, Lipidome, Fertility, Biomarker

Immunology, Health and Disease: Feed Additives

443P Case Study: Boosting vaccine titer production in pullets during stress periods utilizing PHYTOZEN®

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During periods of stress, such as vaccine administration, the immune response in pullets can be significantly compromised, making it harder for them to recover from disease. Recent research has highlighted the potential of phytogenic blends to mitigate stress and enhance overall well-being, suggesting that these natural additives may support immune function and improve resilience in pullets exposed to environmental or physiological stressors. Therefore, a controlled 18-week trial was conducted in three side-by-side barns, each housing approximately 30,000 Dekalb pullets, to evaluate the effects of PHYTOZEN® supplementation (a proprietary blend of neuromodulating monoterpenes) on vaccine titer production under stress conditions. Barn 1 served as the control with no feed or water additives, while Barn 2 received PHYTOZEN® pulsed through the water supply at 200ppm for 7

days (before and after vaccination and human interaction), and Barn 3 was supplemented with PHYTOZEN® at ½ lb/ton in the feed. Throughout the study, birds were exposed to weekly stressors such as vaccine injections, sprays, relocation (at day 16), and beak trimming. All treatments were vaccinated for infectious bursal disease virus (IBDV) at day 1 and infectious bronchitis virus (IBV) at day 31, 35, and 63. To assess the immune response, serum samples were collected from 60 birds per barn at weeks 11 and 18 and analyzed for vaccine titers against IBV and IBDV. Data were statistically analyzed using the MIXED procedure in SAS® studio online program, with means separated via the Tukey-Kramer test ($P<0.05$). Results showed that Barns 2 and 3 exhibited a significant increase in IBV titers from weeks 11 to 18 ($P<0.0001$), suggesting a positive immune response associated with PHYTOZEN® supplementation. Additionally, IBDV titers in both treatment groups approached statistical significance ($P=0.0695$), indicating a potential immunomodulatory effect. These findings suggest that PHYTOZEN®, whether administered via water or feed, can enhance immune resilience in pullets undergoing routine stressors.

Keywords: Pullets, Titers, Stress, Immune response, Phytogenics

444P Effect of dietary inclusion of fermented and non-fermented grape pomace on growth performance and gut function of broilers under chronic inflammation

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The withdrawal of antibiotics growth promoters (**AGP**) from poultry production has increased the chances of exposure to several factors that can induce chronic inflammatory responses in chickens. The poultry industry is always looking for alternative feed additives to mitigate the burden associated with AGP withdrawal. Grape pomace, a byproduct from the wine and juice industry, is rich in bioactive compounds with proven antimicrobial, antioxidant, and immunomodulatory effects. This study aimed to investigate the potential of dietary inclusion of fermented and non-fermented grape pomace as a substitute for AGP in broiler diets. A total of 126 (29 d old) Cornish cross broilers were divided into six treatment groups: i) standard diet wheat-corn-soybean meal (**STD**), ii) 30% rice bran or non-starch polysaccharide (**NSP**), iii) NSP + zinc bacitracin (**AGP**), iv) NSP + 0.5% grape pomace (**GP**), v) NSP + 0.5% *Lactobacillus casei* fermented GP (**LAB FGP**) and vi) NSP + 0.5% *Saccharomyces cerevisiae* EC1118 fermented GP (**YST FGP**). Except for the birds in the STD group, all other birds were fed the NSP diet from day 22 to induce chronic inflammation. Growth performance, breast muscle yield, intestinal tight junction integrity, histomorphology, cecal microbiome, and short chain fatty acid (SCFA) analysis were measured at the end of the experiment. Data were analyzed using one-way ANOVA, with significance at $P<0.05$. NSP inclusion significantly reduced the overall body weight (**BW**), body weight gain (**BWG**), and feed intake (**FI**) and increased the feed conversion ratio (**FCR**) compared to the STD ($P<0.0001$). However, dietary inclusion of either GP or FGP improved the BW, FI, and FCR to levels comparable to the birds fed AGP ($P<0.0001$). The dietary inclusion of the GP or FGP improved the breast muscle yield of chickens at d 42 compared to NSP; however, the yield remained lower than the STD ($P<0.05$) groups. The inclusion of GP and LAB FGP improved the villus surface area of the duodenum compared to the NSP and AGP group ($P<0.05$). Fermented GP reduced the abundance of *Clostridium* and *Klebsiella* and modulated the microbial beta diversity to increase the production of SCFA, namely formate, propionate, and butyrate in ceca ($P<0.05$). These findings suggest that incorporating 0.5% fermented or non-fermented grape pomace into the broiler diet could potentially serve as a novel feed ingredient, an alternative to AGP to improve growth performance, modulate microbial population, and minimize chronic inflammation. However, future research is necessary to understand the possible mechanisms underlying their anti-inflammatory and growth-promoting effects.

Keywords: Grape pomace, fermented grape pomace, non-starch polysaccharide, chronic inflammation, gut function

445P The effect of Tart Cherry Pit Extract (TCPE), at three inclusion rates in feed or water, on growth performance of 1-to-14-day old broiler chicks

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The inclusion of dietary bioactive plant compounds in poultry production has been increasing as the use of antibiotics has been decreasing. Tart cherry pits, a waste product of the industry, contain bioactive compounds. The tart cherry pit extract (TCPE) is abundant in polyphenols and flavonoids that possess antioxidant, antimicrobial, and anti-inflammatory properties. This study was conducted to determine the effect of TCPE on growth performance of 1- to 14-day old broilers. A total of 630 male Cobb 500 broilers were allotted to seven dietary treatments: 1. Control (C, no feed or water additive), 2-4. C+TCPE sprayed on the feed at a rate of 0.5, 1.0, or 1.5%, or 5-7. C+TCPE included in the water at a rate of 0.5, 1.0, or 1.5%. Dietary feed treatments began on d 1 of the trial and water treatments began on day 4. This was a randomized complete block design with nine pens per treatment and 10 birds per pen. As an environmental challenge, broilers were reared in battery cages that were not cleaned after the previous flock was removed. Broilers were fed a standard starter diet for the entire trial. Birds and feed were weighed on days 1, 7, and 14 to calculate average body weight (BW), feed intake (FI), and feed:gain (FG). From day 1 to 14, there were no treatment differences ($P>0.05$) for BW, FI, or FG. Also, there was no effect ($P>0.05$) of treatment on mortality rate. From d 7 to 14, broilers given TCPE in their feed had a greater increase ($P<0.05$) in body weight than broilers given TCPE in their water. On day 7, broilers given TCPE at 1.0% of their feed or water had numerically higher BW (177.24 and 178.21 g/chick, respectively), while those given 0.5% TCPE in their feed had the numerically lowest BW (171.36 g/chick). Chicks fed 1.0% TCPE had the numerically highest BW (429.5 g/chick) on day 14. FC was improved, numerically, for chicks administered TCPE in their water compared to those given TCPE in their feed from d 1-14. For the treatment groups given TCPE in their water, water intake was numerically highest for those given the 1.0% rate (1492 ml/bird) compared to those given 0.5% (1442 ml/bird) or 1.5% (1455 ml/bird) rates. TCPE administered in the feed or water did not have negative effects on broiler growth performance. Future trials should be conducted under a disease or pathogen challenge, as well as a longer grow-out period to determine the efficacy of TCPE antioxidant and anti-inflammatory properties.

Keywords: tart cherry pit extract, antioxidant, anti-inflammatory, polyphenols, broilers

446P Pilot study: Evaluation of the antiprotozoal activity of phytogenic candidates on *Histomonas meleagridis* infection in turkeys

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Histomonosis is a re-emerging protozoal disease critically affecting the turkey industry without commercial treatments available. The purpose of the present study was to evaluate the effect of four phytogenic antiprotozoal candidates on *Histomonas meleagridis*; three monoterpenes (Linalool, Alpha-Terpineol, and Geraniol) and one acetogenins-nanosuspension from *Annona muricata* were screened *in vitro* and *in vivo*. In the *vitro* trial, inclusions of 0.1%, 0.05%, 0.025%, and 0.0125% were tested for the three monoterpenes, while inclusions of 0.01%, 0.006%, and 0.004% were evaluated for the acetogenins-nanosuspension. Viable histomonads were counted 24h post-treatment by triplicate using a hemocytometer. In the *in vivo* experiment, forty-eight Nicholas 700 one-day-old female poults were randomly assigned into four treatments: (1) Positive control, (2) Linalool 0.050%; (3) Geraniol 0.050%; and (4) Acetogenins-nanosuspension 0.01%. All animals were infected on day 10 of age with 100,000 *H. meleagridis* per bird. Each treatment consisted of two replicates with 6-8 poults (n=12-16 poults/treatment). Treatments started to be provided in the drinking water 1 day before challenge. Performance parameters (BW, BGW, FI and FCR) and lesion scores (0 to 3 scale) of the liver and ceca were evaluated at the end of the experiment (28d). The data on performance parameters and *in vitro* viable cells was subjected to a one-way ANOVA in a completely randomized design. The three monoterpenes at 0.1% inclusion caused 100% reduction of histomonads compared to the PC treatment (p<0.0001). Geraniol at 0.05% reduced a 100% viability compared to the rest of the treatments (p<0.0001). Inclusions of 0.025% (p=0.59), and 0.0125% (p=0.36) did not reduce viability. Acetogenins-nanosuspension inclusions of 0.01%, 0.006% and 0.004%, reduce 28%, 24% and 16% histomonads viability respectively (p<0.05). Compared to the PC treatment, no significant improvements were observed in BW, BGW, FI, and FCR in the treated turkey poults. Additionally, no differences in lesion scores were observed in the liver and ceca of turkeys consuming phytogenics (p>0.05). In conclusion, the addition of the evaluated phytogenic candidates did not significantly improve performance parameters or decrease lesion scores. Multiple phytochemical compounds are currently being evaluated in our laboratory as therapeutic or prophylactic alternatives.

Keywords: Turkeys, Histomonas meleagridis, phytogenics, antiprotozoal activity, performance

Immunology, Health and Disease: Health and Disease

447P Not Presented

448P High throughput individualized vaccination improves vaccine take and output in day-old chicks

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Spray cabinet (SC) systems are commonly used for post-hatch mass vaccination of chicks at the hatchery, but they are inadequate because they rely on chicks preening the vaccine off one another, resulting in uneven vaccination. TARGAN has developed a high-throughput vaccination system that delivers vaccine to individual chicks via eye spray. In separate studies, day-old chicks were vaccinated with commercial vaccines for coccidiosis, *Salmonella*, and infectious bronchitis Massachusetts-type using either the individualized vaccination system (TARGAN) or SC and then grown for 7-8 days. Vaccine take and replication were assessed by examining intestinal and cecal contents for *Eimeria* oocysts at 7 days post-vaccination (DPV) for coccidiosis (n=75/group; 1 study), by liver colony counts at 7 DPV for *Salmonella* (n=15/group; 1 study), and by RT-qPCR of oropharyngeal swabs collected at 5 and 8 DPV for infectious bronchitis (n=20/group; 1 study), with statistical analysis performed using t-tests and ANOVA. 81% of TARGAN-vaccinated chicks were positive for *Eimeria maxima* with significantly higher oocyst output, compared to only 33% in the SC group. 100% of TARGAN-vaccinated chicks were positive for *Salmonella* vaccine with significantly higher titers, while 73% of the SC group were positive. Moreover, 100% of TARGAN-vaccinated chicks were positive for infectious bronchitis vaccine by 5 DPV, compared to 80% of SC-vaccinated chicks at that time-point, and although all SC birds became positive by 8 DPV, their vaccine titers remained significantly lower. These results demonstrate that the TARGAN high throughput system not only effectively delivers parasitic, bacterial, and viral vaccines to individual chicks but also significantly improves vaccine take and output.

Keywords: high-throughput vaccination, individualized vaccination, infectious bronchitis vaccine, coccidiosis vaccine, salmonella vaccine

449P Evaluating the Function of *pagP* in avian pathogenic *Escherichia coli* pathogenicity using an embryo challenge model

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Avian pathogenic *Escherichia coli* (APEC) is a leading cause of embryonic mortality and reduced post-hatch performance in poultry due to its ability to vertically transmit from breeding stock to developing embryos. While APEC virulence factors have been extensively studied in post-hatch infections, their role in embryonic infection needs further exploration. The *pagP* gene encodes a lipid A palmitoyl transferase that modifies bacterial outer membrane properties, enhancing resistance to host immune defenses. This study aimed to determine the contribution of *pagP* to APEC virulence in an *in ovo* embryo challenge model. A total of 576 chicken embryos were used for this trial. A strain with *pagP* knockout (APEC Δ*pagP*) was generated from the virulent APEC wild-type strain MS1657 (APEC WT) and inoculated with 0.1 mL of $1x10^3$ CFU/mL into the allantoic fluid on day 12 of incubation. Embryo survival, bacterial colonization, and egg weights were assessed. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test or Kaplan-Meier survival analysis, with significance set at $p < 0.05$.

Survival analysis revealed no significant difference between APEC WT and APEC Δ*pagP* groups ($p > 0.05$), with mortality rates exceeding 50% in both, suggesting *pagP* is not essential for embryonic lethality. The bacterial enumeration indicated APEC Δ*pagP* retained high colonization efficiency in embryonic tissues, yolk, and allantoic fluid, with no significant reduction in CFU counts compared to APEC WT ($p > 0.05$ for all comparisons). The embryo-to-egg weight ratio was significantly lower in the APEC WT group compared to both the APEC Δ*pagP* ($p < 0.05$) and Saline control groups ($p < 0.05$). Additionally, the egg weight ratio showed that the APEC Δ*pagP* group had a significantly higher ratio than the nonpathogenic *E. coli* (NPEC P5) group, suggesting that deletion of *pagP* mitigated the developmental impairment associated with APEC infection. The findings indicate that *pagP* may have minimal contributions to immune evasion in APEC virulence and embryonic infection. Overall, the results suggest that *pagP* contributes modestly to APEC-induced developmental impairment in embryos but is not required for high-level colonization or mortality. These findings highlight the multifactorial nature of APEC virulence and underscore the need to explore additional genetic targets for intervention strategies such as vaccine development or genetic attenuation.

Keywords: Colibacillosis, Lipid A palmitoyl transferase (pagP), Embryo Challenge Model, Vaccine candidate, Virulence Factors

450P Molecular characterization of hydrogenosomal iron superoxide dismutase in *Histomonas meleagridis*

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Histomonas meleagridis, is a parasitic protozoan and the etiological agent of histomonosis or blackhead disease, which has a mortality rate in turkeys up to 80-100%. No approved prophylactic or therapeutic option for histomonosis currently exists due to the ban of arsenic antibiotic in poultry. Therefore, novel drug therapies for histomonosis is urgently needed. However, the lack of knowledge of the biology and transmission of *H. meleagridis* limits this development. A notable feature of *H. meleagridis* is its reliance on a hydrogenosome rather than mitochondria for energy production, likely an adaptation to its anaerobic or microaerophilic growth conditions, a trait shared by other eukaryotic pathogens such as *Trichomonas vaginalis*. Since birds do not have hydrogenosomes, targeting this organelle serves as a promising strategy to treat *Histomonas* infections. In this study we sought to target a specific enzyme in the hydrogenosome for the purpose of developing treatment for histomonosis. Preliminary transcriptome studies showed a high transcription level of a putative iron superoxide dismutase (Fe-SOD), an enzyme largely found in the hydrogenosome. Because of the central role of Fe-SOD in oxidation of superoxide radicals into hydrogen peroxide, inhibition of Fe-SOD is considered as a potential therapeutic strategy. In addition, it also serves as a marker for the hydrogenosome. To test its subcellular location and function, the Fe-SOD of *H. meleagridis* was recombinantly expressed in *E. coli* and purified through FPLC under native conditions using immobilized metal ion affinity chromatography (IMAC). Recombinant *Histomonas* Fe-SOD (HmFe-SOD) was then used to immunize rabbits to generate polyclonal antibodies. Immunoblotting analysis showed that the yielded antibodies recognized the native iron superoxide dismutase of *H. meleagridis*. Additionally, immunofluorescence analysis revealed that the antibody produced a punctate staining pattern within the parasites, similar to the previously observed hydrogenosome staining. Future work will investigate whether HmFe-SOD is localized on the parasite surface or secreted by the parasites, as it is hypothesized to mitigate the host's oxidative burst.

Keywords: Turkeys, Molecular Parasitology, Histomonas meleagridis, Hydrogenosome, Superoxide Dismutase

Immunology, Health and Disease: Immunology

451P Evaluation of a novel electron beam-inactivated vaccine against avian pathogenic *Escherichia coli* in chicken

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Avian pathogenic *Escherichia coli* (APEC) is a leading cause of colibacillosis, a bacterial infection in poultry that results in significant economic losses. Control of APEC has been hindered by the wide genetic diversity among infectious strains rendering conventional vaccines incapable of broad cross-protection. Therefore, there is a need for more effective control strategies. This study examines the immunogenicity and protective efficacy of a newly developed electron beam-inactivated (EBI) vaccine targeting APEC. The vaccine was prepared by exposing an overnight culture of the APEC strain MS1685 to a 6.216 kGy electron beam irradiation dose. The prepared vaccine was stored at 4°C for less than 2 weeks before the study. A total of 160 Hy-Line W36 layer pullets (14 weeks old) were assigned to four groups: (1) Control (non-vaccinated, non-infected), (2) EBI Alone (vaccinated, non-infected), (3) APEC Alone (non-vaccinated, infected), and (4) EBI APEC (vaccinated, infected). After 7 days of acclimation, vaccinated groups ($n = 80$) received 500 µL EBI vaccine intramuscularly (250 µL per breast), while controls received PBS. At 21 days post-vaccination, infected groups were challenged intra-tracheally with APEC strain MS1685 (2.496×10^6 CFU/mL), and controls received PBS. At 2-, 4-, and 7- days post-inoculation (DPI), eight birds per group were randomly sacrificed. Lesion scoring assessed disease severity, and indirect ELISA measured APEC-specific antibody responses to evaluate vaccine efficacy. Lesion score data were analyzed Kruskal-Wallis test followed by Dunn's test with Bonferroni correction, and ELISA antibody titers were analyzed one-way ANOVA followed by Tukey's post-hoc test across groups at each DPI using R (Version 2024.12.1). Statistical significance was determined at $p \leq 0.05$. Our results indicated that birds in the APEC alone group developed severe lesions over time, whereas the EBI APEC group exhibited a measurable immune response but failed to prevent disease formation following challenges with the parent strain. The Control and EBI alone groups remained largely unaffected. ELISA results demonstrated a progressive increase in antibody titers, with the EBI APEC group exhibiting the highest expression at 7 DPI. Pairwise statistical analysis revealed significant differences in both lesion scores and antibody responses among groups, particularly between vaccinated and non-vaccinated birds following APEC infection. These findings indicated that while the EBI vaccine elicited an immune response, it did not provide full protection

against disease caused by the parent APEC strain. Further optimization is needed to enhance its protective efficacy and evaluate its potential under field conditions.

Keywords: APEC, Electron beam-inactivated vaccine, Poultry immunology, Colibacillosis, Antibody response

452P Assessing cell-type population differences in the jejunum of uninfected and coccidia-infected BQ and B19 chickens using spatial and single-cell transcriptomics

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The cellular and molecular mechanisms underlying MHC-I haplotype-associated resistance and susceptibility to coccidia infection remain poorly defined. This study used spatial and single-cell transcriptomics to characterize the immune, stromal, and endothelial landscape of the jejunum during active coccidia infection in an experimental chicken line selectively bred for differential disease resistance (MHC-I BQ-resistant and B19-susceptible haplotypes). We hypothesized that resistance reflects differences in immune cell regulation and intestinal microenvironmental interactions by haplotype. Chickens were bred at West Virginia University, and fertile eggs were shipped to Western University of Health Sciences. Birds were PCR-sexed, genotyped, and assigned to infected or non-infected groups (n=5 per group). Infection was induced at 14 or 28 days of age using Coccivac-D2 (Merck). Seven days post-infection, body weight, jejunal lesion scores, and tissues were collected. Trial 1 utilized fresh-frozen jejunum tissue for spatial transcriptomics (n=4 per haplotype and treatment), while Trial 2 used enzymatic dissociation of jejunal tissue for single-cell RNA sequencing (n=4 per haplotype and treatment). Lesion scores were higher in B19+inf birds, with most showing moderate (score 2) to severe (score 3) lesions, whereas BQ+inf birds had fewer affected individuals and lower scores overall (Mann-Whitney U test, $P > 0.05$). Spatial transcriptomic data were aligned to the broiler genome using SpaceRanger, spatially trained with STAGATE, clustered using Leiden_Voronoi, and annotated with prior cell transcriptomes of chicken and the Tabula Sapiens single-cell datasets as references. Infected birds exhibited increased fibroblast, CD4+, CD8+, and NK cell populations, with a reduction in pericytes and capillary endothelial cells (Two-way ANOVA, Tukey post-hoc, $P < 0.05$). A higher baseline of capillary endothelial cells was observed in B19 birds compared to BQ (Two-way ANOVA, Tukey post-hoc, $P < 0.05$), suggesting pre-existing vascular differences. During infection, significantly elevated CD8+ cell numbers were detected in B19+inf birds compared to BQ+inf birds (Two-way ANOVA, Tukey post-hoc, $P < 0.05$), potentially contributing to increased tissue destruction and more severe disease pathology. Ongoing single-cell analysis with CellRanger will further elucidate gene pathways, cell interactions, cellular heterogeneity, and lineage trajectories across intestinal layers, including the identification of *Eimeria*-infected cells. Together with spatial data, these findings are expected to inform future efforts to enhance disease resistance through targeted breeding strategies, with benefits for flock health and production efficiency.

Keywords: Coccidiosis, MHC-I, spatial transcriptomics, single-cell RNA sequencing, immune response

453P Development and validation of a TaqMan-based multiplex qPCR assay for profiling pro-inflammatory cytokine responses in poultry infected with avian pathogenic *Escherichia coli* (APEC)

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Avian pathogenic *Escherichia coli* (APEC) infection induces a complex immune response, highlighting the need for accurate and efficient methods to analyze cytokine gene expression. In this study, we developed and validated a TaqMan-based multiplex quantitative PCR (qPCR) assay targeting four key cytokine genes—*IFN-γ* (FAM), *IL-1β* (JUN), *IL-8L1* (ABY), and *TNF-α* (VIC)—with *18S rRNA* (FAM) serving as the housekeeping gene. Each probe was labeled with a distinct fluorophore and paired with QSY quenchers to ensure high specificity. To evaluate assay performance, serial dilutions of each target gene-harbored recombinant plasmid DNA were used to generate standard curves for *IFN-γ*, *IL-1β*, *IL-8L1*, and *TNF-α* in both singleplex and multiplex qPCR, whereas *18S rRNA* was assessed exclusively in singleplex qPCR. Additionally, reverse-transcribed RNA from APEC-infected lung tissue or HD11 cells was used to generate standard curves only in singleplex qPCR, with the RNA source selected based on gene expression levels to ensure feasible serial dilutions. Across all conditions, all targets demonstrated amplification efficiencies within the optimal range (90–110%) with R^2 values exceeding 0.98, confirming assay reliability. Further validation of the multiplex assay was conducted using lung tissue RNA from APEC-infected chickens at 1 dpi and 3 dpi, with results compared between singleplex and multiplex qPCR. Gene expression was quantified using the ΔΔCt method, and statistical significance was assessed using the Mann-Whitney U test in SAS 9.4. The results indicated that *IL-1β* and *IL-8L1* were significantly upregulated at 1 dpi ($P < 0.05$) but not at 3 dpi ($P > 0.05$), suggesting an early peak in expression followed by downregulation. In contrast, *TNF-α* and *IFN-γ* did not exhibit statistically significant differences at either time point ($P > 0.05$), indicating more moderate expression changes or greater individual variability. Notably, the trends of gene expression regulation between the APEC-infected and control groups remained consistent across singleplex and multiplex assays, with no changes in statistical significance, further confirming the multiplex assay's accuracy. This TaqMan-based multiplex qPCR assay provides a robust, efficient, and accurate tool for profiling APEC-induced pro-inflammatory cytokine responses in poultry. While lung tissue was used for validation, the assay is broadly applicable for studying immune responses across different tissues, enabling comprehensive investigations of APEC pathogenesis and host immune dynamics.

Keywords: Multiplex qPCR, Cytokine gene expression, Avian pathogenic Escherichia coli (APEC), Assay validation

Metabolism and Nutrition: General Nutrition

454P Comparison of analytical methods for measuring trypsin inhibitor activity in soybean products

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Trypsin inhibitor (TI) is a significant antinutritional factor in soybean products, as elevated trypsin inhibitor activity (TIA) can hinder nutrient digestibility and contribute to gut health challenges in poultry by binding to trypsin and obstructing its digestive functions. Effectively managing TIA in soybean products and complete feed is crucial for optimizing growth and production in poultry. This study compares four analytical methods for measuring TIA, including two wet chemistry, a near-infrared (NIR) spectroscopy technique and Urease Index as indicator of under-processing of soybean products to aid nutritionists in interpreting results and correlating them with poultry performance. A total of 27 soybean samples were collected, comprising 13 mechanical-extracted soybean meal, 2 raw soybeans, and 12 solvent-extracted soybean meal samples sourced from the US and Canada. The samples were analyzed using two wet chemistry methods (AOCS Ba 12a-2020 and USDA-ARS rapid method) and NIR prediction (AMINONIR® RED, Evonik Operations GmbH). 21 out of the 27 samples underwent Urease Index analysis (pH increase). Multivariate analysis using JMP 17 was employed to assess correlations among the results from each method. The findings revealed strong correlations ($P < 0.05$) among NIR prediction, AOCS Ba 12a-2020, and the USDA-ARS rapid method, with R square values of 0.95 between NIR and both AOCS and USDA-ARS methods, and an R square of 0.99 between AOCS and USDA-ARS. In contrast, no significant correlations ($P > 0.05$) were observed between the Urease Index and the other analytical methods. These results suggest that NIR prediction via AMINONIR® RED is a viable alternative for assessing TIA in soybean products, providing poultry producers with timely monitoring capabilities. Conversely, the Urease Index may not accurately represent TIA levels in soybean products.

Keywords: trypsin inhibitor activity, urease index, near-infrared spectroscopy, solvent-extracted soybean meal, mechanical-extracted soybean meal

455P Potential of sow spleen as a sustainable immune and growth promoter for young broiler chickens

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Young broiler chickens, with an immature immune system, are vulnerable to stress and diseases. Spleen, as a central organ processing blood and immune cells, contains immune-protective bioactives that may enhance the health and growth of young broilers. This study aimed to evaluate the potential of sow spleen as a growth promoter in broiler chickens by evaluating the effects of supplementation on growth performance, organ weight, and blood cytokines. Spleen was harvested from 20 sows, freeze-dried, and ground into a powder form. A total of 480 one-day-old male chicks [Cobb500, average body weight (BW): 41g] were allotted to 60 battery cages (8 birds/cage) based on BW. The feeding in the first week (from d 0 to 7) was conducted under a randomized complete block design in 5 treatments (12 replicates/treatment), including a corn-soybean meal-based basal diet as the negative control (NC), the supplementation of a yeast cell wall product as the positive control (PC), and the replacements of corn ingredient with 0.5%, 1.0%, and 1.5% sow spleen (S0.5, S1.0, and S1.5). Afterwards, all chicks were fed the same corn-soy diet from d 8 to 21. All diets were formulated to meet broiler nutrient recommendations and were provided in mash form. At the end of each week, both BW and feed leftovers were measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). On d 7 and 21, two birds per cage closest to the mean BW were euthanized to collect organ (liver, spleen, and bursa of Fabricius) and blood samples. Data were analyzed using ANOVA via the MIXED procedure of SAS, with cage as the experimental unit. The model included treatment as a fixed effect and block as a random effect. Linear and quadratic contrasts were performed to assess the dose-dependent effects of spleen supplementation. Increasing dietary sow spleen levels linearly improved (P<0.05) FCR from d 0 to 7, with the 1.0% inclusion group (S1.0) tending toward a lower (P=0.06) FCR compared with NC and PC. Mortality rates in the S0.5, S1.0, and S1.5 (3%, 1%, and 1%) groups were numerically lower than the NC (4%), while the relative weights of the spleen, bursa of Fabricius, and liver on days 7 and 21were not affected by sow spleen. Moreover, sow spleen linearly reduced (P<0.05) serum interleukin-16, a pro-inflammatory cytokine, on d 7, making its levels in three spleen treatments, as well as in the PC, lower than that in the NC(P<0.05). In conclusion, supplementing sow spleen in starter diet improved growth performance and immune profile of young broiler, warranting more studies for validation and mechanistic insights on its potential as a sustainable immune promoter in poultry production.

Keywords: Sow spleen, immune promoter, Broiler chickens, Feed conversion ratio, Cytokine response

456P Effect of cyclic heat stress and bird strains on performance and egg quality in laying hens

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The objective of this study was to evaluate the simple and interactive effects of cyclic heat stress and bird strain on laying hen performance and egg quality. A total of 240 fifteen-wk-old pullets (120 brown and 120 white) were assigned to treatment in a randomized complete block design in a 2 (white vs. brown hens) × 2 (thermoneutral vs. cyclic heat stress) factorial arrangement with 6 hens per replicate and 10 replicates per strain in each of the two rooms. All birds were fed the same corn-soybean meal-based diet that met or exceeded their energy and nutrient requirements throughout the study. All hens were maintained at 23.9 °C throughout the study, except for those in the cyclic heat stress group, which were exposed to 33.9 °C for 8 hr per day (8:00 AM to 4:00 PM, Monday to Friday). Daily egg production, feed intake, and average egg weight between 17 and 25 wk of lay were determined. These data were used to calculate average daily feed intake (ADFI), hen-day egg production (HDP), and feed conversion ratio (FCR). Egg quality response variables measured include egg weight, percent yolk, percent albumen, percent dried shell weight, eggshell breaking strength, albumen height, Haugh unit, yolk color, and shell thickness. Data were analyzed using the MIXED procedure of SAS, appropriate for a factorial arrangement of treatments. No significant 2-way interaction was observed. At wk 17 and 25, ADFI and FCR were higher ($p < 0.05$) in the brown strain. Hen-day egg production was higher ($p < 0.05$) in brown hens at wk 17. Heat stress reduced ($p < 0.05$) ADFI at both ages evaluated but had no effect on hen-day production. For the egg

quality, the percent albumen was higher ($p < 0.05$) in the brown strain, while the percent yolk was higher ($p < 0.05$) in the white hen at both ages. Heat stress decreased ($p < 0.05$) the albumen height and egg weight at wk 17 and increased ($p < 0.05$) the eggshell thickness at wk 25. In conclusion, breed differences influenced performance and egg quality, with brown hens showing higher feed intake but lower feed efficiency and a greater percent albumen, while white hens consistently had higher percent yolk at wk 17 and 25. Heat stress decreased ADFI at both ages evaluated and increased shell thickness at wk 25, indicating a disruption in feed intake pattern and eggshell integrity under prolonged heat exposure.

Keywords: cyclic heat stress, egg quality, feed conversion ratio, laying hen strain, performance

457P Flaxseed oil improves growth performance and modulates liver metabolomics in broiler chickens

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The degree of fatty acid saturation impacts fat digestibility and the metabolizable energy content of dietary fat. Both saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) undergo mitochondria β-oxidation to produce energy, but UFAs, especially polyunsaturated fatty acids (PUFAs), are more efficiently oxidized. The liver is the main organ for fat metabolism, regulating lipid oxidation, transport, and storage, directly impacting broiler growth and efficiency. It is therefore important to understand how different fat types affect liver metabolism. Thus, this study aimed to investigate the impact of dietary fat composition on liver metabolomics and the growth performance of broilers. In a 3-week experiment, day-old broiler chicks (n = 240, Ross male) were weighed and randomly assigned to 3 dietary treatments, consisting 5 replicate pens of 16 chicks each. The experimental treatments included a basal diet corn-soybean meal with a 3% dietary inclusion of 3 fat types; Poultry fat (CON), Olive oil (OLIV), and Flaxseed oil (FLAX). Growth indices: feed intake (FI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) were recorded and analyzed using One-way ANOVA. Liver samples were collected and subjected to the HPLC-MS metabolomics approach. KEGG and HMDB databases were used to annotate the metabolites, and metaboanalyst 6.0 was used to identify significantly ($P< 0.05$) differential metabolites among treatments using ANOVA. Dietary fat type had significant ($P<0.05$) effect on BW, BWG and FCR, with FLAX having the highest BW (0.9123), and BWG (0.8724), and best FCR (1.1582) compared to CON and OLIV. Out of the 1115 annotated metabolites, twelve (12) metabolites were significantly (FDR < 0.05) upregulated in FLAX. Liver metabolome analysis revealed the upregulation of Eicosapentaenoate (EPA), Lysophosphatidylcholine (LPC) 18:3, Linoleoyl ethanolamide, and sn-Glycero-3-phosphocholine, which play key roles in the reduction of abdominal fat, enhancement of energy consumption, and anti-inflammatory function. Similarly, 6-Hydroxy-3-oxo-1-benzofuran-2(3H) and Tetrahydrochromeno[3,4-b]chromene, known for their antioxidative, antimicrobial, and anti-inflammatory activities, were also upregulated. These metabolic alterations indicate that FLAX modulates hepatic metabolism by promoting lipid oxidation, enhancing energy utilization, and potentially mitigating oxidative stress and inflammation. This study highlights the prospect of flaxseed oil as a nutritional strategy to improve broiler performance and health.

Keywords: Flaxseed oil, fat metabolism, Liver metabolomics, Growth performance

458P Influence of the origin of the soybeans on the composition of the carbohydrate fraction of soybeans meal arriving to European ports

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We studied the composition of the carbohydrate fraction of 27 soybean meals (SBM) from Argentina (ARG), Brazil (BRA), and USA (9 per origin) randomly collected from European crushing plants and feed mills during the 2022/2023 harvest season. The samples were analyzed for sucrose, stachyose, raffinose, total sugars, starch, crude fiber (CF), neutral detergent fiber (NDF), and non-starch polysaccharides (NSP) content in the same laboratory using official methods. The average CP content of all the samples, on 88% DM bases was 46.0 ± 0.97%. Data were analyzed as a completely randomized design by ANOVA, with SBM origin as the main effect. Sucrose (7.93 and 7.26 vs. 6.08%), stachyose (4.96 and 5.41 vs. 4.01%), total sugars (10.3 and 9.30 vs. 8.82%), and starch (0.64 and 0.72 vs. 0.42%) contents were higher for the ARG and USA meals than for the BRA meals ($P < 0.001$). In contrast, NDF (9.95 vs. 8.51 vs. 9.07%), and soluble (4.31 vs. 4.01 vs. 4.23%) and total (16.0 vs. 14.9 vs. 15.7%) NSP contents were higher ($P < 0.05$) for the BRA meals than for the ARG meals, with USA meals being intermediate. However, no differences were detected for CF and insoluble NSP contents. The differences in sugar composition detected in the NSP fraction were numerous, with higher contents in glucose (3.84 and 3.89 vs. 3.11%), xylose (1.39 and 1.33 vs. 1.20%), and mannose (1.10 and 1.12 vs. 0.89%) for the BRA and USA meals than for the ARG meals ($P < 0.01$), whereas, the ARG meals had higher contents in galactose (4.40 vs. 4.14 and 4.04%) and fucose (0.32 vs. 0.31 and 0.30%) than for the BRA and USA meals ($P < 0.001$). A similar composition was detected for the insoluble NSP fraction, with BRA and USA meals had more glucose (3.66 and 3.64 vs. 2.92%; $P < 0.01$) and mannose (0.55 and 0.57 vs. 0.39%; $P < 0.05$) than the ARG meals. In contrast, the ARG meals had more galactose (3.23 vs. 2.97 and 2.88%) and fucose (0.23 vs. 0.22 and 0.21%) contents than BRA and USA meals ($P < 0.001$). Xylose content was higher (1.04 vs. 0.86 vs. 0.98%; $P < 0.01$) for the BRA meals than for the ARG meals, with the USA meals being intermediate. For the soluble NSP fraction, mannose content was higher for the BRA and USA meals than for the ARG meals and glucose was higher for the USA meals than for the ARG and BRA meals, and the galacturonic acid was higher in the BRA meals than in the ARG and USA meals. In conclusion, the carbohydrate composition of the SBM varies according to the origin of the beans. Carbohydrates plays a crucial role, still not well investigated, on physiological and nutritional aspects of poultry nutrition. Consequently, new studies are needed on the carbohydrate fraction of the SBM because of its significant interest for poultry feeding.

Keywords: carbohydrate fraction, non-starch polysaccharides, oligosaccharides, soybean meal origin, sugars

459P Determining the nutrient and anti-nutrient variability in Asia-sourced barley and canola meal

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Poultry diet constituents vary globally based on feed ingredient availability. Understanding alternative feed ingredients' nutrient and antinutrient composition and variability is essential to optimize diet formulations. This study aimed to characterize nutrient and antinutrient compositions of Asia-sourced barley from China (N = 700) and canola meal from India (N = 755) and determine its variability. Two commercial datasets, obtained from ABVista, containing user-submitted near-infrared spectroscopy (NIRS) readings, were analyzed. The nutrient and antinutrient distributions of each ingredient were modeled using JMP 18, and the data were found to be best represented as mixtures of normal distributions. For each ingredient, samples were clustered into three groups (C1, C2, C3) with similar nutrient profiles using the Normal Mixtures platform, based on their proximate nutrient compositions, with all compositions expressed on an 88% DM-basis. One-way ANOVA was applied to determine statistical differences between the clusters, and Tukey's HSD was used for pairwise mean separation. Barley C2, with 38.4% of total samples, exhibited the lowest ($P < 0.001$) starch (49.84%) and phytic P (0.20%) but the highest ($P < 0.001$) total arabinose + xylose (**A+X**; 7.02%), and higher total glucose (5.25%) and fat (2.74%) similar to C3 (5.16 and 2.71%, respectively). Additionally, C2 had higher ($P < 0.001$) fiber fractions for lignin (2.03%), total acid detergent fiber (**ADF**), and neutral detergent fiber (**NDF**). Both C1 (37% of samples) and C2 barley had the highest total non-starch polysaccharide (**NSP**) content (12.88%). Whereas C3 (24.6% of samples) had higher ($P < 0.001$) starch (51.49%), similar to C1 (51.72%), and crude protein (**CP**; 10.90%). For canola meal, C1 (62.38% of samples) had the highest ($P < 0.001$) total A+X (7.17%), total glucose (6.79%), and total NSP (19.32%), and NDF (26.76%), as well as the lowest ($P < 0.001$) CP (37.58%), fat (3.02%), and ADF (16.16%) content. In contrast, C2 (7.95% of samples) had the highest ($P < 0.001$) CP (38.24%), fat (3.67%), and crude fiber (11.03%), coupled with the highest ADF (17.76%), phytic P (0.69%) and lignin (10.34%). Canola meal C3, representing 29.67% of samples, had an intermediate profile between C2 and C3, but the lowest ($P < 0.001$) phytic P (0.60%). In summary, the distinct nutrient and antinutrient profiles observed for barley and canola meal clusters emphasize the importance of understanding ingredient composition. Characterization of composition profiles allows for segregation of feedstuffs, determining additional feed processing, matrixing these alternative ingredients for feed formulation, and deciding on optimum enzyme application, when seeking more precise nutrition.

