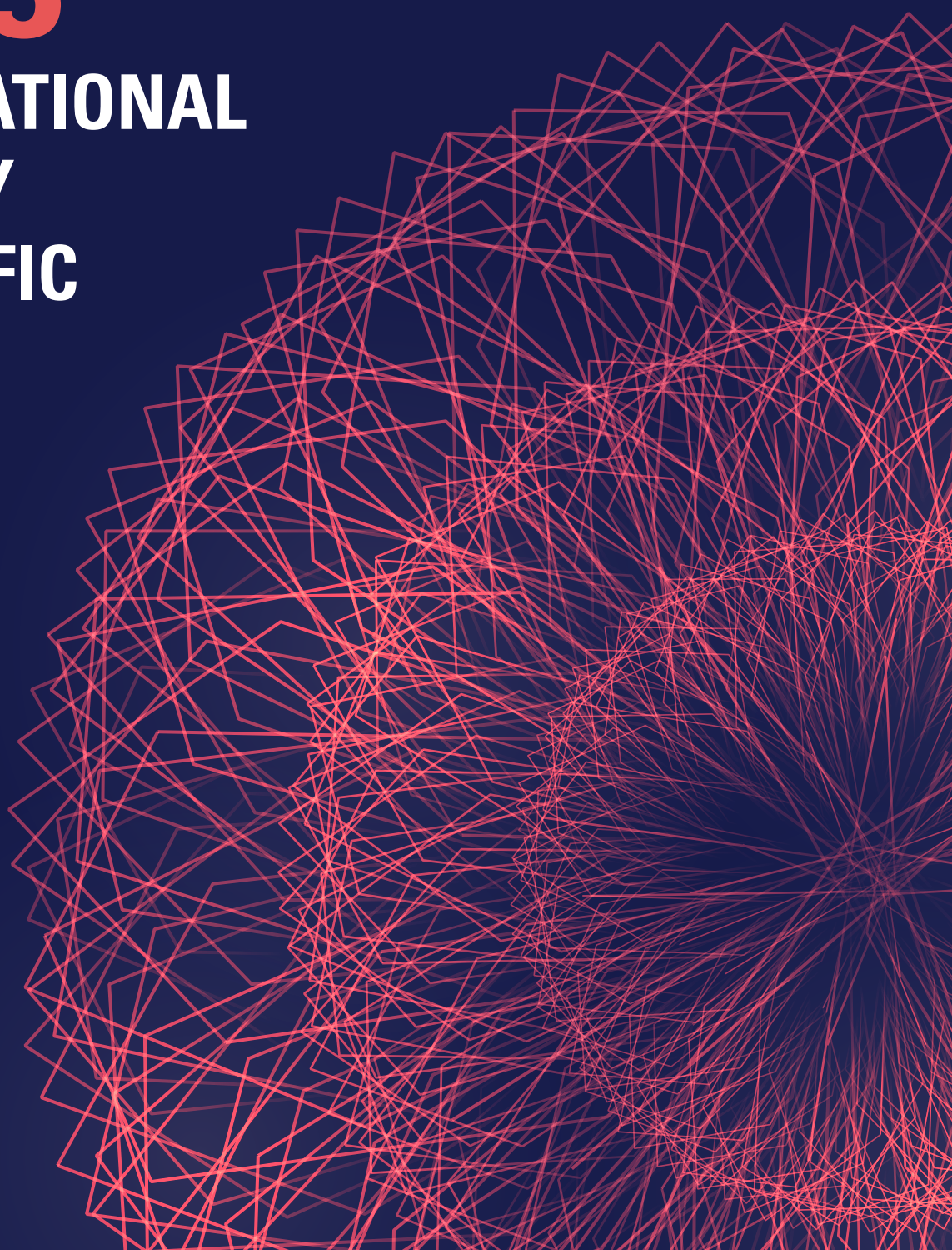




2025

INTERNATIONAL POULTRY SCIENTIFIC FORUM

JAN. 27-28



INTERNATIONAL POULTRY SCIENTIFIC FORUM

FRONT COVER

ABSTRACTS
2025 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 27-28, 2025

Table of Contents

Oral Abstracts

Dendy Keynote Lecture, 3
 Physiology I, 4
 Physiology II, 8
 Environment & Management I, 10
 Metabolism & Nutrition I: Enzymes, 14
 Pathology, 16
 SCAD I, 19
 SCAD II, 21
 Welfare & Behavior I, 25
 Welfare & Behavior II, 28
 Food Safety I, 31
 Processing & Products, 35
 Metabolism & Nutrition II: General Nutrition, 38
 Metabolism & Nutrition III: General Nutrition, 39
 Metabolism & Nutrition IV: General Nutrition, 41
 Metabolism & Nutrition V: Vitamins & Minerals, 43
 Metabolism & Nutrition VI: Amino Acids, Feed Additives, 46
 Metabolism & Nutrition VII: Vitamins & Minerals, 50
 Metabolism & Nutrition VIII: Amino Acids, Enzymes, 52
 Metabolism & Nutrition IX: Feed Additives, 57
 SCAD III, 62
 Metabolism & Nutrition X: Feed Additives, 60
 Welfare & Behavior III, 72
 Metabolism & Nutrition XI: General Nutrition, 74

Metabolism & Nutrition XII: Feed Additives, 76
 Environment & Management II, 78
 Food Safety II, 80

Poster Abstracts – Student Competition

Environment & Management, 84
 Food Safety, 86
 Processing & Products, 91
 Metabolism & Nutrition: Amino Acids, Enzymes, 92
 Metabolism & Nutrition: Feed Additives, 94
 Metabolism & Nutrition: Vitamins & Minerals, 98
 Physiology, 100
 Welfare & Behavior, 103
 Pathology, 105
 SCAD, 108

Poster Abstracts – Non-Competition

Environment & Management, 111
 Food Safety, 115
 Processing & Products, 121
 Metabolism & Nutrition: Amino Acids, Enzymes, 122
 Metabolism & Nutrition: Feed Additives, 124
 Metabolism & Nutrition: Vitamins & Minerals, 138
 Physiology, Welfare & Behavior, 144
 Pathology, 148
 SCAD, 149
AUTHOR INDEX, 153

ABSTRACTS
2025 International Poultry Scientific Forum
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Dendy Keynote Lecture

The History of Our Scientific Societies and the US Broiler Industry Joseph Giambrone*¹ *¹Auburn University, Poultry Science, Auburn, Alabama, United States*

This lecture will follow the histories of the following Educational, Scientific, and Industrial entities: the Milton Dendy Lecture; the Southern Conference of Avian Diseases and Southern Poultry Science Society which make up the International Poultry Scientific Forum; The International Production and Processing Show; and the Integrated United States Broiler Industry. Historical dates, personalities, and the significance of each will be provided.

Keywords: History, Milton Dendy, SCAD, SPSS, US Broiler Industry

*Author presenting paper

GS Denotes Graduate Student Presentation
UG Denotes Undergraduate Presentation

Physiology I

M1 Physiological response of broiler ducks fed Cocoa (*theobroma cacao*) pod husk Muslim K. Ewuola*¹, Kazeem O. Jolayemi¹, Abubakar Musa¹, Florence A. Salako¹, Rasheed A. Hamzat¹ ¹*University of Abuja, Animal Science, Abuja, FCT/Abuja, Nigeria*

High cost of maize has led to incorporation of cocoa pod husk (CPH) as an alternative feed ingredient into ducks' diets to improve performance and overall health. Hence an experiment was conducted to evaluate blood profile of ducks fed graded levels of (CPH) meal. A total of 375-day-old ducklings were randomly monitored on feeding trial for 90-day using CPH meal (CPHM) as partial replacement for maize in diets 1, 2, 3, 4, and 5 at 0%, 5%, 10%, 15%, and 20% levels, respectively. The ANOVA was used to analyse the data after each treatment was replicated five times in a completely randomised design. At the conclusion of the feeding trial, uncoagulated blood samples were taken from the ducks and examined for packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells (RBC), and white blood cells (WBC). The PCV, RBC, and Hb were used to compute the mean corpuscular haemoglobin volume (MCV), mean corpuscular haemoglobin (MCH), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Serum cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined after the blood intended for serological lipid analysis was centrifuged at 1000 G for 10 minutes. Red blood cell counts (2.91 - 3.49), haemoglobin (9.36 - 11.90), and packed cell volume (29.66% - 36.66%) did not differ significantly across treatments. There was no discernible difference in platelets (161333.33 - 214666.66 ul), lymphocytes (61.60 - 65.66%), heterophils (27.66 - 32.00%), and monocytes (2.00 - 3.33%). There was a considerable variation in basophil (0.00-1.00%) between the treatments. The mean corpuscular volume (99.22 - 104.50 fl) and mean corpuscular haemoglobin (31.26 - 32.77%) did not differ significantly amongst the dietary regimens. Serum lipid profile results revealed higher CPH levels were associated with higher levels of triglycerides and cholesterol. The 15% inclusion group had the highest levels of triglycerides (89.66 mg/dL) and cholesterol (197.33 mg/dL). The ducks' serum lipid profile revealed cholesterol levels ranging from 122.00 to 197.33. Adding up to 15% of CPHM to the experimental food had no detrimental effects on the ducks' performance, and there were no health risks to the animals during the trial period.

Keywords: Agro-industrial by-products, Conventional feed stuffs, Poultry diets, Blood profile, Nigeria

M2 Cloacal feather trimming improves reproductive performance of layer breeder roosters Eder O. Barbosa², Regina C. Porto², Felipe L. Kroetz Neto³, Ricardo J. Pereira*¹ ¹*College of Veterinary Medicine and Animal Sciences - São Paulo University, Animal Reproduction, Pirassununga, São Paulo, Brazil;* ²*Mercoaves Comercio de Aves Ltda, Piracicaba, São Paulo, Brazil;* ³*Aviagen America Latina Ltda, Campinas, São Paulo, Brazil*

Brazil is a leader in egg production with ~1.4M layer breeders to support its chain. Although genetics have improved reproductive traits more in layer breeders than in their broiler congeners, male aging is still a critical element in the production of fertile eggs and, consequently, the hatch rate. Here, we assessed the effects of

cloacal feather trimming (CFT) on reproductive parameters of a commercial flock with natural mating. A breeding house accommodating 11,238 females and 1230 males (Bovans White parent stock) was divided into 6 boxes, in which 3 had roosters subjected to monthly CFT and 3 boxes contained intact roosters (trimmed; intact boxes interspersed in the house). From 35-77 weeks of age, 2880 eggs from each group were collected every 40 days, marked and placed back with the rest of the eggs to undergo the company's standard procedures regarding handling, storage, transportation and incubation procedures (both in the farm and in the hatchery). Afterwards, unhatched eggs from the two groups were subjected to embryo diagnosis. Variables were initially tested to determine variance homogeneity and residual data normality, and whenever these assumptions were not validated the data were transformed. The effects of feather management and flockage, as well as their interaction were estimated using the repeated-measures analysis of variance. Comparisons between means of two groups were performed using t-test, whereas Fisher's test was applied for multiple comparisons. A confidence interval analysis was performed under the difference between control and treated groups. Statistical significance for all analyses was set at $P < 0.05$. Aging had an impact on all the parameters evaluated, while CFT reduced infertility and embryonic mortality (0-4 days), improving hatch rate and hatchability. Interactions between CFT and age were observed for infertility and embryonic mortality (0-4 days). From 41 to 77 weeks of age, infertility in the CTF group decreased between 1.14% and 3.26% in relation to control group, whereas hatch rate increased between 2.63% and 4.27%. These findings indicate that a simple management such as CFT can significantly benefit the production of breeding flocks, and can be applied to the routine of parent, gp or ggp stock farms.

Keywords: poultry, chicken, male, fertility, management

M3 Preclinical gene expression alterations in the uterovaginal junction of turkey hens infected with low pathogenic avian influenza Sunantha Kosonsiriluk*¹, Pitchaya Santatvongchai¹, Kent Reed², Milena Saqui-Salces¹, Marissa Studniski³, Ben Wileman³, Kahina Boukherroub¹ ¹*University of Minnesota, Animal Science, Saint Paul, Minnesota, United States;* ²*University of Minnesota, Veterinary and Biomedical Sciences, Saint Paul, Minnesota, United States;* ³*Select Genetics, Willmar, Minnesota, United States*

The turkey industry is often the hardest hit by avian influenza virus (AIV) outbreaks, with even low pathogenicity avian influenza (LPAI) strains leading to significant economic losses. Turkey breeder hens are particularly susceptible to AIV, with weekly artificial insemination potentially serving as an entry route for the virus. This study aimed to identify key genes and signaling pathways affected by LPAI during the early stages of infection in the reproductive tract of turkey hens. We hypothesized that the reproductive tract of infected birds would exhibit gene expression alterations early in the stages of LPAI infection. To understand the initial host response to LPAI, we compared uterovaginal junction (UVJ) samples from presymptomatic and symptomatic LPAI-infected turkey breeder hens ($n = 4$ per group). Total RNA sequencing and differential expression analysis using DESeq2 identified 14,226 genes expressed in the UVJ, with 2,866 genes significantly differentially expressed ($FDR < 0.05$, fold-change ≥ 2) between the presymptomatic and symptomatic groups.