Keywords: canola meal, barley, NIRS, nutrient, antinutrient

460P Influence of the metabolizable energy and amino acid content of the diet on growth performance and fecal biomarkers in broilers from 1 to 35 days of age

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The effects of the apparent metabolizable energy (AMEn) content and the standard ileal digestible Lysine (DLys) level of the diet on growth performance and intestinal health were studied in broilers from 1 to 35 d of age. The experimental design was completely randomized with four treatments arranged as a 2 × 2 factorial with AMEn content and level of DLys of the diet as main effects. The diets were based on corn, wheat and soybean meal, and contained 2,975 vs. 3,075 kcal AMEn/kg and 1.25% vs. 1.05% DLys from 1 to 21 d and 2,900 vs. 3,000 kcal AMEn/kg and 1.05% vs. 1.00% DLys from 22 to 35 d. All other amino acids were formulated according to the ideal protein concept. Each treatment was replicated 16 times, and the experimental unit was a pen with 22 chicks. BW gain, feed and energy (EI; kcal AMEn/day) intake, feed intake (FI), feed conversion ratio (FCR), and energy conversion ratio (ECR; kcal AME/g BWG gain) were measured by feeding phase and cumulatively. In addition, three fecal health biomarkers (ovotransferrin, calprotectin, and aminopeptidase) were analyzed in the colonic content of one broiler per replicate at 21 d of age. From 0 to 21 d of age, a reduction in the AMEn of the diet decreased EI (176 vs. 178; kcal/d; $P < 0.05$) and impaired FCR (1.138 vs. 1.117 g/g; $P < 0.01$) but tended to improve ECR (3.30 vs. 3.32 kcal/g; $P = 0.06$). Also, a reduction in the DLys content of the diet reduced FI (59.7 vs. 60.7 g/d; $P < 0.05$), BW gain (52.7 vs. 54.2 g/d; $P < 0.001$), and EI (175 vs. 178 kcal/d; $P < 0.05$), resulting in an impairment of the FCR (1.134 vs. 1.120 g/g) and ECR (3.33 vs. 3.29 kcal/g) ($P < 0.001$). From 22 to 35 d of age, FI (170 vs. 165 g/d; $P < 0.05$) increased when the AMEn content of the diet decreased whereas a reduction in DLys ($P < 0.05$) impaired FCR (1.547 vs 1.524 g/g) and ECR (4.78 vs 4.63 kcal/g) ($P < 0.05$). From 1 to 35 d of age, a reduction in the AMEn of the diet increased FI (104 vs. 102 g/d; $P < 0.05$) and tended to impair FCR (1.381 vs. 1. 357 g/g; $P = 0.07$) whereas a reduction in DLys impaired ($P > 0.01$) FCR (1.386 vs. 1.352 g/g) and ECR (4.16 vs. 4.06 kcal/g). However, no effects were observed on BW gain for the AMEn content of the diet. In addition, a reduction in AMEn increased ovotransferrin levels (1,586 vs. 914 ng/g; $P<0.01$) whereas an opposite effect was observed with a reduction in the DLys (992 vs. 1,490 ng/g; $P< 0.05$) content of the diet. In conclusion, a reduction of 100 kcal in the energy content of the diets increased FI and FCR without affecting BW gain but increased the concentration of ovotransferrin. In contrast, an increase in DLys improved BW gain, FI, FCR, and ECR, also showing an increase in ovotransferrin concentration of the colonic content.

Keywords: broiler, digestible lysine, intestinal biomarker, metabolizable energy, productive performance

461P Effect of split feeding on nutrient digestibility in Bovans White laying hens

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Conventional feeding systems in laying hens often fail to meet nutritional demands during egg formation, leading to excessive nutrient intake. Split feeding can improve nutrient utilization, making it essential to assess nutrient digestibility. This study evaluated the effect of conventional feeding (CFE) versus split feeding (SFE) on the apparent digestibility (AD) of nitrogen (N), energy (E), calcium (Ca), and phosphorus (P) in Bovans White hens. Sixty-week-old hens, were randomly assigned to two treatments (CFE and SFE), each consisting of 15 replicates with two hens per replicate. The CFE was supplied twice daily (morning and afternoon) and contained 2750 kcal of metabolizable energy (ME)/ kg of feed, 16.5% crude protein (CP), 4.0 % Ca, and 0.3 % available phosphorus (AP). In the SFE, the morning diet (MDi) provided 2950 kcal ME/ kg, 19.5 % CP, 2.0 % Ca, and 0.3 % AP, whereas the afternoon diet (ADi) included 2600 kcal ME/ kg, 5.5 % CP, 5.38 % Ca, and 0.16 % AP. The AD of N, Ca, E, and P was assessed at 60, 68, and 76 weeks of age. Titanium dioxide was used

as an indigestible marker (3 g/ kg) in the experimental diets for eight days, consisting of a five-day adaptation period followed by three days of feces collection. A completely randomized experimental design with a 2×3 factorial arrangement was applied using the SAS Mixed procedure, evaluating two treatments (CFE and SFE) across three periods (60, 68, and 76 weeks of age). AD of N and Ca was significantly influenced by a feeding system × age interaction ($P \leq 0.01$). The SFE treatment resulted in higher ($P \leq 0.05$) N digestibility (60.94 %, 53.96 %, and 53.03 %) compared to CFE (46.90 %, 49.56 %, and 49.98 %) at 60, 68, and 76 weeks, respectively. AD of Ca was higher at 60 weeks ($P \leq 0.05$) with split feeding (SFE; 57.40 %) than with conventional feeding (CFE; 45.60 %), but no significant differences were found at 68 and 76 weeks ($P \geq 0.05$). There was no interaction effect ($P \geq 0.01$) on AD of E and P. However, digestibility of both E and P was significantly higher ($P \leq 0.05$) in the SFE system than in the CFE one, with values of 81.53 % *vs* 79.35 % for E, and 50.93 % *vs* 41.33 % for P, respectively. In conclusion, the findings indicate that the split feeding system enhances digestibility of nitrogen, calcium, energy, and phosphorus. This leads to greater dietary efficiency in laying hens, decreased environmental impact, and increased economic benefits.

Keywords: laying hens, split feeding, apparent digestibility, metabolizable energy, crude protein

462P Gompertz-Laird model prediction of optimum utilization of arginine by the French Guinea fowl broilers

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This study was conducted to assess the influence of dietary arginine on growth parameters of the French Guinea fowl (FGF), a meat-type variety. Using BW data from hatch to 7 wk, the Gompertz-Laird nonlinear mathematical function was employed to estimate growth patterns of the FGF. In five replicates, 200 one-day-old French guinea keets were randomly assigned and fed standard guinea fowl diets containing 0.98, 1.10, 1.22 and 1.30% arginine from hatch to 7 wk. There were 50 birds per dietary treatment and 10 birds per replicate. Birds were weighed weekly and feed and water were provided at free choice for the 7 wk period. Mean instantaneous growth rate, weight at inflection point and asymptotic BW was higher in FGF fed diets containing 1.10, 1.22 and 1.30 % arginine than those fed the 0.98% arginine diet. However, mean differences in exponential growth rate and age of maximum growth were not significant among the arginine concentrations. Therefore, based on the Gompertz-Laird growth model estimates, feeding diets containing 1.10-1.22% arginine seem to be ideal for optimal growth of the French guinea fowl broilers.

Keywords: French guinea fowl, Gompertz-Laird model, Arginine, Growth models, Poultry Performance

Student Competition: Metabolism and Nutrition: Enzymes

463P The effects of feeding a xylanase – beta-glucanase combination on growth performance and energy efficiency in broilers fed a complex diet

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This study evaluated the effects of supplementing a NSPase (a combination of xylanase and beta-glucanase) to a complex diet on growth performance and energy efficiency in male broilers raised to 35 days of age. A complete randomized design was used to test 3 dietary treatments, each containing 10 replicate groups consisting of 30, day of hatch, Ross 308 broilers (260 birds/treatment). The treatments were: 1) A nutrient adequate diet containing higher corn content, wheat, barley, rapeseed meal, and sunflower meal (PC; formulated at ME 2950, 3025, 3100 kcal/kg and digestible Lys at 1.22, 1.11, 0.99% for starter, grower, and finisher respectively); 2) A diet containing high amounts of wheat, barley, rapeseed meal, sunflower meal and rye with 6% ME and 2% digestible AA reduction vs PC (NC); 3) The NC diet supplemented with XB to provide 2440 U/kg xylanase and 304U/kg beta-glucanase (XB). Body weight (BW), body weight gain (BWG), and feed intake (FI) were measured while feed conversion (FCR) and metabolizable energy conversion (MECR) was calculated. Data were analyzed using R 4.4.0 using one-way ANOVA and Tukey HSD means comparisons. On d21, PC (907g) and XB (881g) had heavier BW compared to the NC (824g; $P<0.05$). On d35, PC (2341g) and XB (2253g) were greater compared to NC (2146g; $P<0.05$). On d21, PC (1.39) and XB (1.43) showed significantly lower FCR than NC (1.64, $P<0.05$). Overall, from 0-21d, MECR was significantly lower in PC (3.99 kcal/g BWG) and XB (3.85 kcal/g BWG) than NC (4.28kcal/kg BWG; $P<0.05$). These results illustrated that the use of xylanase and beta-glucanase combination in the diet can increase energy efficiency needed in a complex diet containing high amounts of fiber rich alternative ingredients and a reduced ME and digestible AA. The NSPase allows for the use of more alternative, locally available high fiber ingredients to improve sustainability.

Keywords: Broiler, NSPase, Growth, Energy

Student Competition: Metabolism and Nutrition: Feed Additives

464P Evaluating the impact of a novel Inulin product on 21 day broiler performance

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The poultry industry utilizes prebiotics and probiotics in diets, such as inulin, to reduce the use of antibiotic growth promoters and benefit the immune system and gut microbiota of birds. However, research on its impact on broiler performance remains limited. The objective of this study was to evaluate the effects of an inulin product on broiler performance during a 21-day grow out period. Four hundred and fifty-five Ross 708 male broilers were distributed in 65 cages (7 birds/pen) in an experimental cage house in a randomized complete block design. A mash diet was allocated into 5 treatments, and 3 of the treatments had an inclusion dosage of inulin. This study consisted of the following 5 treatments: Positive Control (PC), Negative Control (NC), NC + 1% inulin inclusion, NC + 2% inulin inclusion, and NC + 3% inulin inclusion. The PC diet was formulated to meet 100% breeder amino acid (AA) recommendation, whereas the NC diets were formulated to meet 85% breeder AA recommendation. Birds and feed were weighed on day 0,7, 14 and 21 days to obtain data to calculate bird feed intake, bird body weight (BW), bird live weight gain (LWG), feed conversion ratio corrected for mortality

(FCRm), and mortality. Statistical analysis was obtained by utilizing SAS 9.4 one-way ANOVA. Significance was set at $\alpha \leq 0.05$. On day 7, there were no significant differences observed from any performance variables. On day 14, the PC treatment demonstrated a significantly lower FCRm than the other treatments ($P < 0.0001$). Significant differences were observed on day 21, with the PC treatment demonstrating a significantly higher bird feed intake ($P = 0.0177$), bird BW ($P = 0.0093$), and bird LWG ($P = 0.0057$), but no significant differences were detected for day 21 FCRm. For the overall study period, the PC treatment had a significantly higher bird BW ($P = 0.0093$) and bird LWG ($P = 0.0100$) than other treatments. Significant differences were observed for the FCRm, with the PC having a significantly lower FCRm than the NC ($P = 0.0121$). The NC + 3% treatment demonstrated a similar FCRm to the PC, NC + 1%, and NC + 2% treatments. No significant differences were detected for bird feed intake or percentage mortality during the overall study period. This study demonstrates that although the PC treatment was smaller from day 0-7, the PC treatment was the largest, numerically on days 14 and 21. From the results of this study, improvement in performance from treatments with the inclusion of inulin was not observed. Future research in this area is needed as the effects of inulin depends on the diet composition, individual animal characteristics, and environmental conditions.

Keywords: Feed, Feed Additives, Prebiotics, Inulin, Broiler

465P Evaluation of supplementing different phytogenic substances on broiler growth and gut health to 35 days of age

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This study evaluated the effect of supplementing different phytogenic substances in the feed on the performance and gut health of broiler chickens reared to 35 days of age. A complete randomized design was used to distribute 12 dietary treatments, each consisting of 10 replicate pens and 20 male cobb 500 day-of-hatch chicks/pen. The treatments were: Control (T1, no supplementation); Betaine (0.2 % inclusion); Green Tea Extract (0.5% inclusion); Grape Seed Extract (0.5% inclusion); Garlic Extract (0.5% inclusion); Oregano Extract (0.5% inclusion); Agmatine Extract (0.5% inclusion); Echineae Extract (0.5% inclusion); Blueberry Extract (0.5% inclusion); Elderberry Extract (0.5% inclusion); Pomegranate Extract (0.5% inclusion); Blue-Green Algae (0.5% inclusion). Body weight (BW) and feed consumption (FC) of birds from 0-35d of age were measured for each pen and mortality corrected feed conversion ratio (FCR) was calculated. On d35, 2 birds/pen had illeal sections collected to determine villi height, crypt depth and their ratio. Data were analyzed using the ANOVA and Tukeys was used for mean separation ($P<0.05$). No differences ($P > 0.05$) were observed between control and other treatments in BW, FC, FCR or mortality during the study with the exception of the Green Tea Extract birds had greater body weights at d35 (2.70 kg) and lower FCR (1.509) than the controls ($P < 0.05$, 2.38 kg and 1.751). The Green Tea Extract birds allow had greater villi height (790.4 um), crypt depth (111.9 um) and ratio (7.73) than the control ($P < 0.05$; 665.8 um, 100.7um, 6.92). These results indicate that supplementation with the majority of phytogenic substances at the rates used in this study had no effect on production or gut health in broilers; however, the Green Tea extract appears to improve gut health which could contribute to the improved growth and FCR observed in this study.

Keywords: Phytogenic, gut health, performance, broiler chicken

466P Impact of Iron Supplement SQM® on microbiome composition and vaccination efficacy against non-typhoidal *Salmonella enterica* in Ross 708 broilers

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Non-typhoidal *Salmonella enterica* pose a threat to poultry health and food safety. Despite advances in vaccines and antimicrobials, these interventions alone are insufficient to control NT-*Salmonella* colonization sustainably in poultry. Alternative nutritional interventions show promising results in depriving pathogens of essential nutrients required for colonization and virulence. Iron supplement SQM® uses innovative PolyTransport® technology to sequester iron from pathogens like *Salmonella enterica* while enhancing delivery to host intestinal cells for absorption. Before implementing SQM® as an anti-*Salmonella* intervention in commercial settings, it is critical to ensure it does not interfere with standard vaccination protocols and to understand its effects on poultry gut microbiota, which plays a key role in colonization resistance against pathogens. This study investigated the potential interactive effects between SQM® and AviPro® Megan® Vac 1 vaccine and assessed the impact of iron supplementation on poultry microbiome composition in the absence of Salmonella challenge. Ross 708 broiler chickens were used to implement a 2×3 factorial design comparing vaccination status (vaccinated/unvaccinated) across three iron supplementation treatments (control, $FeSO_4$, and SQM®) with 5 birds per treatment combination. The birds received phase-specific diets (starter d0-14, grower d14-28, finisher d28-42) supplemented with either control, $FeSO_4$, or SQM® at the optimal concentration of 20ppm. To analyze the effects of vaccination and iron supplementation on gut microbial diversity, we processed cecal 16S rRNA gene sequences through the Qiime2 pipeline with downstream analysis in R. Alpha diversity metrics (Chao1, Shannon, and inverse Simpson indices) revealed no significant differences between vaccination groups ($p > 0.05$), indicating stable microbiome richness and evenness regardless of vaccination status. The Differential abundance analysis revealed that SQM and $FeSO_4$ treatment showed depletion of Lactobacillus and Alistipes while enriching Ligilactobacillus, Streptococcus, Desulfovibrio, and Synergistes. These complex, bidirectional shifts across beneficial and potentially pathogenic taxa suggest that iron supplementation and SQM intervention may influence microbial communities through resource-driven ecological mechanisms rather than selectively promoting beneficial bacteria.

Keywords: Broiler, vaccination, SQM®, Salmonella enterica, pathogen control

Student Competition: Metabolism and Nutrition: General Nutrition

467P Performance impacts of dietary energy in a laying hen strain at late phase of production

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This work was carried out to understand how dietary energy levels impact Ca utilization in laying hens at the late production phase and to understand egg production and shell quality. This study consisted of a total of thirty-six ISA brown laying hens at the late phase of production (~65 weeks) randomly allocated in 36 cages to one of three dietary treatments with low (T1), medium (T2), and high levels (T3) of energy (11.50, 12.75, and 13 MJ/kg, respectively). Treatment diets were formulated to be isonitrogenous and with the same inclusion level of calcium. Each treatment consisted of 6 replicates, considering two consecutive cages as a replicate (1 bird/cage). The experiment was conducted over a 6-week period. The following performance parameters were measured for all treatment diets: egg production %, egg weight, feed intake, feed conversion ratio, body weight gain, and eggshell thickness. The ovalbumin and ovotransferrin concentrations, and calcium related genes (CA2, Calbindin d28k) were measured from the uterus, kidney, and duodenum tissue through the ELISA and qPCR technique, respectively. Results were analyzed using one-way ANOVA through the SAS software (SAS Institute, Inc., Cary, NC, USA). The performance results showed that at the end of the trial, the T2 diet had higher ($p < 0.05$) egg production (92.3%), compared to the T1 diet (71.79% egg production) and T3 diet (78.84% egg production). Further, there were no differences ($p>0.05$) between treatments in feed intake, egg weight, eggshell thickness, or feed conversion ratio. No differences ($p>0.05$) were found in ovalbumin or ovotransferrin amount in tissues, or the differences in the expression of calcium related genes except in CA2 in duodenum ($p=0.0432$) measured among treatment diets. These findings suggested that birds fed dietary energy level at 12.75 MJ/kg had better performance in terms of egg production with shell thickness remaining same as other treatments.

Keywords: dietary energy levels, calcium utilization, laying hens, egg production, late production phase

468P Determination of TME_n, standardized amino acid digestibility and trypsin inhibitor activity in new reduced trypsin inhibitor varieties of soybeans

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Two experiments were conducted to determine nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid (AA) digestibility in 4 new soybean (SB) lines varying in trypsin inhibitor. Two of the 4 SB (HBK-WF and HBK-PF) were developed by crossing a beta conglycinin alpha subunit mutant (allergen) SB and a KTi-3 mutant SB. The other 2 lines were a KTi-3 mutant (LG17), and a BBi mutant (ZB 168-1). All 4 samples of SB were autoclaved for 25 min to reduce antinutritional factors. Raw uncooked HBK-WF and HBK-PF SB contained 30 trypsin inhibitor units (TIU) per mg of sample. The raw LG17 and ZB168-1 SB contained 48 and 43 TIU/mg, respectively. Autoclaving reduced TIU of HBK-WF and HBK-PF to less than 1 TIU/mg and reduced TIU to 3 and 16 TIU/mg for LG-17 and ZB168-1, respectively. Two precision-fed rooster assays were conducted to determine TME_n in conventional roosters and standardized AA digestibility in cecectomized roosters. For both experiments, 20 individually caged White Leghorn roosters were fasted for 26 hours, then crop-intubated with 25 g of each autoclaved SB (5 roosters per sample) and excreta were collected 48 hours post-feeding. The excreta samples were analyzed for gross energy, AA, and nitrogen. Data were analyzed as a one-way ANOVA for a completely randomized design with each individually caged rooster as the experimental unit. Differences among treatment means were considered significant at ($P < 0.05$) using the least significant difference test. The TME_n values ranged from 2.761 to 3.069 kcal/g DM among the autoclaved SB. The TMEn was greater ($P < 0.05$) for HBK-PF than LG17 and ZB 168-1 SB, with HBK-WF being intermediate. Results from Experiment 2 showed that HBK-PF and HBK-WF SB had higher ($P < 0.05$) digestibility of AA than LG17 and ZB 168-1. Digestibility of AA in LG17 was also higher ($P < 0.05$) than in ZB 168-1. The results of this study indicated that reducing trypsin inhibitors in SB by genetic selection can increase TMEn and digestibility of AA in SB for poultry.

Keywords: soybean, rooster, digestibility, energy, amino acid

469P Feeding live black soldier fly larvae (*Hermetia illucens*) affects fatty acid composition in egg yolk of lohman brown laying hens

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This study investigated the impact of feeding live black soldier fly larvae (BSFL, *Hermetia illucens*) as a substitute for soybean meal (SBM) and soybean oil (SO) on the fatty acid composition of the egg yolk of Lohmann Brown laying hens. A total of 144 18-week-old hens were allocated equally (4 pens/treatment, 18 hens/pen, free-range system) to two dietary treatments: SOY and LAR. Birds in the SOY group received a commercial laying meal with 13.5% SBM and 1.5% SO during phase 1 (P1, week 18-39). In the LAR treatment, SBM+SO was completely replaced with BSFL. In Phase 2 (P2, week 40-50), the amount of SBM+SO and BSFL were reduced to 10%. BSFL were provided twice a day, calculated based on the expected dry matter intake. Growth performance and feed intake data were collected weekly. Laying performance data were collected daily. The fatty acid composition in yolk was analyzed at the end of each phase. Data were analyzed using one-way ANOVA ($p<0.05$). Feeding BSFL did not affect hens growth and laying performance (SOY=88% vs. LAR=89%; $p>0.05$). During the whole trial period, BSFL-fed hens showed a significantly reduced overall dry matter intake (SOY=105g vs. LAR=101g/hen/day; $p<0.05$) and a higher average individual egg weight (SOY=60.1g vs. LAR=61.3g/egg; $p<0.05$), resulting in a tendency toward improved feed conversion ratio (SOY=2.5 vs. LAR=2.3g Feed/g Egg mass; $p<0.01$). Due to BSFL feeding, the LAR diet had a higher content of saturated fatty acids (SFA) compared to SOY, while the proportion of polyunsaturated fatty acids (PUFA) was lower. Total FA content and monounsaturated FA (MUFA) levels did not differ between the treatments. This led to an increased SFA content in LAR-fed yolk (P1: SOY=30% vs. LAR=36% SFA of ident. FAs; P2: 29% vs. 33%; $p<0.001$), with a decreased PUFA content (P1: SOY=24% vs. LAR=18%; P2: 26% vs. 22%; $p<0.001$). There were no differences in total FA and MUFA content in the egg yolk. The increase in SFA was primarily due to higher levels of C12, C14 and C16:0, which are the main FAs present in BSFL. Higher levels of SFA may reduce the oxidative susceptibility of the egg yolk and potentially extend shelf

life. Even if higher levels of SFA are considered nutritionally undesirable, C12 and C14 possess antimicrobial properties that may support the immune system. The results of this study indicate that replacing SBM+SO with live BSFL is a suitable and sustainable alternative protein and energy source for laying hens, while maintaining production performance and modifying egg yolk FA composition.

Keywords: Insect, Sustainability, Lauric acid, Egg quality, Alternative feed

470P Determination of TMEn and standardized amino acid digestibility in high-oil corn fed to chickens

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Through over a decade of selective hybridization and dominant trait genetics, a corn hybrid that produces a grain containing 9% oil or higher has been developed. There are no data on the nutritional value of this new high-oil corn for poultry. Therefore, two experiments were conducted to test the null hypothesis that nitrogen-corrected true metabolizable energy (TMEn) and standardized amino acid (AA) digestibility are not different in a new high-oil corn compared with conventional corn. In Experiments 1 and 2, two precision-fed rooster assays were conducted to determine TMEn and standardized AA digestibility in conventional and high-oil corn using conventional and cecectomized roosters, respectively. For each experiment, 12 individually caged White Leghorn roosters (1 year old) were fasted for 26 h and then crop intubated with 25 g of 1 of the 2 corns with 6 roosters per corn sample. Excreta were then collected quantitatively for 48 h post-feeding. Excreta samples were analyzed for gross energy, nitrogen, and AA. Data were analyzed as a one-way ANOVA for a completely randomized design. Differences among treatments were considered significant at $P < 0.05$ using a t test. Results from Experiment 1 yielded TMEn values of 3.970 and 4.432 kcal/g DM for conventional and high-oil corn, respectively. These data indicate that high-oil corn had significantly greater TMEn compared with the conventional corn. The latter is likely primarily because of the higher fat or oil content in the high-oil corn compared with the conventional corn. Results from Experiment 2 showed that the conventional corn contained 7.7% CP and the high-oil corn contained 9.5% CP on a DM basis. In addition, standardized digestible concentrations for several AA in high-oil corn were significantly higher than in conventional corn. For example, digestible concentrations (DM basis) of threonine, lysine and methionine in conventional corn were 0.25, 0.21, and 0.15% compared with 0.33, 0.34, and 0.17% in the high-oil corn, respectively. In conclusion, the results of this study indicated that the high-oil corn had greater TMEn and digestible AA concentrations than the conventional corn.

Keywords: high-oil corn, energy, amino acid digestibility

471P Effects of *in ovo* docosahexaenoic acid supplementation injection on muscle proliferation and antioxidant gene expression in the embryo of broiler chickens

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In ovo supplementation of dietary nutrients enhances the growth and development of the chicken embryo. Among these supplements, docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid (PUFA), can potentially benefit the broiler's development by maintaining muscle proliferation and antioxidant capacity. The development of skeletal muscle in chickens is a crucial determinant of meat yield and quality. Furthermore, DHA provides antioxidant capabilities that mitigate oxidative stress in tissues during major growth phases by scavenging reactive oxygen species and enhancing innate antioxidant defenses. This study aimed to examine the effect of *in ovo* DHA supplementation on gene expression of muscle proliferation and antioxidant of pectoral muscle. A total of 480 Ross 308 broiler fertilized eggs were injected with 98% DHA solution into the yolk sac on embryonic day (ED) 11, and assigned into three groups: non-injected control (CON), 50 µl of DHA (DHA50), and 100 µl of DHA (DHA100). The samples were collected from the pectoral muscle of the CON, DHA50, and DHA100 groups on ED19. Total RNA was extracted from pectoral muscles, and cDNA was synthesized for qPCR analysis. The expressions of the target genes were normalized using the housekeeping gene *TBP*. All data were checked for normality and log transferred where required in JMP® Pro 17 and analyzed using one-way ANOVA followed by the Tukey HSD test. Spearman's test was done to understand the correlation among the variables. Significance was set at $P \leq 0.05$. The results showed that DHA supplementation did not significantly affect muscle proliferation gene expression (MyoG, MyoD1, Myf5, mTOR, MuRF1, MAPbx1, PCNA, and PAX7) among the treatment groups. However, a positive correlation was observed between relative muscle weight and MSTN ($r = 0.6273$, $P = 0.0388$) at ED19. Furthermore, there were no significant differences in the antioxidant genes Keap1, Nrf2, GPX1, and GPX3 among the treatment groups; however, significant changes were observed for the antioxidant gene SOD2 ($P = 0.0427$), with a trend for SOD1 ($P = 0.0905$) and TXN ($P = 0.0818$). There was no difference in hatch weight. In conclusion, *in ovo* DHA supplementation showed no adverse effects on the muscle of broiler embryos, indicating its safety and potential benefits for improving muscle growth and oxidative stability in broiler chickens in post-hatch days.

Keywords: in ovo, DHA, muscle proliferation, antioxidant

472P Impact of varying corn particle size and feed quality during the finisher phase (d 27-35) with and without a subclinical nectrotic enteritis challenge on broiler gastrointestinal measurements challenged with a 10x live coccidiosis vaccine

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The current study was conducted in conjunction with a companion study evaluating the effects of corn particle size (CPS) and feed quality (FQ) on 27-35 d broiler performance from birds in two different experiments (EXP) within an unchallenged and challenged (subclinical necrotic enteritis; SNE) environment (EXP1 and EXP2, respectively). The objective of this study was to determine the impact of a 3 CPS (low, LCPS, 600 µm; medium, MCPS, 900 µm; high, HCPS, 1200 µm) x 3 FQ (low, LFQ, 40% pellets; medium, MFQ, 60% pellets; high, HFQ, 80% pellets)

within a factorial arrangement from birds sampled within the companion study at d 35. Prior to the EXP period, birds were fed diets with MCPS and HFQ crumbles (3200 microns) from 0-17 d and 80% pellets from 17-27 d. For the SNE challenge (EXP2) birds were orally gavaged with a live coccidiosis vaccine on d 14 (10x), and then d 18 with C. perfringens (8.2 log cfu/mL). One bird/pen was sampled at d 35 to measure duodenum (duo), jejunum (jej), and ileum (il) lengths, as well as duo, jej, il, ceca, gizzard, proventriculus (pro), and pancreas weights relative to BW (RWt). Data were analyzed using a two-way ANOVA in SAS 9.4 ($\alpha \leq 0.05$). In EXP1, only a numerical difference was observed (P=0.0551) for FQ, wherein HFQ increased pro RWt. The main effect CPS impacted duo length (P=0.0117), as well as duo and jej RWT (P=0.0108 and 0228, respectively), wherein feeding M and HCPS increased duo length and decreased duo and jej RWt. Additionally, feeding HCPS increased pro RWt vs. LCPS, with MCPS being intermediate (P=0.0396). These data, especially that observed for CPS, support improved gastrointestinal function as verified by EXP1 performance data in the companion study. In EXP2, CPSxFQ interaction were observed for pro RWt (P=0.0366), wherein feeding MCPS at LFQ and MFQ, as well as HCPS at LFQ increased RWt vs. LCPS at LFQ and HCPS at MFQ being significantly lower, with all other treatments being intermediate. Live performance data from EXP2 companion study was more complex in its relationship, wherein MCPS and HFQ demonstrated improved performance. Overall, these data provide physiological insight to help poultry producers determine an optimal CPS and FQ, future research will determine the impact of these treatments on nutrient digestibility.

Keywords: gastrointestinal tract, feed quality, corn particle size, subclinical necrotic enteritis challenge, broiler

473P Fast-growing broilers pair-fed to slow-growing broilers to investigate the effect of feed intake on breast meat quality and oxidant markers

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Modern broiler production is extremely efficient with improved feed efficiency, reduced time to market, and heavier breast muscle yield. Nonetheless, improved efficiency is also correlated with increased incidence of breast myopathies. Wooden breast (WB) causes the most pronounced effects on product quality and reduced functional use. The objective of this study was to determine if nutrient intake affects the growth rate, breast meat yield and quality, and markers of oxidant state in the plasma, serum, muscle, liver, and intestine of fast-growing (Ross 708) and slow-growing (Moyer Robust White SloGrow) broilers. A total of 36 cockerels (n=12/treatment) were individually housed and fed a diet to meet nutrient requirements from 18 days of age to harvest. Birds were assigned to one of three treatments: Ross 708 ad libitum fed (RA), Moyer ad libitum fed (MA), and Ross 708 pair-fed to MA (RR). Tissue and blood samples were collected from birds harvested at a constant weight of 3.86±0.07 kg. The experiment was conducted as a completely randomized block design with individual bird as the experimental unit. Data were subjected to analyses of variance using the MIXED procedures of SAS. Treatment group did not affect carcass weight and cut-up yield ($P > 0.1$). MA and RR dressing percentages decreased by 3.9 and 3.3%, respectively, compared to RA broilers ($P < 0.05$). MA and RR breast yield decreased by 42.8and 5.6%, respectively, compared to RA broilers ($P < 0.05$). Compression force values differed across breast fillets (RA > RR > MA; $P < 0.05$), while shear force values tended to differ (RA > MA > RR; $P = 0.06$). Plasma glutathione peroxidase (Gpx) activity was numerically greater in MA birds compared to RA and RR birds ($P = 0.16$). MA and RR muscle Gpx activity decreased by 61.7 and 31.9%, respectively, compared to RA birds ($P < 0.01$), while liver Gpx activity was numerically greater in MA and RR broilers compared to RA ($P = 0.14$). However, serum diamine oxidase activity increased ~22% in RA birds compared to MA and RR groups ($P < 0.01$), while ileal Gpx activity increased by 36.5 and 13.3% in MA and RR birds, respectively, compared to RA birds ($P < 0.01$). In conclusion, data from the current study demonstrated that broilers with reduced feed intake, whether by normal appetite (MA) or restriction (RR), exhibit slower growth rates, lower dressing percentages and breast fillet yields, and improved breast fillet texture properties, compared to fast-growing broilers that are full-fed (RR). Simultaneously, patterns of oxidant markers across tissues were less apparent and requires further investigation to be fully understood.

Keywords: broiler, breast myopathies, pair-fed, oxidant marker, feed intake

Student Competition: Metabolism and Nutrition: Vitamins and Minerals

474P Comparison of Ca bioavailability based on tibia bone ash and apparent Ileal Ca digestibility in CaCO3 with and without MgO supplementation

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The objective was to compare relative bioavailability (RBV) of Ca based on tibia bone ash with relative apparent ileal digestibility (AID) of Ca in reagent-grade CaCO3 (RCaCO3) ± MgO. In Experiment 1, RBV of Ca was evaluated in commercial broiler chickens. The 5 dietary treatments were Ca-deficient corn-soybean meal-based diets (0.30% Ca, 0.45% non-phytate P). Diets were provided ad libitum from 7 to 21 d-of-age. Diet 1 was a Ca-deficient corn-soybean meal-based diet (0.30% Ca, 0.45% non-phytate P). Diets 2 through 5 were the same as diet 1, except that 2 increasing levels of either RCaCO3 or RCaCO3+MgO were added at the expense of Solka Floc to increase the Ca level to 0.60%. The RBV of Ca was then determined by regressing tibia ash content (mg/tibia) against supplemental Ca intake. In Experiment 2, AID of Ca was determined in broiler chickens. Diets were corn-based (0.84% Ca, 0.45% non-phytate P) supplemented with either RCaCO3 or RCaCO3+MgO; RCaCO3 was the main source of dietary Ca. Diets were provided ad libitum from 19 to 21 d-of-age. Experiments 1 and 2 were run concurrently in the same room with the same batch of chicks, which had high mortality rates during the pre-experimental phase; Escherichia coli were recovered from the birds. Experiment 3 was conducted as a repeat of Experiment 1, to determine RBV of Ca in RCaCO3±MgO in a second set of chickens using the same diet formulations and procedures as Experiment 1. Experiments contained 5 to 6 replicate pens of 5 chickens per treatment. Data were analyzed using a one-way ANOVA. Regression of bone ash content against supplemental Ca intake was also performed in Experiments 1 and 3. The significance value for all analyses was $P < 0.05$. In Experiment 1, tibia ash (mg/tibia) increased linearly ($P < 0.05$) as the dietary Ca level increased. The RBV of Ca in RCaCO3 obtained from multiple linear regression of tibia ash content (mg/tibia) against supplemental Ca intake was increased ($P < 0.05$)

to 136% with the addition of MgO. In Experiment 2, the addition MgO increased (P < 0.05) AID of Ca from 39.4 to 54.5%, which yielded a relative AID value of 138% for RCaCO3+MgO. In Experiment 3, there was no difference (P > 0.05) in RBV of Ca in RCaCO3 from MgO supplementation. In conclusion, RBV of Ca based on bone ash and relative AID of Ca in Experiments 1 and 2 were similar, indicating AID of Ca can be predicted from RBV values as long as AID of Ca in the standard Ca source is known. The increase in AID and RBV of Ca with the addition of MgO in Experiments 1 and 2 may be related to exposure to opportunistic pathogens during the neonatal period as no effect of MgO on Ca RBV was observed in Experiment 3, which warrants further research.