Enrichment analysis of upregulated genes in the presymptomatic group indicated pathways associated with viral infection, such as cell adhesion molecules, focal adhesion, and calcium signaling, as well as host immune responses, including cytokine-cytokine receptor interaction, transforming growth factor-beta signaling, and immunoglobulin A immune network pathways (adjusted p-values < 0.05 for all pathways). Upregulation of genes such as *PTGER2* (prostaglandin E receptor 2), *COX-1* (cyclooxygenase-1), *COX-2*, *IFITM5* (interferon-induced transmembrane protein 5), *IRF5* (interferon regulatory factor 5), and several interleukins (*IL15*, *IL16*, *IL18*, *IL2RB*, and *IL18RAP*) were observed in presymptomatic hens. These changes suggest early immune defense mechanisms and an inflammatory response in the UVJ to LPAI infection, detectable before hens exhibit clinical signs of infection. Such responses may help control the virus before it is fully established in the host. This study provides insights that will guide future strategies to target the early stages of LPAI infection, potentially preventing or slowing viral infection and limiting the spread of LPAI, thus mitigating the economic and welfare impacts of LPAI outbreaks.

Keywords: Avian influenza, Gene expression, RNA sequencing, Turkey, Uterovaginal junction

M4 Hepatic corticosterone binding globulin mRNA expression in chickens of different ages, strains, and genders Coleman J. Hatmaker*¹, Adam J. Davis¹, Martha E. Freeman¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Corticosterone (CORT) is the primary glucocorticoid stimulating the avian stress response. Stress evaluation in birds often relies on plasma CORT concentration as a biomarker. The transport protein corticosteroid binding globulin (CBG), produced and secreted primarily by the liver, binds CORT in circulation with high affinity and delivers it to peripheral tissues. Consequently, the concentration of free CORT available to enter a cell and activate the stress response is directly affected by the amount of circulating CBG. Unlike humans and other production animals, avian species lack sex hormone binding globulin, and therefore CBG also binds and transports steroid sex hormones in birds. The goal of the current research was to determine if CBG expression differed between chickens of various ages, strains and genders. Hepatic tissue was collected from male and female Ross 308 and Athens Canadian Random Bred (ACRB) broilers on the day of hatch and 6 weeks post-hatch as well as from 62-week-old Ross broiler breeder roosters, White Leghorn hens and ACRB hens to assess mRNA expression of CBG. Total RNA was extracted from 6 individual hepatic tissue samples from each gender, strain, and age category. The RNA was then DNase treated in preparation for two-step real-time RT-PCR analysis. Taqman minor groove binding probes and primers for CBG and GAPDH (control) were created using Primer Express (Version 2.0, Applied Biosystems). Relative CBG mRNA expression was analyzed by ANOVA with means separated using Tukey's multiple-comparison procedure. Differences were considered significant when $P < 0.05$. The 6-week-old Ross male broilers had greater ($P < 0.01$) hepatic CBG mRNA expression than the 62-week-old Ross broiler breeder roosters, but there was no difference in expression between 6-week-old and 62-week-old ACRB females. The 62-week-old ACRB hens had greater ($P < 0.01$) hepatic CBG mRNA expression than the White Leghorn hens. However, there was no difference in hepatic CBG mRNA expression between Ross and ACRB broilers on the day of hatch or 42 days of age. The results indicate that factors such as age, strain, and gender influence the hepatic production of CBG; thus measuring both plasma CORT and CBG is necessary to accurately assess a stress response in chickens.

Keywords: stress response, broilers, broiler breeders, laying hens

M5 Impact of heat stress on hepatic lipogenesis in broilers: a comparison of low- and high-water efficient lines Maria Fernandez Cuadrado*¹, Elizabeth S. Greene¹, Sami Dridi¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

Heat stress (HS) presents a significant challenge to sustainability in the poultry industry and has been documented to increase water consumption and fat content in broilers. Recently two broiler lines were divergently selected for high (HWE) or low (LWE) water efficiency. Interestingly, heat-stressed HWE birds had lower fat content compared to their LWE counterparts, and HS reduced abdominal fat content in both lines. The present study aimed, therefore, to delineate the underlying molecular mechanisms involved in this divergent fat content between the two lines. Day-old chicks were placed in 12 environmentally controlled chambers (2 pens/chamber, 20 birds/pen). On D29, daily cyclic heat stress (36°C, 9h/day) was applied to half of the chambers, while the others remained at 25°C, in a 2x2 factorial design. On D49, liver samples were collected for lipid metabolism-associated gene and protein expression analyses. Data were analyzed by Two-Way ANOVA and Tukey's multiple range test using Graph Pad Prism. Data are expressed as mean \pm SEM and were considered significant at $P \leq 0.05$. HWE birds exhibited higher fatty acid synthase FASN protein levels under thermoneutral (TN) conditions ($P = 0.0011$), with both lines showing decreased FASN protein levels under HS. Malic enzyme (ME) protein expression was also significantly higher in HWE compared to LWE birds under both TN and HS conditions, LWE birds showed higher ATP citrate lyase (ACLY) gene expression under HS, the transcription factors SREBP-1 and SREBP-2 expression was generally higher in LWE birds. Although the main enzymes (FASN, ACC) are upregulated in HWE lines, our findings showed that the main transcription factors are also upregulated, suggesting whether there are other main lipogenic protein effectors or high hepatic lipolysis rate in HWE birds that merit further in-depth investigations.

Keywords: broiler, heat stress, water efficiency, liver, fatty acid synthesis

M6 Effect of thermal variation during early-stage incubation on broiler chicken muscle satellite cell populations at transfer and hatch J. Wesley Rogers*¹, Brittany L. Hutson¹, Martha S. Rueda^{1,2}, Jeremiah D. Davis², Katie E. Elliott³, Charlene Hanlon¹, Charles W. Starkey⁴, Jessica D. Starkey¹ ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States;* ²*Auburn University, National Poultry Technology Center, Auburn, Alabama, United States;* ³*USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi, United States;* ⁴*North American Renderers Association, Scientific and Regulatory Affairs, Alexandria, Virginia, United States*

Satisfying the temperature requirements in multi-stage incubators where late-stage broiler chicken embryos must be kept from overheating while ensuring early-stage embryos do not become hypothermic is a challenge. Our previous work revealed that performance and meat yield were negatively affected when early-stage incubation (ESI) temperatures were either decreased to 36.4°C (COLD) or increased to 38.6°C (HOT) from embryonic day (ED) 4 to 11 vs. maintaining 37.5°C (CTL) throughout incubation. The objective of this work was to assess the effect of thermal variation (TV) during ESI on muscle satellite cell (SC) population heterogeneity in broiler chicks at transfer and hatch to improve our understanding of the mechanism underlying the meat

yield effects seen in our previous study. Broiler breeder eggs ($n = 2,160$) were incubated at 37.5°C from ED 0 to 3. On ED 4, incubator setpoints were altered to match the COLD, CTL, and HOT TV treatments described above ($n = 2$ incubators per TV treatment). On ED 11, all incubators were set to 37.5°C until ED 18 when eggs were transferred to hatchers. At transfer (ED 18) and hatch (ED 21), pectoralis major (PM) and biceps femoris (BF) muscle samples were collected ($n = 6$ chicks per treatment). Samples were immunofluorescence stained to facilitate taxonomy of SC populations expressing the myogenic regulatory factors and SC markers, MyoD, Pax7, and Myf5, by fluorescence microscopy. Data were analyzed using SAS (ver 9.4) PROC GLIMMIX as a 1-way ANOVA with the PDIF option at $P \leq 0.05$ to separate means. Tendencies were declared when $0.0501 \leq P \leq 0.15$. Chicks from HOT incubators tended to have the most Pax7+ SC in their BF muscle on ED 18 ($P = 0.1260$). On ED 21, HOT chicks had more MRF- nuclei ($P \leq 0.05$), tended to have more total non-fused nuclei, Pax7+ SC, and myonuclei in their PM muscle vs. CTL ($P \leq 0.15$). These results demonstrate that TV as little as 1.1°C during ESI (ED 4 to 11) altered BF SC populations in modern, high-yielding broiler chicks at transfer and PM SC populations at hatch, though SC from functionally different muscles responded differently which sheds light on the mechanism by which suboptimal (HOT and COLD) incubation conditions negatively impact broiler growth and meat yield.

Keywords: incubation, satellite cell, thermal variation, myogenesis, broiler chicken

M7 Effect of thermal variation during late-stage incubation on broiler chicken body weight gain and Pectoralis major muscle growth characteristics Juan J. Barberena^{*1}, J. Wesley Rogers¹, Brittany L. Hutson¹, J. Enrique Banegas¹, Martha S. Rueda^{1,2}, Jeremiah D. Davis², Katie E. Elliott³, Charlene Hanlon¹, Charles W. Starkey⁴, Jessica D. Starkey¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²Auburn University, National Poultry Technology Center, Auburn, Alabama, United States; ³USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi, United States; ⁴North American Renderers Association, Scientific and Regulatory Affairs, Alexandria, Virginia, United States

The broiler industry commonly uses multi-stage incubators which are difficult to manage and provide optimal temperatures for both early-stage and late-stage embryos at the same time. To assess the effect of thermal variation (TV) during late-stage incubation (LSI; embryonic day (ED) 11 to 18) on *Pectoralis major* (PM) muscle growth characteristics, a randomized complete block design experiment with 3 TV treatments was conducted. Yield Plus \times Ross 708 hatching eggs ($n = 2,160$) were incubated at 37.5°C from ED 0 to 10. From ED 11 to 18, eggs were incubated at 1 of 3 air temperature set points: 37.5°C (CTL), 36.4°C (COLD), or 38.6°C (HOT; $n = 2$ incubators per treatment). On ED 18, all eggs were transferred to baskets in hatchers set to 36.7°C . Chicks were weighed and placed in floor pens ($n = 12$ replicate pens of 30 birds per treatment) and fed a common diet for 28 d. On d 7, 14, 21, and 28 post-hatch, a total of $n = 20$ birds per treatment were weighed, euthanized, and PM weights and samples for cryohistological analysis were collected. Data were analyzed as a 1-way ANOVA using SAS PROC GLIMMIX and PDIF with mean separation at $P \leq 0.05$ and tendencies declared at $0.0501 \leq P \leq 0.10$. On d 7, the chicks from COLD incubators were similar in BW to CTL but were lighter and gained less BW from d 0 to 7 than those from HOT incubators ($P \leq 0.0146$). On d 14, both HOT and COLD chicks tended to be lighter than CTL ($P = 0.0694$) and gained less BW compared with those from the CTL incubators from d 0 to 14 ($P = 0.0746$). On d 7 chicks from COLD incubators had the lightest

PM weight ($P = 0.022$) and on d 21 broilers from COLD incubators had lighter PM weights vs. CTL and HOT birds ($P = 0.033$). Broilers incubated in COLD incubators tended to have the smallest PM fiber cross-sectional area ($P = 0.0564$) on d 14 and the smallest PM muscle cross-sectional area on d 28 which helps explain the reduced breast meat yield observed in our previous work. Overall, these results indicate that sub-optimal LSI temperatures, especially hypothermic conditions, can negatively influence broiler muscle growth characteristics and highlight the importance of achieving optimal incubation conditions for maximizing carcass parts yields.

Keywords: late-stage incubation, thermal variation, muscle growth, muscle fiber cross-sectional area, broiler chicken

M8 Influence of euthanasia methods on broiler blood metabolites Madalyn Jennings^{*1}, Abigail McConnell¹, Matthew Hughes¹, Dianna Bourassa¹ ¹Auburn University, Auburn, Alabama, United States

Euthanasia methods are used by professionals in multiple facets of the poultry industry. Carbon dioxide (CO_2) euthanasia, electrical euthanasia, manual cervical dislocation (CD), and captive bolt are all approved euthanasia methods by the American Veterinary Medical Association (AVMA). Oftentimes, one of these methods is used prior to sample collection for research studies. To investigate the influence of euthanasia method on blood metabolites, five blood samples per method were collected from broilers euthanized by CO_2 , electrical, manual cervical dislocation, and captive bolt. From these blood samples, partial pressure of carbon dioxide PCO_2 (mmHg), pH, partial pressure of oxygen PO_2 (mmHg), HCO_3^- (mEq/L), base excess BE_{ecf} (mEq/L), oxygen saturation sO_2 (%), total carbon dioxide TCO_2 (mEq/L), Na (mEq/L), K (mEq/L), ionized calcium iCa (mmol/L), glucose (mg/dL), hematocrit (%PVC) and hemoglobin (g/dL) were measured using an iSTAT Alinity v blood analyzer. Data were analyzed by General Linear Models through SAS 9.4 University Edition, with significance determined at $P \leq 0.05$. Means were separated by Tukey's HSD. PCO_2 was higher for birds euthanized by CO_2 (115.95) compared to electrical (47.22), CD (37.92), and captive bolt (45.08; $P < 0.0001$). The pH was lower for birds euthanized by CO_2 (6.90) than electrical (7.35), CD (7.42), and captive bolt (7.35; $P < 0.0001$). Na was higher for birds euthanized by captive bolt (148.2) compared to CO_2 (143), electrical (146.8), and CD (147.4; $P = 0.0182$). K was higher in birds euthanized by CO_2 (8.62) than electrical (6.34), CD (6.2), and captive bolt (6.5; $P = 0.0089$). iCa was higher in birds euthanized by CO_2 (1.33) compared to electrical (1.28), CD (1.18), and captive bolt (1.30; $P = 0.0393$). There were no significant differences between the euthanasia methods for PO_2 ($P = 0.5134$), HCO_3^- ($P = 0.7503$), BE_{ecf} ($P = 0.1188$), sO_2 ($P = 0.1200$), TCO_2 ($P = 0.5050$), glucose ($P = 0.1380$), hematocrit ($P = 0.0685$), or hemoglobin ($P = 0.1213$). These findings provide insight into the potential effects of euthanasia methods on blood metabolites, which may influence subsequent samples obtained during research studies.

Keywords: euthanasia, blood, broiler, metabolites

M9 Validation of a Photoelectric Sensor System to Detect Oviposition Timing in Individually Caged Broiler Breeders Lauren R. Sroda^{*1}, Jeremiah D. Davis¹, Kristin Diehl³, Carson M. Edge¹, Madison Berger², Katie E. Elliott³, Brenda Flack⁴, Charlene Hanlon² ¹Auburn University, National Poultry Technology Center, Auburn, Alabama, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ³USDA ARS, Beltsville, Maryland, United States; ⁴Cobb-Vantress, Fayetteville, Arkansas, United States

Understanding the time of oviposition in broiler breeders is an important metric for improving precision animal and feeding management in the U.S. The objective of this project was to validate the use of a system using photoelectric sensors (PES) to quantify the time of oviposition of individually caged broiler breeders. The system was validated using 101 Cobb 500 broiler breeder hens arranged in a completely randomized design over a two-week period. The detection system consisted of a single PES and opposing reflector installed on 3D printed dividers that separated the egg saver for each cage. The PESs were measured with datalogging systems using a 1-min sampling rate. A camera system and staff records were used to validate the time recorded by the PES system using a frequency analysis. For the 1,414 possible oviposition opportunities, events were categorized into one of four categories: detected and observed (D-O), detected and not observed (D-NO), not detected and observed (ND-O), and not-detected and not-observed (ND-NO). The PES system accurately detected the time of oviposition within 3-min for 94.7% of the events (1,339/1,414). Correctable errors, such as staff collection, documentation mistakes, and cage design issues, could improve the accuracy of the system to 98.9%. One limitation of the PES system is that it must be turned off when lights are off as the red light emitted by the PES will overstimulate the reproductive axis of the broiler breeders, resulting in declined production rates and altered timing of oviposition. Post-processing and determination of the time oviposition was efficient using a PES system (< 0.2 h for all data) and cost approximately \$284 per cage.

Keywords: oviposition, photoelectric sensor, broiler breeder, accuracy

M10 Impact of Oviposition Timing on Egg Quality: Translucency, Speckling, and Shell Characteristics Leticia A. Orellana Galindo^{*1}, Cassidy A. Morris², Luis Muñoz¹, Laura Jimenez³, Tania Roman³, Tatiana Salazar³, Josselyn Herrera³, Arley Fernandez³, Jason Cordero³, Luis Castro³, Juan Diego Gonzalez³, Dulman Serrano³, Kenneth Macklin¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Mississippi State University, Poultry Science, Moulton, Alabama, United States; ³Cargill Protein Latin America, Puntarenas, Costa Rica

Oviposition timing and egg position in a clutch have been reported to impact egg quality. Two experiments were conducted to examine how translucency and speckling vary by oviposition time, and whether speckles affect hatchability and other eggshell quality parameters. In Experiment 1, 4,445 eggs from Ross 708 hens (aged 29, 42, and 55 weeks) were evaluated for translucency levels (T1=low, T2=medium, T3=high) and presence of speckling at three oviposition times (8:30 am, 11:30 am, 3:30 pm). In Experiment 2, 3,510 eggs from 42-week-old hens were divided into morning non-speckled, afternoon speckled, and afternoon non-speckled laying groups for hatchability analysis, with 270 eggs analyzed for thickness, breaking strength, and internal quality. Data were analyzed using the GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means. A significant difference was considered between the means when $P \leq 0.05$. Results from experiment 1 showed that regarding translucency, at 8:30 AM and 11:30 AM, T2 had the highest incidence (55.49% and 59.70%, respectively), while T1 and T3 were lower. By 3:30 PM, T3 increased significantly to 77.81%, while T1 and T2 decreased notably ($P=0.04177$). For speckling, the incidence was similar at 8:30 AM and 11:30 AM (16% and 17%, respectively), but by 3:30 PM, it rose sharply to 37% ($P<0.0001$). Results from experiment 2 showed no effect of speckles in hatchability ($P=0.0615$), however there was an effect in eggshell strength ($P=0.0048$) and thickness ($P<0.0001$), where non-speckled eggs

laid in the afternoon were stronger (4.72 Kgf) than those laid in the morning (4.38 Kgf), and both speckled and non-speckled eggs showed greater shell thickness in the afternoon. Speckled eggs laid at 2:00 PM were 26.3 μm thicker compared to non-speckled morning laid eggs. These results suggest that both speckling and high translucency are more prevalent in eggs laid in the afternoon, with afternoon timing contributing to increased shell strength and thickness in broiler breeder eggs.

Keywords: Translucency, Speckles, Eggshell quality, Broiler breeders, Oviposition time

M11 Impact of 28-day storage on translucency score and eggshell quality parameters of W-36 laying hens Cassidy A. Morris^{*1}, Charis A. Waters¹, Pratima Adhikari¹, Kenneth Macklin¹ ¹Mississippi State University, Poultry Science, Moulton, Alabama, United States

Understanding how egg quality deteriorates during storage is useful information to the table egg industry. This study aimed to evaluate how different storage lengths affect the translucency score (TS) and overall eggshell quality of table eggs. To accomplish this, 678 eggs were collected from 25-week-old Hyline W-36 hens. Eggs were washed on day 0 and placed into an egg storage room at 18°C, where they remained for 28 days. On day 0 all eggs had their TS, thickness and L score measurements taken; TS was also measured on day 1. On days 7, 14, 21, and 28, TS and L score measurements were performed; additionally, on day 28, thickness measurements were taken. On days 0, 14, and 28, sixty eggs/day (20/TS) were taken to undergo internal eggshell quality measurements. On day 0 and 28, sixty eggs/day (20/TS) were taken to undergo microbial analysis. For this, eggshells were rinsed with buffered peptone water (BPW) and then aseptically opened into sterile BPW to collect contents. Samples were plated onto Plate Count Agar and MacConkey Agar and allowed to incubate for 24h at 37°C to enumerate total aerobic and coliform bacteria. Samples were also plated on Potato Agar and allowed to incubate for 5 days at 25°C to enumerate fungi. Data was analyzed using PROC Mixed, PROC GLM or Chi Square with $P \leq 0.05$; means were separated using either Tukey's or LSMeans where appropriate. The results showed that as the storage increased, there was a significant worsening of translucency scores with a decrease in TS1 and an increase in TS3 ($P<0.0001$). There was a significant interaction of storage and TS at day 0, with TS1 having the thickest eggs ($P=0.0235$). Yolk color was significantly different for TS on day 0 ($P=0.0481$), but not on days 14 or 28. On day 14, TS1 eggs had a significantly higher breaking strength (5.19 kg) compared to TS2 (4.39 kg; $P=0.0222$). No significant differences were found for total aerobic or coliform bacteria in the shell or contents at any point. Fungi load on the outer shell was higher in TS1 eggs than TS2 & 3 ($P=0.0082$); however, was lower in the contents of TS1 eggs ($P=0.0026$). This study shows that TS and overall eggshell quality does decrease throughout storage. However, further research to understand this relationship needs to be conducted.

Keywords: translucency score, eggshell quality, storage, microbial analysis

M12 Effects of eggshell metrics on hatchability and chick development of two genetic lines Paola Andrea Amaya^{*1}, Edgar Leonardo Molina Villarraga¹, Caleb M. Marshall¹, Cesar Guato Guaman¹, Duarte Neves², Edgar O. Oviedo-Rondón¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²Zinpro Corporation, Eden Prairie, Minnesota, United States

Eggshell properties affect hatchability, but little is known about the impact on chick development or differences among genetic

lines. This experiment evaluated eggshell metrics effects on hatchability, and chick development. Eggs were collected from 33-week-old Ross ($n=800$) and Cobb ($n=1,206$) breeder flocks and individually labeled. All eggs were weighed, eggshell translucency score (TS) from 1 to 3 was completed using the BlueBox[®], and thickness was measured using an ultrasonic gauge. Egg-specific gravity (SG) was obtained in saline solutions. Three incubations were conducted following a single-stage incubation profile. Eggs were transferred to a hatcher at d 18, and eggs representing each TS and SG category were placed in pedigree bags to complete 504 h of incubation. At hatch, hatchability was calculated, and selected chicks were weighed, sexed, and residual yolk, heart, liver, gizzard-proventriculus, and intestines dissected and their weights recorded. Logistic regression was used to analyze hatchability and embryo mortality. ANOVA and regression analyses were used to evaluate the effects of TS and SG categories and other eggshell traits. Results indicated that the lightest eggs had more TS1 ($P<0.05$), and Cobb's lightest eggs had ($P<0.001$) the highest SG. Egg weight loss (EWL) at 18d was as high ($P<0.001$) as 14.2 and

17.3% in Cobb and Ross eggs at 1.060 SG and reduced linearly to 9.7 and 11.0% in eggs with 1.090 SG. EWL linearly decreased ($P<0.001$) as eggshell thickness increased mainly in Cobb eggs ($R^2 = 0.33$). Hatchability was lower ($P<0.05$) in eggs with SG ≤ 1.060 in both lines (50 and 40%) and with 86.2 and 84.6% TS3 ($P<0.01$), respectively. Cobb eggs with SG ≤ 1.065 or 1.090 and Ross eggs with SG ≤ 1.060 or ≥ 1.085 produced ($P<0.01$) the lightest chicks. Cobb eggs with high SG (1.090) and Ross eggs with SG 1.065 produced chicks with reduced heart and gizzard-proventriculus development ($P<0.05$), compared to eggs with SG 1.075 in Cobb or ≥ 1.085 for Ross. The heaviest chicks, in both lines, were observed with TS1. However, no effects ($P>0.05$) of TS were detected on chick organ development. In conclusion, SG and eggshell translucency evaluate different shell properties affecting incubation metrics and embryo development, and optimums vary by genetic line.

Keywords: eggshell translucency, embryo development, hatchability, specific gravity, chick weight

Physiology II

M13 Receptor expression in the shell gland of commercial laying hens indicates it may be a novel target for FGF23 R

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Phosphorus is an important component of the crystalline hydroxyapatite layer beneath the cuticle of the eggshell and may be involved in the termination of eggshell calcification. In mammals, fibroblast growth factor 23 (FGF23) has been shown to mediate renal phosphorus homeostasis; however, its role in avian species has not been fully elucidated, and it could mediate phosphorus utilization by the shell gland. Therefore, this study evaluated the shell gland as a potential FGF23 target based on expression of its receptors across the daily egg formation cycle during early (25w) and extended (95w) production. To this end, mRNA levels of FGF receptors (*FGFR* 1-4 and coreceptor *Klotho* (*KL*)) were evaluated in shell gland collected from Nick Chick hens (H&N International) during times representing bone mineralization (1.5 hours post-oviposition [HPOP]), eggshell mineralization (15 HPOP), and transitions between them (6 and 21 HPOP) ($n=8/\text{age}/\text{HPOP}$). Levels of mRNA were determined by RT-qPCR, and data were analyzed by ANOVA and Fisher's LSD test. An age-by-HPOP interaction was observed for *FGFR2*, 3, and *KL*. At all HPOP, *FGFR2* and 3 were higher in older hens ($P\leq 0.05$). At both ages, *FGFR2* and 3 decreased between 1.5 and 15 HPOP, followed by a 2- or 6-fold increase between 15 and 21 HPOP at 25 and 95w, respectively ($P\leq 0.05$). Levels of *KL* were similar for both ages at 1.5 HPOP, followed by decreased expression at 6 HPOP, where older hens had lower levels ($P\leq 0.05$). At 15 HPOP, *KL* levels did not differ between ages before increasing again at 21 HPOP, with higher expression observed at 95w ($P\leq 0.05$). Main effects of age and HPOP were observed for *FGFR1* and 4, with greater expression in older hens and variation across the daily lay cycle ($P\leq 0.05$). Levels of both decreased between 1.5 and 6 HPOP; however, *FGFR1* increased to its highest levels at 15 HPOP before decreasing at 21 HPOP, while *FGFR4* decreased to its lowest levels at 15 HPOP, followed by a slight increase at 21 HPOP ($P\leq 0.05$). The shell gland is a potential target for FGF23 action, and its sensitivity may increase with age. Elevated *FGFR2*, 3, and *KL* at 21 HPOP indicates FGF23 could regulate phosphorus utilization for the crystalline

layer and cuticle formation occurring during late stages of eggshell formation.

Keywords: Crystalline layer, Hydroxyapatite, Eggshell cuticle, Phosphorus utilization, Eggshell quality

M14 Making a splash: Environmental enrichment has no effect on brain dopamine and serotonin levels or activity in Pekin ducks

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We seek to enhance our understanding on how different forms of water and non-water based enrichment effect dopamine and serotonin levels in the brain. Brain serotonin and dopamine levels have been associated with affective states for decades, but it is not clear the types of behavioral changes resulting from these changing levels in poultry. Thus, we set out to determine if water-based enrichment can impact the affective state of a duck meat breed, the Pekin, through the lens of brain neurotransmitter levels and activity. Our study investigated the link between aggressive 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. 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 by ANOVA 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 states

M15 Orange Corn-based diet improves total antioxidant capacity in layer pullets compared to traditional yellow corn diets Serena Wesley^{*1}, Tyler Lawson², Evan Rocheford², Torbert Rocheford³, Sara Cloft¹, Darrin Karcher¹, Gregory S. Fraley¹ ¹*Purdue University, Animal Science, West Lafayette, Indiana, United States;* ²*Nutr maize, West Lafayette, Indiana, United States;* ³*Purdue University, Agronomy, West Lafayette, Indiana, United States*

Orange Corn (OC) used in our project, developed by Dr. Torbert Rocheford, contains significantly higher carotenoid levels compared to Yellow Corn (YC). Carotenoids have multiple biological functions. Carotenoids are known to increase antioxidant capacity in vertebrates, although the exact mechanism by which this occurs is not clear. We hypothesized that an OC diet will improve laying hen health and performance due to increased antioxidant capacity. For the initial study stages 1400 Bovan Brown chicks across 2 rooms, evenly split into 2 groups were put on diets that were formulated to be equicaloric, varying only in corn source. At 7 weeks of age, each room was split into a total of 4 rooms (350 chicks each) retaining their respective dietary treatments. At 20 weeks of age, 10 birds per group were euthanized by cervical dislocation, and tissues were collected and immediately frozen on dry ice. Supernatant from homogenized spleen, liver, duodenum, jejunum, ileum, brain, heart, uropygial gland, ovarian follicles, or bursa of Fabricius, or diluted blood serum samples were assayed for total antioxidant capacity (TAC) using a commercially available kit and TAC was determined calorimetrically using a spectrometer at 405nm. For tissue samples, a 150-mg sample was homogenized in 1.0ml buffer solution (5 mM K₃PO₄, pH 7.4, 0.9% NaCl, 0.1% glucose). Data were analyzed by Student's t-Test and a $p < 0.05$ was considered significant. Our results showed that OC-fed birds had significantly higher TAC in spleen tissue ($p = 0.0281$), jejunum ($p = 0.001$), brain ($p = 0.0438$), bursa of Fabricius ($p = 0.04$), ovarian follicles ($p = 0.0014$), uropygial gland ($p = 0.003$), and blood serum samples ($p = 0.0017$) when compared to YC-fed birds. No differences in TAC were observed in liver, duodenum, ileum, or heart samples between the YC and OC-fed groups. Our data suggest an OC diet could effectively increase antioxidant capacity within birds, and therefore could improve laying hen performance and welfare.