Keywords: Calcium, Bioavailability, Digestability, Magnesium, Eschericha Coli

475P Evaluation of dietary zinc and manganese source and level on the performance and yield of Ross 708 broilers processed at 8 weeks of age

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Zinc and Mn are essential trace minerals that are often fed up to 120 mg/kg in the finished feed, but the optimal dietary concentrations and sources of Zn and Mn are not well defined. The objective of this study was to determine growth performance, meat yield, and footpad dermatitis (**FPD**) lesion scores of Ross 708 broilers fed graded Zn and Mn concentrations from sulfate or hydroxychloride [IntelliBond (**IB**), Selko USA, Indianapolis, IN] sources. Treatments consisted of 4 diets containing sulfate (30, 60, 90, or 120 mg/kg) Zn and Mn, 3 diets containing IB (30, 60, or 90 mg/kg) Zn and Mn, and 1 "step-down" treatment of IB Zn and Mn at 60 mg/kg in the starter and grower phases and 30 mg/kg in the finisher 1 and 2 phases. All diets were formulated to contain 1,500 FTU phytase and 125 mg/kg IB copper. Treatments were maintained across starter (0 to 14 d), grower (14 to 28 d), finisher 1 (28 to 41 d), and finisher 2 (41 to 52 d) phases, with 12 replicates pens per treatment and 29 birds placed per pen (2,760 total). Feed intake (FI), body weight gain (BWG), and mortality-adjusted feed conversion ratio (FCR) were determined at 14, 28, 35, 41, and 52 d. At 28 and 56 d, 6 birds per pen were selected for FPD scoring, and the same 6 birds were processed at 56 d. Main and interactive effects of source and level (30, 60, and 90 mg/kg groups only) were evaluated by ANOVA, with means separated by post-hoc Tukey's HSD test. Linear (both sources) and quadratic (sulfate only) contrasts of level within source were also performed. Feeding IB Zn and Mn improved ($P < 0.05$) BWG from 0 to 14 d and FCR from 0 to 14 and 0 to 28 d over birds fed sulfate Zn and Mn. In the overall performance trial period (0 to 52 d), there was no influence of Zn and Mn source or level except for a main effect of level on FCR ($P < 0.05$), which was lowest for birds fed 60 mg/kg, highest for those fed 90 mg/kg, and intermediate for those fed 30 mg/kg. The prevalence of FPD lesions was low and unaffected by treatment ($P > 0.05$). Source × level interactions ($P < 0.05$) were observed for 0 to 14, 0 to 28, and 0 to 41 d mortality. Within source, mortality was numerically highest for birds fed 30 mg/kg sulfate Zn and Mn and for those fed 90 mg/kg IB Zn and Mn. There were no effects ($P > 0.05$) of Zn and Mn source or level on carcass or parts weights, except for thigh weight, which tended to be heavier for birds fed sulfate Zn and Mn over those fed IB sources ($P = 0.05$). In conclusion, an advantage of IB Zn and Mn over sulfate forms was observed to 28 d, and no major detriments in performance or yield occurred when feeding Zn and Mn as low as 30 mg/kg under these conditions, although cumulative FCR was best at 60 mg/kg, regardless of source.

Keywords: Zinc, Manganese, Trace mineral, Broiler performance, Processing yield

476P Dose response of novel calcium supplement source in broiler pens

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Calcium deposition represents a significant proportion of the skeletal mass and thus growth in young animals, including market poultry. Maximizing solubility of dietary calcium should increase space in formulation for energy to improve growth rates without sacrificing skeletal integrity. Calcareous marine algae (CMA) represent one such highly soluble calcium source and our hypothesis was that providing this calcium source at decreasing inclusion rates would increase broiler growth rate due to increased energy density of the diet. Ross 708 broilers were allocated in pens of 25 birds to one of four dietary treatments: control (**CON**; a corn-based diet supplying calcium via ground limestone at 1.5% dietary inclusion for the starter diet and 1% dietary inclusion for the finisher diet); 100% replacement of limestone by weight with CMA (**Cal100**) for both starter and finisher diets; 75% replacement of limestone and 25% replacement with ground corn (**Cal75**) for both starter and finisher diets; and 50% replacement of limestone and 50% replacement with ground corn (**Cal50**) for both starter and finisher diets. Starter diets were in crumble form and fed from days 0 to 24, while finisher diets were in pellet form and fed from days 25 to 42. Data were analyzed as a mixed model including fixed effect of treatment and interaction of treatment by week, and random effect of room, with a repeated effect of time (weeks) on feed. Significance was declared at $P \leq 0.05$. While there was no overall effect of treatment on growth rate or efficiency ($P \geq 0.21$), treatments did separate at 6 weeks (terminal days on feed) where Cal100 had greater body weight (+0.13 kg; $P < 0.001$) and average daily gain (+0.02 kg/d; $P < 0.001$) than all other treatments and all CMA treatments exceeded CON for feed efficiency by 0.57 kg fed/kg gain ($P < 0.001$). There were no effects ($P \geq 0.32$) of treatment on measures of bone strength (hardness, brittleness, or toughness) or bone mineral composition (ash, Ca, P, or Mg; $P \geq 0.64$). The present data indicate that while substitution of calcium source improved growth without apparent deleterious effect on skeletal composition, the economical value of the substitution requires further evaluation.

Keywords: broiler, dietary calcium, bone strength, skeletal composition, feed efficiency

Student Competition: Microbiology and Food Safety

477P Genomic characterization of bacterial isolates from broiler chicken cecal microbiota

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Bacterial isolation and characterization are crucial for obtaining live isolates, which enable precise functional analysis and facilitate potential application in poultry. These isolates serve as representative strains for studying microbial interactions, metabolic capabilities, and their functional roles in the gut environment.In our previous study, we employed culturomic approach to isolate bacterial communities from the ceca of broiler chickens, yielding 150 isolates identified by full-length *16S rRNA* sequencing. In this study, 12 isolates were primarily selected for whole-genome sequencing (WGS) using the Oxford Nanopore MinION flow cell. The sequencing data for each isolate was filtered with Filtlong and assembled with Flye. The assembled genomes were annotated with the Prokaryotic Genome Annotation Pipeline (PGAP). Gene Ontology (GO) over-representation for functional analysis was conducted using clusterProfiler, with statistical significance assessed by hypergeometric test with Benjamini-Hochberg multiple testing correction. Antimicrobial resistance genes were detected with ResFinder. The results revealed that these 12 isolates belonged to seven unique species: *Pediococcus pentosaceus*, *Escherichia coli*, *Enterococcus gallinarum*, *Enteroccus casseliflavus*, *Enterococcus faecalis*, *Bacillus pumilus*, and *Bacillus altitudinis*. Gene Ontology enrichment analysis revealed that *Bacillus* species were significantly enriched in spore formation ($P < 0.001$), indicating strong survival mechanisms and environmental resilience. *Enterococcus* species exhibited significant enrichment in carbohydrate transport ($P < 0.001$) and lactose metabolism ($P < 0.05$), suggesting their efficiency in nutrient utilization and gut colonization. *Pediococcus pentosaceus* showed notable enrichment in peptidase activity ($P < 0.05$), potentially aiding protein digestion. ResFinder analysis revealed that *Pediococcus pentosaceus* alone lacked detectable antimicrobial resistance genes. *Bacillus* species carried the cat86 antimicrobial resistance gene, while other species primarily harbored tet(M) and isa(A) resistance genes. These findings confirm the purity of the isolates through WGS and provide functional and antimicrobial resistance gene insights to support the selection of potential beneficial strains. Future research should further validate key functional traits and evaluate species safety for probiotic applications.

Keywords: 16S rRNA gene amplicon sequencing, Bacterial taxonomy, Broiler gut microbiota, Functional gene profiling, Whole-genome sequencing

478P Surveillance of antibiotic-resistant *Salmonella* and *Campylobacter* in turkey carcass rinses across the United States

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The widespread use of antibiotics in humans and agriculture has been linked to increased antimicrobial resistance (AMR) in many pathogens, including foodborne pathogens with potential implication for One Health. This study aimed to characterize the prevalence and resistance profiles of antibiotic-resistant *Salmonella* and *Campylobacter* isolated from turkey carcass rinses in USDA-Food Safety Inspection Service (FSIS) inspected poultry slaughter facilities across the United States. Surveillance data from USDA-FSIS monitoring program of processing plants in the South, West, Midwest and Northeast regions of the U.S. from 2015 to 2023 was evaluated. The data for positive samples from turkey carcass rinses (14,922 samples) for *Salmonella* (0.7%, n=101) and *Campylobacter* (1.1%, n=158) isolation was extracted and analyzed using SAS (v9.4, SAS Institute). Overall, 57% (58/101) *Salmonella* and 70% (111/158) *Campylobacter* were resistant strains (resistant to at least one antibiotic). The regional distribution of antimicrobial resistant *Salmonella* was highest in the South (75%, 18/24) while the other regions were similar, averaging 52% ($P < 0.05$). Resistant *Campylobacter* varied with the highest in the West (93%, 14/15) followed by Midwest (82%, 58/71), South (74%, 23/31) and Northeast (41%, 16/39). Furthermore, the highest AMR for *Salmonella* was observed in 2017 (21%), and *Campylobacter* was lowest with 4% each in 2016 and 2017. Notably, in 2023 and 2017, no AMR was reported for *Salmonella* and *Campylobacter*, respectively in three of the four regions. The incidence of multidrug resistance was generally low for both pathogens at 31% (18/58) for *Salmonella* with similar levels across all regions and 7% (8/111) for *Campylobacter* and Northeast (25%) was the highest. The highest resistance was to tetracycline for both pathogens. Others include sulfisoxazole, ampicillin, and streptomycin for *Salmonella*, while *Campylobacter* had resistance to quinolones (nalidixic acid and ciprofloxacin) and macrolides (azithromycin and erythromycin). *Salmonella* serotype distribution varied across the regions with the highest diversity in Northeast followed by South and serotypes Reading, Hadar, Agona, and Muenchen were the most common. *Campylobacter coli* (66%) was the most common across all regions followed by *jejuni* (26%) and *lari* (4%). This data showed a remarkably low level of both pathogens in turkey from processing plants across the U.S., however the high incidence of antibiotic-resistant strains, especially for *Campylobacter* is of a public health concern. Further evaluation of the data identifying the origin of resistant isolates in terms of historic use of antibiotics is critical to discern the public health implications of the data.

Keywords: Antibiotic resistance, Salmonella, Campylobacter, Turkey, Multidrug resistance

479P Comparing the effects of *Propionibacterium freudenreichii* and *Salmonella* Typhimurium vaccine on cecal colonization, internal organ dissemination, and cecal microbial populations in *Salmonella* Infantis-infected broiler chickens

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Salmonella Infantis (SI) is a top serotype of foodborne *Salmonella* circulating in commercial broiler chickens. Reducing SI colonization in chickens could result in lowering human SI outbreaks. This study evaluated *Propionibacterium freudenreichii* (PF), a dairy-origin probiotic, and a commercial *S.* Typhimurium vaccine (VC) on the cecal colonization, internal organ dissemination of a multidrug-resistant (MDR) strain of SI and the cecal microbiome response to these treatments in 6-weeks-old commercial broiler chickens. Day-old Cornish cross broiler chicks were randomly distributed into five treatment groups (6

birds/treatment group, experiment repeated twice, n=12): Negative Control (NC: -SI, -PF, -vaccine), Positive Control (PC: +SI, -PF, -vaccine), PF (+SI, +PF, -vaccine), VC (+SI, -PF, +vaccine), and the combination of PF and VC (PV: +SI, +PF, +vaccine). PF and PV groups received 10^7 CFU/mL PF via drinking water. VC and PV were vaccinated with the *S.* Typhimurium vaccine. Treatments except NC were challenged with SI in 5th week. Ceca, liver, and spleen samples were collected for analysis seven days after the challenge. One-way ANOVA and Chi-squared tests were used to analyze cecal colonization and organ dissemination data. Metagenomic sequencing was conducted on DNA extracted from the cecal content samples. Shannon index and Aitchison distance matrix were used to determine α and β diversity, followed by pairwise Wilcoxon and PERMANOVA. ANCOM-BC2 was used to determine differentially abundant species (P<0.05). PF, VC, and PV significantly reduced SI populations in ceca and dissemination to the liver (P<0.05). VC and PV significantly reduced *S.* Infantis dissemination to the spleen (P<0.05). β diversity in NC, PF, and PV differed significantly from PC (P<0.05). The abundance of lactic acid fermenting bacteria *Levilactobacillus cerevisiae* was higher in PF compared to PC (P<0.05). The study highlights the potential of PF and *Salmonella* vaccines as non-antibiotic interventions for SI control in broilers.

Keywords: Salmonella, Probiotics, Food safety, Microbiome, Salmonella vaccine

480P Effect of eugenol nanoemulsion in inactivating *Salmonella* Enteritidis survival on broiler chicken skin during refrigerated storage

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Contamination of broiler carcasses with *Salmonella* Enteritidis (SE) during slaughter and subsequent processing is a persistent challenge endured by the U.S. poultry industry. The pathogen colonizes the ceca in high numbers leading to poultry product contamination during processing and subsequent foodborne human infections. Despite several traditional postharvest interventions, the occurrence of SE in broiler carcasses and products suggest the current interventions are not completely effective, and hence, there is a need to innovate novel strategies to control SE contamination on poultry products. Eugenol (EG; phytocompound from clove) has been shown to exert anti-SE efficacy, however, the low water dispersibility of EG hampers its application as a carcass wash treatment. This study investigated the anti-*Salmonella* efficacy of Eugenol in its nanoemulsion form (EGNE) as dip treatments simulating the chill tank application during poultry processing to inactivate *S.* Enteritidis on broiler chicken skin. EGNE was formulated with Gum Arabic and lecithin (GAL) as emulsifiers. Chicken skins were spot-inoculated with a 5-strain cocktail of SE (10^7 CFU/sample), followed by dipping in water (control) or water containing Peracetic acid (PAA) 0.02%, GAL 0.5%, EG, or EGNE at 1.25% for 15 min at 4°C. Post treatment, skin samples were subjected to refrigerated storage for 14 days. Samples were enumerated for surviving SE on chicken skin and in wash water on days 0, 1, 3, 7 and 14. All experiments had triplicate samples, repeated thrice, and analyzed using two-way ANOVA (p<0.05). EGNE had a particle size of ~86 nm, poly dispersity index of <0.3 and zeta-potential of ~-32.3 mV. In baseline (SE inoculated skin, not washed), ~6.79 log CFU/sample SE were recovered on day 0. SE counts decreased to ~5.23 log CFU/sample by day 14 of storage (p<0.05). Immersing SE inoculated skins in water for 15 min did not reduce SE on skin as compared to baseline and ~6.68 log CFU/sample were recovered on day 0. By day 14 of storage, ~5.69 log CFU/sample of SE were recovered on control samples (p<0.05). Washing with water containing GAL or PAA did not reduce SE on skin during storage as compared to control (p>0.05). Washing with EGNE 1.25% for 15 min was more effective than PAA for all storage times, and more effective than EG on day 1, 3, 7 and 14 (p<0.05). In comparison to control, EGNE effectively reduced SE on skin by ~1.32 log CFU as early as day 0, and improved in efficacy by day 14, reducing the pathogen by ~2.76 log CFU (p<0.05). EGNE and EG reduced SE in wash water to below detection limit (p<0.05). Results suggest that EGNE could potentially be used as a natural antimicrobial wash to reduce SE on poultry products.

Keywords: Eugenol, Salmonella, nanoemulsion, emulsifiers, chicken skin

481P Evaluation of the *Enterococcus cecorum* inhibitory effect of cell-free supernatants obtained from different *Bacillus* spp. using an *in vitro* inhibition assay

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Enterococcus cecorum (EC) has been associated with pericarditis and increased mortality in young broiler chicken flocks within the last few years. This pathogen has traditionally been linked to skeletal disease in older chickens. There is a need to identify interventions that can be used to mitigate EC infections in young broiler flocks. *Bacillus* spp. have been shown to produce antimicrobial bacteriocins. Previously, four *Bacillus*-based treatments demonstrated anti-*E. cecorum* efficacy *in vitro*. To confirm the results, two *in vitro* inhibition experiments were conducted to determine the effect of bacteriocins produced from these *Bacillus* species on two *E. cecorum* isolates (EC5 or EC7) isolated from affected chickens in 2022. For the assays, cell-free supernatants (CFS) containing unidentified bacteriocins were obtained from turbid cultures of the following: A - *B. subtilis,* B - *B. amyloliquefaciens,* C - *B. licheniformis,* D - *B. pumilus* and a 1:1:1:1 combination of bacteriocin produced from treatments A-D, also referred to as Combo. CFS from treatments A-D and Combo were individually added to wells of a 96-well plate followed by inoculation with EC7 in Exp 1 or EC5 or EC7 in Exp 2. Combo was only evaluated in Exp 2. Positive and negative control wells and replicates (n=4-8 wells/treatment) were also used. After incubation, a SpectraMax i3 was used to obtain absorbance values (595nm) of each well at respective time points. These values were used to calculate percent inhibition (equation?). For Exp 1, one well plate was incubated statically at 37C for 24h under microaerophilic conditions (static assay). In Exp 2, a static assay and a replicate well plate were incubated at 37C for 24h aerobically with a 10s agitation between each 30m read (kinetic assay). In Exp 1, the inhibition of EC7 after 24h of incubation with CFS from treatments A, B, C, or D ranged from 13-21%. In Exp 2, the bacteriocin inhibition of EC5 ranged from 6-77%, with the Combo treatment being the most effective. A similar trend was observed for EC7 inhibition which ranged from 3-91%, with the Combo once again being the most efficacious treatment evaluated. For the kinetic assay in Exp 2, EC7 growth was completely inhibited by

Combo by 12h and throughout the 24h period followed by Product B which inhibited EC7 growth by 80% at 12h but efficacy declined as there wasonly a 43% reduction by the end of the 24h incubation period. These results suggest that a combination of CFS obtained from a diverse collection of *Bacillus* spp. could improve anti-*E. cecorum* efficacy. Additional research is needed to delineate which compounds in the CFS possess these inhibitory qualities followed by *in vivo* evaluations.

Keywords: Enterococcus cecorum, Bacillus, bacteriocins, cell-free supernatant, assay

482P Population and prevalence of *Salmonella*: A Comparison between conventional floor-pen and silvopasture housing systems in fast and slow-growing broiler birds

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Salmonella, a leading cause of foodborne illness, is commonly associated with poultry during live production and after processing. Alternative rearing systems, like silvopasture, are gaining popularity due to consumer interest in animal welfare and environmental sustainability. However, limited information is available on the microbial food safety of silvopasture poultry compared to poultry raised in conventional floor pens. A total of 500 birds per breed were randomly placed in 5 pens of each housing type, with 50 birds per pen. This study aimed to evaluate the natural occurrence of *Salmonella* in fast and slow-growing broilers (Ross 708 and SASSO) in conventional floor pen and silvopasture systems using a 2×2 factorial design. Ross birds grew for 42 days, whereas SASSO birds grew for 56 days, with both receiving identical conventional diets. At the end of their respective rearing periods, 2 birds per pen were randomly selected from each housing system for microbial sampling. *Salmonella* counts from the whole carcass rinsates and cecal tonsil samples were evaluated using the modified Most Probable Number (MPN) method, and the prevalence of *Salmonella* was evaluated through enrichment and streaking on the selective agar. Data was analyzed using 2-way ANOVA in JMP Pro-17 and the means were separated by Tukey's HSD multiple comparison. In whole carcass rinses, *Salmonella* was detected 100% across all treatment groups by enrichment. The amount of *Salmonella* recovered in whole carcass rinses differed significantly ($P<0.05$) between Ross's housed in conventional floor pens (0.7 log CFU/g) versus those in the silvopasture system (1.4 log CFU/g). SASSOs had significantly lower *Salmonella* counts on carcasses ($P<0.05$, 0.2 log CFU/g in both housing systems) than Ross, with non-significant differences between housing systems ($P>0.05$). In cecal tonsils, all samples were below the MPN detection limit (0.2 log CFU/g), though enrichment detected 10% prevalence in conventionally raised Ross's, while no positive results were found in Ross's raised on silvopasture ($P>0.05$). In contrast, in the cecal tonsils, *Salmonella* was found in 20% of conventionally and 60% of silvopasture-reared SASSOs ($P>0.05$). This study reinforces that both broiler breeds and housing systems can significantly impact the presence of *Salmonella* in poultry. These results suggest that conventional housing is more effective in minimizing external contamination of *Salmonella* in Ross carcasses, which is a food safety goal. In comparison, the level of *Salmonella* contamination in SASSOs was consistently lower, indicating that the breed's genetic resilience may confer a reduced food safety risk regardless of housing conditions.

Keywords: Salmonella, Prevalence, Housing Systems, Ross, SASSO

483P Recovery of salmonellae from chicken enteroids, with or without D-mannose medium treatment

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Enteroids offer a valuable opportunity for *in vitro* disease research using models that more closely resemble their animal of origin while reducing the need for animals to conduct studies. There has been extensive research on mammalian and human enteroids to study host-pathogen interactions and avian enteroid research is ongoing. Establishing efficient methods for enteroid harvest and a reliable method for measuring enteroid infection is paramount for understanding how enteroids can be applied practically to poultry research. The objectives of the present study were 1) to verify a drop-plate method to quantify bacterial invasion of enteroids that can be applied broadly to other experiments and 2) to use this method to determine if D-mannose treated media would reduce *Salmonella enterica* Typhimurium (ST) invasion of enteroids, as previous cell culture research has suggested. Intestinal crypts derived from ceca of 18-day-old chicken embryos were harvested and cultured for 48h until self-assembled, floating enteroids formed. These enteroids naturally repair in the apical-out formation where microvilli face the media and are easily accessed by bacteria or chemicals. A subset of enteroids were suspended in media containing 2.5% D-mannose (Thermo Scientific cat: A10842.22) and infected with ST at approximately 6 Log_{10} CFU/mL while remaining enteroids were infected without D-mannose. After 2h of incubation at 37°C, enteroid-containing media was treated with 100ug/mL gentamicin sulfate for 30min to kill extracellular bacteria. After treatment, enteroids were washed, resuspended in fresh media without antibiotics and fragmented with vigorous trituration for 2min. Enteroid fragments were then serially diluted and drop-plated onto XLT-4 agar plates to determine bacterial recovery. Statistical analysis was performed using a one-way ANOVA with means separated by Tukey's HSD on JMP Pro 17 software. In trial 1 ($n=2$), there was significant ($p<0.05$) reduction of bacterial recovery from enteroids suspended in media containing 2.5% D-mannose (2.40 ± 0.30 Log_{10} CFU/mL) when compared to enteroids without D-mannose (4.65 ± 0.05 Log_{10} CFU/mL) suggesting that the addition of 2.5% D-mannose to the media may provide a protective effect to the enteroids. However, these mannose-associated reductions in intracellular salmonellae recovery were not significantly different in a second replicate trial ($n=3$, 3.13 ± 0.60 Log_{10} CFU/mL recovered D-mannose treated enteroids and 3.31 ± 0.42 Log_{10} CFU/mL recovered from enteroids without D-mannose treatment). These findings suggest that a drop-plating method could sufficiently quantify bacterial invasion in enteroids as shown by the recovery of treated versus untreated enteroid fragments.

Keywords: Chicken enteroids, salmonellae, D-mannose, in vitro, gastrointestinal disease

484P Construction and characterization of bioluminescent avian pathogenic *Escherichia coli* (APEC) strain to understand the pathogenesis of colibacillosis in poultry

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis resulting in significant economic losses in the poultry industry due to mortality, reduced productivity, and carcass condemnation. The APEC strains are characterized by virulence genes, primarily *iroN, ompT, hylF, iutA*, and *iss* which are crucial for establishing infection in poultry, and these genes are less common in avirulent strains. Understanding the dynamics of APEC colonization and pathogenesis is crucial for developing effective control strategies. This study aims to develop bioluminescent strains of APEC for real-time pathogen tracking using an *in vivo* imaging system (IVIS). An APEC strain MS5033 and an avirulent strain of *E. coli* MS1130 engineered to express bioluminescence using the pBS-*slp*GFP*lux*-cat plasmid vector transformation, which harbors the lux operon and chloramphenicol resistance genes. We confirmed the transformation of pBS-*slp*GFP*lux*-cat plasmid vector to both strains by visualizing bioluminescence using IVIS and by testing their resistance to chloramphenicol. These strains demonstrated a high bioluminescence level (2.0×10^4 to 6.0×10^4photons/s/cm^2/s) indicating robust expression of the lux operon following electroporation. They were tested for growth by subculturing in Luria–Bertani medium with and without chloramphenicol. The growth kinetics of the two strains were notably different in the presence of chloramphenicol, indicating successful retention and function of the plasmid. Further testing to determine the stability of the plasmid needs to be conducted. This system allows for real-time, non-invasive monitoring of bacterial localization and proliferation in live animal models. It allows for a direct comparison of colonization efficiency, tissue tropism, and dissemination patterns between virulent and avirulent strains in birds. This research will establish a stable and functional bioluminescent APEC model that could serve as a valuable platform for studying APEC pathogenesis, colonization, and intervention strategies in live hosts.

Keywords: Avian Pathogenic E. coli, Colibacillosis, APEC pathogenesis, Bioluminescence imaging

Student Competition: Processing and Products

485P Inspiring future engineers in next generation poultry processing

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The fields of agriculture and food processing are often thought of as low-technology, without opportunities to utilize advanced technologies. Thus children and young adults interested in STEM may not consider food processing as a career space. For those not involved in STEM, robotics and Artificial Intelligence can instill hesitation over their long-term job security. Robotic Tender Tray Packing is an outreach component of the Center for Scalable and Intelligent-Automation in Poultry Processing (CSI-APP) to develop scalable intelligent solutions to meet the rising global demand for poultry products. Key to developing a resilient industry is an educated workforce able to collaborate with and support automated technologies. This project seeks to engage K-12 students with tools critical to the next generation of food handling by showcasing robotic manipulation and control algorithms. The system uses a Universal Robot UR3 robot arm to manipulate silicone chicken tenders through pick and place procedures. The system implements Finite State Machines to control process flow, HSV masking and classical image segmentation algorithms to locate tenders, and a custom admittance controller to direct robot motions. To increase engagement, manual teleoperation steps were added wherein a user may control the robot arm with a joystick and buttons. Interleaving these manual steps with automatic procedures highlights the roles in which humans and robots can collaborate in effective teams. The system has been presented at two venues, the Georgia National Fair and the Atlanta Science Festival. The first exhibit only included automated components, which led some to unfavorably view robots as replacement workers. The robot's efficiency was questioned, as the UR3 arm moved slowly due to safety limits for unattended operation. The second exhibit with the user controlled segments was more successful in capturing interest. The demonstration improved messaging to effectively convey the goals of CSI-APP, developing scalable poultry processing capabilities through human-robot teams. People enjoyed operating the robot in the crane game format, provided more receptive feedback, and demonstrated curiosity after using the technology. We present Robotic Tender Tray Packing, a tool for STEM education and workforce development in scalable poultry processing. User testing revealed how allowing the human to provide control or decision-level input helps develop a positive attitude toward the system. Future work seeks to improve the system's reliability, speed, and breadth of interactive complexity to showcase a wider set of emerging technologies in the poultry industry.

Keywords: STEM Education, Workforce Development, Robotics, Processing, Automation

486P The use of Peracetic Acid (PAA) in dip application on broiler breast fillet meat quality

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Poultry meat quality and food safety are two very important aspects of the poultry industry, but the safety interventions such as the use of PAA may have some impacts on meat quality. The objective of this study was to assess the meat quality attributes of breast fillets dipped in multiple PAA dips. In this study, broiler breast fillets (n=180) were separated randomly into five groups: negative control (no dip), water control (dipped once in water), PAA Dip 1 (dipped once in PAA), PAA Dip 2 (dipped twice in PAA), and PAA Dip 3 (dipped three times in PAA). Fillets were dipped for 15 seconds in water or PAA, and for treatments with multiple dips, a 90 second wait period was used in between dips. Each treatment was replicated three times. Fillet attributes including weight, pH, and color (L*, a*, b*) were measured before treatment on Day 1 and at Day 3 after treatment and storage (n=24 per treatment over 3 replications). Drip loss, cook loss, and Meullenet Owens Razor Shear (MORS) energy were also assessed. Additionally, a subset of fillets were analyzed for aerobic bacteria using aerobic plate counts (AC) on Day 1 and Day 3 (both after treatments; n=6 per treatment per time over 3 replications). Data in the completely randomized design was analyzed using ANOVA. Means were separated with Tukey's HSD using $P<0.05$ to determine significance. There were no significant differences ($P>0.05$) in initial fillet weight, pH, L*, and b*. After treatments

and storage, drip loss was higher in all treatments dipped compared to the negative control (P<0.05). No differences (P<0.05) among treatments were observed for cook loss. For color attributes, L* value increased with increasing number of PAA dips where PAA Dip 3 had higher (P<0.05) L* value than negative control while other treatments were intermediate. Though differences existed with a* value on Day 3, slight differences among treatments were also noted before treatments were applied. No differences (P>0.05) were noted for pH or b* value on Day 3. Dipping multiple times in PAA resulted in lower (P<0.05) AC on Day 1 and Day 3; PAA Dip 3 had lower (P<0.05) AC than PAA Dip 1 or water on Day 3. Results of this study suggest that increasing the number of dips resulted in some quality changes, but mostly affected lightness of fillets as well as AC. Water holding capacity was not impacted as indicated by no differences in drip and cook loss (among dipped treatments). It is possible that the PAA dips affected primarily the surface properties of the fillets rather than internal (lack of difference in pH and cook loss) due to the size of the fillets. Future research should evaluate fillets of smaller size and/or on tenderloins.

Keywords: peracetic acid, meat quality, broiler breast, color, microbial growth

487P Consideration of multiple feed additives trials and the impact on woody breast frequency

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Muscle myopathies have imposed an economic burden exceeding $1 billion on the poultry processing industry. Among the most prevalent is woody breast (WB), a meat quality defect that leads to downgraded products and reduced profit margins. While WB has decreased, it still represents a significant economic loss to the industry due to the number of factors that can influence its development. Often, in nutrition research, the impact on processing is evaluated and at times, meat quality is assessed. However, the general impact of practical nutrition strategies on meat quality may not be the primary focus. Therefore, this study aimed to identify potential correlations between broiler performance and the occurrence of woody breast using data from four practical feed additive nutrition trials conducted Mississippi State's Poultry Science Department (2018-2024). The comparative analysis included the following measured variables: carcass weight, fat yield (%), wing yield (%), breast yield (%), tender yield (%), thigh yield (%), and the incidence of woody breast (scored 0-3). A total of 336 pen averages from four studies were analyzed. Within each study, data were evaluated using the GLIMMIX procedure in SAS 9.4, with mean separation conducted at a significance level of $P \leq 0.05$. Additionally, control groups from each study were compiled and analyzed similarly. Pearson's correlation coefficient in SAS 9.4 was used to assess the relationships among the measured variables within each study and across the control groups from all four studies, with correlation ranks established at $P \leq 0.05$. Dietary treatments did not directly impact woody breast incidence or broiler performance; though, correlations varied between normal breast meat prevalence and carcass weight, % breast yield, and % thigh yield. In 56-day old broilers processed in 2018, correlation coefficients for normal meat were -0.48, -0.65 and 0.35 for carcass weight, % breast yield, and % thigh yield, respectively (P<0.01). In 56-day old broilers in 2021, only carcass weight showed a correlation (-0.54, P<0.01). Comparing study controls, correlation of normal meat was -0.61, -0.53, and -0.58 for carcass weight, % breast yield, and % thigh yield, respectively (P<0.01). Inclusion of dietary treatments influenced the relationship between woody breast frequency and broiler performance, in some instances resulting in less correlation as compared to controlled commercial diets. Factors such as bird age, breeder flock age, or season were not considered in the current study. However, as more data is incorporated into this model, it may be possible to more accurately link WB incidence to environmental and metabolic factors not previously considered.

Keywords: Woody Breast, Broiler Nutrition, Meat Quality, Correlation, Broiler Performance

Metabolism and Nutrition: Feed Additives

488P Effects of Betaine supplementation on growth performance, nutrient digestibility, egg quality, water loss, gas emissions, and blood profile in laying hens under heat stress

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During the summer season, laying hens are particularly vulnerable to heat stress, which can adversely affect their growth performance, nutrient digestibility, egg quality, and overall health. Betaine, a naturally occurring compound, has been explored for its potential to mitigate the effects of heat stress and improve various performance parameters in laying hens. However, to date, limited studies have focused on evaluating the impact of betaine supplementation in laying hens. Therefore, in this study we aimed to investigate the effects of betaine on growth performance, nutrient digestibility, egg quality, egg water loss, gas emissions, and blood profile in laying hens subjected to heat stress during the summer season. A total of 216 Hy-Line brown laying hens (28 wk old) were randomly assigned to one of three dietary treatments. The test diets were as follows: control (CON, basal diet) and a basal diet supplemented with 0, 0.075% and 0.15% betaine. Each treatment had 6 replications with 12 hens per replication. The relative temperature of the rearing house was fixed at 31°C. All data were examined statistically in a completely randomized design using mixed procedures of SAS (SAS Inst. Inc., Cary, NC, USA) with pen as the experimental unit. Orthogonal comparisons were conducted using polynomial regression to determine the linear and quadratic effects on the graded level of betaine. $P < 0.05$ was considered as significant. The findings indicated that laying hens' fed betaine supplementation had significantly enhanced egg production during weeks 6 to 8. Additionally, a linear improvement in Haugh Units (HU) was observed by the end of week 8. Furthermore, dietary betaine led to a linear reduction (P<0.05) in NH_3, H_2S, and methyl emissions, and blood cortisol levels by week 8. Collectively, these findings suggest that incorporating 0.15% betaine into the diet of laying hens during the summer season would be beneficial to enhance the egg production, egg quality, and to reduce gas emissions and cortisol levels.

Keywords: betaine, blood profile, egg production, growth performance, organ weight

489P Impact of *Pediococcus acidilactici* CNCM I-4622 supplementation on reproductive performance in commercial turkey breeders

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Novel feed additives, including probiotics, offer a sustainable and cost-effective approach to improving turkey health and production efficiency while supporting animal welfare. Supplementation with *Pediococcus acidilactici* CNCM I-4622 (PA) has been shown to enhance growth performance in turkeys. This study aimed to evaluate its effects on the reproductive performance of commercial turkey breeders. A commercial breeder farm in Brittany, France, housed 4,540 Nicholas turkeys in two identical barns with straw bedding. One barn received PA supplementation at 5.1×10^9 CFU/kg of feed for the first two weeks, followed by 1.1×10^9 CFU/kg thereafter, while the other barn served as the control. Birds were monitored from 28 to 57 weeks of age to assess reproductive performance, including lay rate, egg production, egg quality, mortality, litter condition, and feathering quality. Data were analyzed using SPSS v29.0. A paired t-test was used to compare performance parameters between groups, while binary logistic regression was applied to analyze egg classification and mortality outcomes. Supplementation with PA significantly increased lay rate by 0.4% compared to the control (CON) (P<0.05) and improved total egg production (PA: 253,625 eggs vs. CON 249,860 eggs; P<0.01). PA supplementation increased Category A eggs and reduced Category B eggs (P<0.01), with fewer broken eggs compared to control (PA: 29% vs. CON 31%). PA supplementation was associated with a trend towards lower mortality (P=0.10) and improved litter quality, with 5.2% less degraded litter. Additionally, supplemented birds exhibited better feathering quality. In conclusion, PA supplementation improved reproductive performance and reduced mortality, resulting in an 8:1 return on investment. These findings highlight its potential as a valuable tool for enhancing productivity and welfare in commercial turkey management.

Keywords: probiotics, turkeys, reproduction, performance

490P Microbial supplementation in turkey production: three decades of practical applications and key learnings

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Poultry production has long relied on in-feed antimicrobials to enhance health and performance, but growing regulatory and consumer pressures have accelerated the search for alternatives. In modern turkey production, physiological and metabolic challenges necessitate effective non-antibiotic strategies to improve gut health and optimize performance. A meta-analysis was conducted to assess the effects of two probiotics, *Saccharomyces boulardii* CNCM I-1079 (SB) and *Pediococcus acidilactici* CNCM I-4622 (PA), on growth performance, feed conversion ratio (FCR), mortality, and gut histology in turkeys, compared to a negative control (NC). Data were compiled from 16 trials, with 66% conducted in research institutions and 44% in field evaluations. Statistical analyses were conducted using general linear mixed models and non-parametric Mann-Whitney tests in SPSS 29.0, with significance set at P<0.05. Results demonstrated that probiotic supplementation significantly improved body weight (BW) and FCR in turkeys. Overall, probiotic-fed turkeys exhibited an average BW increase of +1.1% and FCR reduction of -2.0% (P=0.015 and P=0.004, respectively). Among the probiotic treatments, PA resulted in the greatest BW increase (+1.0%, P<0.05) with an FCR reduction of -1.9% (P<0.05) compared to the NC. Conversely, SB demonstrated the most pronounced FCR improvement (-3.7%, P<0.05) while also enhancing BW by +0.7% (P<0.05) compared to NC. These findings demonstrate that probiotic supplementation improves growth performance in turkeys, enhancing body weight and feed efficiency. This supports their potential as a sustainable, non-antibiotic strategy for optimizing turkey production.

Keywords: probiotics, turkeys, production, performance, efficiency

491P The effect of pigment saponification and dispersing agent on egg yolk pigmentation

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ORO GLO® 50 Dry from Kemin is a stable, highly concentrated source of yellow pigments including lutein derived from marigolds that allows egg producers to efficiently and cost effectively meet yolk color and lutein level targets. The development of the product included assessment of unsaponified marigold oleoresin, instead of a saponified source. To determine the effect of saponification and dispersant in marigold derived pigment formulations, a 2-month layer trial was conducted using an untreated control (no lutein supplementation) plus a 2x2 crossed factorial design (main effects saponification and dispersant), ORO GLO 50 (unsaponified, added dispersant), ORO GLO® 30 (saponified, no added dispersant), ORO GLO 30 + dispersant (saponified, added dispersant), and Prototype II (unsaponified marigold oleoresin, no added dispersant). Feed was manufactured every 14 days. Treatment groups and the control consisted of 7 replicate pens each comprised of two cages containing 7 birds, 14 birds per pen. Every two weeks, 6 eggs were taken at random from each pen and yolks tested for Roche score (DSM Yolk Color Fan), Lutein, Zeaxanthin, and Total Xanthophylls. Week 0 eggs were collected after all birds were on the negative control diet with no pigment feed additive. The treatment groups were then switched to diets with an inclusion rate normalized to 30 mg pigment additives per 1 kg feed, and the control group continued receiving the non-pigment treated baseline feed. Pigment analysis of yolks was examined in a linear mixed effects model (R package *lmer*) and the ordinal Roche score data was analyzed in a Cumulative Link Model (*clmm*, ordinal R package). Yolks of the untreated control had a Roche score (DSM Yolk Color Fan) of 7. This improved to 9 for supplemented treatments. A small improvement in Roche scores for the saponified treatment was observed but was only a ~0.5 increase. However, pigment supplementation resulted in an increase of 7.9 ± 0.24 µg/g lutein in yolk that was further improved by 0.9 µg/g with dispersant. No significant effect on yolk lutein from the saponification treatment (P=0.08) nor dispersant/saponification interaction effects were observed. The zeaxanthin content increased by 1.2 ± 0.13 µg/g and further improved by 0.23 ± 0.1 µg/g by dispersant; however, saponification did not have a significant effect (P=0.37). Total xanthophylls showed a similar trend with an increase of 21.9 ± 0.61 µg/g with the dispersant improving it by 1.8 ± 0.46 µg/g and no significant saponification effect (P=0.2). Therefore, products based on unsaponified marigold oleoresin are equivalent to saponified marigold oleoresin if the formulation includes a dispersing agent.