Keywords: zeaxanthin, lutein, glutathione, peroxidase, superoxide dismutase

M16 The effect of Eurycoma longifolia (Longjack) on semen characteristics on aging broiler breeder roosters Jacob C. Wesson^{*1}, Emmillie Boot¹, Beverly McLendon², Skye Freeland², Tatyany S. Mani², Rebecca Wysocky², Ramon Malheiros² ¹*North Carolina State University, Poultry Science, Raleigh, North Carolina, United States;* ²*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Broiler Breeder reproduction has deteriorated in the last 15 years. With the reduction of the number of chicks from each hen, it is estimated that the poultry industry is losing millions of dollars each year. As the global population increases, the demand to produce meat has also increased resulting in investigations of the effectiveness of broiler breeder roosters. To ensure that every egg laid has the highest chance to be a fertilized one we have to

maintain semen quality to the best of our ability. The objective of this study was to determine if the inclusion of Eurycoma longifolia (Longjack) at two different amounts in the diets of broiler breeder roosters would improve sperm quality parameters. To evaluate semen quality, 16 broiler breeder roosters were randomly assigned to the three diet treatments and placed into individual pens. The first treatment diet had 0 mg/kg of Longjack, the second had 75 mg/kg, and the third had 150 mg/kg added to the same base diet. Semen was collected and analyzed 8 times from each rooster between 38 and 47 weeks of age for a total of 128 samples. Immediately after collection, 100 microliters of each semen sample were diluted into 2mL of semen extender and kept at 40C until testing was completed with the SQA-Vt™ Automated Sperm Quality Analyzer with semen volume, sperm concentration, total sperm count, and sperm motility being measured. Data was analyzed via one-way ANOVA in JMP Pro 17, with a significance level of $P \leq 0.05$. At the end of the study, body and testis weights were recorded for each rooster. Total sperm count was the highest in the 150 mg/kg supplementation treatment (T3) ($P = 0.0153$). This suggests that higher amounts of Longjack supplementation can improve semen concentration. Testis to body weight ratio was not significant among all three treatments. However semen motility increased with age for all treatments, peaking at 41 weeks at 78% ($P < 0.0001$). This data provides evidence that Longjack can improve the efficiency of the testis function and the rooster aging which could have the potential to cause benefits to broiler breeder reproduction.

Keywords: Semen, Fertility, Rooster, Broiler Breeder, Quality

M17 Impact of feeding regimens during rearing on sperm quality and fertilization capacity of broiler breeder hens and roosters through sexual maturity Madison Berger^{*1}, Kristin Diehl², Brenda Flack³, Patricia Quino¹, Olivia McGuire¹, Kristian Almendares¹, Charlene Hanlon¹ ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States;* ²*USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Maryland, United States;* ³*Cobb-Vantress, Siloam Springs, Arkansas, United States*

Chick shortages in the broiler industry necessitate improvements in reproductive performance of broiler breeder flocks. Feed restriction strategies are implemented to improve body weight and flock uniformity leading up to sexual maturation. While these regimens have previously been assessed regarding their impact on fertility and hatchability rates, this study aims to evaluate sperm quality and fertilization capacity under various feeding regimens. At 3 weeks of age (woa), 90 Cobb 500 FF pullets and 9 Cobb cockerels were randomly placed into individual cages pre-assigned to one of three treatments: (1) daily control diet (CON), (2) skip-a-day control diet (SKIP) or (3) daily high fiber diet (ALT). Treatments were initiated at 4 woa, and birds received individual allocations corrected for caloric intake. SKIP birds transitioned to a daily allocation at 19 woa. Rearing photoperiod was maintained at 8L:16D. Birds were photostimulated at 21 woa with 12L:12D, increasing by 1 h/week to 23 woa (14L:10D). Eggs were collected daily, and breakouts were performed weekly. Artificial insemination (AI) and semen analysis were conducted weekly, allowing each hen to receive 1×10^9 sperm/dose. Sperm penetration (SP) assays were completed at 40, 47, 50, and 55 woa, with sperm viability assessed at 40, 45, 50, and 55 woa. A two-way ANOVA was conducted using the MIXED procedure SAS v9.4, with age and treatment as the fixed effects. There was an effect of age on SP ($P < 0.01$), with the number of holes declining between 40 and 50 woa ($P < 0.01$). There was also an effect of treatment on SP ($P < 0.05$), as ALT and SKIP eggs had the largest number of holes, while CON had the least. An interaction was also

observed ($P < 0.01$). ALT eggs had a greater number of holes at 40 woa than any other age in this treatment, yet CON and SKIP showed no difference across ages. This was consistent with egg breakouts, which demonstrated an effect of treatment on fertility ($P < 0.01$), as ALT demonstrated higher fertility rates than CON, while SKIP rates were intermediate. Interestingly, no effects of treatment on sperm viability were found. In conclusion, this study has demonstrated that feeding regimens can influence fertility rate, potentially by altering the success of sperm storage and penetration in the hen.

Keywords: Sperm Penetration, Sperm Viability, Feeding Regimens, Fertility, Artificial Insemination

M18 Reference point methodology with chicken tibiae Dylan W. Seets^{*1}, Sara Cloft¹, Brittney Emmert¹, Darrin Karcher¹ ¹*Purdue University, Department of Animal Sciences, West Lafayette, Indiana, United States*

Reference Point Indentation (RPI) is a minimally invasive technique of assessing bone properties and has been established for mammalian use. The recommended settings, 10 N, previously have broken some chicken bones; thus, we need to determine the best settings for replicable data with poultry. We tested 8 indentation forces of 10 to 4, and 2 N each test used 10 loading cycles and 2 Hz frequency. Right and Left tibiae from 50 45-week-old laying hens were cleaned and marked at 25, 50, and 75% length, denoting proximal (P), middle (M), and distal (D) locations. Each bone was then inserted into an anti-twist apparatus and indented at each mark and 2mm on either side of the mark. We focused on 3 outputs that translate to bone biomedical properties: 1st -cycle Indentation Distance (FID), the depth of the 1st loading cycle; Total Indentation Distance (TID), the total depth after last cycle; Indentation Distance Increase (IDI), the difference between 1st and last cycle depth. A repeated measures ANOVA reported FID and TID values increased in a stair-like fashion with increasing indentation force ($P < 0.0001$). At all forces, except 8 N, there were no differences in FID or TID between locations ($P > 0.05$). At 8 N, the P location had higher FID and TID values than M and D ($P < 0.001$). IDI continually increased with increasing indent force until 7 N ($P < 0.0001$), when IDI plateaued and was similar through 10 N. The M location had the lowest IDI values compared to the P and D locations, regardless of indent force ($P < 0.01$). All forces seemed to have similar coefficient of variabilities (CV) within each parameter, but IDI had the largest variability overall with a CV range of 0-50%, while FID and TID ranged from 0-20%. Taken together, an indentation force of 6 N can be used for weaker bones as FID, TID, and IDI were similar to higher forces, but 9 N appears to be ideal as this force resulted in the most

consistent and replicable data through all parameters at the highest force possible between all locations.

Keywords: Reference Point Indentation, Tibiae, Bone Quality, Methodology, Laying hens

M19 Comparative analysis of body composition and bone microarchitecture in wild-type and restricted ovulator hens Deependra Paneru^{*1}, Fatemeh S. Mahdavi¹, Venkata Prathap Reddy Keshavareddy¹, Sai Kumar Reddy Ragyari¹, Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Restricted ovulator (RO) hens lack the ability to lay eggs due to a point mutation in oocyte very-low-density lipoprotein (VLDL) receptor gene, which inhibits the uptake of yolk precursors and serve as a valuable model animal for studying avian bone metabolism in relation to metabolic demands of egg production. In contrast, their wild-type (WT) siblings, which possess a functional VLDL receptor gene, undergo regular ovulation, and represent typical production hens. A study was conducted to investigate how ovulation in laying hens affects the body composition and bone turnover rate comparing RO and WT hens. A total of 20 (8 WT and 12 RO), 42-week-old hens were analyzed for body composition and bone microarchitecture, with genotype confirmation via PCR targeting the VLDL receptor gene. Dual-energy X-ray absorptiometry (DEXA) and micro-computed tomography (microCT) were employed to measure body composition, bone mineral density (BMD), and microstructural properties across the total body, femur, keel, and humerus bones. Statistical analyses were performed using Welch's t-test to assess group differences, with significance set at $P < 0.05$, and trends were considered at $0.10 < P < 0.05$. RO hens showed significantly higher total body BMD ($P = 0.0067$), femur BMD ($P = 0.0015$), and keel BMD ($P = 0.0348$), and tendency for higher humerus BMD ($P = 0.0603$) compared to WT hens, with increased bone volume-to-tissue volume ratios (BV/TV) in the femur ($P = 0.0068$). RO hens also had lower porosity and more compact trabecular architecture in these regions, indicating denser bone structure. Additionally, RO hens had significantly higher body fat percentage ($P = 0.0232$) and body fat mass ($P = 0.0351$), with no significant differences in lean mass between the groups. In conclusion, our results suggest that metabolic demand of egg production in laying hens is associated with decreased body fat and loss of bone microarchitecture, particularly affecting the femur, followed by the keel bone, and least in the humerus bone.

Keywords: restricted ovulator hen, wild-type hen, bone metabolism, body composition, bone microarchitecture

Environment & Management I

M20 Effects of synchronizing body weight and photostimulation on cage-free pullets to maximize reproductive capacity Olivia McGuire^{*1}, Regina Halm², Ana Rentsch^{2,3}, Sam Maasant², Grégoy Bédécarrats², Bethany Baker-Cook¹, Charlene Hanlon¹ ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States;* ²*University Of Guelph, Department of Animal Biosciences, Guelph, Ontario, Canada;* ³*University of Guelph, Campbell Centre for the Study of Animal Welfare, Guelph, Ontario, Canada*

As the laying industry transitions to cage-free rearing, body composition of pullets in these environments has changed, altering their entry into lay. While photostimulation (PS) has been the primary tool to stimulate reproduction, this study aims to evaluate synchronization of body weight (BW) and PS to enhance sexual maturation. At 12 weeks of age (woa), 600 Lohmann LSL-Lite

pullets were randomly assigned to 4 rooms, each with 6 aviary-style pens ($n=25/\text{pen}$) reared under 9L:15D. At 13 woa, pens were assigned to 2 feeding regimens ($n=3$ pens/room): *ad libitum* (AL) and 10% restriction from guidelines (R). PS (14L:10D; $n=2$ rooms/PS) was based on average BW of the treatments reaching a pre-established threshold (1.45-kg; AL at 18 woa or R at 20 woa). This resulted in 4 groups ($n=6$ pens each; 150 hens): (1) AL+synchronized PS (AL-S), (2) AL+late PS (AL-L), (3) R+synchronized PS (R-S) or (4) R+early PS (R-E). Feed restriction was lifted at 21 woa. BW was recorded weekly from 12 woa and egg production daily. Egg analysis was conducted at 21, 23, and 25 woa. One bird/pen was euthanized ($n=6/\text{trt}/\text{timepoint}$) *via* cervical dislocation at 14, 16, 18, 19, 20, 21, 22, and 26 woa to gain abdominal fat pad (AFP) and ovary weight. Linear mixed effect models were fitted using the MIXED

procedure SAS v9.4. As expected, R birds were lighter in BW from 15 woa on ($P<0.01$). Feeding and PS affected entry into lay ($P<0.001$) as AL-S reached 50% production first and R-S last. Age, feeding, and PS affected egg production ($P<0.001$), as AL-S had higher egg production than R-E at 19 woa. At 20 and 21 woa, AL-S had the highest egg production and R-S the lowest, and R-S remained lower until 23 woa. Age and feeding affected ovary weight ($P<0.001$), as AL ovaries were heavier than R at 20 and 21 woa. PS also contributed ($P<0.05$) as AL-S ovaries were heavier than R-S at 20 woa. At 21 woa, R-S maintained the smallest ovary ($P<0.05$), but these differences dissipated. Relative AFP was larger in AL birds from 16 to 20 woa ($P<0.05$) until feed restriction was lifted. Egg weight was not affected by PS, but AL birds had heavier eggs than R at 21 and 23 woa ($P<0.001$). This study has demonstrated that synchronizing PS and BW can significantly improve sexual maturation and cumulative eggs in cage-free hens.

Keywords: Laying Hens, Photostimulation, Body Weight, Reproduction, Sexual Maturation

M21 Effects of the shift from caged to aviary housing systems on commercial laying hen egg grade and quality Taylor O'Lear Reid^{*1}, Rebecca Wysocky¹, Ishab Poudel¹, Kenneth Anderson¹ ¹*North Carolina State University, Raleigh, North Carolina, United States*

The purpose of the field trial was to determine the effect of housing type on egg grade and quality. Approximately 70,000 Hy-Line W-36 hens were studied in both aviary and cage systems. Eggs were sampled at 22, 32, 52, and 82 weeks in a completely randomized design, graded according to current USDA standards, with grade% calculated by flat. Physical egg quality was conducted on AA and A-grade eggs, 150 eggs/period. Quality parameters included egg weight (EW), shell strength (SS) and elasticity, vitelline membrane strength (VMS) and elasticity (VME), shell color, albumen height (AH), Haugh unit (HU) and yolk color score (YCS). Statistical analysis was conducted via JMP Pro17. Two-factor fixed ANOVA tests were conducted to determine interaction with age. Contrasts were utilized to evaluate within period. One-way ANOVA was used to compare housing. Significance was set at $P\leq 0.05$. There was an increase ($P<0.01$) in AA% in eggs from caged birds compared to aviary. Grade A% saw a significant difference based on period ($P=0.036$). Grades B, check, and toe holes showed a significant interaction effect between age and housing ($P<0.01$, $P\leq 0.01$, and $P\leq 0.01$). Younger aviary hens had significantly lower B grades compared to caged. Checks and toe picks were significantly lower in caged birds. Meat spot% noted a significant increase based on interaction, with aviary at 82 weeks being highest ($P=0.027$). Total EW significantly increased due to the interaction ($P<0.010$), with caged at 82-week weights being highest ($P<0.01$). SS had a significant decrease due to interaction effect (P), with elasticity decreasing based on age ($P=0.048$). VMS ($P<0.01$) and VME ($P<0.01$) decreased with age. Shell color, for the aviary at 52 weeks was significantly different from caged at 32 and 82 weeks ($P<0.01$). AH, HU, and YCS noted a significant difference via interaction ($P<0.01$). Caged at 22 weeks had a higher AH and HU compared to all other groups. These findings suggest that with the changing of the housing types, producers need to be aware of the increase in unsellable eggs due to damage from the hens within the aviary system, but that egg quality parameters may not differ significantly. However, as this study implemented one commercial farm, additional studies need to occur with other farms.

Keywords: Housing, Laying hen, Egg quality, Aviary, Caged

M22 Enhancing Mortality Detection in Broiler Production: Evaluating a Rail-Mounted Robotic System's Accuracy and

Limitations Tanner Thornton^{*1}, Shawn Hawkins², Yang Zhao¹, Tom Tabler¹, Robert Burns² ¹*University of Tennessee, Animal Science, Knoxville, Tennessee, United States;* ²*University of Tennessee, Biosystems Engineering and Soil Sciences, Knoxville, Tennessee, United States*

The aim of this study was to evaluate the performance of a rail-mounted robotic mortality identification system in a commercial broiler production house and to assess the challenges of implementing such systems in real-world environments. This research is relevant as it addresses the labor-intensive process of manually identifying mortalities in broiler houses, which the robotic system aims to streamline through computer vision. The experiment followed a randomized complete block (RCB) design, with enclosures strategically placed under the rail/robot, between feed and water lines, and near sidewalls as treatment blocks. Mortality detection was evaluated both within these enclosures and in open areas outside them. Data collection occurred across two production flocks, with robotic mortality detection rates compared against manual counts. Statistical analysis included ANOVA to determine the effect of enclosure placement and camera positioning on detection rates, as well as chi-square tests to compare detection frequencies between enclosure and non-enclosure groups. T-tests were applied to assess differences in performance between the bottom and side cameras. Results indicated that the robot achieved a significantly higher mortality detection rate within enclosures under the rail (57%) compared to unenclosed mortalities in the same areas (19%; $p < 0.001$). The bottom camera demonstrated superior detection accuracy (42.3%) compared to side cameras (20.0%), with statistical significance ($p < 0.001$). Challenges such as occlusion by live birds, house infrastructure (feeder and drinker lines), and lighting variability limited system performance. ANOVA confirmed that both mortality location and enclosure use significantly influenced detection efficacy. These findings highlight critical barriers to the commercial viability of automated mortality identification systems. While the robotic system shows potential, adjustments to camera positioning, lighting, and software to mitigate occlusion are necessary to enhance performance under commercial conditions.

Keywords: Mortality detection, Rail-mounted robot, Broiler production, automated monitoring, Precision Livestock Farming (PLF)

M23 The effect of silvopasture environment and genetic strain on broiler performance Athena D. He-DeMontaron^{*1}, Jesse L. Grimes¹, Yan L. Campbell¹, Lin L. Walker¹, Allison N. Pullin¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Slower growth rates and/or alternative housing systems are management strategies presumed to improve broiler welfare. However, their effect on broiler productivity is understudied, particularly in the US. We aimed to evaluate the effect of housing environment and genetic strain on the growth performance, feed efficiency, and mortality of broilers. A total of 500 Ross 708 (fast-growing) and 500 SASSO (slow-growing) male chicks were randomly placed in either indoor conventional (I) or outdoor silvopasture housing (O; $n=5$ pens/strain/system, 50 chicks/pen). To achieve a similar target weight, Ross 708 were reared to 6 wks and SASSO to 8 wks. Body weights (BW) were measured at 1, 14, 28, 42, and 56 d. Average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were calculated on a per-pen basis. Mortality (including culls) was recorded as it occurred. Data were analyzed with linear and generalized linear mixed effects models in R software. There were no interactions between genetic strain and housing ($p>0.05$). The Ross 708 birds were 0.87 kg heavier

than the SASSO birds on average ($p < 0.0001$; $R: 2.13 \pm 0.06$, $S: 1.26 \pm 0.05$ kg, mean \pm SE). Ross 708 birds also had nearly double the ADG ($p < 0.0001$; $R: 81.51 \pm 2.47$, $S: 42.28 \pm 1.92$ g) and greater FI than SASSO ($p < 0.0001$; $R: 72.09 \pm 2.65$, $S: 45.54 \pm 2.05$ kg) As a result, Ross 708 birds showed a lower FCR than SASSO ($p = 0.0035$; $R: 1.28 \pm 0.08$, $S: 1.57 \pm 0.06$). Mortality was not affected by strain ($p > 0.05$). There were no differences for BW or ADG between housing types ($p > 0.05$). Birds reared in I had a greater FI than the birds reared in O ($p = 0.0345$; $I: 62.00 \pm 2.28$, $O: 55.63 \pm 2.28$ kg), but I maintained a lower FCR than O birds ($p = 0.0035$; $I: 1.31 \pm 0.07$, $O: 1.53 \pm 0.07$ kg). Mortality was greater for O than I ($p = 0.036$; $I: 1.29 \pm 0.43$, $O: 3.20 \pm 0.86$ birds/pen). As predicted, the Ross 708 birds had better production performance than SASSO in both housing environments. Birds in O may have supplemented FI with foraging on the range, but the energy expended with behavioral activity likely increased FCR. Weather events were the primary driver of higher mortality in O. Broiler production is optimized with indoor housing and a fast-growing strain, but mitigating variability in outdoor environments (e.g., weather, terrain) warrants further investigation.

Keywords: performance, broiler, housing, feed conversion ratio, silvopasture

M24 Effect of litter condition on the duodenal proteome of young broiler chickens Gerardo A. Abascal-Ponciano^{*1}, Melissa D. Boersma², A. Jake Keel¹, Charles W. Starkey³, Jessica D. Starkey¹ ¹*Auburn University, Poultry Science Department, Auburn, Alabama, United States;* ²*Auburn University, Department of Chemistry and Biochemistry, Auburn, Alabama, United States;* ³*North American Renderers Association, Scientific and Regulatory Affairs, Alexandria, Virginia, United States*

The term “new house syndrome” refers to the commonly observed decrease in performance of broiler flocks raised on completely new litter (NL) compared with that of subsequent flocks raised on reused litter (UL). Limited research has shown that broilers reared on UL can exhibit improved performance by harvest age vs. NL, though the underlying causes remain largely unexplored. Changes in the ileal and cecal microbiota in broilers raised on UL vs. NL have been reported, and in our previous work more mitotically active cells were observed in the small intestine of 3, 15, and 21-d-old broilers raised on UL vs. NL. The objective of this experiment was to explore the duodenal proteomic profiles in broilers raised on NL vs. UL. A randomized complete block design experiment was conducted with broilers reared on either NL or UL for 15 d. On d 15, 6 birds per treatment from 6 different blocks (total $n = 12$) were randomly selected for sampling. Duodenal tissue samples were collected 1.5 cm distal to the duodenal loop and snap-frozen in liquid nitrogen until processing. Duodenal samples were homogenized using a total protein extraction reagent and analyzed through liquid chromatography-tandem mass spectrometry. Spectral data were processed using Proteome Discoverer software (ver. 2.5.0.400) and searched with Sequest HT and Mascot against chicken databases. Data was further processed using the Bioconductor pipeline in R (ver. 4.3.1). Proteins were considered differentially expressed (DE) with a significance threshold of ≤ 0.05 and a \log_2 fold change ≥ 2.0 . Nine proteins met the DE criteria, all of which were upregulated in the NL treatment: AT-III, FGA, FGB (coagulation), KHK and GNPDA1 (sugar metabolism), RAB5B (endocytosis), PAFAH2 (inflammation), EPHX1 (detoxification), and AGMAT (amino acid and urea metabolism). Although immune related proteins were DE expressed, major immune pathways such as STAT3 and MHC-I were not DE. Rearing broilers on NL vs. UL appears to upregulate proteins associated with intestinal immune modulation and other metabolic functions providing some insight into the

intestinal physiological mechanisms impacted when broiler flocks experience “new house syndrome” growth performance issues.

Keywords: new house syndrome, poultry litter, proteomics, duodenum, broiler chicken

M25 Ambient and conditioning temperature interactions alter steam dynamics during the pelleting process Alexis Renner^{*1}, Emily B. Estanich¹, Elizabeth A. Lynch¹, Kristina M. Bowen¹, Lucas E. Knarr², Staci Cantley³, Joseph S. Moritz¹ ¹*West Virginia University, School of Agriculture of Food Systems, Morgantown, West Virginia, United States;* ²*West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States;* ³*Huvepharma, Nutritional Product Information, Peachtree City, Georgia, United States*

Pelleting involves steam conditioning mash, extruding the conditioned feed through a pellet die, and cooling. Steam conditioning applies heat and moisture which aids in pellet formation and production rate. This study aimed to determine how ambient temperature interacts with varying steam conditioning temperatures to influence moisture addition throughout pelleting, and the pelleting process. A split plot design was implemented using two whole plot units (Ambient temperature: -1 and 16°C) and three subplot units (Conditioning temperature: 66 , 74 , and 82°C), with three replications. Main effects and interactions were determined using two-way ANOVA, and means were separated using Fisher’s LSD Test ($\alpha = 0.05$). Increased conditioning temperature increased conditioned mash and hot pellet moisture ($P = 0.0002$ and 0.0013 respectively), regardless of ambient temperature. Pellet production rate was affected by an interaction with ambient and conditioning temperatures ($P = 0.0357$). Production rate did not change across conditioning temperature at -1°C ambient temperature. However, production rate increased 4% from 74°C to 82°C at 16°C ambient temperature. Pellet mill motor load decreased with increasing conditioning temperature ($P = 0.0002$) and numerically decreased with 16°C ambient temperature ($P = 0.0647$). Moisture determined on pellets cooled for 12 minutes demonstrated an interaction between ambient temperature and conditioning temperature ($P = 0.0476$). Pellet moisture did not change at 16°C ambient temperature across conditioning temperature but increased incrementally with increased conditioning temperature at -1°C ambient temperature ($P < 0.05$). The 16°C ambient conditions produced steam that improved production rate at 82°C and contained less moisture after cooling, likely due to more water being utilized for lubrication at the pellet die. It is possible that the thermodynamic steam traps in the pilot feed mill opened more frequently at -1°C ambient temperatures thus, creating a dryer steam. Conditioned mash moisture was not observed to increase at 16°C ambient temperatures, likely due to a greater potential for steam flash off during measurement. These data suggest ambient temperatures may alter steam dynamics that ultimately influence the pelleting process.

Keywords: Feed Manufacture, Steam, Pellet Production, Steam Trap

M26 Antimicrobial resistance in the different stages of commercial poultry production environment Pankaj P. Gaonkar^{*1}, Alinne Santana-Pereira¹, Alyssa Lambert¹, Reed Golden¹, Courtney Higgins¹, Yagya Adhikari², Matthew Bailey², Kenneth Macklin³, Laura Huber¹ ¹*College of Veterinary Medicine, Auburn University, Pathobiology, Auburn, Alabama, United States;* ²*Auburn University, Poultry Science, Auburn, Alabama, United States;* ³*Mississippi State University, Poultry Science, Starkville, Mississippi, United States*

Antimicrobial resistance (AMR) is a threat to the poultry industry, resulting in economic losses. AMR transmission can occur between poultry, humans, and the environment, yet environmental AMR remains understudied. Our study aimed to determine AMR in the environment in different stages of commercial poultry farms. We included commercial poultry farms (n=29) in different stages of production practicing restricted antimicrobial use. Litter samples from inside and soil samples from outside the poultry house were collected. Carcass rinses were collected from the processing plant at post-pick and post-chill stages. The frequency of 3 mobile genetic elements (MGEs) and 14 antimicrobial resistance genes (ARGs) was assessed using qPCR. Shotgun metagenomics was performed on litter and soil to examine microbiome and resistome compositions. AMR to majority of antimicrobial classes was found in litter samples, and it was higher in broiler compared to breeder and pullet farms. AMR was lower in soil compared to litter and there was no difference among farm types. MGEs were most frequently found in litter and were consistent across the farm types. In the processing plant, AMR was comparable between post-pick and post-chill stages. Distinct microbial and resistome composition was observed between litter and soil (PERMANOVA=0.001). Pullet and breeder had similar profiles (PERMANOVA > 0.05), while broiler had distinct microbial (PERMANOVA < 0.002) and resistome (PERMANOVA < 0.01) compositions compared with other farm-types. Litter microbiome and resistome shifted along the production chain, with increased frequency of *Staphylococcus* and bacitracin resistance in broiler farms. Two broiler farms had soil composition similar to litter, indicating possible AMR contamination between inside and outside poultry houses. Restricted AMU alone does not prevent AMR persistence in poultry production environments. Historic AMU may contribute to ARGs persistence. Cross-contamination between litter and soil poses a risk of AMR dissemination. The presence of ARGs in carcasses in the post-chill stage indicates a potential public health risk. Understanding AMR spread in the environment is essential to maintain poultry, human, and environmental health.