Keywords: Marigold, Pigments, Layers, Yolk, Lutein

492P Assessing brown and white mushroom stem powders as sustainable substitutes for soybean meal in poultry nutrition

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This study investigated the potential of brown mushroom stem (BMS) and white mushroom stem (WMS) powders as sustainable alternatives to soybean meal in poultry diets. It evaluated their effects on chick growth performance, internal organ development, gas emissions, and blood biochemistry, offering insights into their viability as feed ingredients. A 2x4 factorial design was employed, with two mushroom types (BMS and WMS) and four supplementation levels (0, 2, 4, and 6%). A total of 320 three-week-old Lohmann LSL Lite chicks were randomly assigned to eight treatment groups, each with five replicates, conducted over five weeks. Mushroom stems were cleaned, washed, and freeze-dried to reduce moisture content below 5%, improving shelf stability. The freeze-dried BMS and WMS were ground and incorporated into the chicks' feed. Feed intake, body weight, feed conversion ratio (FCR), and internal organ weights were monitored and measured weekly. Additionally, gas emissions, blood biochemistry, and mineral retention were measured to assess the nutritional and environmental impact of mushroom stem supplementation. A two-way ANOVA was used to analyze the effects of mushroom stem type, supplementation level, and their interaction on chicks' performance, with statistical significance set at $P < 0.05$. Results showed no significant differences in body weight, weight gain, or FCR between mushroom stem types or supplementation levels. However, chicks fed BMS exhibited significantly higher feed intake than those fed WMS. WMS supplementation led to increased CO2, CH4, and NH3 emissions, with variations based on mushroom type and supplementation level. Regarding internal organ development, WMS supplementation resulted in a higher heart weight compared to BMS, with 4% and 6% supplementation levels further increasing heart weight. No significant differences were found in liver, spleen, or gizzard weights. Blood biochemistry showed that WMS had higher levels of glucose, total protein, globulin, and amylase than BMS, with higher supplementation levels generally reducing these parameters. Mineral retention analysis revealed that WMS enhanced sodium and chloride retention, while higher supplementation levels decreased phosphorus, calcium, and potassium retention. In conclusion, BMS and WMS show potential as partial replacements for soybean meal in poultry diets, impacting feed intake, gas emissions, internal organ weights, blood biochemistry, and mineral retention. The significant interaction between mushroom variety and supplementation level underscores the complexity of their effects on physiological parameters. Further research is required to optimize inclusion levels to maximize their benefits in poultry nutrition.

Keywords: Chicks, Gas emissions, Growth performance, Health status, Mushroom stem

493P *Spirulina platensis* supplementation reshapes gut microbiota and functional metabolic pathways in laying hens

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Spirulina platensis (SP), a bioactive-rich microalga, supports poultry health through its probiotic, antioxidant, and immunomodulatory properties, but its effects on microbial diversity, composition, and function in laying hens remain unclear. This study evaluated SP supplementation's impact on gut microbiota in laying hens. A completely randomized design (CRD) assessed SP's effects on fecal microbiota in 192 White Leghorn hens (39 weeks old, 1670±66 g). Birds were randomly assigned to four dietary groups: Control (0 g/kg SP), T1 (2.5 g/kg), T2 (5 g/kg), and T3 (10 g/kg) for six weeks, with eight replicates (six birds/replicate). Fecal samples from one bird per replicate (eight per group) were collected for microbial DNA extraction and 16S rRNA sequencing using the FastDNA™ Spin Kit for Feces. The V3-V4 hypervariable regions of the 16S rRNA gene were sequenced on the Illumina platform. Reads were processed with QIIME2 (Silva 138.1 database), and functional profiling was conducted using PICRUSt2 with KEGG pathway analysis. Alpha diversity was analyzed via the Kruskal-Wallis test, beta diversity was visualized using PCoA and NMDS, and differential abundance was determined using LEfSe (LDA>4.0). Pathway alterations were assessed through heatmaps, volcano plots, and confidence intervals. SP supplementation led to a microbial shift, increasing the Firmicutes while reducing the Bacteroidota. The abundance of Lactobacillus significantly increased in the T3 group, while Fusobacterium declined. Alpha diversity showed a non-significant reduction in microbial richness and evenness, whereas beta diversity analysis indicated significant microbial composition shifts ($p<0.05$). KEGG pathway analysis revealed enrichment in carbohydrate metabolism, nucleotide biosynthesis, and lipid metabolism-related pathways. Notable upregulated pathways included glycolysis, peptidoglycan biosynthesis, and microbial fermentation pathways. KEGG Orthology analysis showed increased expression of genes for nutrient transport, cell wall biosynthesis, and stress adaptation, while genes linked to lipid metabolism and oxidative stress were downregulated. Enzyme Commission analysis indicated higher activity in carbohydrate metabolism and nucleotide biosynthesis, while enzymes related to lipid metabolism and oxidative stress modulation were reduced. PFAM domain analysis identified enriched protein families linked to microbial resilience in the T3 group. Dietary SP supplementation influenced the gut microbiome of laying hens by enhancing beneficial bacterial populations and modulating key metabolic pathways. These findings suggest that SP could serve as a functional feed additive to improve poultry gut health and microbial metabolic efficiency.

Keywords: Spirulina platensis, gut microbiota, laying hens, functional pathways, microbial metabolism

494P Evaluation of organic KEM GLO™ product in laying hen diet

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Organic KEM GLO™ (OKG) from Kemin is a USDA-certified organic carotenoid used to enhance yolk pigmentation, primarily through capsanthin. Formulated with organic paprika and dried kelp, OKG provides a natural source of xanthophylls that support consistent and vibrant yolk coloration. This study aimed to evaluate the impact of OKG supplementation on yolk pigmentation, specifically capsanthin concentration and Roche color score. The trial was conducted at University of Illinois with Hy-Line® W-36 hens (30 weeks of age), which were randomly allocated to one of six dietary treatments (14 hens per replicate; 7 replicates per treatment). Hens were housed in conventional cages (two adjacent cages per replicate, 7 hens per cage). All diets were fed continuously throughout the 3-month trial (30 to 46 weeks of age) with feed provided ad libitum. Experimental diets included formulations with varying levels of OKG (0.1%, 0.2%, 0.4%, 0.6%) combined with 0.1% Organic ORO GLO® (a lutein source from marigold (OOG)), as well as a treatment with 0.6% OKG alone. Eggs and feed samples were collected biweekly and analyzed by the Kemin Customer Laboratory Services. Individual egg yolks were scored by the DSM Yolk Color Fan (dsm-firmenich, Netherlands). In addition, egg yolks and feed samples were further analyzed for lutein, capsanthin, and total xanthophylls by HPLC and UV-Vis spectrometry. Data collected were analyzed by a repeated-measures ANOVA test (JMP® v16.0.0 software, SAS Institute Inc., Cary, NC), which compared the effect of Organic KEM GLO levels on the DSM Yolk Color Fan score, capsanthin, lutein and total xanthophylls. Results demonstrated that hens receiving 0.6% OKG + 0.1% OOG exhibited the highest DSM Yolk Color Fan score ($P < 0.001$). This treatment also yielded significantly greater capsanthin (12.30 µg/g yolk ± 0.90), lutein (205.76 µg/g yolk ± 3.09), and total xanthophylls (27.4 µg/g yolk ± 0.06) with ($P < 0.001$) compared to other treatments. These findings confirm the effectiveness of OKG as a natural pigmenting agent in organic layer diets. The observed improvements in yolk color intensity provide valuable insights for optimizing carotenoid supplementation strategies to meet consumer preferences in organic egg production.

Keywords: carotenoids, capsanthin, lutein, laying hens, roche score

495P Evaluating the effects of a blend of short- and medium-chain monoglycerides on intestinal functions using an apical-out chicken enteroids model

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Gut health is essential to poultry nutrition and production, as it directly influences nutrition absorption, growth performance and disease resistance. Short- and medium-chain monoglycerides (SC- and MC-MAGs) have gathered significant attention due to their benefits on intestinal barrier function and antioxidant function in poultry. The present study was aimed to evaluate the effects of a blend of SC- and MC-MAGs on intestinal functions using an *in vitro* apical-out chicken enteroids model. Apical-out enteroids were isolated from the small intestine of Cobb 500 broiler chicken embryos at age E18. At day 3 in culture, the enteroids were treated with a serial dose of MAGs blend. The enteroids' viability was measured at 24 and 48 hours post the treatment. The enteroids were then challenged with either 15 µg/mL lipopolysaccharides (LPS) to induce inflammation, or with 400 µM menadione to induce oxidative stress. Fluorescein isothiocyanate-dextran (FD4) was used to measure enteroids' permeability. Oxidative status was measured by the generation of reactive oxygen species (ROS) after 1 hour of menadione (MD) challenge. Gene expression of tight junction proteins (CLDN1, CLDN2, CLD3, ZO1, ZO2, OCLN, MUC2) and antioxidant enzymes (Nrf2, Catalase, GPx4, SODs) were measured via RT-PCR at 6 hours post treatment. Data were analyzed using Student's t-test. The results showed that the blend of SC- and MC-MAGs improved barrier function by reducing FD4 permeability under the LPS challenge ($P \leq 0.05$). Under LPS challenge, adding the SC- and MC-MAGs at 5 ppm improved tight junction CLD 1 and CLD 3 gene expression ($P \leq 0.05$). Additionally, the MAGs blend significantly reduced the ROS generation at 5 ppm under the MD challenge. In conclusion, results from the current *in vitro* enteroids model confirmed the beneficial effects of the blend of SC- and MC-MAGs on intestinal barrier and antioxidant function in poultry.

Keywords: Monoglycerides blend, Intestinal Barrier, Antioxidant, Enteroids, Poultry

496P The effects of supplementing a bacterial xylanase and a multi-component protease on growth performance, fermentation metabolites, and economic benefits in broiler chickens

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Optimizing feed formulations with exogenous enzymes is important for maximizing productivity in broiler production. This study evaluates the effects of supplementing bacterial xylanase (XYL; 100g/t) and a multi-component protease (PRO; 125g/t) on growth performance, hindgut fermentation metabolites, and economic benefits in broiler chickens. A total of 720 day-old male broiler chicks were allocated to five dietary treatments: 1) Positive Control (PC), 2) Negative Control 1 (NC1; ME-reduced diet formulated with energy matrix equivalent to 90, 100, and 110 kcal/kg in starter, grower, and finisher, respectively), 3) NC1 + XYL, 4) Negative Control 2 (NC2; ME and AA-reduced diet formulated with the same energy matrix as NC1, combined with an additional energy (10 kcal/kg) and digestible AA (2.5%) matrix from PRO), and 5) NC2 + XYL + PRO. Each treatment included 12 replicate pens, with 12 chicks per pen. Performance data were collected on day 42. The data were analyzed using one-way ANOVA, followed by Tukey's test for mean comparisons. Statistical significance was set at $P \leq 0.05$. Supplementing XYL in the NC1 diet resulted in a 2.2% increase ($P<0.05$) in body weight (BW) over the NC1 group, with BW similar to the PC group. Birds fed the NC2 diets had the lowest ($P<0.05$) BW. Supplementing XYL + PRO to the NC2 diet led to a 7.1% improvement ($P<0.05$) in BW. The NC2 group had the lowest and worst ($P<0.05$) average daily gain and feed conversion ratio (FCR), respectively, among all treatments. Supplementing XYL + PRO in the NC2 diet led to a 4.8% improvement ($P<0.05$) in FCR similar to the PC group. The improvement in FCR with the combination of enzymes over the NC2 was higher than with the XYL alone over the NC1. Birds fed NC1 and NC2 had increased ($P<0.05$) lactic acid concentration in the cecum compared to the PC group. Supplementing XYL + PRO in the NC2 increased ($P<0.05$) lactic acid concentrations and reduced ($P<0.05$), p-ethyl phenol concentrations in cecum of birds versus other treatments, suggesting improved gut health and environment. Additionally, XYL and XYL + PRO

supplementation improved (P<0.05) flock uniformity compared to other treatments. The NC1 + XYL and NC2 + XYL + PRO diets showed lower (P<0.05) feed costs per kg body weight gain compared to the other treatments. In conclusion, supplementing XYL in energy-reduced diets improved broiler growth performance, while the combination of both XYL and PRO in energy and amino acid-reduced diets provided even greater benefits. This combination led to enhanced growth performance, feed efficiency, fermentation profiles, and economic returns, emphasizing the potential of enzyme supplementation to optimize poultry production under suboptimal feeding conditions.

Keywords: fermentation metabolites, performance, broilers, protease, Xylanase

497P Effects of *Trisetum flavescens* extract in feed on production performance and eggshell quality of laying hens at 94 weeks of age

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This study aims to assess the effects of *Trisetum Flavescens* extract in feed on old egg brown laying hens performance, and eggshell quality. 1,25-Dihydroxycholecalciferol plays a crucial role in the metabolism of calcium (Ca) and phosphorus (P), facilitating the absorption of these minerals, bone mobilization, and mineralization. It is also involved in growth regulation and cellular differentiation within bone tissue. 1,25-Dihydroxycholecalciferol, the active form of vitamin D3, enhances eggshell quality by improving calcium absorption and metabolism. This promotes efficient calcium deposition, resulting in stronger and more resilient eggshells. Furthermore, it may help reduce eggshell defects and breakage. A total of 400 94wk of age, Hy-Line Brown laying hens, were distributed in a completely randomized design with five treatments, for four weeks. The treatments were five levels of *Trisetum Flavescens* extract (Active D®, Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany), T1:0, T2: 50g/ton, T3: 100 g/ton, T4: 150 g/ton and T5: 200 g/ton. Eggs were collected daily, and egg quality was assessed by analyzing six eggs per replicate during the last three days of the fourth week. All data were analyzed using polynomial regression, and differences were considered significant at P < 0.05. No differences in performance were observed during the adaptation period, which lasted 14 days. Egg production showed a quadratic behavior with levels of Active D inclusion (P= 0.013). The regression equation was EggProduction = 52.416 + 13.888ActiveD - 6.5292ActiveD^2, R^2: 0.55. Maximum production was expected with an addition of 106 g/ton. For eggshell quality were measured eggshell percent and eggshell thickness, for both a quadratic behavior was observed. For eggshell percent (eggshellP= 8.9117+1.1945ActiveD-0.5132ActiveD^2, R^2: 0.95, were maximum eggshell percent expected with an addition of 105 g/ton. For eggshell thickness (eggshellT= 0.4254+0.0753ActiveD-0.0337ActiveD^2, R^2: 0.95, were maximum eggshell thickness expected with an addition of 111 g/ton of Active D. In conclusion the addition of *Trisetum Flavescens* extract in level around 110 g/ton improve the egg production and eggshell thickness on old laying hens.

Keywords: Egg production, Active D, Laying hens, Eggshell Thickness

498P Does butyric acid enhance the effects of a blend of organic acid and essential oil blends? Findings in broilers facing a gut challenge

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This study aimed to evaluate the effects of a blend of protected organic acids + essential oils [P(OA+EO)], a similar blend containing butyric acid [P(OA+EO+BA)], and an antibiotic growth promoter (AGP) on intestinal health, nutrient digestibility, and growth performance in broilers subjected to an intestinal challenge. A total of 1,350 male broilers were allocated in 50 floor pens and randomly assigned to five treatments (10 replicates, 27 birds/pen), for a 42-day trial. Treatments included: T1: unchallenged control (UC), T2: challenged control (CC), T3: AGP (Enramycin) at 10 ppm, T4: P(OA+EO) at 300 g/t, and T5: P(OA+EO+ BA) at 500 g/t. All birds were challenged with Eimeria spp. on day 1 and Clostridium perfringens from days 11-13, except from those in the UC group. Intestinal permeability was assessed on day 17 using fluorescein-isothiocyanate dextran (FITC-d) as a marker. Intestinal alterations (I See Inside methodology - ISI) and ileal digestibility were measured on day 21. Growth performance was evaluated weekly. Data were submitted to ANOVA and Tukey's test. Birds from P(OA+EO) group had lower (P<0.05) FITC-d recovery in the blood compared to the CC and UC groups, indicating enhanced intestinal integrity, not differing from the P(OA+EO+BA) and AGP groups. Birds on P(OA+EO) group had the lowest ISI histological score (P<0.05) among all treatments, meaning reduced intestinal lesions and inflammation. The P(OA+EO) improved ileal dry matter digestibility by 3% and digestible energy by 106 kcal/kg when compared to CC (P<0.05), having similar results than UC, AGP, and P(OA+EO+BA) groups. Broilers in the P(OA+EO) group had a 6.9% higher body weight (BW) (P<0.05) and a 4.7% improvement in feed conversion ratio (FCR) compared to the CC group. Their BW was also 3% higher (P<0.05) when compared to the AGP group, with overall performance recovering to levels similar to the UC group. In conclusion, the P(OA+EO) enhanced intestinal health and nutrient digestibility, leading to better growth performance in broiler chickens facing an intestinal challenge and achieving results comparable to or better than those of an AGP. Also, adding butyric acid in the P(OA+EO) formula did not bring any additional benefits.

Keywords: Feed additives, Gut health, Intestinal barrier, Intestinal lesions, Nutrient digestibility

499P Are all "total polyphenols" really the same? A comparative study of two sources of polyphenols on the antioxidant defenses in broiler chickens

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Polyphenols, a wide family of plants' secondary metabolites, are of high interest for animal production due to the ability of some of these compounds to exert a strong antioxidant activity to counteract the negative effects of oxidative stress. Whilst many natural solutions exist on the market with varying compositions in terms of polyphenols, it is getting increasingly difficult to select those of interest to formulate feed. The aim of the present study was to compare 2 sources of polyphenols for their effect on the antioxidant system of broiler chickens. 1356 day-old broiler chickens were randomly divided in 3 groups: Control group (CTL,

n= 442), Reference group (NG, n=442) supplemented with a well-documented source of polyphenols (Nor-Grape®, Nor-Feed, France), and Alternative group receiving an alternative source of phenolic compounds (ALT, n=472). Both NG and ALT supplementation provided 24g total polyphenols/T feed for the whole production period without stressor event. Blood samples were drawn from 52 birds per groups on D5 and on D35 to analyze plasma antioxidant parameters: total antioxidant capacity (FRAP), oxidative damages (TBARS) and modulation of the endogenous antioxidant system (catalase [CAT] and total reduced glutathione [GSH]). Statistical analysis were performed using t-test (R-Studio software). Mean value were considered different when p value was < 0.05. On D5, NG chickens had all their parameters (FRAP, TBARS, CAT and GSH) significantly improved compared to ALT broilers ($p<0.01$). ALT levels of GSH and TBARS were respectively significantly lower ($p<0.05$) and higher ($p<0.01$) than CTL, where NG broilers had significantly higher levels of FRAP and GSH ($p<0.001$) than CTL. On D35, NG broilers had significantly higher FRAP ($p<0.001$) and lower TBARS ($p<0.001$) level than both CTL and ALT birds, and a higher level of CAT than CTL broilers ($p<0.05$). ALT treatment only reduced TBARS compared to CTL group ($p<0.001$), but did not impact any other antioxidant parameter. These results show that, despite being supplemented with the same level of total polyphenols, the physiological response in broiler chickens differed, evidencing the fact that not all phenolic compounds may provide the same level of antioxidant protection *in vivo*. Thus, it underlines that "total polyphenols" might not be a sufficient criterion in the selection of the adequate plant-based antioxidant solution for broilers' diets.

Keywords: Antioxidant, broiler, polyphenols, grape extract, oxidative stress

500P Effect of a natural phosphatidylcholine source on layers' performance and egg composition

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The aim of this study was to compare the effects of supplementing a layer diet with or without a synthetic or natural source of choline on performance and egg composition. The trial was conducted at the experimental farm Cori EARL 3l, France. In total, 57 Lohman layers (37 wk-old) were randomly distributed into 3 treatments: **CON-**, diet without supplemented choline (total choline: 957 mg/kg); **CON+**, CON- plus 1.2 kg/t of synthetic choline chloride 50% (CC); **BC**, CON- diet plus 200g/t of a poly-herbal containing 1.6% of phosphatidylcholine (BioCholine™, Nuproxa Switzerland). BC contains *Trachyspermum ammi*, *Achyrantes aspera* and *Andrographis paniculata*. Diets were based on horsebean, corn and oats with > 1400 mg/kg choline in the pre-trial phase. During the 5 week-trial, egg weight and feed intake were recorded daily. Between days 28-35, 60 eggs per treatment were collected and split into two groups of 30 eggs, and analyzed for fat, protein and choline. A panel of 23 people measured yolk color and conducted an organoleptic test on eggs from the CON+ and BC treatments. Laying rate and egg size distribution were analyzed by Chi square. Egg weight, nutrient composition and organoleptic tests were analyzed with one way ANOVA and contrast analysis. Data were analyzed using SAS. Laying rate was not different at the start of the experiment ($p>.05$; avg. 89.1), however during the study, it significantly improved with BC (77.7%, 81.8% and 94.1%, for CON-, CON+ and BC, respectively; $p<.001$). Feed intake did not differ between treatments (avg. 157 g/h/d; $p>.05$). Egg weight was higher for BC (58.4g, 58.3g and 61.7g, for CON-, CON+ and BC, respectively; $p<.001$). A difference was observed in the percentage of small eggs (4.69%, 2.23%, 1.86%, for CON-, CON+ and BC, respectively; $p=.027$). The content of choline in the eggs did not differ significantly, however it was 4% lower in hens fed the CON- compared with the supplemented birds ($p= .44$). BC increased the protein level of eggs compared to CON- and CON+ (12%, 12.2% and 12.8%, for CON-, CON+ and BC, respectively; $p=.008$). Both supplements reduced the eggs' fat content, compared to CON- ($p=.026$). The egg yolks from BC hens had a higher color score compared to CON- and CON+ (6.04, 5.24 and 7.57, for CON-, CON+ and BC, respectively; $p<.0001$). The organoleptic test showed no difference between CON+ and BC eggs in all parameters tested. To conclude, this study demonstrated that synthetic CC can be completely replaced by BioCholine™ without affecting the hens' performance or the nutritional characteristics and organoleptic traits of the eggs, while also increasing yolk color.

Keywords: layer performance, egg composition, poly-herbal, natural choline, yolk color

501P Effect of corn particle size and essential oils on broiler live performance, digesta retention time, and meat quality of broiler chickens

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The objective of this trial was to evaluate the effect of corn particle size and essential oils on growth performance, meat quality, and digesta retention time of broilers raised to 32 days. A total of 616 one-day-old mixed-sex Ross 308 broiler chicks were randomly distributed in 28 pens and assigned to 4 treatments with 7 replicates per treatment and 22 birds per replicate. Diets were corn-SBM-based and contained 2 levels of essential oils (0 and 150 g/MT) and 2 levels of coarse corn (CC) particle size (0 and 5%(starter)/10% grower). The coarse corn particle size was 1241 µm, while remaining corn was at 924 µm. Birds were fed starter (1-14 d) and grower (15-32 d). Body weight (BW), feed intake (FI), and mortality adjusted feed conversion ratio (FCR) were measured weekly. Feed retention time was measured on d 32 using indigestible food grade dye. Carcass and parts yield, organ weights, and meat quality attributes were determined on d 32. Data was analyzed as two-way ANOVA using GLM procedure for the main effects of particle size and essential oil and the 2-way interaction. No significant differences were observed in BW and BW gain among treatments. The FCR was improved ($P<0.05$) by the combination of either 0% CC without essential oils or using CC with essential oils during 0-7 d only with an improvement of 6 point compared to other treatments. The retention time was longer by about 25 minutes for birds consuming diets with CC regardless of essential oil level, compared to birds consuming 0% CC. At the age of 32 days, the combination of CC with essential oils improved ($P<0.05$) breast meat color by increasing redness by about 0.5 point, and yellowness by about 1 point, compared to other treatments. It can be concluded that the inclusion of CC in diets of broilers can be utilized with the combination with essential oils to improve FCR during the first week of life, while using CC with essential oils throughout the production cycle can improve breast meat color.

Keywords: Broiler, Particle size, Essential oils, retention time

502P Supplementation of organic trace minerals and prebiotics in broiler diets with varying levels of soy

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An experiment was conducted to determine the effects of supplementing a novel blend of organic trace minerals and mannan-rich-fraction (APK; Advantage PakTM; Alltech, UK) with varying levels of soy extract on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), carcass yield (CY), and litter nitrogen (LN), phosphorus (LP) and calcium (LCa). A total of 336 1-d old male Ross 308 chicks were used in a 35-d study. Pens of 7 chicks were randomly allocated to 1 of 4 dietary treatments with 12 replications per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of APK supplementation (0 or 0.075%) and soy level (moderate or low). Diets were wheat/soy based, in low soy diets, 50% of the soy was replaced by peas, field beans, lupin and sunflower extract. The feeding program consisted of starter (d0-14), grower (d15-28) and finisher (d28-35) and offered ad libitum in mash form. Data were analyzed using SPSS v.28 (IBM, Armonk, NY) in a randomized complete block design with pen serving as the experimental unit. Main effects of APK and soy level, as well as their interaction, were tested. APK supplementation improved BWG (2,136 vs 1,887g [P<0.001]), FCR (1.53 vs 1.66 [P<0.001]) and increased FI (3261 vs 3120g [P=0.047] . Low soy increased FI (3324 vs 3057g [P<0.001]) and tended to increased FCR (1.63 vs 1.53 [P=0.061]). Furthermore, APK supplementation improved CY (394 vs 379 g/kg BW [P=0.007]), total breast (P=0.004), thigh (P=0.001) and drum weights (P<0.001), without evidence for differences associated to soy levels. In terms of litter measurements, low soy diets significantly reduced LN (3.4% vs 3.3% [P=0.001]) and LP (7.9 vs 7.5g P /kg [P=0.043]) contents of the litter. There was no effect of APK on LN or LP, however it did reduce LCa (16.9 vs 23.8g/kg [P<0.001]). These data suggest that APK supplementation could improve broiler BWG, FCR, and CY irrespective of soy level and that broilers are able to compensate for the possible reduction in nutrient digestibility associated with replacing soy with other plant protein sources by increasing FI. Moreover, these results show consistent improvement in production efficiency and meat yield of all birds fed diets containing APK.

Keywords: broiler, soy level, organic trace minerals, mannan rich fraction

503P *In ovo* docosahexaenoic acid supplementation and its effects on serotonin and neuropeptide Y-related genes during chicken embryogenesis

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Docosahexaenoic acid (**DHA**), an essential omega-3 fatty acid, is crucial for the development of the central nervous system and for reducing stress. Therefore, supplementation of an adequate dose of DHA is critical. Serotonin (**5-HT**) and Neuropeptide Y (**NPY**) act as morphogens influencing embryonic brain development and as neurotransmitters regulating multiple biological functions. These molecules have lifelong effects on animals' physiological, and mental health, especially during the rapid growth phase prior to birth when embryos face many challenges to reach structural and functional completion. The serotoninergic system may play a critical role in forming the 5-HT neural circuit during chicken embryogenesis. To our knowledge, no studies have investigated the effects of *in ovo* DHA feeding on the brain development in relation to 5-HT-related genes (serotonin reuptake transporter (**5-HTT**); tryptophan hydroxylase 2 (**TPH2**); 5-HT1A receptor (**HTR1A**)) and Neuropeptide Y-related genes (Neuropeptide Y (**NPY**), Neuropeptide Y receptor Y2 (**NPY2R**)). This study aimed to investigate the effects of *in ovo* DHA supplementation on the expression of the 5-HT-related and Neuropeptide Y-related genes in the brain. A total of 796 fertile eggs of Ross 308 broilers were divided into five groups: non-injected control (**CON**), 50µL of DHA (**DHA50**), 100µL of DHA (**DHA100**), 200µL of DHA (**DHA200**), and 300µL of DHA (**DHA300**). The eggs from the DHA-treated groups received a 98% DHA solution, injected into the yolk sac on the 11th embryonic day. On the 19th embryonic day, the whole brain was collected from each group (n=4). Total RNA was extracted from brain samples, and cDNA was synthesized for qPCR analysis. The expressions of the target genes were normalized using the housekeeping gene GAPDH. Statistical analysis was conducted using SAS (version 9.4) with a Kruskal-Wallis test and Spearman's correlation test, and significance was reported at $P \leq 0.05$. There were no significant differences among the treatments in brain weight or in the expression of the 5HTT, TPH2, HTR1A, NPY, and NPY2R genes. However, there were positive correlations between 5HTT and TPH2, HTR1A, NPY, and NPY2R ($r^2 = 0.94$, P <.0001; $r^2 = 0.51$, P = 0.02; $r^2 = 0.71$, P <.001; $r^2 = 0.54$, P = 0.01, respectively). Additionally, TPH2 and HTR1A were also positively correlated with NPY and NPY2R. In conclusion, *in ovo* supplementation of DHA had no adverse effects on brain development. Furthermore, 5-HT-related and Neuropeptide Y-related genes may play a crucial role in embryo brain development in broiler chickens, potentially positively impacting post-hatch health and performance.

Keywords: in ovo, Docosahexaenoic acid, serotonin, brain, embryo development

504P Effect of feeding a novel phytogenic feed additive on growth performance and clinical outcomes of broilers administered a mixed *Eimeria* spp. challenge

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Coccidiosis is a protozoal disease in poultry that results in significant economic loss to the industry due to mortality and reduced productivity. The objective of this study was to evaluate a novel blend of phytogenics and its effect on growth performance and intestinal lesion scores of broilers administered a mixed *Eimeria* spp. challenge. A total of 750 birds were placed into 30 pens on re-used shavings from a previous coccidia challenge study. At the start of the trial, birds were randomly assigned to one of three treatment groups: 1) birds fed a control diet without coccidiosis vaccination or *Eimeria* inoculation (NC); 2) birds fed the control diet and administered a commercial coccidiosis vaccine and *Eimeria* inoculation (PC); or 3) birds fed the control diet with128 g/ton of a novel phytogenic feed additive, administered a commercial coccidiosis vaccine, and inoculated with *Eimeria* (Phyto). *Eimeria* inoculation consisted of 100,000 *E. acervulina*, 50,000 *E. maxima*, and 75,000 *E.*

tenella oocysts inoculum mixed into the feed on day 22. Body weight (BW), feed intake (FI), and feed conversion ratio adjusted for mortality (mFCR) were evaluated at 20, 28, and 42 days of age. On day 28, three birds from each pen were sacrificed and examined for the presence and degree of coccidia lesions. Statistical evaluation of the data was performed using a STATISTIX for Windows program (Analytical Software, Tallahassee, FL). The procedures used were general linear procedures using ANOVA with a comparison of means using least significant difference at a significant level of $P < 0.05$. At the end of the 42-day trial, birds fed the Phyto treatment had significantly greater weight gain (2.41 kg) compared to PC (2.28 kg) and the same weight gain as NC (2.41 kg). Feed intake was significantly greater in NC birds (92.27 kg/pen) compared to PC birds (87.82 kg/pen), with birds fed the Phyto treatment being intermediate (90.62 kg/pen). There were no differences in mortality or mFCR during the entire 42-day period. Lesion scores indicated there was background *Eimeria* cycling from the reused litter in the unvaccinated and uninoculated control (0.22); however, there were significantly greater lesions in the vaccine challenge control (1.10). Birds fed the Phyto treatment had numerically lower overall lesions than the PC birds. These results found that feeding a novel phytogenic feed additive increased total weight gain in broilers compared to a challenged control (PC), despite having minimal impact on *Eimeria* lesions or shedding. These findings indicate this novel phytogenic feed additive supports overall bird productivity during a coccidiosis challenge.

Keywords: feed additive, broilers, coccidiosis, phytogenic, botanicals

505P The effect of feeding new *Bacillus* probiotic strains on the growth of Ross 708 male broilers

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Since the implementation of the Veterinary Feed Directive, antibiotic alternatives have been sought to prevent enteric infections and improve growth parameters. This study evaluated NCH *Bacillus* probiotic strains on Ross 708 by-product male broiler performance. A total of 1,800 birds were randomly assigned to 90 floor pens with 9 treatments (trts) using random-blocking and fed a corn-soy diet for the 42-day grow-out (20 birds/pen; (0.084 m^2/bird). A common basal diet was formulated to meet breeder's recommendations and was used to create 9 trts, which were: Negative control (NC) basal diet with no additive; Positive control (PC) basal + Bacitracin methylene disalicylate at 50ppm; basal + commercial triple-strain probiotic (TS); basal + dormant *Bacillus amyloliquefaciens* + *Bacillus licheniformis* (DD1); basal + dormant *Bacillus subtilis* + *B. licheniformis* (DD2); basal + dormant *B. licheniformis* (DS1); basal + dormant *B. subtilis* (DS2); basal + dormant *B. amyloliquefaciens* (DS3); and basal + dormant 60% *B. licheniformis* + 40% *B. amyloliquefaciens* (DD3). Chicks were spray vaccinated on d0 with a live coccidiosis vaccine (Coccivac-B52®) at a 1X dose. Body weight (BW), BW gain (BWG), feed intake/bird (FI), and mortality-adjusted feed conversion ratio (FCR) were recorded on d14, 28, 35, and 42. Results were analyzed using GLM SAS 9.4 ANOVA, and means were separated using Tukey's HSD and Fisher's LSD, with P-value set at ≤0.05. No significant differences were found from d0-14. From d15-28, DS1 had a more efficient FCR (P=0.0164) compared to DS3 but was similar to all other trts. DS1 resulted in heavier d28 BW (P<0.0001) compared to DD2 and DS3 but performed similarly to the rest of the trts. DS1 resulted in greater d15-28 BWG (P<0.0001) than DD2 and DS3 and was comparable to the other trts. On d35, birds fed DS1 had higher BW (P=0.001) than DD2 and DS3 but was similar to the other trts. On d42, birds on DS2 diets had higher BW (P=0.0062) than PC, DD2, and DS3 but were similar to the remaining trts. Overall, birds fed NC trt had higher FI (P=0.0168) compared to those fed DD2 and were similar to all other trts. DS2 and TS birds had higher BWG (P=0.0064) than PC, DD2, and DS3, but were similar to the remaining trts. These results indicated that inclusion of dormant *B. licheniformis* may help reduce the negative effects of coccidiosis on performance during the grower phase. The overall results suggest that inclusion of *B. subtilis* may serve to improve FI and BWG during coccidia infection from vaccination.

Keywords: Bacillus, Coccidia, Probiotics, Antibiotic Alternatives, Growth Performance

506P Prolonged subinhibitory exposure to a phytogenic feed additive does not induce resistance in *Escherichia coli* strains

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Although there is a consumer push towards no antibiotics ever, the use of antibiotics classified as no antibiotics important to human medicine (NAIHM) is present in the poultry industry. The interest in the use of NAIHM classified antibiotics is to lower the potential for antibiotic resistance to drugs commonly used in human medicine. Natural alternatives to antibiotics are also commonly used in the poultry industry. These alternatives such as phytogenics, also have anti-microbial properties. However, the potential for resistance to phytogenics has been given little attention. Therefore, the objective of this study was to evaluate the impact of prolonged exposure to subinhibitory concentrations of a phytogenic feed additive, derived from a proprietary blend of botanical secondary metabolites [ALTERNA®Poultry, (AP) Probiotech International] on the resistance of *Escherichia coli* strains. Three strains of *E. coli* were tested: a nalidixic acid-resistant control; an avian pathogenic strain (O78 serotype); and a reference strain used as a negative control. AP was diluted by 2 between 1% to 0.0625% until minimum inhibition concentration (MIC) was reached. Two ml of each dilution were added in 18mL of medium, mixed and poured into Petri dishes, added inocula incubated for 20h at 35°C±2°C at aerobic conditions in triplicate for each *E. coli* strain. Subinhibitory level was determined as 0.5× initial MIC AP. Twenty daily passages were conducted; aliquots were plated and stored for further analysis. Bacterial growth curves were generated by measuring optical density at 600 nm every 30 minutes over six hours to determine optimal incubation conditions. Bacterial concentrations were verified through serial dilution and colony counting. MIC was determined before and after the experiment to assess any changes in bacterial susceptibility. MIC values remained unchanged for all three strains, potentially indicating a lack of resistance to subinhibitory levels of AP. Growth kinetics and population densities remained stable throughout the study. Statistical analyses using one-way ANOVA confirmed that there were no significant differences in MIC values before and after exposure. These results suggest that AP may not induce resistance in *E.coli* when used at subinhibitory levels over

prolonged periods. These initial findings support the use of natural alternatives in place of traditional growth promoting antibiotics due to the reduced potential for antibiotic resistance. However, more research is warranted on other pathogenic bacteria that commonly present challenges to modern poultry production

Keywords: Antimicrobial Resistance, phytogenics, Escherichia coli, broiler, NAIHM

507P Bile acids improve energy digestibility in broilers

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The present study aimed to evaluate the effect of bile acids (BAs), specifically cholic and deoxycholic acids, on the digestibility of apparent metabolizable energy corrected for nitrogen in broilers. The study was conducted at the Poultry Technology Research Center/Federal University of Lavras, in Lavras, Minas Gerais, Brazil. A total of 192 one-day-old male Cobb 500 chicks were randomly assigned to four treatments with eight replicates of six birds each, totaling 32 metabolic cages. The treatments consisted of T1 – basal diet (BD), T2 – BD + 100 g/ton of BAs, T3 - BD + 150 g/ton of BAs, and T4 - BD + 200 g/ton of BAs. The diets were formulated according to the nutritional levels recommended by the lineage manual for the initial (1-21 days of age) and final (22-39 days of age) phases. The diets were based on corn and soybean meal and were offered in the form of bran. Both BAs and emulsifier were added as a replacement for the inert material (kaolin). Total excreta collection was performed in the last three days of both the initial phase (21 days of age) and the final phase (39 days of age), to evaluate the digestibility of apparent metabolizable energy corrected for nitrogen (AMEn), based on formulas described by Matterson et al. (1965). The results were statistically analyzed using the SISVAR program (2016) to perform a linear regression analysis. A data set containing the four levels of bile acids tested (0, 100, 150 and 200 g/ton) and their influence on the digestibility of metabolizable energy was used. The dependent variable was defined as the increase in AMEn, while the independent variable corresponded to the different levels of bile acids. In the initial phase, it was found that, for each gram of bile acids added per ton of feed, there was an increase in AMEn of 0.4354 kcal/kg of feed (Y = 0.4354 X + 1.5143, r2 = 0.99). In the final phase, this increase was 0.6274 kcal/kg of feed (Y = 0.6274 X + 0.0857, r2 = 0.99). Therefore, it can be concluded that the use of bile acids in broiler diets promotes better digestibility of apparent metabolizable energy corrected for nitrogen balance (AMEn).