Keywords: Antimicrobial resistance, Resistome, Antimicrobial use, Commercial poultry production

M27 Transportation elicits a decrease in serotonergic activity, but not dopaminergic activity, in 5-day-old grow-out Pekin ducklings Erica R. Roge^{*1}, Isabella von Seggern², Jenna M. Schober³, Gregory S. Fraley⁴ ¹Purdue University, Lafayette, Indiana, United States; ²Purdue University, Animal Sciences, Columbia City, Indiana, United States; ³Purdue University, Animal Science, Lafayette, Indiana, United States; ⁴Purdue University, West Lafayette, Indiana, United States

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 (D). We used mass spectrometry to evaluate serotonin and 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 (p = 0.0030) and the RM (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: Affected state, stress response, brain chemistry, welfare

M28 Play that funky music Pekin duck: How auditory enrichment affects Pekin duck body condition, production, and fear response Jenna M. Schober^{*1}, Jordan Curry¹, Grace Ayres¹, Gabriella Chambers¹, Brynn Peterson¹, Emma Stuart¹, Gregory S. Fraley¹ ¹Purdue University, Animal Science, Lafayette, Indiana, United States

Few studies have looked at the effects of auditory enrichment on the health, production, and welfare of poultry species, and even fewer have looked at Pekin ducks specifically. We utilized 400 grow-out Pekin ducks obtained on day-of-hatch from a commercial hatchery and evenly and randomly placed into 4 pens in 3 rooms at Purdue University. Each room was assigned an audio treatment, Mozart music (MOZ) or pond sounds, (POND) and a control (no audio; CON). Ducks were housed to closely approximate industry standards for density, 30 ducks/pen. The POND and MOZ audio started on day 7 and was played starting at 0300h (lights on) until 2100h (lights off) one hour on, one hour off, in a cyclic manner and ranged from 65-75dB. A novel object test (NOT) was conducted on each pen every week. Body condition scores were taken on 10 birds/pen/week (final N = 80 ducks/treatment/week) using a published rubric. Production data were collected weekly. On days 16, 30, and 42, 2 ducks/pen were euthanized using pentobarbital and their spleen, Bursa of Fabricius, liver, uropygial gland and body weights were recorded (final N = 16 ducks/treatment/week). 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. All other 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 ≤ 0.05 considered significant. There were no significant treatment effects for weekly body weights, FCR, or any necropsy measurements. There was a significant DOA x treatment interaction for feather cleanliness, where on day 19 POND ducks were cleaner than MOZ (p=0.0248) and CON (p=0.0248). On day 26, MOZ ducks were cleaner than CON ducks (p=0.0046) and on day 33, CON ducks were cleaner than MOZ ducks (p<0.0001). For the novel object test, there were no significant differences among treatments for approach and fear, but the POND ducks were significantly more passive than the CON ducks (p=0.0068). Our data suggests that auditory enrichment may be a viable alternative compared to no auditory enrichment, as the auditory enrichment ducks were cleaner on days 19 and 26, and POND ducks were more passive overall during NOT.

Keywords: Pekin duck, auditory enrichment, novel object test

M29 Exploring the use of computer vision and machine learning for bodyweight estimation in turkey hens Mireia Molins*¹, Luis Giron¹, John Boney¹, Enrico Casella¹ ¹*Pennsylvania State University, Animal Science, State College, Pennsylvania, United States*

Optimal body growth in turkeys is crucial for achieving the objectives of the poultry industry: minimizing bird losses, maintaining flock uniformity, and increasing meat yield with high feed efficiency. However, monitoring requires frequent body weight measurements, which is costly, labor-intensive, and time-consuming. Computer vision (CV) and artificial intelligence (AI) have emerged as powerful tools for predicting individual bodyweights (BWs) in poultry. However, existing studies relied on experimental setups that did not reflect realistic commercial environments. Thus, in this work, a longitudinal observational study was carried out to evaluate whether CV could be used to estimate bodyweight in turkey hens under realistic commercial settings. Color and depth images of 25 hens housed in a single pen were captured using a depth camera with a top-down view installed on the ceiling. A similar setup enables consistent measurements without obstructions caused by animals walking in front of each other, and is robust against the orientation of the animals with respect to the camera. Animals were followed from age 40d to 106d. Manual measurements of chest width (CW), chest length (CL), back length (BL), and BW were recorded three times a week. A correlation of 0.98, 0.97, 0.94, 0.95 was found between BW and Age, CW, BL, and CL respectively. Since BL has a correlation of 0.97 and 0.95 with CW and CL, we evaluate the performance of an AI-based model for regression called Gradient Boosting Trees (GBT) to predict BW using only Age and BL as input features. Validation of results followed typical AI settings, such as a 10-fold cross validation, using 70% of animals for training, and the remaining 30% of animals for testing. AI metrics for validation were calculated, showing a root mean squared error percentage of 9.47%±1.19%, mean average percentage error of 7.48%±1.09%, and R-squared of 0.96±0.01. Given the encouraging results, future work will explore automated BW estimation using CV. Thanks to the use of depth cameras, we can extract three-dimensional features across the animal back, this providing even more details than BL alone. Preliminary results on the automatic detection of birds from images using CV show a precision of 0.9 and f1-score of 0.84.

Keywords: Computer Vision, Bodyweight estimation, Growth, Machine Learning

M30 Integrating Computer Vision and Data Science in Egg Science towards Advancing Poultry Education and Extension Program Alin Khaliduzzaman*¹ ¹*University of Illinois, Department of Agricultural and Biological Engineering, Urbana, Illinois, United States*

Integration of computer vision (e.g., multispectral, hyperspectral images) and data science (machine learning, deep learning algorithms) into egg and poultry science may open a new avenue for improving precision and efficiency in table and hatching egg industry and others (e.g., healthcare, biomedical engineering). Recent advances in machine learning (ML) algorithms, (PLSR, PLS-DA, LDA, and CNN based deep learning (DL) models such as VGG (Visual Geometry Group), InceptionNet, ResNet (Residual Net) have shown potential for classification, regression and detection of eggs. These DL models, particularly CNN-based methods, and machine learning models such as PLS-DA, have demonstrated high accuracy in fertility and mortality detection at early incubation. Evaluation metrics such as precision, recall, specificity and confusion matrix are commonly used for the performance evaluation of those DL and ML classification models. In ML-based classification models, PLS-DA performed well in fertility and mortality detection of incubated chicken eggs with 100% accuracy at d4 of incubation. CNN-based models showed excellent performance for embryo and fertility detection with the accuracy of 98%. Considering all metrics, InceptionNet performed well compared to other CNN based models in fertility detection. Architecture of ResNet is deeper than VGG. VGG performs well for smaller datasets and simple tasks but computationally expensive and prone to overfitting due to the high parameter count. Deep learning and bio-inspired algorithms (e.g., U-Net, Siamese Network) might precisely be used for smart incubation management in the future, helping maintain high-quality standards and reducing the labor for manual inspection. Thus, this state-of-the-art approach for the egg industry may enable precise control and improved decision-making in incubation and precision hatchery management. With this trend toward automation and data-driven techniques, addressing ML, DL and CV in egg science education and extension programs for graduate students has become essential. Integrating these advanced technologies in poultry curricula will prepare future professionals to harness ML and artificial intelligence (AI) for industry challenges, fostering progress in the egg and poultry sector.

Keywords: Deep learning, image segmentation, egg industry, incubation management, poultry education and extension

Metabolism & Nutrition I: Enzymes

M31 Effects of novel protease from *Bacillus subtilis* K-5 on low protein and low energy guar (*Cyamopsis tetragonoloba*) meal based diets on performance and nutrients digestibility in broiler chickens Aqeel Ahmed Shad*¹, Dr. Tanveer Ahmad¹, Muhammad Farooq Iqbal¹, Dr. Muhammad Javaid Asad^{2,3} ¹*Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, Livestock Production and Management, Rawalpindi, Punjab, Pakistan;* ²*Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, University Institute of Biochemistry and Biotechnology (UIBB), Rawalpindi, Punjab, Pakistan;* ³*Pir Mehr Ali Shah - Arid Agriculture University, Rawalpindi, National Center of Industrial Biotechnology (NCIB), Rawalpindi, Punjab, Pakistan*

The supplemental effects of novel protease produced from *Bacillus subtilis* K-5 and beta-mannanase were evaluated on growth performance, carcass characteristics, nutrients digestibility, blood profile and intestinal morphometry of broilers fed guar meal (*Cyamopsis tetragonoloba*) based diets with reduced crude protein (CP), essential amino acids (EAAs), and

metabolizable energy (ME) contents. A total of 360 one-day old broiler chicks were randomly assigned to 36 experimental units, having nine dietary treatment, each replicated 4 times, with 10 birds per replicate for 35 d. Three basal diets (0GM: without guar meal, 5GM: 5% guar meal and 10GM: with 10% guar meal) were formulated with reduction of 5% CP, 5% EAAs and 80 Kcal/kg ME. These diets were supplemented with novel protease (PROT) and commercial beta-mannanase (Beta-M). At the end of 35 d of experimental period, four birds per experimental unit were randomly selected for blood samples collection and later slaughtered for ileal digesta, intestinal tract and carcass trait sampling. The data on overall performance (day 1 to 35) indicated improved ($P<0.05$) BWG and feed:gain in birds supplemented with PROT (1.41% and 1.67) and Beta-M (2.79% and 1.64) than non-supplemented groups. Improved ($P<0.05$) carcass yield, breast meat yield and thigh meat yield were noted with the supplementation of Beta-M compared to non-supplemented group. However, non-significant ($P>0.05$) effect on carcass traits was noted in broiler fed guar meal based PROT supplemented diets.

Crude protein digestibility, nitrogen retention (*Nret*) and apparent digestibility coefficient for nitrogen (ADCN) were improved ($P<0.05$) only with PROT. The improvement in apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEN) was noted ($P<0.05$) with both supplemented enzymes. However, no effect ($P>0.05$) of enzyme addition was noted on blood parameters. Improved villus height (VH) of duodenum, jejunum and ileum was noted ($P<0.05$) with the addition of both enzymes. The EAAs digestibility was improved ($P<0.05$) only with PROT. In conclusion, beta-mannanase and protease supplementation better improved the overall bird performance in guar meal-based diets with reduced CP and ME than non-supplemented diets.

Keywords: Novel protease, Low protein diets, Low energy diets, Nutrients digestibility, Intestinal morphometry

M32 A high-dose of protease in the starter diet improves growth performance and intestinal morphometrics of broiler chickens challenged with *Escherichia coli* Olufemi O. Babatunde^{*1}, Glenmer Tactacan¹, Ludovic Lahaye¹, Miguel Barrios¹, Derek Detzler¹, Mariana L. de Moraes¹ ¹*Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada*

High doses of protease may have extra-proteinaceous effects beyond protein digestion, including positive effects on intestinal health and structure, which could be beneficial during enteric disease challenges in poultry. This study investigated the effects of high-dose protease supplementation in the starter phase, followed by regular doses in the grower and finisher phases, on growth performance and intestinal morphometrics of broiler chickens challenged with *Escherichia coli* (*E. coli*). A total of 480 one-day-old Cobb 500 chicks were randomly assigned to two treatments for 42 days. The treatments were a control corn-soybean meal-based diet, or the control diet supplemented with protease (Jefo Protease) at 400 g/t (starter phase) and 125 g/t (grower and finisher phase). Each treatment had 12 replicate floor pens with 20 birds per pen. All treatments were challenged by placing day-old birds in pens containing litter sprayed with *E. coli* at 100 mL/pen. Body weight (BW), BW gain, and feed intake were measured to calculate feed conversion ratio (FCR). At day 14, duodenal, jejunal, and ileal tissue samples were collected (1 bird/pen) to measure villus height (VH), crypt depth (CD), and their ratio (VH:CD). Data was analyzed using T-test, with significance set at $P < 0.05$ and trends at $P < 0.1$. The inclusion of the high-dose of protease improved ($P < 0.05$) the final BW of broiler chickens at day 42, with enhanced ($P < 0.05$) overall BW gain and FCR observed at the end of the trial period compared to the control group. There was a tendency ($P < 0.1$) for high-dose of protease to improve the duodenal VH in birds as compared to the control group. Though not significant, birds supplemented with the high-dose of protease had reduced CD by up to 12% and increased VH:CD by up to 21% in the duodenum, jejunum, and ileum when compared to the control birds. These findings suggest that the benefits of protease may go beyond its traditional use of improving protein and amino acid utilization in poultry by actually playing a role in reducing the impact of pathogenic bacteria while supporting intestinal structure and health, ensuring that birds can thrive even under enteric disease conditions.

Keywords: broiler chickens, growth performance, high-dose protease, intestinal health, intestinal structure

M33 Optimal phytase use enables complete removal of inorganic phosphate in young broiler chickens Xavier Rousseau^{*1}, Gilson Gomes¹, Sudipto Haldar², Amrita K. Dhara² ¹*AB Vista, Marlborough, United Kingdom*; ²*Agrivet Research and Advisory, Kolkata, India*

Formulating broiler diets without inorganic phosphates to address environmental and economic pressures has become a standard practice and phytase is one of the primary tool to achieve this objective. This study aimed to evaluate the graded effect of phytase supplementation in phosphate-free diets on broiler performance and bone mineralisation from 6 to 21d. A total of 900 Ross 308 male birds were randomly assigned to 9 treatments with 10 replicate pens each. Birds were fed with a nutrient adequate diet from 0 to 5 days of age which was followed by distribution of the chicks into 5 treatments consisting in gradual levels of available phosphorus (avP) and calcium (Ca) (avP at 4.2,3.5,2.7,2.0 and 1.2 g/kg and Ca at 8.4, 7.6, 6.8, 5.9 and 5.1g/kg, respectively). Four levels of phytase (250,500,1000 and 2000 FTU/kg, Quantum Blue, AB Vista, UK) were added on top of the 1.2g/kg avP diet which contained no inorganic phosphates. One-way ANOVA (JMP Pro 16.2) was used for statistical analysis with means separated by Student's T-Test ($P \leq 0.05$). Polynomial orthogonal contrasts (linear, logarithmic, and quadratic) were assessed and P equivalency of phytase calculated. Average daily gain (ADG), feed intake (ADFI) and mortality corrected feed conversion ratio (mFCR) were reduced with lower avP and Ca levels (logarithmic, $p<0.001$) while phytase supplementation progressively improved ADG, ADFI and mFCR (logarithmic, $p<0.001$). Tibia ash percentage and weight increased with higher avP and Ca (logarithmic, $p<0.001$) and with phytase supplementation (logarithmic, $p<0.001$). Phytase P equivalencies, averaged across the 4 parameters were 1.31, 1.78,2.27 and 2.80 g/kg of avP for 250, 500, 1000 and 2000 FTU/kg, respectively. The highest dose of phytase (2000FTU/kg) achieved similar performance and bone mineralisation than the highest avP and Ca treatment (ADG =65.6 vs 63.2g/d; ADFI = 81.8 vs 82.5g/d; mFCR = 1.26 vs 1.29g/g; Tibia ash % 42.9 vs 45.3%; Tibia ash weight = 1.42 vs 1.36). Results indicate that complete removal of inorganic phosphates from 6 to 21d is feasible with high dose of phytase supplementation without compromising performance or bone mineralisation that potentially translates in feed cost reduction and reduce reliance on inorganic phosphates.

Keywords: inorganic phosphates, phytase

M34 Comparison of two commercial proteases on *in vitro* peptide cleavage profiles and apparent ileal digestibility of amino acids using protein-rich ingredients and a complete diet Abiodun Bello^{*1}, Jacob Lum², M Baxter², Susan Arent³, Karsten M. Kragh³, Kirsty Gibbs¹, Ceinwen Evans¹ ¹*Danisco Animal Nutrition & Health - IFF, Oegstgeest, South Holland, Netherlands*; ²*Ozark Avian Research, Gravette, Arkansas, United States*; ³*Danisco Animal Nutrition & Health, IFF, Brabrand, Denmark*

The efficacy of two commercial serine proteases, a *B. licheniformis*-produced (PRO L) and a *B. subtilis*-produced (PRO S) were evaluated *in vitro* by comparing cleavage profiles of soybean meal (SBM) and canola meal (CM). Also, *in vivo*, apparent ileal digestibility (AID) of AA complete, SBM, and CM diets were assessed. *In vitro*, each ingredient was digested with PRO L or PRO S. The protease-hydrolyzed products were run through mass spectrometry and fragments searched against the ingredient-specific protein database using Proteome Discoverer Software, for protease cleavage profiles, expressed as the relative abundance of a given AA. Cleavage profiles were overall similar for both PRO L and PRO S; however, PRO L showed higher cleavage frequency for SBM Gln, Arg, and Ser; CM Gly, Met vs. PRO S; while PRO S showed higher cleavage frequency for SBM Lys, Thr, Ile, and Val; CM Lys, Leu, Val vs. PRO L. *In vivo*, 384 male Ross 308 broilers (8 birds /cage) were all fed a standard diet from 1 – 17 d followed by one of 6

TiO₂ (0.5%)-supplemented experimental diets (3 diets × 2 Proteases × 8 cage reps) from 18 – 21 d. On d 21, all birds were euthanized and the distal 2/3 ileal digesta collected and freeze dried for AID of each AA. Data was analyzed for protease effect in each diet using the Fit Model function of JMP 16.1. $P < 0.05$ was held statistically significant. With complete diet, PRO S showed higher ($P > 0.05$) AID of Thr (6.13% p), Trp (5.27% p), Ile (5.35% p), Leu (4.71% p), Val (5.6% p), His (4.61% p), and Phe (4.82% p) than PRO L. With SBM diet, PRO S increased ($P > 0.05$) AID for all 18 AAs vs. PRO L, with the lowest and highest increase observed for respectively Met (6.27% p) and Cys (16.92% p). With CM diet, only Trp tended to be higher ($P = 0.056$) by 6.06% p for

PRO S vs. PRO L. Overall results indicate that both PRO L and PRO S exhibit similar cleavage profiles, but subtle differences do exist *in vitro*. These differences may go some way in explaining the differences in AID observed *in vivo*. In complete feed as well as in SBM diets, PRO S consistently exhibited higher AID for most AA relative to PRO L, indicating a higher bio-efficacy for the *B. subtilis*-sourced protease to improve protein digestibility and AA availability for absorption in broilers.

Keywords: amino acid digestibility, *B. subtilis*-produced protease, *B. licheniformis*-produced protease, broilers, cleavage profile

Pathology

M35 The effects of incremental inclusions of a novel blend of yeast cell wall, proteinate, and flavor on growth performance and oocyst count of coccidiosis vaccinated broiler chickens Lauren Nolan², Brett Lumpkins¹, Greg F. Mathis¹, Jose A. Soto^{2*}, Dulmelis Sandu², Roy Brister², Curtis Novak² ¹*Southern Poultry Feed & Research, Inc., Athens, Georgia, United States*; ²*Alltech Inc, Nicholasville, Kentucky, United States*

An experiment was conducted to determine the effects of incremental levels of a novel blend comprised of yeast cell wall, proteinate, and flavor (YPF; Alltech, Nicholasville, KY) on growth performance and fecal oocyst count of coccidiosis vaccinated broiler chickens. A total of 1,750 1-d old male Cobb chicks were used in a 42-d study. Floor pens of 50 chicks, reared on fresh pine shavings, were randomly assigned to 1 of 5 dietary treatments with 7 replications per treatment. Dietary treatments consisted in 5 levels of YPF (0, 0.023, 0.045, 0.091, or 0.182%). The diet was a standard corn soybean meal and fat-based and the feeding program comprised of a starter (d 0-21), grower (d 22-35), and finisher (d 36-42). Feed was offered *ad libitum* in crumble/pelleted form. Pens of chickens were weighed and feed disappearance was measured on d 0, 21, 35, and 42 to calculate ADG, ADFI, and mortality adjusted FCR. Additionally, fresh fecal samples were collected on d 14, d 21, and d 28 to quantify oocyst per gram. Fecal oocyst count included the summation of *E. acervulina*, *E. maxima*, and *E. tenella* species. Data was analyzed using the PROC GLIMMIX procedure in SAS (v 9.4) with pen as the experimental unit and location as a blocking factor. Dietary treatments were the fixed effect and block served as the random effect in the analysis. Preplanned contrasts were used to evaluate linear and quadratic effects of incremental levels of YPF. From d 0 to 42, increasing YPF supplementation improved (quadratic, $P = 0.019$) ADG (2,213, 2,403, 2,422, 2,387, and 2,383 g/bird) and (quadratic, $P = 0.030$) adjusted FCR (1.71, 1.64, 1.65, 1.66, and 1.67), with highest improvement at 0.045 and 0.023% of YPF supplementation, respectively. Moreover, increasing YPF supplementation marginally reduced (quadratic, $P = 0.098$) fecal oocyst count at d 14 (3,541, 1,283, 1,015, 775, and 440 oocyst/g), and (quadratic, $P = 0.048$) d 21 (23,517, 12,606, 6,623, 6,997, and 5,437 oocyst/g), without evidence for differences at d 28 (1,589, 2,039, 546, 1,340, and 622 oocyst/g). Results from this experiment suggest that dietary supplementation of YPF improved ADG and adjusted FCR, and reduced fecal oocyst counts at d 14 and d 21 when supplemented at 0.023 to 0.045% in coccidiosis vaccinated broilers.

Keywords: Eimeria, Broilers, Yeast cell wall, proteinate

M36 Combating Necrotic Enteritis: A Breakthrough with *L. reuteri* Vectored Nanobodies Simmi Manuja^{*1}, Dharanesh Gangaiah¹, Desmond Adeniyi¹, Jacob Shields¹, Ali Camara¹, Amy

McMillan¹, Alecia Godoy¹, Eric Nungester¹, Michael Hicks¹, Emily Helmes¹, Arvind Kumar¹, Nathan Augspurger¹ ¹*BiomEdit, LLC, Fishers, Indiana, United States*

Necrotic enteritis (NE), caused by *Clostridium perfringens*, severely impacts poultry health and industry economics. Our platform technology introduces engineered probiotic *Limosilactobacillus reuteri* to deliver nanobodies that specifically target and neutralize NetB and α toxin, the primary drivers of NE pathology. A vaccine consisting of two *L. reuteri* strains, each engineered to express nanobodies against either NetB or α toxin, was evaluated against a virulent NE challenge in two randomized, partially blinded studies. Broiler chicks were housed in battery cages with 11 birds/cage. Equal number of chicks received either Investigational Veterinary Product (IVP) at a dose of 1.0E+08 CFU or distilled water (challenge control) on the day of hatch via coarse spray, and on day 13 via drinking water. All birds were subjected to NE challenge consisting of a predisposing oral dose of *Eimeria maxima* on day 14 followed by a virulent oral challenge with *C. perfringens* on days 19 and 20. Chickens were observed daily until day 28 and mortality due to NE evaluated. The primary variable of efficacy was mortality due to NE, with statistical analysis focusing on comparing vaccinates to challenge control birds using the Prevented Fraction (PF). Mean and 95% confidence interval (CI) estimates of PF were derived using logistic regression and the Delta Method. In Study 1, the IVP reduced NE mortality from 20.9% to 10.2%, resulting in a PF of 52% (CI > 0 at $P < 0.05$). In Study 2, which experienced a more virulent challenge, the IVP reduced NE mortality from 39.1% to 29.7%, resulting in a PF of 24.5% (CI > 0 at $P = 0.06$). A meta-analysis on mortality data from both studies showed that the IVP effected a PF of 30.6% (CI > 0 at $P < 0.05$). Further efficacy data may be presented based on ongoing studies. These findings validate the use of live *L. reuteri* engineered to deliver preformed nanobodies in the gut of a chicken as an effective technology for dealing with NE, and it opens new possibilities for combating other complex diseases. This dual-purpose solution combines immunoprophylactic value of the nanobodies with the probiotic value of the *L. reuteri* in a single product, representing a paradigm shift in enhancing both animal health and productivity.

Keywords: Necrotic enteritis, *Clostridium perfringens*, Engineered probiotic, Nanobodies, *Limosilactobacillus (Lactobacillus) reuteri*

M37 Leveraging autofluorescence for accurate enumeration of *Eimeria tenella* oocyst viability Philip Yeboah^{*1}, Demilade Ibiwoye², Muhammad Wajeed², Thaciane Amaral², Renata Gomes², Lisa Bielke² ¹*North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United*

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One significant challenge in the development of control methods against *Eimeria* is a lack of *in vitro* viability assays, critical for ensuring consistent vaccine efficacy and experimental challenge performance. Previous studies demonstrated that broilers inoculated with *Eimeria maxima* oocysts exhibiting varying levels of autofluorescence (AF) correlated to infectivity and lesion scores after infection, suggesting AF as an indicator of non-viability. Building on this preliminary data, the present study aimed to validate and extend the methodology to *Eimeria tenella*, establishing an approach to determine oocyst viability using fluorescence microscopy. Two independent experiments were conducted with two treatments: room temperature (RT, control) and accelerated aging at 45°C under anaerobic conditions, each with five replicates. Each test tube contained 1ml of approximately 4.6×10^5 sporulated *E. tenella* oocysts, those under 45 °C, stored in the anaerobic chamber to eliminate favorable storage conditions for eight days. Oocysts were visualized under Revolve inverted fluorescence microscope using FITC and trans filters, with AF and non-fluorescent oocysts enumerated by counting 5 images obtained from each replicate. Excystation of oocysts was completed to confirm non-viability. Data were analyzed using a two-tailed t-test in JMP software (version 17.0). In both experiments, oocysts subjected to 45°C exhibited significantly higher ($p < 0.001$) mean AF percentages (82.70 ± 1.9 and 82.46 ± 1.7 in experiments 1 and 2, respectively), which corresponded to a significant increase ($p < 0.001$) in the count of non-infective oocysts, which includes AF and non-sporulated, (60.55 ± 4.0 and 46.44 ± 2.5) compared to RT controls (6.24 ± 1.4 and 5.68 ± 1.8). Conversely, RT oocysts with low AF percentages (3.57 ± 0.1) exhibited significantly higher ($p < 0.001$) percentage of infective oocysts (42.76 ± 2.0 and 16.24 ± 1.2) than the 45°C group (6.35 ± 1.0 and 3.68 ± 1.7). Excystation confirmed the presence of motile sporozoites in RT samples but not in those under 45°C, validating non-viability in high rate of AF oocysts. These findings confirm that increased AF is strongly associated with non-viability in *Eimeria* oocysts, thus providing a reliable tool for assessing oocyst viability.