Keywords: Cholic acid, Deoxycholic acid, Energy, Digestibility

508P Effect of prebiotic and organic acid supplementation on productive performance, carcass yield and skin pigmentation of broiler chickens

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Currently, there is a need to find viable alternatives to the use of antibiotic growth promoters, such as prebiotics (PB) and organic acids (OA). Seaweed (SW), yeast cell walls (YCW) and OA have gained popularity as functional ingredients in poultry diets due to their beneficial effects on animals. The objective of this study was to evaluate the effect of supplementation with SW, YCW, or their combination with OA on live weight (BW), feed conversion (FC), carcass yield (CY) and colorimetry of live chickens (LC) and carcasses (CC). 2000 one-day-old male broiler chickens (Ross 308) were randomly distributed among five treatments (eight replicates/treatment): T1 or control, 0 ppm PB or OA; T2, 1250 ppm SW; T3, 2500 ppm SW; T4, 1250 ppm SW + 2000 ppm of OA, and T5, 1000 ppm YCW. A three-phase feeding program was used: starter, 0 to 20 d; grower, 21 to 34 d; finisher, 35 to 42 d. BW, cumulative feed intake and FC were recorded at 42 d. At the same age, 16 birds per treatment (two per replicate) were humanely euthanized, following local guidelines. Individual BW was recorded and then, the hot carcass (without head, legs and viscera) and abdominal fat were weighed. The carcasses were stored in ice-packed containers for 24 hours, and the weights of the following variables (including skin and bones) were recorded: breast, thighs, wings, legs, and backbone. The colorimetric variables were L* (lightness), a* (redness), and b* (yellowness). Data were analyzed using the GLM procedure in SAS and the mean test was performed using Tukey's HSD procedure (alpha, 0.05). No significant differences ($P \geq 0.05$) were found in BW (2766.23 to 2810.76 ± 11.22 g) and FC (1.54 to 1.57 ± 0.01) at 42 d. Similarly, no statistical differences ($P \geq 0.05$) were observed in the yield of eviscerated carcass (72.00 to 73.47 ± 3.28%), breast (28.23 to 29.05 ± 1.91%), abdominal fat (0.97 to 1.02 ± 0.06 %), legs (9.14 to 9.39 ± 0.26 %), thighs (11.46 to 11.99 ± 0.51 %), wings (7.17 to 7.43 ± 0.11 %) and backbone (9.40 to 9.75 ± 0.67 %). Regarding L*, significant differences ($P \leq 0.05$) were found in CC (T1, 64.90^{b}; T2, 64.61^{b}; T3, 65.89^{ab}; T4, 65.62^{ab}; T5, 68.00^{a}; SEM, 0.36), however, in L* of LC (65.86 to 66.27 ± 0.25), a* (LC, 1.76 to 2.85 ± 0.19; CC, 2.23 to 3.39 ± 0.21) and b* (LC, 17.70 to 19.74 ± 0.32; CC, 42.96 to 45.73 ± 0.45) no statistical differences ($P \geq 0.05$) were found between treatments during the evaluated period. It is concluded that the inclusion of YCW, SW, or their combination with OA, did not improve productive performance, carcass yield, or the colorimetry variables evaluated in broiler chickens at 42 d of age (except for L* in CC), however, more research is needed to better understand the effect of PB.

Keywords: broiler chickens, seaweed, prebiotics, organic acids, yeast cell walls

Metabolism and Nutrition: Enzymes

509P A multicomponent protease improves the productive performance of laying hens reared in high temperature and humidity conditions

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A multicomponent protease can hydrolyze a wide range of proteins sources improving the digestion process and the digestibility of amino acids. Because the physiological digestion process generates metabolic heat, the multicomponent protease could reduce the deleterious effect of high temperatures and humidity on the performance of laying hens. This study investigates the effects of a multicomponent protease on the performance of laying hens fed low-density diets (LD) and reared in a region with high temperature and humidity. A total of 240 Bovans White laying

hens 22-w-old were randomly assigned to three treatments, with 20 replicates (4 birds/cage), during 28 weeks in Yucatán, Mexico. Treatments were as follows: T1: Control; T2: LD (6% reduction in crude protein and amino acids); and T3: LD+Protease. A two feeding phases program were used: Booster (22-28w) and Layer (29-50w). Diets were offered ad libitum in mash form. Averaged temperature and humidity were 30°C and 72%, respectively. The following performance data were evaluated: feed intake, egg production, egg weight, egg mass, and feed conversion (FCR) per egg mass and per dozen eggs. Data were submitted to ANOVA and means compared by Fisher's test. From 22-28w, the laying hens on LD+Protease presented lower ($P < 0.05$) feed intake than those in LD (92.2 vs. 95.2 g/d), being similar to Control. Egg production tended to be higher ($P < 0.07$) for LD+Protease when compared to LD (97.6% vs. 96.0%), not differing from Control. The FCR per egg mass of the LD+Protease group was improved ($P < 0.05$) and Control when compared to LD (1.69 and 1.71 vs. 1.75, respectively). LD+Protease had improved ($P < 0.05$) FCR per dozen eggs when compared to LD (1.13 vs. 1.19), being similar to Control. From 29-50w, FCR per dozen eggs tended to improve ($P < 0.07$) for LD+Protease when compared to Control (1.20 vs. 1.21), not differing from LD. From 22-50w, egg production tended to improve ($P < 0.07$) with LD+Protease when compared to LD (97.6% vs. 96.4%), not differing from Control. The LD+Protease and Control had improved ($P < 0.05$) FCR per egg mass when compared to LD (1.70 and 1.70 vs. 1.73, respectively), while the FCR per dozen eggs tended to improve ($P < 0.06$) with LD+Protease when compared to Control and LD (1.18 vs. 1.20 and 1.20, respectively). Egg mass and egg weight did not differ among treatments. In conclusion, this multicomponent protease improved the productive performance of laying hens fed low-density diets and reared under high temperature and humidity conditions, achieving better or comparable results to those fed a standard diet.

Keywords: Laying rate, Heat stress, Production performance, Protein utilization, Enhanced feed conversion ratio

Metabolism and Nutrition: Amino Acids

510P Effects of a low crude protein amino acid-balanced diet on performance, egg quality, nutrient digestibility, and gas emissions in Hy-line brown laying hens

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Ammonia emissions from commercial poultry farms significantly contribute to atmospheric pollution, making their reduction a critical global challenge for the poultry industry. This study evaluated the effects of a low crude protein amino acid-balanced diet on performance, egg quality, nutrient digestibility, and manure gas emissions in laying hens. A total of 252 Hy-Line Brown laying hens (43 weeks old; average egg production 93%) were randomly assigned to one of three dietary treatments for 12 weeks with seven replicates per treatment and 12 birds per replicate: 17% crude protein (CP; control, CON), 16% CP (low protein, LP), and 16% CP supplemented with amino acids (LPS). The amino acids including methionine, lysine, threonine, and tryptophan were supplemented to maintain equal amino acid concentration in CON and LPS diets. Growth data were recorded at the start, 2nd, and 12th weeks of the trial, while egg production and quality were analyzed weekly. Fecal samples collected in the 7th week were used for nutrient digestibility and gas emission analysis. Data were analyzed as a randomized complete design using one-way ANOVA in the General Linear Model (GLM) procedure of SAS (SAS Institute, Cary, NC, USA). Reducing dietary CP from 17% to 16% (LP group) significantly decreased egg production and feed conversion ratio (FCR) during later weeks of the trial (weeks 5-12 and 7-12, respectively), whereas the LPS diet-maintained performance comparable to the CON group. Egg quality traits, including shell color, Haugh unit, yolk color, albumin height, shell strength, and shell thickness, were unaffected by dietary treatments. Similarly, nutrient digestibility (dry matter, nitrogen, energy, calcium, phosphorus) remained unchanged ($P > 0.05$). Notably, the LPS diet significantly reduced ammonia (NH3) emissions from manure, while hydrogen sulfide (H2S) emissions were unaffected. Collectively, these findings demonstrate that reducing dietary CP with supplemented amino acids can mitigate ammonia emissions without compromising production performance or egg quality in Hy-Line Brown laying hens.

Keywords: low protein, amino acid balance, gas emission, environment, laying hen

Metabolism and Nutrition: Vitamins and Minerals

511P In ovo supplementation of combined chelated minerals (Zn, Fe, & Mn) enhances chicken embryonic development and nutrient absorption, and modulates immune and antioxidant responses

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Studying in ovo administration of chelated minerals offers a promising strategy to enhance immunity, mitigate stress, and boost nutrient absorption in embryos, potentially optimizing post-hatch health and performance in poultry. This study evaluated the combined effects of amino acid-chelated zinc, iron, and manganese on embryonic development, nutrient absorption, immune responses, and oxidative stress in broiler embryos, comparing these with their inorganic counterparts. Individual and optimal doses were determined prior to this experiment. 140 fertile eggs were randomly divided into seven treatments, with 20 eggs per treatment. Treatments included a control group, three inorganic minerals (IM), and three organic chelated minerals. The three levels of inorganic and chelated minerals were 0.5× (20 µg Zn, 250 µg Fe, and 10 µg Mn per egg), 1× (40 µg Zn, 500 µg Fe, and 20 µg Mn per egg), and 2× (80 µg Zn, 1000 µg Fe, and 40 µg Mn per egg). Minerals were injected into the amnion on embryonic day 15. Tissue samples from the liver, spleen, and intestine were collected on embryonic day 20, and extracted mRNA were analyzed for gene expression using quantitative real-time PCR (qRT-PCR). Statistical analysis was conducted using two-way ANOVA in GraphPad Prism 8, with Tukey's post hoc test for multiple comparisons ($p < 0.05$ considered significant). Findings from the current study showed that organic minerals at 0.5× and 1× levels significantly upregulated mTOR in the liver ($p < 0.05$) and enhanced PepT1 and SGLT1 expressions at 1× and 2× levels in the intestine ($p < 0.05$). The inorganic minerals at 0.5× indicated an upregulation of mTOR, while a higher dose of 2× was necessary for PepT1 expression and had no significant effect on SGLT1 at any tested levels. Organic minerals increased IL-22 expression at 1× and 2× in the intestine ($p < 0.05$), supporting intestinal immunity and tissue regeneration, while inorganic minerals had no effect. IL-1β remained unchanged, whereas a high dose of

inorganic minerals showed a pro-inflammatory trend. IL-2 was upregulated only at the highest inorganic dose (2×) in the spleen ($p < 0.05$), indicating T-cell activation. No significant embryonic mortality or oxidative stress was observed ($p > 0.05$). In conclusion, organic minerals at lower doses may promote embryonic development, nutrient absorption, and immune modulation without stress or inflammation.

Keywords: In ovo chelated minerals, Embryonic development, Nutrient absorption, Immune and antioxidant responses, In ovo inorganic minerals

512P Niacin supplementation effects on broiler growth performance

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Previous research concluded that supplementation of niacin (NA) up to 140 mg/kg in a mash diet improved FCR of broilers from d 0-18. It is known that adequate NA levels in broiler diets plays a crucial role in enhancing the growth performance and efficient utilization of energy. This study aimed to evaluate the effects of supplementing broiler chick diets with NA on growth performance. Presently, available recommendations range 35-85 mg/kg NA in the diet. Day-old male COBB by-product breeders (420 broilers; initial BW 39.9 g) were used in a 21-d study where five dietary treatments were randomly assigned to 60 cages (7 birds/cage) within 12 location blocks across 3 Petersime batteries. This resulted in 12 replicates per treatment. Diets were fed in crumble form and consisted of basal diets that had 28, 88, 148, 208, or 268 mg/kg NA. The experimental design was a RCBD, and data were analyzed using GLIMMIX procedure of SAS 9.4, with cage as the experimental unit, and cage location as blocking factor. There was no evidence of differences in BWG, FI or FCR across all dietary treatments ($P > 0.05$). Therefore, increasing NA levels above 28 mg/kg did not improve growth performance of broilers housed in battery cages for 21 days. Further research is warranted in floor pens or a commercial environment to better understand the economic advantages supplementing NA may have.

Keywords: niacin, broilers, vitamins

Metabolism and Nutrition: Microbiology and Food Safety

513P Prevalence, serotyping and antimicrobial resistance analysis of *Salmonella* isolated from the pastured poultry farm

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Pastured poultry farms, managed without antibiotics, can provide a valuable baseline for studying *Salmonella* ecology and intrinsic antimicrobial resistance (AMR) profiles. This study investigated the impact of different broiler breeds on *Salmonella* prevalence and further characterized AMR and serotype distribution from 94 *Salmonella* isolates. Two different breeds, fast-growing Cornish Cross (CC) and slow-growing Freedom Ranger (FR), were used and samples were collected from broilers (ileum, ceca, fresh feces, feces collected from pasture, whole carcass rinses at processing (WCR)) and their environment (feed, water, flies and wild bird feces) at multiple time points from d3 post hatch through carcass processing (d56 and d77 for CC and FR, respectively). *Salmonella* prevalence was determined using traditional cultural methods with selective enrichment, and positive samples underwent whole-genome sequencing (WGS) and antimicrobial sensitivity testing (AST). One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to assess statistical differences in *Salmonella* prevalence between two breeds (α=0.05). Serotyping based on core genome multilocus sequence typing (cgMLST) was performed using the SISTR package and AST was performed using the CDC-NARMS protocol. *Salmonella* prevalence in CC (15.6%) was significantly higher than FR (7.5%, $p<0.05$), although *Salmonella* was only recovered from the WCR samples from the FR broilers. While low levels of *Salmonella* were recovered from water and wild bird feces samples (9.4% and 8.3%, respectively), flying insects was the greatest sampled environmental reservoir of *Salmonella* throughout the study (60%). WGS analysis identified 10 different serotypes including Bareilly (24.3%), Senftenberg (23.0%), Kentucky (20.3%), Typhimurium (9.5%), and Infantis (6.8%), with serotypes differences based on sample type and broiler breed. Even when raised without exogenous antibiotics, these isolates possessed resistances to various antibiotics with tetracycline and cefoxitin being most prevalent (42.6% and 26.6% of isolates, respectively). Multidrug resistance (MDR; ≥3 antibiotics) was observed from 20.2% of isolates, dominated by Senftenberg and Infantis (10 and 5 isolates, respectively), with >3 times more MDR isolates from CC compared to FR (11 and 3 NDR isolates, respectively). These findings demonstrate a significant breed effect on *Salmonella* prevalence and provide valuable insights into serotype and AMR diversity under a pastured poultry farming system, which can be critical for enhancing food safety and mitigating AMR risks for the poultry industry.

Keywords: Salmonella, Pastured poultry farming, AMR, MDR

514P Synbiotic nutritional additives reduce *in vitro* growth of avian pathogenic *E.coli* (APEC)

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Nutritional supplements promoting animal health and well-being have become progressively more popular through the increased use of prebiotics and/or probiotics. Moreover, recent research has led to the use of additional biotics such as postbiotics, synbiotics and even paraprobiotics. Each can have a role to play in supporting intestinal function and the gut microbiome. Avian Pathogenic *E.coli* (APEC) is one of the most significant bacterial pathogens in terms of animal health within poultry production, impacting both meat and egg sectors. A range of strategies to reduce the prevalence and transmission of APEC such as vaccines are currently in place however, control through feed additives such as probiotics represent a cost-effective increasingly popular means to combat APEC within the poultry industry. This study was conducted to evaluate the antimicrobial efficacy of eight synbiotic preparations to restrict and inhibit growth of three APEC chicken isolates. Synbiotic preparations comprised of Bacillus and

Lactobacillus derived probiotics in combination with yeast mannan-rich fraction were cultured overnight in MRS media (0.1%, w/v). Cell-free supernatant (CFS) was collected following overnight incubation at 37°C (4000rpm, 0.22µm filter). Antimicrobial activity was tested by broth microdilution method overnight using a Varioskan LUX Multimode Microplate Reader (OD@595nm). Statistical analysis was performed using GraphPad Prism version 5. One-way ANOVA with Dunnett's multiple comparison was carried out to test for significant differences compared to bacteria control, at a 95% confidence level. Growth restriction of APEC following exposure to synbiotic preparations resulted in significant restriction of APEC microbial growth ($P \leq 0.05$). With differing synbiotics comprised of different combinations of probiotic mixtures impacting APEC growth significantly ($P \leq 0.05$). Growth analysis with synbiotic preparations determined that *in vitro* APEC growth was reduced by up to 80% compared to relevant bacteria control ($P \leq 0.05$). In conclusion, synbiotic preparations effectively restricted microbial growth of APEC *in vitro*. These findings suggest that synbiotics may facilitate new disease control methods by reducing growth and transmission of pathogenic species with potential to reduce antibiotic use. The use of functional dietary components, such as synbiotics, could represent an innovative approach to combat prevalence of APEC in poultry production.

Keywords: APEC, Probiotics, Poultry Nutrition, Antimicrobial Activity, Feed Additives

515P Microbial Volatile Organic Compounds (mVOCs) as early indicators of contamination during incubation of SPF White Leghorn eggs

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This study aims to identify and characterize microbial volatile organic compounds (mVOCs) produced by *Salmonella* enteritiditis (SE) in contaminated poultry eggs during incubation, with the goal of improving early detection methods and differentiating contamination location in ovo (yolk vs albumen). Microbial contamination in eggs can reduce hatch rates, impair embroyonic development, and increase post-hatch mortality. Traditional detection methods, such as candling and post-incubation egg breakouts, are invasive and time-consuming. Our research proposes a non-invasive, rapid detection method using mVOCs as biomarkers for contamination. In identifying specific compounds associated with the contaminated eggs, we plan to develop a sensing technology that enables timely removal and reducing cross-contamination risks in hatcheries. SPF white leghorn fertile eggs were sourced and randomly assigned to six groups (10 eggs/group with an additional for 12 eggs/group with three per sampling day): control albumen, control yolk, 10^2 SE albumen, 10^2 SE yolk, 10^4 SE albumen, and 10^4 SE yolk. Eggs were inoculated with 100 µL phosphate buffered saline (PBS) or PBS containing SE at 10^3 or 10^5 CFU/mL and cold stored for two days. VOCs were collected on days 0, 5, 11, and 18 of incubation using a sorption extraction device in sterile glass jars over 90 minutes. On day 18, embryo viability was assessed via breakout analysis. Egg contents and embryos were diluted with 1% BPW, plated on BGS, and incubated for 48 hours. Egg contents and embryo recovered SE counts were used as ground truth data for collected VOC profiles. For both experiments, all SE inoculated yolk eggs were nonviable while most of low inoculum (10^2) SE albumen and some of the high (10^4) SE were viable at breakout. VOCs were analyzed using two-dimensional gas chromatography-mass spectrometry with a non-targeted approach, and compounds were identified using the NIST 2017 mass spectral database. Statistical analysis of the data was performed using Principal Component Analysis (PCA) for the mVOCs and one-way ANOVA for recovered SE counts. Results reveal that amine- and sulfur-containing compounds are significantly associated with contamination. PCA showed the greatest separation in the low-inoculum albumen group, supporting previous studies suggesting that albumen has a protective effect against contamination at lower SE concentrations. These findings highlight the potential of mVOC profiling as an efficient and non-invasive tool for early detection of contamination in poultry eggs, offering enhanced biosecurity for hatcheries.

Keywords: Non-invasive detection, Contamination, Salmonella, Volatile Organic Compounds

516P Functionality of liquid smoke fractions as natural antimicrobials against human foodborne and poultry bacterial pathogens

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Smoke has been used historically to preserve foods and to impart desirable color, flavor, aroma, and textures. Liquid smoke, with different levels of carbonyl, phenolic, and organic acid compounds, can be produced from the condensation, purification, and fractionation of pyrolyzed wood vapors and have been demonstrated to have various antimicrobial and antioxidant activities. More recently, we have demonstrated the ability of liquid smoke condensates to mitigate effects of necrotic enteritis challenge in broilers. In this study, we determined the minimum inhibitory concentrations (MIC) of three liquid smoke fractions with varying carbonyl concentrations (low, med, high) for the human foodborne pathogens *Salmonella* enterica (*Typhimurium*, *Enteritidis*, and *4,[5],12:i:-*) and *Campylobacter jejuni* and poultry pathogens avian pathogenic *Escherichia coli*, *Clostridium perfringens*, and *Enterococcus cecorum* in order to inform the design of future studies to be conducted in live poultry. The MIC of liquid smoke fractions were determined using two methods, agar well dilution and microbroth dilution, according to standard procedures of the Clinical & Laboratory Standards Institute over a two-fold dilution range from 16% to 0.06% with gentamicin and ampicillin controls as appropriate. One assay was conducted using each method for each organism. Overall, the MIC were consistent between the two methods used, and all three smoke fractions were observed to inhibit all the organisms they were tested against. In the event that results did not match across the two methods used, we report the greater MIC value. For each organism, the MIC were highest for the low carbonyl fraction and lowest for the high carbonyl fraction. For *C. jejuni* the MIC were 0.25%, 0.12%, and ≤ 0.06% for the low, medium, and high carbonyl fractions, respectively. The MIC of the low, medium, and high carbonyl fractions were 0.5%, 0.25%, and 0.12%, respectively, for the remaining organisms except *E. cecorum* for which the MIC of the high carbonyl fraction was ≤ 0.06%. The MIC determined for the human foodborne and poultry pathogens in this study demonstrate the antibacterial functionality and suggest potential applications of these liquid smoke condensates to promote animal health and food

safety in poultry production. Additionally, these data will be used to inform the design of future studies evaluating the effects of these natural antimicrobials to be conducted in live poultry.

Keywords: liquid smoke, natural antibacterial, natural antimicrobial, food safety, animal health

517P Pangenome analysis reveals distinct evolutionary clusters and transmission patterns of avian pathogenic *Escherichia coli*

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Avian pathogenic *Escherichia coli* (APEC) infection is one of the major causes of economic losses in poultry production worldwide and poses potential zoonotic risks, underscoring the need to understand its genomic diversity and transmission dynamics for effective disease control. This study aimed to characterize the genomic diversity and transmission patterns of APEC through a pangenome analysis. As an observational genomic study utilizing computational approaches for comparative analysis, this research does not employ experimental designs or inferential statistics. We conducted genomic analysis of 65 newly sequenced APEC isolates, 396 previously sequenced APEC isolates, and 3,562 publicly available *E. coli* complete genomes to identify distinct evolutionary clusters and their associated genomic features. Newly sequenced APEC isolates were assembled using filtered Illumina and Nanopore reads with Unicycler, while the previously sequenced APEC isolates were assembled using Spades. The newly assembled genomes were annotated using Prokka. The pangenome was constructed with all genomes using PIRATE with identity thresholds ranging from 45% to 98%. Pairwise genomic distances were calculated using Mash, and multidimensional scaling (MDS) was employed to visualize genomic relationships, complemented by K-means clustering to delineate distinct *E. coli* populations. Seven distinct *E. coli* clusters were identified, with Cluster 5 representing the largest group (53.6% of isolates, 2,156/4,023). The pangenome comprised 33,341 gene families, categorized into core (2,976), shell (2,724), and cloud (27,641) genes. Based on the metadata of our lab-collected APEC isolates and reference strains, two clusters exhibited particularly notable characteristics. Cluster 1 comprised predominantly highly pathogenic APEC strains associated with severe clinical manifestations. Cluster 5 displayed greater sequence type diversity and contained both disease-related and environmental isolates, with 90% of APEC strains in this cluster harboring a distinctive set of genes encoding proteins involved in carbohydrate metabolism (sugar efflux transporter SetA, mannosylglycerate metabolism), aromatic compound degradation (phenylacetate-CoA ligase, phenylacetate degradation proteins), and bacterial adhesion (fimbrial proteins), indicating possible functions in directly facilitating bacterial transmission between environments and hosts by enhancing survival in diverse conditions and enabling efficient attachment to new surfaces. This study highlights the genomic diversity of *E. coli* populations through the identification of distinct evolutionary clusters and reveals the unique genomic signatures associated with APEC transmission capability.

Keywords: Avian pathogenic E. coli, Genomic epidemiology, Pan-genome, Transmission

518P Not Presented

519P Evaluation of interior quality and microbiological aspects of eggs stored for 28 days at different relative humidity levels

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A study was conducted to evaluate the interior and microbiological quality of eggs stored at room temperature with three different levels of relative humidity (20, 60 and 100%). Fresh medium-sized eggs were randomly collected from those laid by 60-week-old Lohmann Brown hens (n=30). The eggs were positioned inside an airtight chamber to maintain the relative humidity levels for 28 d. Egg weights were determined on a weekly basis. The internal quality characteristics of the eggs were determined after 28 days of storage. The surface of each egg was swabbed with a sterile swab, and the total populations of aerobic bacteria, and yeasts and molds were determined. A one-way ANOVA was conducted for normally distributed data, while the Kruskal-Wallis test was applied to non-normally distributed data using SPSS. The highest egg weight loss (5.9%) was recorded for 28 days of storage in eggs stored at 20% relative humidity, whereas the lowest egg weight loss (0.39%) was observed at 100% relative humidity ($P<0.05$). The redness of the yolks from eggs stored at 20% relative humidity was higher than that of yolks stored at 60% relative humidity ($P<0.05$). After 28 days at 100% relative humidity, only 37% of the eggs were deemed consumable, whereas in the 60% and 20% relative humidity groups, the respective consumable egg percentages were 97% and 100%. Egg albumen height and Haugh unit values were significantly lower in eggs stored at 60% relative humidity for 28 days compared to those stored at 100% and 20% relative humidity. The egg albumen length and width were found to be significantly lower in eggs stored at 20% relative humidity compared to those stored at 60% and 100% relative humidity. The pH level of the egg yolk was not influenced by relative humidity. The egg yolk index decreased as the relative humidity level increased ($P<0.05$). The highest levels of mold-yeast and total bacteria were found on the eggshells of eggs stored at 100% relative humidity. The total mold-yeast level on the eggshells of eggs stored at 20% relative humidity was found to be lower than those stored at 60% and 100% relative humidity. No significant difference was found in the total aerobic bacteria and mold-yeast present on the eggshells stored at 20% or 60% relative humidity. In conclusion, eggs stored at 20% relative humidity maintained better internal quality. In contrast, eggs stored at 100% relative humidity experienced considerable quality deterioration, with a substantial reduction in consumable eggs and an increase in microbial contamination. Overall, low relative humidity storage (20%) appears to be more beneficial in preserving egg quality, while higher humidity levels may compromise both microbial safety and egg quality.

Keywords: Eggshell microbial activity, Relative humidity, Interior Quality, Egg yolk color, Egg

520P Effect of passage of an attenuated phenotype of *Salmonella enterica* serovar Typhimurium through embryos on parameters of virulence

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Salmonella enterica exhibits phenotypic and genotypic variability among serotypes, resulting in diverse infectivity rates, colonization capacities, and broad adaptability to various hosts. *In vitro* passage alters the phenotype of *S. enterica*, adapting it to laboratory conditions. These adaptations induce an attenuated state attributed to losing transcriptional factors, virulence plasmids, mutations, and regulatory factors. However, these phenotypes can be reversed in the case of interaction with a host. Thus, a deep understanding of factors that contribute to the effect of reversion to a state of virulence could lead to the development of effective control strategies for *S. enterica,* this being the principal objective of the current study. A previous analysis in *S. enterica* serovar Typhimurium (ST) from infected chickens and its lab-adapted strain showed significant differences ($P < 0.05$) for *FlhD* (motility) and *sdiA* (QS and virulence). For this investigation, we compared the phenotypic differences of the lab-adapted strain of ST passaged 1, 5, or 15 times through tryptic soy broth (TSB) medium (ST1, ST5, and ST15) with corresponding ST passaged through chicken embryos (STCE1, STCE5, and STCE15). Subsequently, biofilm formation, motility, and infectivity capacity in the chicken macrophage HD11 cells (HD11 cells) model in all passages were evaluated in triplicate. The optical density (OD 600 nm) of 1% crystal violet staining was measured to assess biofilm formation. Motility was evaluated by the relative diameter in a semi-solid medium. Infectivity was determined in HD11 cells after 30 min incubation (37 °C). The data obtained from the results were analyzed using one-way ANOVA. Biofilm formation increased ($P < 0.05$) in passages ST1, ST5, and ST15 in comparison to STCE1, STCE5, and STCE15. Motility increased in all passages (5 and 15) regardless of the source (TSB or embryo; $P < 0.05$). HD11 cell infectivity decreased ($P < 0.05$) at passages ST5 and ST15 compared to ST1. In embryo-inoculated samples, HD11 cell infectivity decreased ($P < 0.05$) in STCE1 and STCE5 versus ST1 and ST5. Results indicate ST passages in TSB boosted motility and biofilm formation but not HD11 cell infectivity. In conclusion, ST passages in TSB or chicken embryos showed different virulence factor responses. Passage of ST in chicken embryos did not increase the capacity of this isolate to form biofilm, motility, or infect macrophages. Future research will evaluate gene expression of selected virulence genes associated with *S. enterica* pathogenicity after passage on chickens.

Keywords: Salmonella Typhimurium, Chicken embryo, Biofilm, Motility, HD11 cells

521P Ultra fine ozone bubble water wash reduces *Salmonella* Enteritidis without negatively impacting meat quality and sensory attributes of chicken breast

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Salmonella Enteritidis (SE) poses significant food safety risks during carcass chilling due to potential cross-contamination via reused potable water or ice. This study evaluated the antimicrobial efficacy of ultrafine ozone bubble (UFOB) water as a natural intervention against SE on chicken carcasses and assessed its impact on chicken meat quality and sensory attributes. UFOB was generated in water maintained at 4°C using an ozone nanobubble generator. Chicken skin samples (4 × 4 cm) were spot-inoculated with a 4-strain cocktail of SE (7 log CFU/sample) and allowed to attach for 120 min at room temperature. One inoculated skin and nine uninoculated skins were submerged in 500 mL of either refrigerated deionized (DI) water (Control) or UFOB water (Treatment) for 30 min at 4°C. By including one SE inoculated skin among ten total skins (10%), our design closely models, USDA FSIS *Salmonella* performance standard, which allows a maximum of 9.8% positive samples in broiler carcasses. In a parallel experiment, non-inoculated chicken breast fillets (n = 24/group) were dipped in either DI or UFOB water for 30 min at 4°C and analyzed for pH, surface color (L*, a*, b*), thaw loss, cook loss, and sensory attributes by a descriptive trained panel. All experiments included duplicate samples, repeated in triplicate, and data were analyzed using Student's t-test with statistical significance set at $p < 0.05$. The dissolved ozone concentration in the UFOB water was approximately 9 ppm, with bubble concentrations of ~10^8/mL and diameters ranging from 100 to 200 nm. Cross-contamination analysis showed that dipping SE inoculated skin samples alongside non-inoculated samples in deionized (DI) water resulted in 100% cross-contamination (10 out of 10 samples positive). In contrast, treating with UFOB water significantly reduced cross-contamination by 50% (5 out of 10 samples positive; $p < 0.05$). Additionally, treatment with UFOB water resulted in ~1 log CFU/sample reduction in SE load on chicken skin compared to the control group ($p < 0.05$). No significant differences were observed in pH value, surface color parameters (L*, a*, b*), thaw loss (~3%), or cook loss (~33%) following UFOB treatment of samples ($p > 0.05$). Descriptive sensory analysis indicated no significant differences between control and UFOB ($p > 0.05$) treated samples in appearance, aroma, flavor, texture, basic taste, or feeling factors, suggesting that UFOB treatment did not negatively affect the sensory quality of chicken meat. These findings demonstrate that UFOB water effectively reduces SE without negatively impacting chicken meat quality or sensory attributes, supporting its potential as a sustainable antimicrobial intervention in poultry processing.

Keywords: Ultrafine Ozone bubbles, Salmonella, Chicken breast, Meat quality, Sensory attributes

Processing and Products

522P Growth performance and breast myopathies in broiler chickens fed different oils

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Chicken breast myopathies, including woody breast (WB), white stripping (WS), and spaghetti meat (SM) have caused substantial product quality defects which have negatively impacted the poultry industry worldwide. Though exact etiologies for these myopathies are unclear, lipid metabolism associated with energy production has been hypothesized to be a potential factor in breast myopathy development in chickens. In this study, the effects of using differential dietary oil sources were determined on growth performance, meat yield, and breast myopathy occurrence in

broiler chickens. A total of 420 0-d-old male Cobb 500 broilers were allotted to 3 treatments with 7 replicates. The three treatments were as follows: (1) Soybean oil without distilled dried grains with solubles (DDGS) control group **(N)**; (2) High Soybean oil with DDGS **(HSOY)**; and (3) High Coconut oil with DDGS **(HCCO)**. Energy levels were equivalent among treatments. In the early growth phase at d 7 and d 14, **HSOY** and **HCCO** treatment showed significantly beneficial effects (P<0.05) on body weight gain compared to **N**. No significant differences in body weight at d 42 and d 49 were observed. In the finisher phase (d 28 – d 42), feed conversion ratio (FCR) for **HSOY** and **HCCO** were significantly greater compared to **N** (P<0.05). The **HCCO** group exhibited significantly lower skeleton weight (P<0.05), and greater relative breast yields compared to **N** (P<0.05). With regards to organ weights, the **HCCO** group, but not **HSOY**, showed heavier heart weight compared to **N** (P<0.05). Regarding breast myopathies, **N**, **HSOY**, and **HCCO** groups had 40.48%, 34.78 %, and 22.86 %, respectively, of birds with severe WB scores (scores greater than 2 out of 3), indicating that the **HCCO** group exhibited decreased WB severity compared to the **N** group (P=0.077). Incidence of WS and SM were not significantly different between groups (P>0.05). These data indicate that the dietary use of coconut oil, which enriches medium chain fatty acid, may reduce chicken breast myopathy severity. Results suggest that use of different kind and level of oils, which possibly regulate lipid metabolism, may be an effective method to mitigate the severity of chicken breast myopathies.

Keywords: breast myopathy, coconut oil, chicken breast, lipid metabolism

523P Effect of in-water supplementation of trans-cinnamaldehyde nanoemulsion in layer chickens on quality and consumer acceptability of eggs

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Egg quality and consumer acceptability are critical factors in the poultry industry, influencing marketability and profitability. Natural compounds, such as phytochemicals, have gained attention as alternatives to antibiotics due to their antimicrobial properties. Trans-cinnamaldehyde (TC), a major component of cinnamon oil, is classified as Generally Recognized as Safe (GRAS) and exhibits strong antibacterial activity against *Salmonella* in layer chickens. However, the impact of TC supplementation on egg quality and consumer acceptance has not been extensively studied. Therefore, this study aimed to evaluate the effect of in-water supplementation of TC oil and its nanoemulsion (TCNE) form on egg quality parameters and consumer acceptability in layer chickens. A total of 48 seventy-week-old White Leghorn laying hens (12 birds/treatment) were randomly allocated to four groups: control, emulsifier control, TC-0.005, and TCNE-0.005. Birds in the TC and TCNE groups were supplemented with TC oil or TCNE at 0.005% in drinking water for nine weeks. Egg samples were collected bi-weekly, and egg quality characteristics were analyzed, including egg weight, shell thickness, shell strength, yolk color, and Haugh unit (HU). A consumer panel (n = 60 participants) assessed sensory attributes, such as aroma and flavor liking, texture, and overall impression, using a 9-point hedonic scale. Statistical analysis was performed using one-way ANOVA, with $p < 0.05$ considered statistically significant. Additionally, body weight gain, feed intake, and water intake were measured. In the final week of the study, control birds had an average egg weight of 65 g, with shell thickness and shell strength measuring 0.45 mm and 4.5 kg/cm, respectively. Similarly, yolk color and HU values were recorded at 9 and 75, respectively. In TC oil- and TCNE-supplemented birds, the average egg weight remained around 65 g ($p > 0.05$), with shell thickness and shell strength at ~0.45 mm and 4.5 kg/cm, respectively ($p > 0.05$). Likewise, yolk color and HU values were ~9 and 75, showing no significant differences compared to the control. Consumer panel evaluations for overall impression, aroma and flavor liking, texture, aftertaste, and purchase intent also revealed no significant differences in TC, TCNE, and control groups ($p > 0.05$). Additionally, body weight gain, feed intake, and water intake remained statistically similar across all treatments ($p > 0.05$). The results suggest that TC and TCNE supplementation did not negatively impact egg quality parameters or consumer perception. Therefore, TC and TCNE could be incorporated as natural antimicrobials to control *Salmonella* in layer chickens without compromising egg quality or consumer acceptability.

Keywords: Trans-cinnamaldehyde, Egg quality, Consumer perception

524P Reduction of *Salmonella* in immersion cChillers using Peracetic Acid (PAA) and sonication

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The poultry industry utilizes peracetic acid (PAA) as an antimicrobial agent in an effort to ensure reduce microbial contamination. Previously, we have shown that PAA's efficacy is highly dependent under varying chiller and dip tank conditions, where the impact on PAA of the organic loads present are poorly understood. This study builds upon a multi-year project examining PAA's chemical stability and antimicrobial properties against varying organic loads during processing. This year, ultrasonication combined with PAA was investigated in an effort to maximize *Salmonella* reduction in dip tank conditions. A bath sonicator was used for all related sonication studies. Each experiment used wings, drumettes, and drums, which were inoculated by submerging (for 10 seconds) into a *Salmonella Typhimurium* (10^5 cfu/mL) phosphate buffered saline (PBS) solution. The parts were drained for two minutes, placed in a bag, and incubated at 37 C for 60 minutes, allowing for surface attachment. After incubation, parts were randomly placed into four groups with n=15 per group: control, sonication+PAA, PAA only, or water sonication only. This experiment was replicated three times. Each part was exposed to its assigned condition for 15 seconds. PAA levels were maintained at 600 ppm with no pH adjustment. After exposure, samples were individually placed in a plastic bag containing 50 mL neutralizing buffered peptone water (nBPW) to deactivate any residual PAA. *Salmonella* was recovered by hand shaking each bag for 60 seconds and all samples were serially diluted in PBS. Three dilutions were plated (duplicates per dilutions) on 3M aerobic Petrifilm® in 1 mL volumes. Colony doc-it imaging station was utilized for all plate counts using the same identification template for each plate. Plates best fitting the counting range of 10-150 cfu/mL were selected

from the three dilutions per sample. Counts were averaged and the dilution factor was included in all final counts and log transformed. The results were averaged from fifteen samples per group. All statistical analysis of the data was performed using a One-Way ANOVA model followed by Tukey-Kramer HSD. The sonication only group, had an average 0.16 log reduction in *Salmonella* compared to the PAA only group which showed an average reduction of 0.93 log with a p-value of <.0001. The highest amount of pathogen reduction was seen in the PAA+sonication group, an average 1.08 log reduction in *Salmonella,* but was not statistically significant in reduction compared to PAA treatment. These results indicate that there is a synergetic effect between PAA and sonication on the reduction of *Salmonella* under dip tank conditions.

Keywords: Peracetic acid, Salmonella, antimicrobial, sonication, Salmonella reduction

525P Effects of duck breeds and chicken production types on egg quality characteristics through fourteen days of egg storage

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In recent years, duck egg consumption in the US has increased due to the availability of duck eggs. This study aimed to evaluate how egg quality differs between different duck breeds and chicken eggs found at retail. Duck eggs from 8 breeds (Jumbo Pekin-JP, Pekin-P, Rouen-R, Khaki Campbell-KC, Mallard-M, Duclair-D, Fawn and White Runner- FWR, and Silver Appleyard-SAY) were obtained from commercial duck breeder hens. Chicken eggs from 2 production types (conventionally raised, white shelled-CW and pasture-raised, brown shell-PB) were obtained from a local grocery store. Eggs were packed into pulp egg flats and stored in cardboard egg boxes at 3°C/93% RH during the study. Eggs were divided into 3 sampling groups (0, 7, 14 d of storage). On each day of sampling, 12 eggs were used for egg quality measurements of shell color (LAB), egg shape index (ESI), shell strength, shell thickness, whole egg and individual component weights, moisture loss, yolk to albumen ratio, yolk centering score, Haugh unit, yolk index, DSM yolk color, and vitelline membrane strength. Statistical analysis was performed using the General Linear Models procedure in SAS with means separated using Tukey's HSD. For shell L*, CW (94.52) was the lightest and PB (61.85) was the darkest (P<0.0001). PB eggs had a positive a* value (red), while all other breeds and production types had a negative value (green) (P<0.0001). Eggshells from PB (30.26) were more yellow (b*) than all other breeds and production types (P<0.0001) and CW had the lowest b* value (1.61). For ESI, eggs from JP and KC were classified as pointed, CW were round, and all others were normal shaped (P<0.0001). SAY had the highest (4.81 kg) shell strength and R had the lowest (3.65 kg) (P<0.0001). JP, P, D, and SAY (0.45 mm) had the thickest shells while CW had the thinnest (0.36 mm, P<0.0001). All duck breeds had heavier eggs and albumen than chicken eggs except for M (P<0.0001). All duck eggs had heavier yolks than chicken eggs (P<0.0001). All duck eggs had a larger yolk percentage and yolk to albumen ratio compared to chicken eggs (P<0.0001) while chicken eggs had a larger albumen percentage than duck eggs (P<0.0001). The largest moisture loss came from R (0.21%) and the smallest from JP and D (0.11%, P<0.0001). Yolks from chickens were more centered than from ducks (P<0.0001). CW had the highest Haugh unit (86.17), and R had the lowest (63.74, P<0.0001). Yolk index was higher, and yolks were darker in chickens compared to duck breeds (P<0.0001). All duck breeds had stronger vitelline membranes compared to chickens except KC (P<0.0001). These observed differences could influence storage stability and their suitability for functional properties.

Keywords: duck, chicken, egg, quality, storage

526P Field test study of prechiller dwell time variance

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Primary immersion chilling involves reduction of core temperatures of poultry WOGs (whole chickens w/o giblets) to prevent microbial and pathogen growth within the product. The pre-chiller stage has been widely accepted by industry as serving to initial clean WOGs of excess organic matter as a major part of HACCP (Hazard Analysis & Critical Control Point) disinfection plan and provide an initial chilling effect. However, due to the nature of the conventional "rocker-style" pre-chiller, there are apparent variations in WOG dwell times. The present study has been conducted to gain insight into the aforementioned phenomenon and quantify sample set averages and standard deviations of WOG pre-chiller dwell times in comparison with nominal facility dwell times. One sample set was taken at each of three facilities in the study with two sample sets taken at the fourth facility as there were multiple chillers in operation. The five sample sets of WOGs were tagged with color-coded, distinguishable IDs and passed through five separate pre-chillers with dwell times recorded for each WOG. Empirical results indicate notable pre-chiller dwell time variation amongst the five sample sets. Facility A's nominal pre-chiller dwell time was noted to be 17.5 minutes with the corresponding sample set of 20 WOGs determined to have an average dwell time of 12.4 minutes with a standard deviation of 5.8 minutes. The sample set of 20 WOGs for Facility B with nominal dwell time of 17.5 minutes resulted in an average dwell time of 21.5 minutes with standard deviation of 17.8 minutes while the sample set of 10 WOGs for Facility C with nominal dwell time of 30 minutes indicated an average dwell time of 29.5 minutes and standard deviation of 29.6 minutes. Facility D had a nominal dwell time of 30 minutes for each of their two parallel chiller operations and the two sample sets of 12 WOGs passed through each chiller resulted in average dwell times of 29.3 minutes and 25.4 minutes and standard deviations of 17.0 minutes and 18.4 minutes, respectively. From study results, it can be gleaned that in traditional pre-chiller operation, there is significant variance in individual WOG dwell times for a particular chiller operation even if the sample average does not indicate significant deviation from the facility's nominal dwell time. For example, in one sample set from Facility D, the sample average dwell time is 29.3 minutes while there was one WOG that experienced an 8-minute dwell time and another WOG that experienced a 68-minute dwell time within the same set. Overall, the variability and inconsistency in plant pre-chiller operations is notable and apparent and can have implications on desired uniform chemical and chilling exposure.

Keywords: Chilling, Process Control

SYMPOSIUM PRESENTATIONS

WPSA Lecture: Legal challenges in animal agriculture: Implications for the poultry industry

527S Legal challenges in animal agriculture: Implications for the poultry industry

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This lecture will explore legal issues facing the poultry industry, with a focus on laws and regulations affecting animal welfare and food safety. Attendees will gain insights into how legal frameworks influence scientific research and the broader agricultural landscape.

528S Chicken gut microbiota profiling: History and future directions

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In the last 15 years, the gut microbiome has been shown to play crucial roles in poultry nutrition, growth performance, and disease resistance. In coming years, technological advances in DNA sequencing and data analysis will continue to deepen our understanding of the mechanisms underlying these phenomena, leading to advancements in basic science and commercial applications. This presentation will speculate on future directions in the field and synthesize key findings from various collaborative efforts my research group has been involved with to design and apply new tools to poultry microbiome research. Highlights will be shared from several recent studies using now common tools such as 16S rRNA gene profiling, metagenomics, and genomics. In multiple studies we have shown that exposing day-of-hatch chicks to adult microbial communities significantly alters the composition and function of the gut microbiota, leading to improved weight gain, feed efficiency, and pathogen resistance. Metagenomic comparisons of birds with and without early-life exposure found that the major difference between low and high-performing birds was the presence of microbial hydrogenase genes in the cecum, suggesting an important role for fermentation reactions in bird performance. Another recent study from my research group has applied modern sequencing techniques to a historical product shown to be effective against *Salmonella* and *Campylobacter* colonization. By comparing the microbial gene content of the historical product to typical commercial broiler microbiomes, we were able to identify several metabolic pathways that may explain the mechanisms of action of this previously patented product. Specifically, we found statistical enrichment of genes involved in biosynthesis of phenylpropanoids, glycosphingolipids, and secondary bile acids. These pathways may contribute to pathogen exclusion by interfering with bacterial adhesion and immune signaling. Several *Bacteroides* species were identified as possible contributors to these protective effects. These results and others underscore the potential of microbiome-based interventions to improve food safety and performance goals. As microbiome science continues to evolve, particularly in the areas of rapid diagnostics, gut microbiota profiling will become more widely adopted and allow new insights into this important component of poultry science. By integrating these insights, this symposium will explore practical applications of microbiome engineering in poultry production, discuss the challenges of implementation, and highlight future research directions for more sustainable and efficient poultry farming.

Keywords: Microbiome, 16S rRNA, metagenomics, bioinformatics

529S Next-Generation Sequencing (NGS) technologies for rapid microbiome profiling

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The intestinal microbiome in animals is a complex system influenced by diet, genetics, and environmental factors, playing a vital role in the health, performance, and sustainability of poultry flocks. Manipulating this microbiome presents unique opportunities to develop strategies that enhance animal resilience against enteric stress, improve intestinal function, optimize nutrient utilization, promote animal welfare, and reduce environmental emissions. By focusing on the functional aspects of the microbiome, researchers can explore the activities of microorganisms and the beneficial metabolic by-products they produce for the host. A significant portion of substances in the bloodstream of animals originates from intestinal microbes, linking key physiological processes, such as digestion and fermentation, to overall health and welfare. Additionally, the removal of Antibiotic Growth Promoters (AGPs) from poultry feed has driven the development of alternative feed additives that support the establishment of functional, stable, and reproducible microbial communities. To achieve this, novel technologies, including omics approaches, are being integrated into both poultry research and commercial applications, with the potential to transform poultry production as these technologies become more affordable. For example, Next Generation Sequencing (NGS) technologies have revolutionized microbiome research in poultry, providing detailed insights into the microbial communities that influence poultry health, growth, and disease resistance. Techniques such as metagenomic sequencing (16S and whole-genome sequencing) and metatranscriptomics, utilizing platforms like Illumina Sequencing and Oxford Nanopore, are at the forefront of these advancements. However, these innovations present challenges, including data complexity and standardization. Despite this, real-time microbiome profiling for more personalized management strategies in poultry production is now becoming a reality. In conclusion, NGS is transforming poultry production by providing crucial insights into microbiome composition and functionality, helping to improve health, productivity, and sustainability in the industry.

Keywords: chicken, microbiome, sequencing, metagenomic

530S Deep learning models for understanding microbial communities

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Microbiomes, the complex communities of microorganisms residing in the gut, intestines, and other environments, serve as vital indicators of health in humans and animals. Traditional microbiome studies often focus on characterizing alpha diversity (the richness and evenness of microbial taxa within a single sample) and beta diversity (the similarity or dissimilarity in microbial composition among samples). However, these approaches alone may not fully capture the dynamic interactions between microbes, pathogens, and their hosts. Our study adopts a combinatorial approach, integrating advanced machine learning techniques with 16S rRNA gene sequencing microbiome data to predict the presence of zoonotic food-borne pathogens, Salmonella, Campylobacter, and Listeria, in pastured poultry systems. Additionally, we investigate the role of microbiota in modulating pathogen prevalence, and the emergence of antibiotic resistance, a growing global health concern. Our dataset includes soil and fecal samples collected from 42 distinct poultry farms across the southeastern United States over a six-year periodobtained at multiple stages of poultry development. To contextualize the microbial data, detailed farm management practices were also recorded. Using this rich dataset, we developed predictive models to identify "ideal" microbiome communities

tailored to specific objectives, such as minimizing antimicrobial resistance (AMR) and reducing pathogen prevalence. These models leverage machine learning to uncover complex patterns in microbial interactions that are not readily apparent through traditional statistical methods. Unlike conventional machine learning frameworks, which often assume that input features (e.g., microbial taxa or environmental variables) are uncorrelated, our approach incorporates more sophisticated deep learning architectures, such as graph neural networks and transformers. These models can integrate prior knowledge of feature associations, including those derived from external or seemingly unrelated datasets, to enhance predictive accuracy and biological insight. Our current research is focused on constructing large-scale microbiome models, analogous to large language models in natural language processing. These models are designed to encapsulate the vast complexity of microbiome data across diverse ecosystems and hosts. By fine-tuning these models with a small number of targeted samples, we aim to address specific research questions, such as identifying microbial signatures of health or disease. Furthermore, these advanced modeling techniques facilitate the development of more explainable models, which can elucidate the biological mechanisms underlying microbiome-pathogen interactions.

Keywords: deep learning, microbiome research

Informal Nutrition Symposium: Dynamic feed formulation in commercial poultry nutrition

531S Dynamic feed formulation in commercial poultry nutrition

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Historical approaches to poultry feed formulation have been rooted in static information, both in terms of setting nutrient specifications for stages of production, and assumptions regarding nutrient content and availability in feedstuffs. Dynamic feed formulation attempts to integrate real-time analysis of feedstuff nutrient composition and availability with more precise nutritional requirement information based on age, body weight, productivity, health status and other factors. Real-time adjustments to feed formulation to accurately meet the nutritional needs of poultry will increase efficiency and productivity, but advanced and complex information on the supply (feedstuffs) and demand (bird nutrient requirements) must be integrated accurately and quickly. The 2025 Informal Nutrition Symposium will bring together an outstanding panel of speakers to address the major topics that must be addressed in order to adopt a dynamic feed formulation approach.

Keywords: dynamic feed formulation, nutritional requirements, feedstuff nutrient composition

532S Key issues to address to successfully implement dynamic feed formulation

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This presentation will address several key issues to consider when implementing Dynamic Feed Formulation, in particular the manufacturing of the resulting diets and the efficient delivery of the diets to the classes of poultry they are developed for. After leaving the nutritionist's desk, the feed mill becomes a critical factor in the success of accurately making the diets as well as the accurate delivery to the birds. Every feed mill is different, which seems like an obvious statement, however a more accurate statement would be every feed mill has different capabilities and potential to implement programs. While critically important, feed mills and feed milling are considered as only a "cost" of production. Thus, implementing new or updated equipment and software will require an increase in cost(s) in the capital budget, which can be a challenge. Key areas to address are: 1) the number of ingredient bins, finished feed bins and micro-bins the mill currently has or that may be needed to implement Dynamic Feed Formulation. 2) The system that is used to manufacture diets needs to be addressed *i.e.,* is it a batch system or in some cases an in-line system. 3) The sensitivity of the scales weighing out the ingredients needs to be noted and considered, whether it is a major-, minor- or micro-scale in a batch system or the accuracy of determining ingredient weights in an in-line system. This will also include liquid systems. 4) Warehouse space must also be considered. 5) The personnel involved in the process of manufacturing of the various diets sent to the feed mill is another key issue. The ability of personnel to properly manufacture and deliver the various feeds needs to be assessed. Training of personnel is critical and important, especially if complexity is introduced. More tech-savvy personnel may need to be hired to effectively implement Dynamic Feed Formulation in a feed mill. Once again this will involve an increased cost to the feed mill. 6) In addition to bin space at the feed mill, bin space (numbers) on the farm to deliver Dynamically Formulated feed must be considered *i.e*., does the farm have enough bins per house to feed multiple diets. Dynamic Feed Formulations can only be effective if properly delivered to the bird. In conclusion, as stated above, every feed mill is different both in size and capability. In summary, in the effective implementation of Dynamic Feed Formulation, a "one size will fit all" program will not fit all situations and feed mills. The concept of "Reverse-Engineering" a program would be encouraged. Adapt a Dynamic Feed Formulation program that can be both efficiently and economically implemented at a given feed mill and company.

Keywords: Dynamic, Formulation, Feedmill

533S Opportunities and challenges for expanding NIR use in dynamic feed formulation

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Near-infrared infrared NIR has been used since the 1970s to quickly and cheaply estimate nutrient levels of raw materials and final feeds. The potential of NIR is broader than its current application of measuring nutrient levels in batches of feed. Recently, not only nutrient levels but also amino acid digestibility values of raw materials were estimated. For a wide range of protein-rich raw materials, amongst others soybean meal, *in vitro* protein digestibility assays were developed which well correlate with *in vivo* digestibility coefficients. NIR lines were developed based on the *in vitro* assays and were used to predict protein and amino acid digestibility coefficients and performance validation trials were performed. This digestibility prediction allows for a quicker and cheaper alternative to characterize raw material quality for more precise feed formulation, resulting in reduced variation in bird performance due to a more consistent dietary nutrient supply. Recent developments have shown that NIR tools with a specific wavelength can be used to estimate abdominal fat content in hens. Body composition during rearing can have significant long-term effects on laying performance and persistency in both laying hens and broiler breeders, and body composition during lay may also influence liver condition. Using NIR allows to select and measure a large number of hens from a flock non-invasively, allowing feed adjustments to maintain an optimal abdominal fat content to improve overall laying performance. Trials with laying hen flocks in the field are confirming the findings about the effect of body composition on laying persistency observed in research trials. Another application of NIR is to develop and use NIR lines for excreta analyses. NIR lines were piloted to analyze excreta composition in the field, initially focusing on wet litter conditions. Subsequently, these lines were extended to provide information about nutrient digestion and nutrient utilization efficiency. This can help to adjust feed composition to optimize performance and to reduce nutrient excretion. However, collecting good quality excreta samples in the field is still a limitation and further progress in NIR equipment may help to overcome this. In future, integrating rapid NIR analyses of raw materials, feeds, body composition and excreta can help to optimize feed formulation for different conditions in the field, to

reduce nutrient excretion, and to improve efficiency and profitability. The main challenges will be about changing and linking digestibility and utilization coefficients in formulation with performance model systems and about sampling frequency and accuracy.

Keywords: near-infrared, raw materials, in vitro assays, body composition, excreta

534S Dynamic formulation for amino acid and energy

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In general, broiler chicken diet formulations are based on feed composition and nutritional requirement tables for different production phases. It is important to always use data from the same source. However, it is also important to understand that feed intake per phase determines the actual fulfillment of the animals' nutritional needs. A well-formulated diet with lower-than-expected consumption during a phase does not benefit broiler development. Moreover, if we aim to be more precise in broiler nutrition, increasing the number of diet phases can lead to better performance results, improved gut microbiota development, and reduced nitrogen and phosphorus excretion, among other benefits. In formulation, a major advancement is when the nutritionist begins to use non-linear models. These models integrate nutritional requirements with certain production indicators, such as density, downtime, genetics, and others. The goal is no longer only diet performance and cost but the cost per kilogram of chicken produced before processing or the business's economic margin. Another innovation in formulation is the use of "in-line NIR," where diet formulas are adjusted in real-time according to ingredient composition variations.

Keywords: diet formulations, broilers

535S Beyond LCF - formulating for broiler performance, yield, and profitability

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Least cost formulation (LCF) software is a resource for nutritionists to generate formulas that are least cost considering a set of constraints. However, LCF does not aid nutritionists in determining nutrient requirements, and thus constraints, or determine whether its outcomes will be the most profitable feeds for an operation. Profitability modeling requires additional information that standalone LCF programs cannot interpret such as feed efficiency, yield, sales programs, meat prices, and operational constraints. Systems that are more complex exist that consider a greater number of factors than feed cost, but their implementation in the United States seems to be limited. It is the job of the nutritionist to consider all of these factors to maximize profitability as opposed to minimizing the cost of a feeding program.

Keywords: Least Cost Formulation, Poultry Nutrition, Profit Optimization

536S Putting the pieces together: the use of production datasets for predictive modeling within an integrated system

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Commercial broiler nutritionists are often tasked with multiple objectives when building feeding programs for operations. This juggling act attempts to satisfy the needs of the bird for optimal live performance while balancing the economic impact of dietary density, feeding schedule, flock age, and health. Nutritionists have many tools at their disposal to accomplish this feat. NIR allows for a cost effective solution to observe nutrient quality, ie proximates and amino acids, of in-bound ingredients to the feed mill. Implementing a vigorous monitoring program allows for the construction of robust datasets that can be used for routine updates of nutrient specifications in feed formulation. Although an extensive amount of research has been conducted to establish optimal levels and "requirements" for energy, lysine, and ideal protein ratios, the level utilized will be dictated by the performance goals of the individual complex, whether that be a fixed live body weight, or WOG or breast meat yield at the processing plant. However, the cost to produce this bird should be considered against the return relative to the finished meat market. Modeling of broiler responses to nutrient intake provides an opportunity for nutritionists to predict performance outcomes due to changes in dietary programs, such as changes in diet nutrient density, feed schedule, and age. The impacts of mortality can also be analyzed to predict not only changes in performance, but the feed intake and subsequent feed conversion ratios associated with these changes. Model inputs should include dietary nutrient densities, the feeding schedule, as well as the average flock mortality and age, with body weight, feed intake, feed conversion ratio, and yield serving as outputs. Dietary cost can also be included to measure the returns netted or lost due to program changes Previous observations of a nutrient response model developed for use within an integrated system indicated the ability of the model to predict performance of consecutive datasets assessed for program changes. The model is mechanistic, employing several empirical models representing the impact of diet, age, and mortality on performance responses; stochastic, taking into account the variation of responses observed within a commercial broiler operation, with contract grow-out farms serving as replicates; and dynamic, considering changes in age as well as ingredient pricing and diet composition in assessing the impact on predicted performance.

Keywords: broiler, nutrient response model

The many facets of glucocorticoid actions from stress and beyond

537S Historical and present understanding of glucocorticoid research in poultry: A case for reevaluation of the entire avian hypothalamo-pituitary adrenal axis

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An undergirding principle across vertebrates is that stress is the reciprocal of welfare and is mediated by glucocorticoid hormones. In mammals excluding rodents, cortisol is the dominant glucocorticoid hormone. While predominantly in rodents, the glucocorticoid is cortisosterone (guinea pigs being an exception). A similar state-of-the-art viewpoint with corticosterone as the glucocorticoid existed in poultry species until recently. There is increasing evidence that domesticated birds have both corticosterone and cortisol. In poultry species, this contention will be advanced, as will, the need for further investigations. The issues of appropriate assays, standards, validation and both inter-laboratory and intra-laboratory issues will be addressed with recommendations of what is needed. Again, across the vertebrates, the hypothalamo-pituitary-adrenal axis controls the synthesis and release of glucocorticoids. In birds, the hypothalamic neuropeptides are corticotropin releasing hormone together with arginine vasotocin controlling release of adrenocorticotropic hormone (ACTH) from the pituitary gland. In turn, ACTH stimulates the synthesis and release of adrenal glucocorticoids. This simple system provides rapid amplification of signal when a bird experiences stress. However, it is unstable and, arguably, an over-simplification. An additional control point is binding of glucocorticoid to a corticosteroid binding globulin such that the glucocorticoid is trapped in a reservoir or sink with only free glucocorticoid being able to bind to specific nuclear receptors. There is now evidence for additional neuropeptides influencing glucocorticoid synthesis and release. These include neuropeptide W, Met-enkephalin, possibly other opioid peptides and parathyroid hormone related peptide. Neuropeptide W acts at the level of the anterior pituitary gland. Not only are multiple stressors followed by increases in plasma concentrations of Met-enkephalin but also opioids affect plasma concentrations of corticosterone. The site of action and of synthesis of Met-enkephalin are both the anterior pituitary gland and adrenal gland. Cross talk between the chromaffin and cortical cells of the adrenal and the corticotrophs, other adenohypophyseal secretory cells and folliculo-stellate (neural) cells are likely to be viewed as of increasingly importance. As progress is made, our understanding of the avian hypothalamo-pituitary adrenal axis becomes more profound and complex. This is to be expected.

Keywords: Cortisol, Corticosterone, Adrenal, Pituitary

538S What wild bird studies can tell us about how to interpret corticosterone responses in poultry

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Corticosterone measurement is a popular technique to assess stress in birds. Although data are typically interpreted in reference to biomedical data from humans or laboratory rodents, data from wild free-living birds often contradicts those interpretations. This talk will use results from ecological research on wild birds to address two major areas where corticosterone results are often misinterpreted. **Interpretation 1**: Corticosterone levels can diagnose stress in a bird. Although this is often claimed, the literature does not support such a statement. Because corticosterone is needed to survive stressors, high acute levels of corticosterone might indicate a bird that is successfully coping with a stressor rather than a bird in distress. Conversely, low corticosterone concentrations might indicate a bird unable to cope rather than a healthy bird. Furthermore, studies from wild populations indicate that chronic stress cannot be diagnosed from sustained elevated corticosterone alone. Finally, interpretations of corticosterone levels greatly depend upon the tissue in which corticosterone is measured (e.g., plasma, feces, or feathers) When taken together, it is clear that corticosterone levels by themselves are not diagnostic of stress. **Interpretation 2**: Corticosterone mobilizes glucose to survive short term stressors. Corticosterone has two primary effects on glucose. First, it removes glucose transporters from cell surfaces, meaning that measurable increases in plasma glucose during stress reflect decreased, not increased, glucose utilization. Second, corticosterone induces gluconeogenesis, but this is on the time scale of hours. The conclusion is that corticosterone mobilizes energy to recover from stressors, not to fuel the coping response itself. In summary, proper consideration of these common assumptions will greatly aid in using corticosterone data to interpret stress in birds, and hence welfare.

Keywords: corticosterone, stress, feathers

539S Glucocorticoids are not just about stress: from metabolism to epigenetics and the rheostatic control of baseline

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Glucocorticoids are among the most commonly measured hormones in poultry science to determine birds' stress, welfare, or reaction to different environmental conditions. However, in vertebrates glucocorticoids primary physiological role is in energetics and metabolism. The activation of the septo-hypothalamic-adrenal-axis during a stressor is an allosteric response. Allostasis is an organism's programmed response to maintain homeostasis and is a relatively short term response to increase glucocorticoid levels in order to cope with a stressor. There is a hierarchical model of responses to cope with a stressor that can exist at the genetic, cellular, neuronal, and organismal levels but again, at a relatively short time frame. For long term changes in baseline, another model exists referred to as rheostasis. Rheostasis is a regulated change in set points over time within a broader range that still maintains stability; may also be thought of as a resetting of physiological zero. This seminar will first define and go over examples from the Fraley and from the literature about each type of homeostatic control. Second, we will discuss the relative roles of corticosterone and cortisol in homeostasis in poultry using data from the Fraley lab and others. Third, we will review potential epigenetic effects of glucocorticoids to pass along traits to offspring and the implications of these actions for poultry.

Keywords: glucocorticoids, homeostasis, allostasis, rehostasis

540S Developmental and metabolic impacts of glucocorticoids in poultry

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In addition to their role in mediating the acute and chronic stress response, glucocorticoids have developmental and metabolic impacts that ultimately influence avian growth and body composition. Studies in broiler chickens that have been genetically selected for efficient growth and meat production have highlighted an essential role for corticosterone in functional differentiation of growth hormone (GH)-producing pituitary somatotrophs and point to differences in corticotropic axis activity that contribute to the potential for growth and muscle accretion. Injection of eggs with corticosterone during mid-embryonic development stimulates pituitary GH production and appears to alter post-hatch nutrient partitioning in a way that increases total meat yield without affecting overall body weight. On a molecular level, rapid activation of ERK1/2 MAPK signal transduction pathways appears necessary for maximum induction of GH expression, indicating that non-genomic signaling mechanisms play an important role in the glucocorticoid induction of somatotroph differentiation during embryogenesis. In two separate studies, broiler chickens with higher growth potential were shown to have lower circulating corticosterone and predicted to have reduced glucocorticoid receptor activity in anterior pituitary and muscle, leading to decreased expression of genes associated with the immune response, inflammation, and other cellular stressors. This difference in corticotropic axis activity also appears to alter glucose metabolism in response to elevated ambient temperature. Recently, we have investigated a potential role for yolk sac tissue in the endocrine regulation of embryonic growth and development. Corticosteroid binding globulin exhibits a marked increase between early and mid-embryogenesis, suggesting this might be a tissue that facilitates both yolk and systemic glucocorticoid utilization by the developing embryo. Expression of corticosteroid binding globulin is also high within the ovary before and after the onset of egg production, indicating that it may facilitate deposition of maternal glucocorticoids into the yolk. Together, these results demonstrate a critical role for glucocorticoids beyond the stress response and highlight their importance in mediating avian growth, development, and metabolism.

Keywords: corticosterone, embryonic development, growth, muscle accretion, yolk sac tissue

541S Physiological responses of wild birds to climate change- and urbanization-related temperature variation

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Ambient temperature (T_a) is increasing worldwide due to climate changes and urbanization. This increase has important consequences to avian populations including on their geographical range, biodiversity, and species assemblage composition. The current T_a changes occur at a rate such that most organisms may be capable to adjust to them phenotypically within limits, but cannot keep up with them through genetic adaptations. Understanding the consequences of T_a increase, therefore, requires that we identify the limits of phenotypic plasticity. Research on this subject in wild birds exists, but is challenging as changes in environmental factors (e.g., T_a, day time, and activity of arthropods that birds consume) often covary and their effects can be difficult to isolate. Thus, manipulative experiments in controlled laboratory settings are also necessary. Defining reaction norms (ReNo), i.e., the sensitivity of a group of genotypically identical organisms to a particular environmental factor, is also fundamental to understanding phenotypic plasticity. These norms are physiologically- and biochemically-based, but as yet poorly understood for wild birds. To address these questions, we measured blood glucose (GLU), uric acid, the stress hormone corticosterone (CORT), and heat shock proteins (Hsp) levels in wild House Sparrows, *Passer domesticus*, sampled during the day along a latitude gradient of T_a, and at baseline as well as in response to acute (30 min) handling/restraint stress. We hypothesized that daily variation in physiological parameters would change latitudinally, driven either by T_a or by time of day, which are associated. Some parameters showed temporal plasticity of ReNo during the day (Hsp) and in response to daily T_a changes as well as to stress (GLU and CORT). However, we found little evidence for similar plasticity in ReNo across the latitudinal gradient, indicating that birds can adjust phenotypically to a wide range of thermal conditions. In complementary laboratory work, we measured the energy intake and blood parameters (GLU, uric acid, ketones, and oxidative stress) of House Finches, *Haemorhous mexicanus*, exposed to cool or warm T_a near the thermoneutral zone boundaries. Few differences between groups were observed and changes in the above parameters during stress were not T_a-related. If applicable to other taxa, these and other results indicate that birds exhibit considerable resilience to variation in thermal conditions and so may adjust easily to limited climate change- and urbanization-related warming.

Keywords: Corticosterone, Glucose, Heat shock proteins, temperature, Reaction norms

The feed milling process: A focus on pelleting, liquid application, and their effects on animal production

542S History of the American Feed Industry

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The American feed industry has evolved over the last 150 years to meet the needs of livestock and poultry producers. The industry started in the late 1800s by supplying feed for horses and mules. Early feed mills included Blatchfords, 1875, H.K. Webster Company, 1868, Moorman Manufacturing, 1885, Purina Mills, 1894. These feed mills went through a number of different ownerships and are now parts of companies such as Blue Seal Feeds, ADM Alliance Nutrition and Land O Lakes/Purina. The American Feed Manufacturers Association was established in 1909 to create standards and collaboration throughout the industry. The industry continued to grow with the construction of larger feed mills, that included grinders, mixers, micro-ingredient systems, pellet mills, and bulk delivery. The development of integrated poultry and swine industries in the 1950s changed the landscape of the feed industry as new feed mills were built for single species feed production. The industry is now comprised of multi-species commercial feed mills, toll mills, and integrated feed mills. The early feed mills formulated feed with minimal information on the nutrient values of ingredients and nutrient requirements of animals. In the late 1800s farmers relied on grains and roughages raised on the farm to feed their livestock and poultry. The develop of proximate analysis techniques and feed standards by Henry and Morrison and National Research Council helped propel the industry forward. It was discovered that by-products from the milling, meat packing, and oil seed processing industries had feeding value for animals. Molasses, corn gluten, brewer grains, and meat scraps were some of the earliest ingredients used in livestock feeds. The production of SBM in 1922 created a shift in diet formulation, SBM soon became the major protein source in feed. The development of vitamin and mineral premixes, medications, and synthetic amino acids helped balance diets to meet nutrient requirements of animals for improved growth and feed efficiency. The use of computers in the 1980s for least cost formulation of diets created a major shift in how animals were fed to meet their nutrient requirements. The modern feed industry is faced with many challenges but these challenges are not unique to today's feed industry, in 1961 Dr. Robert Schoeff, a long time extension leader from Kansas State University wrote, "The challenge facing the formula feed industry today is formulating, manufacturing, and distributing quality feeds to the livestock and poultry farms that will enable them to provide quality products at the lowest possible cost".

Keywords: Feed Industry, Feed Mills

543S Perspectives on pelleting: What is too little and too much in regard to nutrition?

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The decision to manufacture pellets may be simple. The process of making high quality feed that maximizes poultry performance efficiency is complex and may be met with resistance. This presentation will address those complexities and will highlight recent work conducted to support the manufacture of high-quality poultry feed. The five key points of this presentation will provide data that supports improving feed quality. The recently conducted studies will provide new perspectives on improving feed quality to enhance nutrient uniformity throughout the house. **Feed form** – A brief review of the literature that compares performance of mash fed vs pellet fed broilers. Recent data comparing light and heavy hen turkey performance when fed mash vs pellets. **Feed manufacturing strategies** – Reviewing strategies to improve the physical quality of feed. Considering the implications to nutrient availability, enzyme efficacy, and subsequent poultry performance. **Pellet quality** – A review of recent literature on performance effects from varying ratios of pellets and fines in complete feed. Recent performance data on heavy toms, light hens, and heavy hens when fed diets varying in ratios of pellets and fines in the feed pan. **Nutrient segregation** – A review of replicated field studies considering how nutrient profiles change and how nutrients are presented in feed pans throughout the poultry house. **Poultry Performance** – Using findings from the field and better understanding their impacts to poultry performance with replicated pen studies at University. When considering these five key points, decision makers will be better informed on the importance of using feed manufacturing strategies that allow a more uniform distribution of nutrients and feed particles throughout the poultry house. Using centrally located feed bins and augering high P:F feed to either end of the house can eliminate nutrient segregation, ultimately allowing for efficient and uniform flock production. While the challenges of manufacturing a high-quality feed are numerous, both field studies and replicated pen trials at universities continue to demonstrate the opportunity to improve production efficiency.

Keywords: Pellet quality, nutrient segregation, uniformity

544S Liquid application in today's feed mill environment and importance

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As feed technology changes and demands on feed production increases liquid ingredient applications become more and more important. Early on, there would have been one liquid included in the feed mix. That has drastically changed with as many as 7 - 8 liquids being added in a single batch. With more liquids being added, technology had to change to allow production standards to keep up as well as mixability and pellet/feed quality. As diets change and mixer sizes increase, the need for post pellet liquid application started manifesting. This also allowed the addition of heat sensitive liquid applications with post pellet processes. The proper function and calibration of all of the liquid application equipment; mixer/batch as well as post pelleting, has been proven in real-world applications to provide better feed quality, animal performance and cost savings.

Keywords: Liquid, post-pellet, flow meters, pumps, mixer

545S Pellet quality from a production perspective: What is measured and why is it important

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Pelleting broiler feeds has been a well-established endeavor to improve feed handling and palatability and thus broiler performance outcomes. There are several ways to measure and interpret pellet quality, but within any operation, the measurement technique should be consistent and relative. While the benefits have been consistently reported in the scientific literature, there

remains physical and nutritional limitations that should be considered while attempting to improve pellet quality. From a production perspective, inconsistencies in raw materials, weather, and management make it difficult to draw definitive conclusions regarding slight improvements or reductions in pellet quality. However, the notion that improving pellet quality will improve broiler performance outcomes seems to be well accepted and operators should proceed cautiously to ensure that a desire for more pellets in the pan does not lead to a reduction in the nutrients available to broilers.

Keywords: Corticosterone, Glucose, Heat shock proteins, C541, Reaction norms

546S Feed Milling Dynamics: What should we monitor and what can we improve?

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With the rise of AI, the focus has shifted to utilizing the compounding data that is generated through the feed manufacturing process. This would allow feed manufacturers a better insight into their process and allow them to make real-time decisions that would improve their finished products. With the end goal to be safer, more competitive and more cost-effective. While this technology may be at our fingertips there is a cost barrier that companies will have to realize. Until that point proven practices in the art of feed milling will continue to serve and push companies forward. With a focus on Safety, Customer confidence, Regulation, and Cost companies and producers can continue to monitor and improve their process In this presentation we will explore when monitored and utilized these four areas can impact a company's performance and productivity. Safety: The most important and dynamic process of any company should be safety. Creating safe practices will maintain not only worker safety but environmental safety leading to a more sustainable outcome. Customer confidence: Customers are at the heart of everything we do. Maintaining a positive reputation and brand leads to company longevity in the marketplace. Regulations: A target that is constantly evolving and we must meet the demand. Whether governmentally imposed or customer requested, regulations must be monitored and met to continue to conduct business. Cost: Many costs go into the production of food animals. As one of those costs, feed can account for 60%-80% of production cost. Factors that concern producers and add to feed cost can be off spec product, the time it takes to rework, downtime, employee morale, and damage to brand. While covering these areas of focus, this presentation will highlight the need to instill a proactive approach to safety, quality assurance and maintenance. As the pace of information continues to build it is these fundamental duties that allow for continued improvement in feed milling.

Keywords: Feed milling, metrics

Current perspectives on the control of Avian Influenza in the poultry industry

547S Environmental dynamics and transmission of high pathogenicity avian influenza in poultry production

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Disease transmission among poultry, such as highly pathogenic avian influenza (HPAI), can very complex and depending on the situation (e.g., initial transmission from wild waterfowl vs farm-to-farm transmission) different factors may be important and identifying the source of an infection can be difficult. Salient features of transmission pathways for HPAI include biological components associated with the virus and host, and also needs a mechanism that provides an interface through which transmission can occur. Notably, transmission pathways are similar among many viral diseases and measures that can reduce the transmission of other diseases will be relevant for HPAI. Here, known factors that contribute to risk of infection based on epidemiological studies, surveillance, and animal studies are described.

Keywords: avian influenza

548S Transmissibility of avian influenza from dairy cattle to poultry

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Wild birds serve as the primary reservoir for avian influenza viruses (AIVs) with periodic spillover events leading to outbreaks in domestic poultry and mammalian species. Recent incursions of highly pathogenic avian influenza (HPAI) H5N1 into novel hosts, including domestic cattle, have raised concerns regarding interspecies transmission dynamics. In early 2024, HPAI H5N1 clade 2.3.4.4b was detected in dairy cattle across multiple U.S. states, marking the first known sustained transmission of AIV in bovine species. Transmission is occurring within and between herds, with potential implications for cross-species spread to poultry. In response to this outbreak, we initiated high containment research focusing on susceptibility and transmission routes of an influenza A H5N1 isolate, A/Bovine/Ohio/B24OSU-439/2024, in peak lactation dairy cattle. Our objective was to define the epidemiology of IAVs in livestock, providing critical data on the potential transmission from dairy cattle to poultry. We hypothesized that HPAI clade 2.3.4.4b would infect dairy cattle experimentally challenged with high dose virus intranasally and would transmit the virus to cohoused chickens.Two peak lactation Holstein dairy cattle were inoculated intranasally (IN) with A/Bovine/Ohio/B24OSU-439/2024 HPAI using 2.5 mL of 1 x 10^8 TCID50/mL per nostril via a nasal mucosal atomization device. Twenty-four hours post inoculation 8 spent laying broiler chickens were placed into open air caging between the two infected cow pens. In addition to cow sampling, the chickens were monitored for clinical signs and swabs of the cloaca and oropharynges were taken daily. Chickens were euthanized on day 6 and 17 post exposure and necropsied. Samples were screened by polymerase chain reaction for the presence of HPAI viral RNA. Cows challenged IN with HPAI had detectable viral RNA in nasal swabs for 5 days post inoculation. No severe clinical signs were detected in these animals out to 18 days post infection. HPAI viral RNA was not detected in chickens co-housed with IN inoculated cows. Despite A/Bovine/Ohio/B24OSU-439/2024 being reported as highly virulent to chickens, we were unable to reproduce efficient cow to chicken spread in the high containment facility. These results may stem from inefficient HPAI replication in the bovine respiratory tract and high containment engineering measures, including directional airflow (>15 air exchanges/hour) and HEPA filtration of milking pump exhausts, limiting aerosol spread. While HPAI remains a serious threat for transmission between cattle and poultry, appropriate engineering controls, biosecurity, and the introduction of vaccination programs can potentially mitigate the spread of HPAI.

Keywords: Influenza A, H5N1 virus, HPAI, dairy cattle, high containment

549S A One Health approach to avian influenza: Interrelationships between poultry, dairy, and humans

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Highly Pathogenic Avian Influenza A (H5N1) is an adaptable virus that has decimated poultry in the United States since early 2022. The 2024 recognition that it had spilled over into dairy cows, followed quickly by identification of growing numbers of cases in dairy and poultry workers, complicates prevention and control efforts. The objective of this scientific and policy analysis is to apply a One Health approach to evaluate the interconnectedness of the poultry and dairy sectors—with particular emphasis on risks to workers, consumers and the general public—as these relate to transmission risks and mitigation strategies. The poultry and dairy sectors are connected in many ways—via movement of wild birds and other wildlife, through shared farmworkers or workers in the same household or community, and when poultry and dairy cows are produced on the same facility or in close geographic proximity. A United States Department of Agriculture evaluation of early cases of farm-to-farm transmission among dairy farms identified shared personnel and equipment as potential risk factors for transmission. The potential for peridomestic rodents (field mice) and airborne (plume) transmission are emerging concerns that require further study. Of high concern is the potential for this virus to continue to evolve (or worse, reassort) resulting in more severe disease, human transmission chains, and resistance traits—all of which could complicate control efforts and further the pandemic potential of the virus. This is balanced by carry-on impacts within the industry and to consumers from ongoing animal disease. Mitigation strategies to introduce strong biosecurity practices to dairy production and enhance existing efforts in the poultry industry require consideration of human, animal and environmental factors that influence introduction (spillover) and transmission (lateral movement) among farms. For example, protection of workers from exposure may help to prevent lateral spread among facilities and reduce risk of human transmission chains and further virus adaptation. Attention to both biosecurity and biocontainment through attention to the facilities themselves, to how humans move through the system, and to points of incursion from wild birds and rodents can both prevent new introduction from wild animal reservoirs and safeguard neighboring facilities. This persistent virus may require novel mitigation strategies, such as attention to the ecology of the virus outside of the affected industries, to improve control.

Keywords: One Health, avian influenza, bird flu, H5N1, workers

550S Vaccination strategies and surveillance of high pathogenicity avian influenza in poultry

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Biosecurity, surveillance and stamping-out are the primary methods used in the prevention and control of high pathogenicity avian influenza (HPAI) in U.S. poultry. However, the current H5N1 2.3.4.4b clade virus has challenged the completeness and sustainability of this strategy. Vaccination is an additional tool that can reduce susceptibility of poultry to HPAI virus infection, diminish replication and shedding of virus, greatly reduce the virus quantity in environmental contamination and decrease the number of outbreaks. An effective, sustainable HPAI vaccination program should follow the principles of Avian Influenza Vaccine Stewardship which includes: antigenically matching the vaccine hemagglutinin to the circulating HPAI virus, especially critical for inactivated vaccines, monitoring the vaccinates for a protective immune response to assess herd immunity and booster vaccinate when required, and conduct appropriate surveillance for HPAI virus. The United States Department of Agriculture-(USDA)-licensed H5 vaccines are fit-for-purpose by being safe, pure, potent and efficacious against specific H5 HPAI viruses, and can fit into existing laying chicken and meat turkey vaccination programs for endemic diseases. The inactivated H5Nx oil emulsified, an RNA particle and the two recombinant herpesvirus of turkeys with H5 gene insert vaccines have been shown to be efficacious against the current H5N1 2.3.4.4b clade and to be effective in stopping poultry transmission in USDA and European experimental laboratory and field studies. In addition, a H5 subunit protein vaccine and a self-amplifying RNA vaccine have been successfully used in France to protect domestic ducks. The principles for Detecting Infections in Vaccinated Animals (DIVA) can be fulfilled by either virological or serological surveillance. Virological surveillance uses the USDA-approved quantitative real-time reverse transcriptase polymerase chain reaction assays (qRT-PCR), and targeted samples of daily mortalities, supplemented by sick poultry. These qRT-PCR assays are highly specific and sensitive for detecting the H5N1 2.3.4.4b clade HPAI virus and have been used extensively for diagnosis and surveillance in non-vaccinated poultry and other animals. These qRT-PCR assays and targeted samples have the same utility for use in vaccinated poultry to detect HPAI viruses. However, serological surveillance is of limited value because of false positives that require further investigation, and for the need for specially designed inactivated vaccines with heterologous neuraminidase and companion neuraminidase tests for serological DIVA strategies. Infections in a well-vaccinated flock will be uncommon, and any transmission will not be sustained.

Keywords: Avian influenza, H5N1, High Pathogenicity Avian Influenza, Surveillance, Vaccination

551S Controlling avian influenza in turkey flocks

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Avian Influenza (AI), in all its forms, is an important disease of turkey flocks. Because of turkeys' susceptibility, both low pathogenicity avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) can have dramatic effects the turkey industry. LPAI can lead to decreased production, increased condemnation and elevated mortality. HPAI results in entire flock loss, increased downtime for both the affected farms and neighboring farms, as well as costs associated with depopulation, disposal and cleanup. Understanding the history of AI in turkeys in the United States is important to the control of AI and the different regional approaches. This history and sometimes a lack of history will be discussed as well as veterinary experiences with LPAI and HPAI incidents in their flocks. Biosecurity and surveillance methods, as well as innovative trials and strategies from experienced veterinarians will be shared. The efforts of the turkey industry in decreasing the impact of avian influenza will be highlighted: what is working, what is not working and where research could help in control efforts.

Keywords: Avian Influenza, turkey

552S Future research on Highly Pathogenic Avian Influenza (HPAI) for the development of superior control strategies for poultry production

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Highly Pathogenic Avian Influenza (HPAI) remains one of the most devastating infectious diseases affecting poultry worldwide, posing a serious threat not only to animal health and food security but also to public health and global trade. Recent outbreaks, particularly those involving clade 2.3.4.4b viruses, have demonstrated a significant shift in virus ecology, with increasing host range, environmental persistence, and transboundary spread. These evolving characteristics highlight the urgent need for innovative and interdisciplinary research to develop more effective and sustainable control strategies. Future research must prioritize enhanced surveillance systems, particularly at the wild bird–poultry interface, to detect and respond to emergent strains rapidly. Genomic studies will be critical in tracking virus evolution and understanding host-pathogen interactions, including mechanisms of pathogenicity and immune evasion. Equally important is the advancement of next-generation vaccines capable of providing broad and durable protection against diverse HPAI strains, as well as rapid diagnostics for early detection and containment. The integration of One Health approaches, considering the interconnectedness of animal, human, and environmental health, is essential for comprehensive risk mitigation. Furthermore, research should explore novel biotechnological tools, such as CRISPR and RNA-based platforms, to target viral replication or enhance host resistance. Socioeconomic analyses and behavioral studies are also necessary to evaluate the feasibility and adoption of control measures by stakeholders. Ultimately, a coordinated global effort, supported by scientific innovation and strong policy frameworks, is vital to reduce the burden of HPAI and protect the future of poultry production.

Keywords: Highly Pathogenic Avian Influenza (HPAI), control strategies, poultry, one health, Policy and Regulation

The inflammation saga: Breakthrough nutritional insights for poultry

553S The nutritional impact of gut inflammation

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The intestinal tract is one of the largest, if not the largest surface area exposed to the environment of poultry. Although contained within the body, the lumen of the digestive tract is actually outside of the bird. Therefore, although the function of the intestinal tract is usually thought of as being the uptake of nutrients, it has another important function: preventing access of microbes and toxins to the tissues of the bird. The intestinal tract has multiple layers of defense including passive mechanisms such as mucin, tight junction proteins and the epithelial surface. Additionally, the immune system has a large presence in the intestinal tract, monitoring the presence of commensal and pathogenic microorganisms, and detecting proliferation of potential pathogens within the gut. If a microbe is able to breach the passive intestinal defenses, localized inflammatory responses are initiated to limit the translocation of the pathogen. If necessary, cells of the adaptive immune response are recruited and activated, and if a more vigorous response is necessary, the inflammation can become systemic. The activation of a systemic inflammatory response reduces poultry performance through a number of different mechanisms. The primary effect, particularly in young birds, is a dramatic reduction in feed intake, thus reducing the supply of building blocks for growth and egg production. However, additional mechanisms such as the cost of increasing protective barriers at the level of the gut, a mobilization of muscle protein to support protective acute phase protein production, fever, and reduced efficiency of nutrient absorption also contribute to a loss of performance. The use of antibiotic growth promotors (AGP) allowed growing birds to achieve a greater proportion of their genetic potential for growth. The global move away from AGP requires that we both understand the nature of intestinal inflammation, and develop effective alternatives to replace AGP in poultry diets.

Keywords: nutrition, cost of immunity, inflammation, intestinal health

554S The impact of mineral chelation and potential considerations for nutritional immunity and inflammation

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Owing to headwinds in raw material availability and quality, as well as legislations on the use of antibiotics and environmental impact there is more focus on precisely optimizing diet formulations in production. Interestingly, trace minerals (TMs; Zn, Cu, Fe, Mn) are typically over-supplied given their importance in biological processes compounded by their relative affordability. TMs are critical to immune function. For example, Zn is important in the formation of reactive oxygen species formation in innate immune cells, such as Heterophils, as well as playing. That said, animals and avian species have evolved mechanisms to limit the availability of TMs to reduce toxicity and prevent utilization by pathogens. This is known as Nutritional Immunity (Hood et al, 2013). Recent publications demonstrate the ability of phytase to help remove inorganic phosphate from diets, reducing environmental impact and feed costs whilst maintaining bird performance (Dersjant-Li et al, 2022 & Marchal et al, 2021). However, phytate also has a strong affinity for TMs at intestinal pH, forming difficult to digest complexes. Consequently, this reduces the bioavailability of TMs in raw ingredients, otherwise found in sufficient abundance to meet requirements. A fast-acting phytase with activity at low pH prevents such inositol complex formations, thus reducing the need to supplement TMs (Dersjant-Li et al, 2025). This approach has been shown to positively impact the microbiome. Recent work involving 500 Ross 308 broilers challenged with the *C. jejuni* JB strain (0.1 ml of ~1.0 x 10^6 CFU/ml) at 7 days of age demonstrated a 0.87 Log_{10} reduction (P=0.12) in mean *Campylobacter* caecal load and a 1 Log reduction in highest threshold (4.9 x 10^7 CFU/g vs 3.8 x 10^8 CFU/g). *Campylobacter* is a leading cause of gastroenteritis globally, and poultry is a primary source. Such a reduction was achieved by removing added iron from a sulfate-based premix, removing inorganic phosphate and including a consensus 6-bacterial phytase (Axtra® PHY GOLD) at 3000, 2000 and 2000 FTU/kg feed in starter, grower, finisher diets respectively. This illustrates the importance of iron and other TMs in microbial pathogenesis. Avian pathogenic *E. coli* (APEC) causes a systemic syndromic infection in poultry, responsible for both early mortalities and condemnations later in life. APEC are defined by an array of virulence-associated genes which they harbor. Many of these genes, which support their pathogenesis are iron-acquisition mechanisms (Kemmett et al, 2013). Overall, it is important to consider the additional benefits optimized dietary formulations can have to support lower cost of production in a reduced antibiotic era.

Keywords: inflammation, trace minerals, phytase, micro-organisms

555S The role of fiber in gut inflammation in poultry

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Gut inflammation in poultry, driven by pathogens, stress, or dietary imbalances, significantly impacts overall health and production efficiency. Proper nutrition programming can play a critical role in maintaining intestinal homeostasis. Dietary fiber was traditionally considered an anti-nutritional factor due to its potential to reduce nutrient digestibility in poultry. However, it is now recognized as a functional component for its pivotal role in modulating gut physiology, microbial composition, and inflammatory responses. This presentation explores the multifaceted role of fiber in mitigating inflammatory responses within the poultry gastrointestinal tract. The impact of fiber on gut inflammation in poultry is highly dependent on its physicochemical properties, including solubility, fermentability, and structural integrity. Different fiber types exert distinct effects through various mechanisms. Soluble fibers undergo microbial fermentation to yield short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate. These SCFAs exhibit potent anti-inflammatory properties by enhancing gut barrier integrity, modulating immune responses, and reducing oxidative stress. Insoluble fibers, conversely, influence gut motility and microbial diversity, reducing pathogen exposure and enhancing gut morphology, and may also contribute to mechanical irritation, excessive mucus secretion, and inflammatory cytokine production based on fiber source, structure, and inclusion level. Also, fiber enhances gut barrier function by upregulating tight junction proteins and mucus production, thus limiting pathogen

translocation. The dynamic interplay between fiber composition and the gut microbiome is a critical determinant of inflammatory homeostasis in poultry. Fiber influences gut microbiota composition, promoting beneficial bacteria while suppressing pathogenic populations and reducing inflammation-associated dysbiosis. However, an imbalance in fiber intake or improper fiber source selection can lead to disrupted gut microbiota, increased gut permeability, and heightened immune activation, predisposing chickens to enteric disorders. Thus, a balanced approach to fiber inclusion, leveraging both soluble and insoluble fractions in appropriate ratios, is essential for optimizing gut health, minimizing inflammation-related performance losses, and reducing reliance on antimicrobial interventions. Future research should focus on identifying the optimal fiber sources and inclusion levels that maximize gut health benefits while mitigating potential pro-inflammatory effects.

Keywords: Dietary fiber, Gut health, Gut inflammation, Intestinal homeostasis, Intestinal microbiota

556S It's not just about digestibility: the role of amino acids in inflammation

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Enteric diseases and environmental stress are important economic and health issues for the poultry industry. Enteric diseases, such as coccidiosis and necrotic enteritis, damage intestine and cause inflammation and oxidative stress, considerably affecting growth performance, gut and bone health, and overall health of poultry. Minimizing inflammation and oxidative stress during infection and environmental stress periods is critical to alleviating the detrimental effects and maintaining efficient gut and skeletal integrity and organ development in poultry. Thus, identifying nutritional strategies to reduce the negative effects of enteric diseases and environmental stress in poultry. Animo acids are important building blocks of muscle protein synthesis. Beyond their role in muscle growth, certain amino acids have functional roles which may directly or indirectly manage inflammation and oxidative stress caused by enteric diseases and environmental stress. There are several amino acids with functional roles in poultry: arginine, methionine, threonine, glutamine, and branched chain amino acids (BCAA: Leucine, Valine, and Isoleucine). First, methionine is an essential amino acid, which is important for muscle accretion and DNA methylation. Methionine is a precursor of glutathione and taurine which have anti-inflammatory and antioxidant properties in the body. Second, threonine also has functional properties. Under enteric disease conditions, threonine requirements may be elevated to maintain gut integrity and functionality by promoting mucin secretion from goblet cells. Glutamine plays a role in maintaining gut health and control inflammation under enteric disease conditions; it can be utilized as an energy source for enterocytes and alleviate inflammation by reducing pro-inflammatory cytokines. BCAAs are essential amino acids whose balance in diets is critical for efficient production and good health. BCAAs also have antioxidant and anti-inflammatory properties to improve gut health and maintain efficient growth in poultry. Arginine is a multifunctional amino acid, which is involved in protein synthesis, nitric oxide production, antioxidant mechanism, anti-inflammatory process, angiogenesis, proline production, and polyamine production. Arginine supplementation has been showed beneficial effects on growth performance and gut health in poultry under enteric disease and environmental stress conditions. Strategic use of such nutritional approaches and understanding of the mode of actions of specific functional amino acids in poultry under enteric disease and environmental stress condition will maintain efficient production and improve gut health in poultry, promoting sustainable poultry production.

Keywords: Amino acids, Inflammation, oxidative stress, Enteric diseases, Gut health

557S Managing subclinical inflammation from a commercial perspective - a Nutritionist POV

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I think one could argue that there exists subclinical inflammation in the best of poultry flocks just due to the nature of the animal. By ingesting not only feed provided to the bird but also the litter containing bugs, excreta, all nature of bacteria and viruses poultry are almost constantly being inoculated not only with vaccines but with all manner of natural disease causing organisms. Fortunately they are equipped with a robust immune system capable of handling most of these insults, however, if possible we should intervene when we can to help them come to homeostasis faster and with less energy expended. While we don't have clear guidelines on how to make this happen in spite of over 45 years of research, I think that we do have a better picture of how different nutrients affect the immune system and maybe we can make decisions that help to increase an immune response, or modulate the immune system when we can save those nutrients from being wasted. We can separate those compounds into two areas for ease of discussion, one, feed additives such as probiotics, essential oils, etc., and two, nutrients such as Vitamins, trace elements, etc. Research surrounding each of these compounds and their influence on the immune system is growing each year. I think that we will soon be able to look at how our formulation may be modified depending on the type of health program we are using and feedback on the upcoming health challenges in the flocks. We will discuss several of these options and how we might be able to affect the birds response to insult in a more positive manner.

Keywords: Nutrition, Inflammation, subclinical

Emerging technologies in poultry genomics: Unlocking innovation for the future of sustainable production

558S Functional annotation to support interpretation of genomic analysis

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The process of acquiring and assembling genomic sequence and identifying functional elements within that genome have well established workflows and standards, and are the foundation required to support genomic analyses. However, the interpretation of genomic datasets so that they can be effectively applied to poultry systems also requires information about gene function. Functional annotation is the process of linking genes and their products to information about molecular function, physiological process and pathways. This presentation will review the process of functional information, including workflows we have developed to provide this information and how manual biocuration enables us to identify poultry- and bird-specific genes and their role in key function related to immunity and reproduction. With help from experts in the field, we have systematically curated the MHC regions for broiler and leghorn, identified chicken immunoglobin receptors and classified the expanded olfactory receptor repertoire in chicken. We work closely with researchers and stakeholders to identify additional gene sets which need to be manually reviewed and encourage scientists to contact us with suggestions for further review.

Keywords: genomics, functional annotation

559S CRISPR-based in vitro approaches for investigating chicken genome function

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Although genome editing has greatly advanced biological research, its use in poultry science has been limited. To address this, we developed a user-friendly CRISPR-based in vitro platform for studying gene functions and regulatory elements in the chicken genome. Using DF1 (embryonic fibroblast), DT40 (B-cell), and LMH (liver epithelial) cell lines, we established stable expression of Cas9, CRISPR activation (CRISPRa), and CRISPR interference (CRISPRi) systems. The platform allows precise gene manipulation for knockout, activation, or repression, with minimal user input—only the addition of a gRNA is needed. This system also enables the validation of non-coding regulatory regions and genome-wide association study (GWAS) variants in the chicken genome, turning statistical predictions into functional insights. By linking these regions and variants to specific phenotypes, we move beyond correlation-based findings to uncover the underlying gene regulatory mechanisms.

Keywords: CRISPR, tool development, genome function

560S A census of immune and non-immune cell types of the adaptive immune systems for the bursa and thymus of chicken

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With billions of chickens hatched annually, they are among the world's most essential food-producing animals. Any decline in their resistance to pathogens poses a significant threat to global food security, as demonstrated by recent outbreaks of highly pathogenic avian influenza (HPAI). Understanding the genetic and molecular mechanisms underlying disease resistance requires a comprehensive catalog of immune cell types, their transcriptomic profiles, and their responses to pathogen challenges. Single-cell RNA sequencing has revolutionized our ability to dissect the complexity of immune cell populations and their molecular functions. Here, we present a survey of immune cell types from the two primary immune organs of layer chickens: the thymus and bursa. In the adult thymus, we identify 96% of all cells as various T cell subtypes, encompassing all major classifications observed in other species. In the bursa, we capture distinct stages of B cell development, offering key insights into their maturation process. A fundamental question in avian immunogenetics is identifying the most critical immune cell types and their gene regulatory networks in response to an ever-evolving array of pathogens. Our inferred intercellular communication analyses reveal intricate and previously unappreciated crosstalk between B and T cells, as well as interactions with non-immune cell types. In the bursa, we show how B cell subtypes exhibit unique molecular responses to a bacterial infection mimic, underscoring the importance of cell-type-specific functional profiling. Across both T and B cell subtypes, we identify distinct gene expression patterns that could serve as valuable reference datasets for future characterization of avian immune cell populations. This comprehensive census of adaptive immune cells in the thymus and bursa provides a foundational resource for studying avian immunogenetics at the single-cell level, offering critical insights into the transcriptional landscape governing immune function and pathogen resistance in chickens.

Keywords: genomics

561S Harnessing the poultry microbiome: Enhancing health, productivity, and sustainability in modern production

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The gut microbiome is a complex and dynamic community of microorganisms that play a pivotal role in poultry metabolism, immune function, and disease resistance. Advances in microbiome research have highlighted the potential of targeted microbiome interventions to improve poultry health, productivity, and sustainability. Our research focuses on elucidating the role of commensal bacteria in disease resistance and production performance in broiler chickens. By analyzing broilers with divergent residual feed intake and weight gain, we have identified bacterial taxa that are strongly correlated with improved feed efficiency and growth. Furthermore, we have demonstrated that cecal microbiota transplantation from disease-resistant breeds, such as Fayoumi chickens, confers significant protection to Cobb broilers against necrotic enteritis (NE), a severe enteric disease caused by *Clostridium perfringens*. Currently, we are systemically characterizing the protective roles of differentially abundant bacteria in Fayoumi chickens against NE. Additionally, we have identified multiple commensal bacterial species exhibiting potent inhibitory activity against *C. perfringens, Enterococcus cecorum*, and other important poultry and zoonotic pathogens. Notably, oral administration of *Megasphaera stantonii* to day-of-hatch broilers improved the survival rate to 98% in a chicken model of NE, while 52% mortality was observed in untreated birds. Moreover, *M. stantonii* significantly alleviated the severity of intestinal lesions. The antimicrobial properties and mechanisms of *M. stantonii* and

other beneficial bacteria are under further investigation to assess their potential for disease mitigation. Our findings contribute to the growing body of evidence supporting microbiome-based strategies to enhance poultry health and productivity. The identification and application of beneficial commensal bacteria provide a promising antibiotic-free strategy for disease resistance and performance enhancement. This approach aligns with global efforts to reduce antimicrobial dependence in animal agriculture while promoting sustainable and profitable poultry production.

Keywords: Microbiota, Necrotic enteritis, Antibiotic-free production, antimicrobial resistance, disease resistance

562S Integrative omics in poultry genomics: Unraveling complex traits for enhanced production and health

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The chicken is an essential agricultural species serving as a major global source of nutrient-dense and lean protein. Although it was the first non-mammalian species to have its genome sequenced, functional characterizations of genomic regulatory elements and comprehensive catalog genetic variations affecting gene regulation across the chicken genome remains incomplete. To address this knowledge gap, we performed an integrative omics analysis combining extensive genomic, transcriptomic, and epigenomic datasets, including genome-wide sequencing data from 23 adult chicken tissues (377 datasets). Our systematic analysis identified approximately 1.57 million regulatory elements representing distinct chromatin states, predicted over one million enhancer-gene interactions, and annotated thousands of super-enhancers. In addition, we initiated chicken GTEx project by combining bulk RNA sequencing (7,015 samples), single-cell RNA sequencing (127,598 cells), and whole-genome sequencing (2,869 genomes) across 28 tissues. Through this approach, we systematically identified millions of regulatory effects influencing primary gene expression (protein-coding genes, long non-coding RNAs, and exons), and discovered extensive post-transcriptional modifications, including alternative splicing and alternative polyadenylation. Integrating functional annotation of regulatory elements with genetic regulations across tissues in chicken genome significantly enhanced our ability to pinpoint causal genetic variants influencing economically important complex traits, such as growth rate, productivity, and health. We further highlighted distinct molecular mechanisms underpinning these regulatory variants, their tissue- and context-dependent manners, and their utility for interpreting genome-wide associations across economically important poultry traits. Ultimately, this integrative atlas provides a comprehensive resource to accelerate precision poultry selective breeding and advances fundamental understanding of gene regulation in poultry.

Keywords: Omic data, Regulatory elements, Functional annotation, GTEx, Complex traits

Honoring Dr. Dibner: Pioneering poultry science and lasting influence on early nutrition, gut health, and cellular Metabolism

563S Building strong foundations: Advances in early nutrition for poultry

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Early nutrition in poultry is a critical factor that shapes the growth, health, and overall performance of birds throughout their life. The first few days of a chick's development are particularly sensitive, and proper nutritional management during this period have long-lasting effects on their ability to grow, develop immunity, and achieve optimal production. Dr. Julia Dibner was one of the first few researchers who started to explore this phase and made significant contributions to understand the specific nutritional needs during this early stage, focusing on key factors such intestinal development, protein and energy requirements, vitamin and mineral supplementation, and the timing of feed introduction. Pioneering studies identified how early feeding practices can enhance gut development, improve nutrient absorption, and support the energetic statues of the hatchlings. Along the 30 years of research worldwide, the knowledge was converted to practical progresses including the development of starter diets tailored to improve post-hatch performance, the exploration of novel feed additives and the use of new products that specifically contribute to support the hatchling. The knowledge, along with the continuous development of more specialized feed ingredients, led to products which provide essential tools to optimize the early feeding process and are today an integral part of the poultry diet. As poultry farming continues to evolve, the research buds, in early nutrition concept, become a fundamental part in poultry nutrition practice and a key role in improving productivity, in reducing disease susceptibility, and in enhancing the welfare of poultry.

Keywords: intestinal development , early feeding, hatchling

564S A legacy of scientific influence: Intestinal physiology measures and approaches with meaningful impact

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Dr. Dibner was instrumental in making sure the scientific questions we asked were the drivers for research and not solely allowing the question to be driven by the measurement tools we have. In doing so, she was one of the earlier ones in our scientific community to use histology to tell the story of outcomes. Utilizing 5-bromo-2'-deoxyuridine as a proliferating cell marker within tissues allowed for a fundamental understanding of the ramifications of tissue turnover rates and how this affects growth, maturation, and resulting perturbations by different stressors (e.g. oxidized fat) by the poultry science community. Her scientific approach laid the groundwork for subsequent generations and was fundamental to our studying the modes of action of antibiotic replacement strategies and consequences of other stressors on the intestine.

Keywords: Intestinal physiology, enterocyte turnover, histology

565S That is so cool! I know right! To glutathione and beyond.

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From the first time we met (thanks to Bob Wideman), I realized that Julia was not your basic run of the mill deep thinking cell biologist. I was amazed by the depth and scope of understanding on a wide range of knowledge of fundamental biology as well as the poultry and animal industries. She'd pull me over to her trademark microscope ("*Oh, you've got to take a look at this!)* always prominently situated on her office desk. Looking at a section of intestine or embryo in her fundamental science tool box she'd say, "*That is so cool, right?!* Shortly after arriving at University of Arkansas, I was extremely fortunate to receive an NIH grant focused on a major cellular antioxidant, glutathione (GSH). I accumulated two filing cabinets full of papers (that I still have) on 'everything GSH'; GSH was the center of my research universe. When I met Julia a few years later, I had a PhD student/post doc (Suiying Wang) following the path of 'all things GSH' (with an added dash of Julia). Suiying conducted a study showing an antioxidant sparing effect of GSH in liver and intestines of birds provided Santoquin (with or without oxidized fat in the diet). Suiying's PhD work on interorgan circulation of GSH used a method to repeatedly sample blood samples from the hepatic portal vein, hepatic vein, and carotid artery. This sampling method demonstrated an apparent high rate of hepatic clearance of Aliment® (2-hydroxy-4-methylthiobutanoic acid) when infused directly into the hepatic poral vein. Alimet® was just one of Julia's near and dear 'babies' in her career. Other PhD students, post-docs, visiting professors in my lab were investigating oxidative stress, mitochondrial function, and reactive oxygen species (ROS)l production . They too received a couple of dashes of Julia effect. Julia gave me two thumbs up to look into mitochondrial function and reactive oxygen species (ROS) associated with feed efficiency. *"That would be so cool!"* In short, Dr. Dibner had a profound effect on my research for many years both directly and indirectly. She was a dedicated scientist who also had a very effective and dry sense of humor. If her interactions and positive influence on my program is any indicator of impact, undoubtedly the 'Julia ripple effect' has had, and continues to have, a very wide range of positive impact on research conducted by PSA members and on the poultry industry.

Keywords: Julia Dibner, that would be so cool , glutathione, antioxidants

566S Dr. Julia Dibner: A lifelong dedication to advancing poultry science – accomplishments, milestones, and lasting impact

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Dr. Julia Dibner had a distinguished career in scientific research, contributing significantly to various crucial topics in animal nutrition and metabolism. Her work focused on monogastric animals and addressed significant issues such as metabolic processes, gut microflora interactions, and the nutritional needs of animals during critical developmental stages. One of her key areas of research was the impact of early feeding on the immune system development in neonatal poultry. She demonstrated that providing an optimal balance of nutrients immediately after hatch significantly enhances disease resistance, growth performance, and the structure of the small intestine. Dr. Dibner also investigated the effects of feeding oxidized fats to broilers and swine. Her research revealed that oxidative stress from these fats

can increase cell turnover in the gastrointestinal epithelium, which may have downstream effects on animal performance and health. In addition, her studies on trace minerals like zinc, copper, and manganese highlighted their essential roles in bone development and repair in poultry and swine. She suggested that optimizing the availability of these minerals could enhance the resilience and structural integrity of bones in these animals. Among her most influential publications, "Antibiotic Growth Promoters in Agriculture: History and Mode of Action" published in *Poultry Science* in 2005, garnered 1,496 citations. Other notable works include "Use of Organic Acids as a Model to Study the Impact of Gut Microflora on Nutrition and Metabolism," published in *The Journal of Applied Poultry Research* in 2002 with 643 citations, and "Early Feeding and Development of the Immune System in Neonatal Poultry," from 1998 with 229 citations. These publications underscore her pivotal contributions to poultry science and animal nutrition, shaping both academic research and practical applications in the field.

Keywords: metabolism, nutrition, health

Inflammation is not a 4-letter word: How to influence immunity while not compromising performance

567S The essential Evil: Inflammation's role in forging long-lasting immunity

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In animal production, particularly poultry and swine, inflammation serves a dual role: it's a crucial defense against microbial infections and a necessary driver of tissue repair. A well-regulated inflammatory response is essential for initiating adaptive immunity, providing long-term protection through memory T and B cell development. While pro-inflammatory mediators can enhance resistance to key pathogens, excessive or prolonged inflammation can lead to tissue damage and nutrient diversion, negatively impacting growth and productivity. This duality necessitates careful management. Strategies aimed at mitigating the negative effects of inflammation, such as anti-inflammatory feed additives, must be implemented judiciously, as overly suppressing the immune response can compromise an animal's ability to combat infections, especially in young animals. The optimal strategy focuses on promoting a rapid, acute inflammatory response to control infection, followed by a timely resolution that facilitates tissue repair and homeostasis. Producers must carefully evaluate the impact of nutritional and management interventions, including direct-fed microbials, essential oils, enzymes, and immune-modulating products, to achieve this delicate balance, ensuring robust disease resistance without compromising productivity.

Keywords: inflammation, immune modulation, inflammatory homeostasis, Adaptive Immunity, Animal Production

568S The Good, the Bad, and the Ugly of Gut Inflammation

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Inflammation is traditionally considered a defense response induced by infection or injury. However, because infection and injury-induced inflammation are the most prominent and most studied forms of the response, our understanding of inflammation are skewed according to these extreme conditions. Inflammation can be induced by tissue stress and malfunction in the absence of infection or overt tissue damage. The common denominator of all inflammatory responses is the deviation or disruption of homeostasis (a state where the host functions operate normally). In the poultry gut, homeostasis is regarded as a major pre-requisite for zootechnical performance responses, but the reductionist view of inflammation (infection or injury induced) and the use of antibiotic growth promoters (AGPs) in poultry feeds limited our understanding of the complete physiological roles of inflammation in maintaining and monitoring tissue homeostasis. The efficacy, duration, and consequences of an inflammatory response is dependent upon the type of trigger that is recognized by the innate immune receptors. Because of different triggers, there are multiple phenotypes of inflammation: (1) Physiological is the homeostatic balance between tolerance of the microbiota and the reactivity to pathogen invasion; (2) Pathologic is an acute response to toxins and infection that damages surrounding tissue and increases metabolic energy use; (3) Sterile is a low-grade, chronic inflammation in the absence of an infection, in response to chemical, physical, and metabolic stimuli; and (4) Metabolic is a chronic low-grade inflammation generated by excessive nutrient intake or non-nutritional components in the diet that fosters metabolic dysfunction by integrating signals from both the immune and metabolic systems.

Keywords: inflammation, gut homeostasis, innate immunity

569S Balancing inflammation and immunity to optimize performance

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Immunity is a complex interaction between the host, pathogens, and environment. There is not a practical US broiler production context devoid of immune system stimulation and consideration. Broiler producers have vaccination programs which are not designed solely to protect the parents, but to help defend the offspring by enriching the eggs with protective, maternal-derived antibodies for the first few weeks of life. This early protection is imperative for many reasons, but especially in US broiler industry where chicks are placed on reused litter. Following placement, these chicks are exposed to a plethora of viral, bacterial, and protozoal organisms. Immune stimulation is frontloaded in most vaccination programs across species. Most obviously, chicks are exposed to pathogens early and need to be protected. Less evident is by frontloading the immune response, the cost of the stimulation can be compensated for over subsequent weeks. In late exposures, broilers do not have time to recover from the performance suppression which may occur due to disease or immune stimulation. Enteric health and intestinal immunomodulation provide an optimal example of immunity in broiler production because tangible outcomes on performance can be observed. For example, coccidiosis vaccination is a common means to protect broilers against *Eimeria* challenges. Ideally, immunization is inexpensive, fully protective, and has no detriment to the host. In practice, tradeoffs are managed when considering protective immunity and energetic/nutrient expense. Too much immunostimulation may cause unnecessary damage to the host (elevate FCR) and too little immune stimulation may leave the birds inadequately protected from the protozoa (late cycle coccidia exposure). In addition to vaccines, some alternative products seem to work, in part, through direct or indirect immune stimulation. Further understanding the mechanism of these products could help refine broiler immunity programs as they may complement existing intervention strategies. “Equipoise between contrasting, opposing, or interacting elements” is one of the definitions from Merriam-Webster for “Balance.” In a sense, this is the conundrum broiler producers face regarding immunity for common diseases. Too little immune activity can result in mortality and morbidity from viruses, bacteria, fungus, and protozoa the chicks are exposed to and alternatively, too much immune stimulation can negatively impact bird performance. This interaction may be considered more of a “verb” than a “noun”. It requires monitoring and action, “balancing,” to achieve the proper level of immune stimulation to protect our flocks without pushing them so hard that performance is reduced.

Keywords: Veterinary, Immune, Enteric Health, Coccidia, Homeostasis

570S Immune modulation using microbiota-centric feed additives

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The poultry industry applies microbiota-centric feed additives like probiotics, prebiotics, and synbiotics to improve production performance and reduce gut infections and foodborne pathogen loads and thereby replace antibiotics. The gut of a chick is sterile at hatch and is rapidly colonized by microbes from the environment. The gut microbiota of poultry is predominantly composed of Firmicutes, Bacteroidetes, and Proteobacteria, and small numbers of mycobiota, including Aspergillus and Candida. Gut microbiota significantly affects host physiological functions and immune responses. Probiotics, such as Lactobacillus and the yeast Pichia guilliermondii, modulate gut microbiota by competitive exclusion, secreting antimicrobial compounds like bacteriocins and organic acids, and strengthen epithelial barrier integrity through mucin gene upregulation. Prebiotics that include yeast cell wall mannans and β-glucans are nondigestible carbohydrates that are resistant to digestion. Beneficial bacteria can selectively ferment prebiotics to increase the Bifidobacterium loads in the gut. Probiotics also secrete short-chain fatty acids that can improve gut epithelial integrity and health. Synbiotics are a combination of probiotics and prebiotics and are extensively applied to in poultry industry to promote gut health and improve feed efficiency. Yeast-based probiotic supplements consist several long chain polysaccharides that can modulate host immunity and have been routinely applied to reduce gut pathogen loads and decrease the severity of enteric infections in poultry. Microbiota-centric feed additives show beneficial effects by improving immune cell activity, cytokine production, and antibody levels, shifting T-cell responses toward Th1-mediated immunity. Dysbiosis, induced by stressors, can disrupt gut homeostasis, and applying microbiota-centric feed additives can reverse dysbiosis. However, high variability in microbial composition because of individual variations in diet, environment, and host factors complicates establishing clear host-microbiota relationships Understanding gut microbiota-mycobiota-immune crosstalk is essential to develop microbiota-centric strategies that can improve poultry health and productivity and replace antibiotics.

Keywords: Vaccine, Gut pathogen, Killed vaccine, Nanoparticle

571S Immune modulation using host directed additives

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The immune system is a complex physiological system that relies on homeostatic balance for optimum functionality. Stimulation is required for development and differentiation of immune cells and tissues. At the same time, keeping a cap on inflammatory responses and preventing runaway immune processes, such as a cytokine storm, is necessary to avoid immune-induced pathologies. Unlike in humans, where immune-mediated inflammatory diseases are a major health concern, in poultry there is often a desire to activate the immune system to enhance disease resistance. Immune training, priming, activation or stimulation are often the goals in animal production disease resistance. However, in studying the mechanism of action in the host for some of the most promising feed additives, we often see a mixture of classically “pro” and “anti” inflammatory responses. Scientists have come to refer to these additives as immune modulatory. Immune modulatory additives may stimulate or suppress the immune system, often generating signals indicative of both simultaneously. Feed additives including, prebiotics, probiotics, postbiotics, yeast fractions, essential oils, organic acids, algae, enzymes, and others have shown promise in poultry production and in modulating immune responses. The containment and resolution of an immune response is an essential part of the process itself, in order to limit pathology and returning to homeostasis. Similarly, an effective immunomodulatory feed additive would both activate and control immune responses to optimize immunity.

Keywords: inflammation, additives, feed, immunity, disease

572S Improving animal health through antibody-driven targeted immune modulation

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The prevalent use of antibiotics is a major concern in animal production due to the risks of antimicrobial resistance, environmental impact, and regulatory restrictions. As the need to reduce antibiotic dependence grows, researchers and industry professionals are seeking alternative, antibiotic-free methods to maintain animal health and productivity. Among the newer disease control strategies, antibodies and their derivatives have emerged as promising solutions, offering targeted approaches to neutralize disease causing agents or pathogenic modulators and therefore, supplementing host immune response. One such breakthrough is the use of egg yolk-derived immunoglobulins (IgY). Extracted from the egg yolk of immunized hens, IgY antibodies have been explored as feed additives to improve gut health and disease resistance in poultry and swine. Unlike mammalian antibodies, IgY do not trigger inflammatory responses in the intestine, making it a favorable option for passive immunization strategies. By neutralizing specific pathogens, these antibodies help reduce the need for antibiotics while enhancing overall animal health. Another promising advancement is the nanobody technology. Nanobodies are camelid-derived single-domain antibodies. Due to their small size, high stability, and strong binding affinity, nanobodies offer unique advantages over traditional antibodies. They are being developed as therapeutic agents and diagnostic tools in veterinary medicine, particularly for the control of viral and bacterial diseases. Their ability to bind specific microbial targets with high precision makes them a viable alternative to antibiotics, offering a more targeted approach to disease prevention and management. Complementing these approaches, genetically engineered plants have emerged as an innovative platform for antibody production. Plants such as corn, and soybeans have been engineered to express specific antibodies that target pathogens affecting livestock. These plant-derived antibodies provide a cost-effective and scalable alternative to traditional antibody production methods. Additionally, some of these plant-based compounds function as immunostimulants, enhancing the animal's natural immune response and further reducing reliance on antibiotics. Overall, the continuous evolution of antibody technologies has played a pivotal role in modern animal production. By providing precise, effective, and sustainable solutions for disease prevention and treatment, these advancements contribute to enhanced animal health, welfare, and productivity, while reducing the industry's reliance on antibiotics.

Keywords: Poultry, Inflammation, Antibody, Gut health, Immune

A new focus on fiber in poultry: Is it a nutrient or antinutrient?

573S Maximizing the benefits of fiber in poultry nutrition: insights and ingredient evaluation

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Fiber plays a complex role in poultry nutrition, and ongoing research continues to refine strategies for its optimal utilization. Once considered an anti-nutritional factor due to its limited digestibility and potential to hinder nutrient absorption, fiber is now recognized for its functional benefits, including improved intestinal integrity, enhanced gut health, and greater digestive efficiency. To fully harness these benefits, it is essential to accurately characterize fiber composition in feed ingredients. Poultry diets predominantly consist of cereal grains and plant-based ingredients each containing diverse fiber fractions. Thus, distinguishing and measuring these fractions is increasingly important. Traditional methods, such as crude fiber (CF) analysis, quantify insoluble fiber components such as cellulose and lignin, but fail to account for hemicellulose and soluble fiber. This can lead to an underestimation of the total fiber content present. In contrast, neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses provide a more detailed assessment, with NDF measuring total cell wall fraction (cellulose, hemicellulose, and lignin) and ADF capturing only cellulose and lignin. While NDF and ADF offer insights into the structural fiber of the ingredient, these methods fail to capture the soluble fiber components most likely to impact digestion in poultry. Total dietary fiber (TDF) provides a more comprehensive evaluation, encompassing both insoluble (cellulose, hemicellulose, and lignin) and soluble fiber (pectins, β-glucans, and oligosaccharides). This broader classification offers greater insight into fiber's effects on gut health, fermentation, and nutrient absorption. As fiber analysis becomes more complex, so do the associated costs and time requirements. To mitigate these challenges, near-infrared spectroscopy (NIRS) may offer a rapid and cost-effective alternative to traditional wet chemistry methods for fiber characterization. As our understanding of fiber's role in poultry nutrition advances, so must our ability to quantify and evaluate fiber fractions. Achieving the right balance of fiber types is key to optimizing feed formulations across different poultry species and production stages. A deeper understanding of fiber's influence on nutrient digestibility, energy utilization, and gut health is critical for developing cost-effective and sustainable feeding programs. A well-managed fiber strategy can enhance feed efficiency, promote gut health, and support the long-term sustainability of poultry production.

Keywords: Fiber, Fiber Analysis

574S From pigs to poultry: The evolution of fiber in monogastric nutrition

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In current practice, dietary fiber (DF) is not properly accounted for when formulating monogastric diets. This is because historically only anti-nutritional effects of DF have been considered, and inaccurate DF values are commonly still used. However, recent research has presented the advantageous effects of DF on feed digestion, health and behavior. Despite this, DF remains one of the most challenging nutritional components to define, analyze, and apply effectively. Our research in pigs has highlighted the importance of understanding fiber type, source, and context, and matching this to the animal's physiological stage and production environment. Poultry nutritionists could potentially use these insights to advance use and study of DF to support productivity, animal welfare, and sustainability in poultry systems. For example, insoluble DF can act as a nutrient diluent and physical barrier to enzymes, but can also maintain gastrointestinal motility and digesta flow, and stimulate gizzard and proventriculus function in poultry. Feeding insoluble DF has also been shown to reduce feather pecking and cannibalism in laying hens, induce satiety in feed-restricted broiler breeders, and improve nutrient digestibility and performance in broilers. Soluble DF has a high water-holding capacity, increasing digesta viscosity when in excess, but it can also be fermented by beneficial microbiota, resulting in production of valuable short chain fatty acids and improved microbiota composition. In pigs, dietary inclusion of fermentable DF sources has been shown to increase satiety, improve insulin sensitivity, reduce lameness-related mortality in pen-housed sows, and reduce negative hunger behaviors during gestation. Increasing DF intake has also been shown to alleviate constipation, shorten farrowing duration and reduce stillborns and pre-weaning mortality during the peripartum period, and enhance milk yield during lactation. Similarly, in both laying hens and broilers, improved bird performance has been observed because of supplementing low DF diets with fermentable DF, attributed to modulated gut microbiota. However, DF is not universally beneficial. For example, recent U.S. studies indicated that including fiber-rich ingredients in nursery pig diets under enteric disease pressure did not consistently improve performance, fecal scores, or gut health. Likewise, a recent review of broiler breeder responses to DF revealed variable responses, with cases of poorer performance or no response when feeding fiber-rich diets. Collectively, a cross-species approach is needed to better understand and optimize DF utilization in monogastric diets and will be to focus of this presentation.

Keywords: Dietary Fiber, Lameness, Layers, Physiology

575S Fiber and the microbiome: What's all the hype?

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Efficient food animal production relies on the overall maintenance of health in the animal and promotion of a balanced and efficient gastrointestinal tract. This can be quite complicated because balance in the gut depends on a healthy microflora balance. There are more than 1,000 bacterial species in the guts of food production animals, and these species are naturally balanced under normal conditions. However, in modern production the establishment of a healthy microbiome is often impeded by confinement and lack of beneficial microbes typically present in nature during development. To address these challenges, feed additives are often used to promote the establishment of beneficial microbes. Dietary fibers used in food animal production include a diverse range of different combinations of monosaccharides to form oligosaccharides, intended to target specific microbes capable of breaking down these sugars. The basic concept of using fibers to modulate the gut microbiome is that fibers can be developed to promote the expansion of specific beneficial groups of bacteria. This, in turn, promotes competitive exclusion of potential pathogens in the gut. At the same time, these beneficial bacteria

positively stimulate the immune system and produce byproducts such as short-chain fatty acids which are used by the host and accelerate energy utilization. There is evidence that these interactions could also influence the gut-brain axis and have physiological effects. It is generally believed that dietary fibers and other prebiotics have positive effects on the establishment of important beneficial bacteria in the gut, such as *Lactobacillus* and *Bifidobacteria*. There is evidence to support this theory, but there is less evidence regarding ancillary effects of dietary fibers on the gut microbiome. It is also challenging to differentiate between bacteria which are truly commensal and provide some benefit, versus those that are opportunistic and have potential to exacerbate disease problems. For example, *Escherichia coli* comes in many forms, ranging from beneficial to deadly in the host. There is little to no evidence at the bacterial strain level regarding specificity of dietary fibers, and in general dietary fibers should not be specific to certain strains within the same species. Overall, more high-resolution microbiome studies (including functional) are needed, and platforms to assess multiple products in different combinations are desperately needed. There is immense potential in using dietary fibers to modulate the microbiome, and the current peer-reviewed publication landscape inhibits true dissemination of knowledge in this area.

Keywords: microbiome

576S Intestinal integrity and key biomarkers for performance

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Maintenance of intestinal health, or more precisely gut homeostasis, is critical to successful poultry production and one of the goals of the poultry production industry. Gut homeostasis, described as a state where the host physiological functions operate normally, is maintained by the gut ecosystem that consists of multiple crucial host elements within the intestine their interactions with the gut microbiota. The abundance of immune cells that reside together with trillions of beneficial commensal microorganisms in the intestinal tract requires barrier and regulatory mechanisms that conserve host–microbial interactions and intestinal homeostasis. This homeostasis depends on the diverse functions of intestinal epithelial cells (IECs), which include the physical segregation of commensal bacteria and the integration of microbial signals. Thus, IECs are crucial mediators of intestinal homeostasis that enable the establishment of an immunological environment permissive to colonization by commensal bacteria. IECs form a physical and biochemical barrier to commensal and pathogenic microorganisms. Intestinal integrity is dependent on this barrier function of the IELs, but numerous environmental stressors (infectious and none-infectious) can contribute to the disruption of these barriers and compromise intestinal integrity. During the last decade, assessment of intestinal permeability has evolved from invasive techniques to noninvasive biomarkers with a particular effort on identifying reliable biomarkers able to assess intestinal permeability in blood and feces but challenges remain in achieving consensus about the best testing methods and their accuracy.

Keywords: biomarkers, calprotectin, intestinal integrity

577S Fiber and performance: Connecting the dots for optimal performance

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Dietary fiber (DF) was first described in 1953 in relation to pregnancy toxemia in women between 1940 and 1950. Interestingly, today we are still finding relationships between DF and reproductive performance. For example, in sows DF has been linked to satiety, insulin sensitivity, reducing constipation and farrowing duration, and improving milk yield during lactation. In addition, beneficial effects of DF on microbial fermentation, gizzard function, intestinal morphometry, immune function, satiety, and behavior in layers, breeders, or broilers have been observed. However, the beneficial effects of DF are not unequivocal. DF is also considered an anti-nutrient with impacts on digesta viscosity, entrapment of nutrients and thereby reducing digestibility, and diluting the nutrient density of the diet. Some of this confusion is related to the complexity of DF and the lack of precision when using the term DF. As analytical techniques improve, so does our knowledge of DF and the major components that are described within the term (i.e. total, soluble, and insoluble non-starch polysaccharides [NSP], and lignin) and their concentration within cereals, protein meals, and by-product meals used in animal feed. The use of accurate, reliable, and cost-effective near-infrared (NIR) spectroscopy to measure the NSP and lignin content in feed ingredients will enable real-time, accurate feed formulation, targeting ingredient concentrations to provide beneficial concentrations of these fractions in the diet, as well as improve enzyme efficacy and recommendations. This means diets could be formulated to include feed ingredients or additives with specific NSP or oligosaccharide fractions to promote beneficial bacterial fermentation, gut function through production of short-chain fatty acids, and minimize protein fermentation and pathogenic bacterial growth. As well as measuring the benefits of the DF fractions on animal performance and microbial populations, the use of non-invasive biomarkers may provide an insight into the effects of DF fractions on intestinal integrity, nutrient utilization, and immune function and resilience, especially under enteric challenges such as coccidiosis, E coli, and Clostridium perfringens. As our understanding and ability to measure DF and its fractions improves, so does our ability to harness the beneficial effects of DF, mitigate detrimental effects with optimal feed formulations, exogenous enzymes, and feed additive supplementation, and maintain intestinal homeostasis and function for optimal performance.

Keywords: biomarkers, dietary fiber, non-starch polysaccharides

Genetic bases for resistance and immunity to avian diseases

578S Why understanding the poultry enteric nervous system is critical to poultry health and production

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The safeguarding of poultry gastrointestinal (GI) health is a critical preventive measure to reduce foodborne pathogen carriage at the preharvest stage. Researchers have focused for decades on avian intestinal epithelial alterations following *Salmonella* and *Campylobacter* infection, yet foodborne pathogen carriage remains a problem in poultry production. Like in mammals, the avian intestinal epithelium is extensively innervated by three different neuronal pathways. Vagal and sympathetic neuronal terminals innervate the intestinal epithelium and originate from ganglia (or groups of neuronal cell bodies) outside the gut wall. By sending information to the brain, these two branches of the peripheral nervous system modulate visceral sensitivity, appetite and gut homeostasis. The third nervous system is a complex network of neurons, ganglia and nerve fibers completely embedded in the gut wall, and it is called the enteric nervous system (ENS) or "brain in the gut" for its capability of functioning independently from the brain. The ENS coordinates essential physiological GI functions, including intestinal motility and peristalsis, as well as digestion and absorption of nutrients and water. Recent studies conducted in mammals demonstrate that enteric neurons can detect bacterial pathogens (e.g., *Salmonella*) in the gut lumen, thus stimulating local immune cells to release antimicrobial peptides against that specific pathogen. Hence, ENS-dependent neuroimmune interactions can be leveraged to identify novel biomolecular mechanisms for reducing foodborne pathogens load within the chicken GI tract. Furthermore, the pivotal role of the ENS in coordinating intestinal motility patterns and fluid dynamics through aquaporin water channels can be used by poultry scientists to develop novel strategies for facilitating foodborne pathogen clearance at the preharvest stage of production. This presentation will provide an overview of the next generation techniques to study the ENS in chickens, with particular focus on the characterization of different neuronal populations and structures within the chicken gut wall by using tissue clearing and 3D imaging without tissue sectioning. Discussion will also include the effect of early life stress in altering the development of intestinal motility patterns and aquaporin water channels, thus affecting the bird's capability of foodborne pathogen clearance later in life. This research sets the basis for the development of novel ENS-driven pathogen-specific intervention strategies that will ultimately enhance preharvest food safety in poultry.

Keywords: enteric nervous system, poultry, foodborne pathogens, neuro immunity, intestinal motility

579S What the kinome reveals about resistance to foodborne pathogens

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While the genetic similarities between vertebrates have been remarked on with each new complete genome sequenced, the physiological and phenotypic differences between agricultural species remains clear even to the casual observer. As one moves along the central dogma of molecular biology, from DNA to RNA to protein, the distinctions between species increases. The separation between species further increases when the active sites of protein are considered. Phenotype is dictated by gene expression patterns, response to environmental stimuli, epigenetic alterations, protein translation, protein folding and interactions, post-translational modifications, and a host of other mechanisms. Studying active protein kinase changes is as close as we can come to studying phenotype and remain at a molecular level. Intracellular metabolic responses, specifically, depend on these kinases switches and are often transcriptionally independent, an organism must respond to the availability of nutrients and does not have time to engage the transcription and translation machinery in order to take up and consume sources of energy. Thus, studying kinase activity allows us to study metabolism alongside the classic gene expression dependent responses such as innate immunity. Over many years of study of poultry in the context of foodborne pathogens, such as *Salmonella* species and *Clostridium perfringens,* a few kinase dependent pathways and energy sensors have emerged as critical to resistance or susceptibility in poultry. The central immunometabolic signaling hubs such as PI3K-AKT-mTOR have been shown to be part of the immunometabolic physiological responses key to resistance or susceptibility in chickens. *Salmonella* and *C. perfringens* infections have a significant impact on kinases in these signaling hubs, while the most important interventions target these hubs for modulation. Directing selection criteria or feed additive interventions to these hubs and critical activating/deactivating control points is a promising direction in controlling these foodborne pathogens in poultry.

Keywords: kinome, immunity, disease, metabolism, Food safety

580S Functional perspectives of host-microbiome interactions in poultry production: Where we are and where we need to go

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Over the last two decades, scientific interest in the poultry microbiome has skyrocketed with a role ascribed for the microbiome in virtually every aspect of poultry health and production. While substantial progress has been made in revealing important correlations that can be used as a roadmap to utility in a production setting, much remains to be understood in way of causality of the microbiome for the bird. In other words, correlation does not mean causality and we need to identify causal mechanisms if we are going to implement new microbiome-based modalities for improving poultry production. Therefore, the present symposium discusses the need for function-driven hypotheses that provide an evidence-based framework when approaching poultry microbiome research. Considering that the impact of stress on both bird and microbiome is well-appreciated across poultry species, pre-harvest stress will be taken as a prototypical example of where the use of function-driven hypotheses can help bridge basic to applied microbiome-based strategies to benefit poultry production. This will extend from areas of foodborne pathogen carriage to performance. To accomplish this, I will employ the emerging interdisciplinary field of Microbial Endocrinology as it represents the intersection of microbiology with neurophysiology. According to Microbial Endocrinology, stress-related neurochemicals can serve as mechanistic routes of host-microbe interaction, providing an evidence-based platform for identification of causal mechanistic relationships between microbial taxa and the host. By identifying

these mechanistic routes, interventions can be designed to modulate these relationships to benefit the bird. While emphasis will be given towards the gut microbiome, discussion will also include less explored, but highly relevant, microbial communities including those in the respiratory tract and litter. The need for widespread adoption of best practices methodologies in microbiome experimental design will also be highlighted. In conclusion, it is anticipated that the cooperative use of directed hypotheses and the implementation of robust experimental design will accelerate the application of microbiome-based solutions in poultry production of which the use of Microbial Endocrinology will serve as a lens in this talk.

Keywords: Microbiome, Stress, Disease, Foodborne pathogen, Host-microbe

581S Poultry microbiota's influence on disease: A breeder perspective

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The poultry gut microbiota plays a pivotal role in maintaining host health, influencing nutrient absorption, immune modulation, and pathogen resistance. This complex microbial ecosystem establishes a symbiotic relationship with the host, shaping its physiological and immunological responses. Characterizing and investigating this complexity has become commonplace with approximately 49,900 publications containing "Poultry" and "Microbiota" as keywords since 2010, with nearly 55 percent of publications in the last five years alone. Pathogenic bacteria such as *Salmonella spp.*, *Clostridium perfringens*, *Escherichia coli*, and *Campylobacter spp.* exploit microbiota perturbations, breaching mucosal defenses and exacerbating inflammatory responses. Environmental stressors, dietary changes, antibiotic interventions, and pathogen exposure significantly alter microbial homeostasis, impairing competitive exclusion mechanisms that typically suppress pathogenic colonization. Notably, *Salmonella* and *Campylobacter*, major zoonotic pathogens, leverage microbiota imbalances to enhance their gut colonization, leading to increased fecal shedding and subsequent transmission risk. Host-microbiota interactions regulate immune responses, with commensals promoting mucosal barrier integrity and pathogen recognition. Disruptions in these interactions can lead to immune dysregulation, fostering conditions such as necrotic enteritis, colibacillosis, and dysbiosis-associated enteropathies. Microbiota modulation strategies, including prebiotics, probiotics, synbiotics, and competitive exclusion cultures, have emerged as promising alternatives to antibiotics, aiming to restore microbial equilibrium and enhance disease resistance. Advances in metagenomics and multi-omics approaches provide deeper insights into microbiota-pathogen-host interactions, offering novel strategies for disease mitigation and poultry health optimization. However, the cost of implementing a multi-omics approach along with large-scale, multi-year investigations of the various modulation strategies complicate the utilization in the breeding system. From a breeder's perspective this poses a unique challenge as an emerging field with enormous opportunities. The real question remains how to generate, scale, and incorporate this knowledge base into a breeding program?

Keywords: Microbiota, Multi-Omics, Disease, Breeding

582S Identifying chicken alloantigens to understand their influence on disease and production traits

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Self-recognition system components including red blood cell antigens and the major histocompatibility complex (MHC) are alloantigens found on multiple cell types. Reactivity with specific polyclonal antisera classified fourteen chicken erythrocyte alloantigens (*A, B, C, D, E, H, I, J, K, L, M, N, P, R*). Multiple associations between variation within these blood systems and disease and/or production traits were reported. Blood group B, the MHC, was the first system whose genes and function were identified. Recent investigations sought to find the chromosomal location and gene identity for other alloantigen systems. DNA from pedigree and non-pedigree chickens having known alloantigen types were pooled according to phenotypes followed by genome-wide association (GWAS) analyses using genotype information from either a 600K or a 54K SNP panel. DNA from independent samples was then used to confirm candidate gene associations. Genome sequences from elite production and experimental lines with identified alloantigen alleles were also analyzed. Candidate genes within strong GWAS peaks were bioinformatically screened for cell surface expression and co-segregation of non-synonymous SNP with serologically defined haplotypes. Specific genes and their variants have now been identified for four of these alloantigen systems; *A* = complement component 4 binding protein, membrane (C4BPM) and *E* = Fc fragment of IgA and IgM receptor (FCAMR), both on chromosome 26; *D* = CD99 gene on chromosome 1; and *I* = RHCE equivalent to the human Rh blood group locus on chromosome 23. Gene deletions covering > 6,000 bp found in RHCE were predicted to affect the last seven RHCE gene exons. The chromosomal location for three additional systems was determined, though small sample size and problematic SNP alignment restricted definitive gene identity. Two independent sample sets confirmed the *L* system gene was located on chromosome 4. The candidate gene region for alloantigen *H* was on chromosome 24, and the alloantigen M candidate gene region was on chromosome 26, near to C4BPM and FCAMR genes. The *C* and *P* blood systems' samples analysis failed to identify any genomic regions associated with alloantigen serological differences. In summary, four alloantigen genes with allele associated variants have been identified plus the chromosomal location for three other systems. Genes identified have regulatory roles in immune-related activities including the complement system, leukocyte migration and maternal/fetal antigenic differences. Identification of specific genes and their variants responsible for the blood system alleles provides tools to study relationships between these chicken alloantigens and disease and production traits.

Keywords: alloantigen, C4BPM, FCAMR, CD99, RHCE

583S Host genetics associated with coccidiosis and necrotic enteritis

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The chicken has served as a valuable model organism for studying various biological systems including the development and delineation of the various arms of the immune system. Central to more recent discoveries have been the characterization of the major histocompatibility complex (MHC) and the sequencing of the chicken genome. Even prior to those events, disease resistance

in poultry had been linked to bird genetics, particularly in the context of the MHC and other alloantigen systems. To this end, chicken congenic lines have provided an invaluable resource for immunogenetics research in our efforts to better understand the specific genetic components associated with specific phenotypic traits. Such congenic lines could differ only in the B haplotype, thus allowing scientists to interrogate the role of different MHC B haplotypes in traits such as immune responses underlying resistance or susceptibility to specific diseases. Disease outbreaks pose significant challenges across the global poultry industry. Enteric challenges such as avian coccidiosis and its 'sidekick' necrotic enteritis (NE) are two critical diseases affecting chickens and threatening sustainable poultry production. As early as the 1950's, scientists documented the role of genetic components in resistance and susceptibility to coccidiosis in chickens. Later studies reported that specific B haplotypes were associated with either resistance or susceptibility to coccidiosis. in response to a mixed *Eimeria* spp. infection, we found that the MHC B21 haplotype was associated with improved feed conversion and increased severity of coccidial lesions. Further, those studies assessed the association between haplotypes of the alloantigen systems A, D, E, and I with resistance or susceptibility to mixed *Eimeria* infection. The E-H02/H02 genotype exhibited the lowest lesion scores compared to other genotypes of the E alloantigen system. Similarly, the I-H01 and I-H03 haplotypes showed positive and negative associations, respectively, with jejunal and cecal gross lesions showing improved resistance to this mixed *Eimeria* infection model. Other studies investigating similar associations influencing birds' responses to NE highlighted the importance of host genetics in resistance to this impactful enteric disease. Yet, much of the research focused on phenotypic outcomes among different genetic lines without in-depth characterization of the specific genotypic variations. Therefore, upcoming research could utilize the genetic markers linked to responses to coccidiosis to investigate their involvement in NE and any potential resistance in the context of host genetics.

Keywords: disease resistance, immunity, coccidiosis, necrotic enteritis, genetics

584S Commercial breeding for disease resistance

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Breeding for disease resistance in poultry is crucial for enhancing productivity and sustainability within the industry. Both broilers (raised for meat production) and layers (raised for egg production) face significant challenges from various infectious diseases, which can lead to increased mortality, reduced growth or egg-laying performance, and considerable economic losses. While traditional disease control methods, such as vaccination and biosecurity measures, are essential tools in commercial operations, they should be supplemented by selective breeding to improve flock performance. Recent advancements in genetic selection and genomic technologies provide promising opportunities for breeding disease-resistant poultry. By identifying and selecting genetic markers linked to resistance against specific diseases, breeders can develop strains of chickens that are more resilient and tolerant to infections. This approach not only enhances the overall health and welfare of the birds but also reduces reliance on antibiotics. This addresses concerns about antibiotic resistance and contributes to safer and more sustainable food production. Key strategies for breeding disease-tolerant poultry include evaluating relatives under disease challenge conditions, known as sib-testing. This can be done using specific, controlled pathogen challenges or by observing their performance under commercial conditions. Additionally, molecular tools such as genome-wide association studies (GWAS) can help identify gene markers that improve tolerance to specific diseases, which can then be incorporated into breeding programs. Furthermore, advanced genetic techniques, including gene editing, can also be used to promote specific alleles associated with disease resistance. These alleles can be integrated into commercial lines to produce chickens that can withstand field challenges from particular pathogens. The integration of precision livestock farming technologies, such as digital phenotyping facilitated by artificial intelligence (including computer vision systems, RFIDs, and various sensors), further enhances disease management. These technologies facilitate real-time monitoring and early detection of health issues in chicken flocks, providing abundant and accurate phenotypic data for genetic improvement. Overall, these multifaceted strategies leverage genetic advancements and innovative technologies to improve poultry health, productivity, and sustainability. This holistic approach not only addresses current challenges but also sets the stage for a more resilient, efficient, and sustainable poultry industry, ready to meet the growing global demand for poultry products, including meat and eggs.

Keywords: Disease resistance, Breeding and genetics, Poultry

Student Workshop: Where can poultry science take you? Career pathways, preparation tips, and profile-building strategies

585S What it takes: Building a career as a poultry nutritionist in an integrated or consulting company
J. T. Pope*[1] [1]*House of Raeford, Mebane, North Carolina, United States*

Abstract not required.

586S Climb, Swing, Leap: Embracing the jungle gym career path with a curious mindset
Kayla Price*[1] [1]*Alltech, Guelph, Ontario, Canada*

Abstract not required.

587S From grad student to faculty: A field guide to academic survival
Chongxiao Chen*[1] [1]*University of Georgia, Poultry Science, Athens, Georgia, United States*

Abstract not required.

588S Technical services or marketing careers in poultry: Insights from an industry leader
Milan Hruby*[1] [1]*ADM, Chicago, Illinois, United States*

Abstract not required.

589S Roles and opportunities for poultry science students at the USDA
Deana R. Jones*[1] [1]*USDA Agricultural Research Service, US National Poultry Research Center, Athens, Georgia, United States*

Abstract not required.

590S From the corporate to the entrepreneurial world
Federico Etcheverry*[1] [1]*Anpro Marcom, Jacksonville, Florida, United States*

Abstract not required.

Presidential Symposium: Precision poultry farming

591S Precision poultry farming: A purposeful endeavour
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Farmed poultry are an animal category that represents 70% of all birds on the planet. According to the OECD-FAO Agricultural Outlook 2023-2032, the demand for poultry proteins will continue growing with income countries, while animal protein consumption will become more strongly linked with the sustainability of the livestock sector. Citizen-driven initiatives such as End the Cage Age in the EU or the Better Chicken Commitment indicate how western society values the lives of farm animals. Furthermore, increasing the welfare standards will have a positive influence on the welfare standards in lower income countries. Consequently there is a growing need for better technologies to support farmers in in managing environmental emissions, animal feeding, health and welfare. Precision Farming (PF) is a field where the aim is to exploit Information and Communications Technologies (ICTs) to facilitate high resolution planning and management of farming operations. PF has seen great success in the crop production sectors, where it can be used to maximize the spatial-temporal productivity of the crop within the certain environmental constraints. However, the application of precision farming to livestock production, i.e. precision livestock farming (PLF), has been more challenging to implement with the same level of success. As a result while there are various PLF technologies under research for poultry health, welfare and productivity monitoring and management, the translation to functioning robust farm technologies that capture and act on information is still taking significant time. Part of the problem is the difficulty in demonstrating the value for the farmer and the animal alike. This requires an answer to how PLF technology can balance sustainability while at the same time farmers have a livelihood worth pursuing. This talk will look to the opportunities provided by PLF for the poultry sector, the recent state of the art as well as some pitfalls to consider when translating research into functioning solutions.

Keywords: Smart systems

592S Real-time monitoring and management of broiler health and welfare
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Modern broiler production is defined by the use of fast-growing genetic strains that achieve market weight in a matter of weeks. While this rapid growth supports production efficiency, it also presents significant challenges for maintaining bird health and welfare under commercial conditions. Broiler chickens are complex, individually-different, time-varying and dynamic live organisms. Current precision technologies provide multiple options to promote early detection, proactive intervention, and continuous improvement of broiler health and welfare outcomes. The section focuses on discussing hand-on experiences of applications of different sensors including computer vision, acoustics, depth, thermography, and environmental sensing, which continuously collect data on bird behavior, movement, vocalizations, and housing conditions. Discussions of how these data streams are interpreted using machine learning, computer vision, and edge computing are delivered to provide actionable and corrective insights for producers and enable timely adjustments to management practices. Cases studies of the sensor applications are provided, regarding heat stress status determination, avian disease detection, multiple bird tracking, body weight regression, and actual eating events identification. Emphasis is placed on practical integration of these precision tools into existing farm infrastructure and their potential to support both manual and automated decision-making processes.

Keywords: real-time monitoring, animal welfare, precision agriculture, artificial intelligence

593S Smart poultry systems: Commercializable insights from a sensor-equipped poultry house
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The advancement of the U.S. broiler industry in the 20th century occurred primarily with improved breeding and nutrition, with minimal improvements in housing. Towards the end of the 20th century, two housing changes that were implemented were: closed water systems and solid-sided housing. The closed water systems, e.g., nipple drinkers, resulted in improved flock health and improved water efficiency. The removal or change in construction from curtain-sided housing to solid-sided housing improved flock performance and health via improved house tightness and air flow, and improved energy efficiency with higher insulation values. At present, the implementation of broiler management tools, e.g., smart housing, has allowed for real-time evaluation of data from broiler houses resulting in increased decision-making tools and overall management flexibility. Real-time information data captured on farms (e.g., metered water intake, bin scales for feed intake, bird scales for BW, and environmental sensors) can be analyzed for making flock changes or predicting performance. Recent advancements in equipment have allowed for robust water and feed intake precision, but broiler BW measurements warrant improvements as affected by bird scale choice, especially at heavier broiler weights. As a result, our work has focused on a computer vision technique to measure broiler BW. Briefly, the proposed system takes videos from cameras (i.e., color and depth) as input and processes them through multiple modules to isolate individual broilers along with their poses, track their movements, and reconstruct their three-dimensional volumes. The broiler BW from hatch to market weights is then estimated by regressing from the reconstructed three-dimensional volume. Indeed, real-time "smart" data assessment will continue to improve flock performance, health, and resultant yields.

Keywords: Smart systems

594S What will a smart poultry farm look like?
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The rapid advancement of smart technologies can revolutionize modern poultry farming, introducing new levels of efficiency, animal welfare and well-being, reducing the use of resources, and minimizing negative impacts on the environment. This presentation explores the concepts and technologies and their implementation into a Smart Poultry House - an intelligent, automated system designed to optimize poultry farming

operations. Integrating sensors, artificial intelligence, and cloud-based data analytics, the smart poultry house monitors and controls key parameters such as temperature, humidity, lighting, feed, water supply, and bird behavior in real-time. Those smart technologies will enhance bird health, reduce mortality rates, improve feed conversion ratios, and minimize labor costs. This presentation also highlights Smart poultry farming innovation conceptualized and implemented at the Georgia Tech Research Institute's Agricultural Technology Research Program. Technologies being developed at ATRP and presented as a part of this talk will include: a raised broiler house flooring system, inflatable system, in-house autonomous robot platform, ammonia sensing, and modeling and simulation of broiler behavior based on house environmental conditions. Sensing can advise poultry farmers on the condition of the house and flock but it would not be as impactful as if we can make knowledge gained actionable. Several of the systems described in this talk go beyond sensing and utilize cyber-physical nature to allow for much more direct and targeted interventions to produce the best living conditions for broilers. By embracing and implementing Smart automation and data-driven decision-making, the poultry industry can meet rising demand while promoting sustainable farming practices.

Keywords: Smart systems

595S Precision feeding to reduce environmental impact of poultry production

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Today, poultry growers take advantage of specific dietary formulations, phase feeding, and advanced feed mill processing to meet poultry nutritional requirements. Despite enormous improvements over the past decades, concerns regarding the environmental impact of poultry feeds have recently increased. The environmental impact of poultry feeding practices is mainly associated with the excretion of nitrogen, phosphorus, and greenhouse gas emissions. While methods to mitigate the impact of poultry waste can be applied to manure and litter, strategies to decrease nitrogen and phosphorus excretion can be achieved before the digestion and metabolism of feeds. Thus, meeting the nutritional requirements of poultry is the primary goal to prevent unnecessary waste. Nonetheless, chicken genetics has increased tremendously, with rapid growth of meat-type chickens and enormous reproductive performance of egg-type chickens. Thus, nutrient requirements of poultry change daily (e.g. lysine for broilers) or hourly (e.g. Ca for reproducing birds). In this context, precision feeding and precision nutrition are relatively new technologies used in research and have started to be implemented in other animal industries (beef and swine) to increase economic outcomes and decrease environmental impact. Among the available strategies, daily phase feeding and split feeding programs have the potential to meet nutrient requirements more efficiently than common commercial practices. Daily phase feeding has been demonstrated to improve production efficiency through matching nutrient supply to nutrient demand, ensuring a constant supply of nutrients to the dynamic requirements; this method avoids under- and over-supply of nutrients in a commercial phase feeding program. A step further to precision feeding is split feeding, which involves the provision of different dietary feeds throughout the day, meeting specific diurnal requirements in laying hens and broiler breeder hens. This strategy provides nutrients during specific times during daily egg formation, reducing dietary requirements of amino acids, calcium, and phosphorus. Examples of precision feeding from other industries showed promising results. With the possibility of real-time monitoring of individuals, precision feeding will match nutrient supply to nutrient requirements not only in real-time, but also to individuals of a flock. Financial application, logistics, and paradigm shift are implementation challenges of precision feeding technology. However, with increasing sustainable benefits, precision feeding is expected to be gradually implemented in commercial poultry production.

Keywords: efficiency, precision livestock nutrition, smart agriculture, sustainability

596S A poultry value proposition: Precision feeding to eliminate spiking of broiler breeder roosters

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The use of smart sensing devices and systems to monitor the state of livestock and their environment to implement or inform optimal management and feeding decisions in real-time is known as precision livestock farming (PLF). While these technologies offer improvements in efficiency, animal health monitoring, and resource optimization, they typically require considerable upfront investment in development and training. The significant investment in commercial implementation of a technology like a precision feeding system demands that the system pay for itself in a reasonably short time. In the poultry sector, this challenge is amplified because the economic value of individual animals is low compared to cattle and pigs. A precision feeding system was designed to improve flock uniformity and egg production in broiler breeder females. Though the system and method eventually worked as intended, there was no immediate path to commercial adoption for the original implementation vision. Serendipitously, we discovered that precision feeding increased fertility because rooster uniformity increases breeding success, resulting in a 4 to 6% fertility increase. Coinciding with worldwide concerns about uniformity and hatchability, the timing of this discovery is somewhat of a perfect storm. Thus, after a decade of precision feeding research, a commercial adoption pathway has emerged. This presentation will tell the rollercoaster story of the disappointment and thrills leading to a commercial value proposition for precision feeding for broiler breeder males.

Keywords: Smart systems, precision feeding

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