Keywords: autofluorescence, *Eimeria tenella*, Viability, oocysts

M38 Effective oral vaccination with *Zea mays*-expressed antigens against *Eimeria maxima* Jeffery Escobar*¹, Richard Sibbel¹, Tracy Raines¹ ¹Mazen Animal Health, Ames, Iowa, United States

Coccidiosis is a highly prevalent and costly disease in poultry that is currently controlled using ionophores, chemicals, or purposeful infestation of sporulated oocysts. This study evaluated the effectiveness of a novel oral vaccine using *Eimeria spp.* antigens expressed in *Zea mays*. Eighty wire floored cages from 8 battery units with feeders and waterers were used; bottom cages were not used. Eight one-d-old Cobb 500 male chicks were randomly allocated to cages. A complete randomized design with battery as block was used. Batteries were placed in a temperature-controlled room fitted with vertical fluorescent lighting. Treatments were placebo (i.e., unvaccinated), and vaccination doses consisting of 6.4, 63.5, 95.3, and 145.4 mg of antigen per bird. Ground corn in the diet was replaced with antigen-containing corn to achieve the different doses in complete feed. Placebo and vaccine diets were offered for two periods of 72 h at cage placement and d 11 of age; the rest of time birds consumed placebo diet. Birds were fed starter (d 1 to 14) and grower (d 14 to 27) diets and had *ad libitum* access to feed and water. Birds were orally challenged with 12,500 oocysts of *Eimeria maxima* on d 21 and 6 d later were euthanized to blindly evaluate lesion scores according to VSM No. 800.123

(CVB, USDA). Lesion score data were analyzed using Fit Model of JMP (version 17.2, Statistical Discovery, LLC) using cage as the experimental unit and block as a random effect. Prevented fractions were calculated in RStudio (2023.12.0+369) using binomial GLM with a logit link according to STATWI0007.01 (CVB, USDA) considering lesion scores 0 and 1 as negative and 2, 3, and 4 as positive for coccidiosis. Average lesion scores in vaccinated birds ranged from 0.51 to 0.78, depending on dose, and were lower ($P < 0.001$) than placebo at 1.69. Prevented fractions for the different vaccine groups ranged from 0.861 to 0.989 with lower limit for 95% confidence interval ranging from 0.537 to 0.923. In conclusion, oral administration of this novel vaccine using corn-expressed antigens was effective at significantly reducing lesion scores and coccidiosis incidence in broiler chickens.

Keywords: Oral vaccine, coccidiosis, lesion score, *Eimeria maxima*, broiler chicken

M39 Designing and testing PCR primers for *Cochlosoma anatis* detection in commercial turkey poults Justin Lowery*¹, Christina S. Sigmon¹, Katherine Rysdon¹, Krista Chang¹, Caiti Heil², Lin L. Walker¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²North Carolina State University, Department of Biological Sciences, Raleigh, North Carolina, United States

Cochlosoma anatis is a flagellated protozoan parasite of young turkeys and waterfowl, causing cochlosomiasis, a disease characterized by increased mortality, enteritis, and non-uniform growth with reduced weight gain. Cochlosomiasis often co-occurs with other enteric pathogens, such as viruses, bacteria, and protozoa, exacerbating disease severity. Symptoms are typically observable 5-11 days post-exposure, delaying producer response. Altogether, limited understanding of *C. anatis* persistence in the environment and delayed recognition hinder proactive disease management. This study aimed to develop PCR primers specific to *C. anatis* DNA. Genomic DNA from infected commercial turkey poult gut mucosa was sequenced using long-read nanopore technology, with contigs filtered to remove host and microbiome DNA. The remaining contigs were screened using NCBI BLAST for sequences with over 60% similarity to *Trichomonadidae*, the family of *C. anatis*. Primers were designed from selected contigs using NCBI Primer-BLAST and tested against *C. anatis* DNA from commercial farms and our cryo-preserved collections. Two primers produced over 99% amplicon alignment to their targets with no cross-reactivity to uninfected poults or other protozoa. Of 47 samples (15 positive and 32 negative), all negative samples returned no PCR amplicons, while 13 out of 15 positive samples (determined by light microscopy) returned PCR amplicons, achieving 87% accuracy with 0% false-positive results. Statistical significance in this study was measured through the E-value provided by NCBI BLAST, indicating the probability of finding an alignment as similar as, or more similar than the tested alignment. In this study, the average E-value was 0.0, indicating a very high probability of the PCR amplicon matching the intended amplicon. This study successfully identified PCR primers with high specificity for *C. anatis* DNA; full assay validation and refinement are in progress. Future research should employ the intended PCR assay to identify potential environmental reservoirs of *C. anatis*. Ultimately, this assay is expected to support *C. anatis* diagnostics and targeted interventions for producers managing cochlosomiasis in their flocks.

Keywords: *Cochlosoma anatis*, Turkeys, PCR, Diagnostics, Intervention

M40 *Castanea sativa* tannin reduces coccidiosis intestinal lesions in broilers Eveline Berwanger¹, Fabrizio Oristanio*²,

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The aim of this study was to evaluate the effect of hidrolisable tannin from *Castanea sativa* (Kingbrown[®]) as a coccidiostat on broiler's intestinal health. The trial was conducted at a experimental facility in the state of São Paulo, Brazil. A total of 192 male Cobb chicks were assigned to four treatments, T1 negative control diet, T2 positive control diet, T3 salinomycin + 3nitro4hydroxyphenylarsonic acid diet, T4 Kingbrown[®] tannin diet. Kingbrown[®] was added at 1kg per ton of feed. At 14 days of age all treatments but the negative control were challenged with *Eimeria maxima* (1ml with 12,000 sporulated oocysts/bird). At 20 days of age jejunum fractions were sampled and scored for lesion from zero to four. Lesion score means were compared by Kruskal-Wallis test and lesion score frequencies were compared by Kolmogorov-Smirnov test. Less than 7% of the birds not challenged with *E. maxima* showed any intestinal lesion, whilst all challenged birds showed intestinal lesions. Birds fed salinomycin + 3nitro4hydroxyphenylarsonic acid diet reduced significantly the lesion score when compared to the positive control group (2.1 to 1.67; *p*-value = 0.0075). Birds fed Kingbrown[®] tannin also reduced significantly the lesion score (2.1 to 1.73; *p*-value = 0.0075) when compared to the positive control group. Both feed additives had the same effect over the lesion score (1.67 to 1.73; *p*-value > 0.05). In conclusion, data from this study indicates that the supplementation of Kingbrown[®] tannin to broilers reduces intestinal lesions caused by *Eimeria maxima*.

Keywords: *Eimeria maxima*, tannin, coccidiostat, intestinal lesions, chicks

M41 Environmental Monitoring of the IBV-G1-23 Variant in Uruguayan Poultry Flocks Using the CAPTUS Air Sampling Tool Martin Masner^{*1}, Luis-Miguel Gomez-Osorio¹, Andres Pio¹, Pia Fernandez¹, Facundo Rocha Calvette¹, Ignacio Gonzalez^{2,3}, Franco Bazzi⁴, William Serna¹, Laura Maccio¹ ¹metaBLX Biotech, Montevideo, Montevideo, Uruguay; ²Avicola El Campo, Tala, Uruguay; ³UDELAR, Veterinary Faculty, Montevideo, Uruguay; ⁴3 Arroyos, Canelones, Uruguay

Monitoring poultry environments for pathogens presents several challenges, including ensuring representative sampling, overcoming limitations of non-quantitative methods, and addressing the emergence of new pathogen variants that impact poultry production. This study investigates the use of CAPTUS, an air sampling device, to detect and quantify the IBV-G1-23 variant in Uruguayan poultry flocks across multiple production facilities. Sampling was conducted weekly or *ad libitum* on breeder and laying hens in countryside Uruguayan areas. Using the CAPTUS air sampler, 30-minute air samples were collected and subsequently analyzed in a molecular biology laboratory. Following nucleic acid extraction, targeted qPCR assays were performed for generic IBV and the specific IBV-G1-23 variant on individual or pooled samples. Additional pathogens, including other IBV variants, Metapneumovirus, Reovirus, Gumboro, Salmonella, and Mycoplasma G/S, were concurrently tested via qPCR on the collected samples. Most were also positive except Metapneumovirus. The IBV-G1-23 variant was detected in flocks with respiratory signs, particularly those lacking serotype-specific vaccination. A marked decrease in egg production (21-34%) was observed over 8-12 weeks during acute infection phases. Environmental detection of the virus persisted for at least ten weeks, with peak viral loads reaching hundreds to thousands of copies per cubic meter. Elevated serological levels for IBV

confirmed field challenges by non-vaccine strains. In conclusion, CAPTUS air sampling provides a reliable method for pathogen surveillance, offering critical insights into environmental infection pressure and pathogen dynamics. The method's adaptability allows for multi-pathogen detection, enabling rapid response strategies against the IBV-G1-23 variant, thus supporting poultry health and productivity.

Keywords: Air Sampling, Bronchitis, IBV, CAPTUS, Environmental Pressure of Infection

M42 Direct RNA sequencing with the Minion platform enables sequencing of nearly full-length viral RNA reads from *Orthoavulavirus javaense* Megan Mears^{*2}, Quentin Read³, Abhijeet A. Bakre¹ ¹USNPRC, Exotic and Emerging Avian Viral Disease Research Unit, Athens, Georgia, United States; ²USNPRC, Exotic and Emerging Avian Viral Disease Research Unit, Athens, Georgia, United States; ³USDA-ARS Southeast Area, USDA, Raleigh, North Carolina, United States

Newcastle disease (ND) is caused by virulent strains of *Orthoavulavirus javaense* (OAVJ). OAVJ can infect many avian species but outbreaks with virulent strains can result in significant economic losses for the poultry industry. Rapid identification of virulent OAVJs in field samples is thus crucial to ND outbreak containment. In this study, we hypothesized that Direct RNA Sequencing using an Oxford Nanopore technologies (ONT) MinION device and custom adapter would enable sequencing of the full-length (~15.2kb) viral RNA genome of OAVJ. RNA was extracted from biological triplicate samples of sucrose purified virions of the LaSota strain of OAVJ, then sequenced using the two different chemistries and associated workflows available from ONT. Statistical differences between the two groups was tested using an unpaired t-test with Welch's correction. The data demonstrate that a custom OAVJ-specific adapter paired with the ONT DRS kits enables capture and sequencing of OAVJ viral RNAs. Further, the new ONT SQK-RNA004 chemistry and flow cells, paired with the associated super accurate base calling workflow improves on read quality and length compared to the previous SQK-RNA002 chemistry. This is the first report of a method to sequence the near full-length viral RNA genome of a member of the *Paramyxoviridae* family. While additional improvements in DRS are needed before widespread adaptation of this method for rapid field sequencing, DRS of OAVJ has the potential to enable further studies into the viral epitranscriptome and its role in infection and pathogenesis.

Keywords: Newcastle Disease virus, Direct RNA sequencing, MinION, viral RNA, Paramyxovirus

M43 Development of an assay to detect cytolyisin and a presumptive *Enterococcus faecalis* pathogenicity island associated with chicken embryo mortality Jessica Foote^{*1}, Enid T. McKinley¹, Claire Podvin¹, Zachary Zawada¹, Jen Key¹, Jodi Delago¹, Alexandra H. Smith¹ ¹Church & Dwight, Arm & Hammer Animal Nutrition, Waukesha, Wisconsin, United States

The United States broiler hatchability rate has been steadily decreasing since 2012. One potential causative agent of this trend is *Enterococcus faecalis*, infection with which has been hypothesized to cause early embryo mortality in chickens. The purpose of this study was to determine the ability of *E. faecalis* to cause chicken embryo mortality, identify genes present in strains that caused significantly increased mortality, and to develop a high-throughput assay to detect these genes in field isolates. To achieve these goals, an embryo lethality assay was performed. Fertilized chicken embryos were injected with different strains of *E. faecalis* that had previously been isolated from poultry

complexes and whose genomes had been sequenced. The mortality rate of each treatment group was recorded daily. After seven days, the cumulative mortality rates of each strain and control group were compared using Fisher's exact test. Each strain had a significantly higher mortality rate than the control group ($p < 0.01$), demonstrating that *E. faecalis* can cause embryo mortality. Strains containing the *Enterococcus* toxin cytolysin had significantly higher mortality than strains without (71% vs 21%, $p < 0.0001$). A 20 kilobase pair pathogenicity island (PAI) containing multiple cell wall, capsule, and O-antigen genes was only found in strains that caused greater than 60% mortality. Strains containing this PAI had significantly higher mortality than strains without (78% vs 31%, $p < 0.0001$). Primers were designed for three genes in the PAI and one gene in the cytolysin operon. These primers and an *E.*

faecalis species-specific primer set were used to develop a multiplex PCR assay. This assay was successfully used to screen *Enterococcus* isolates from poultry complexes experiencing health issues. Cytolysin was detected in 2% of the *E. faecalis* isolates and the PAI was detected in 12%. In conclusion, an mPCR assay was developed to detect genetic markers in *E. faecalis* that are associated with increased chicken embryo mortality. This assay provides a valuable tool to monitor pathogenic *E. faecalis* in hatcheries to determine if highly virulent strains are associated with the current hatchability issues in the field.

Keywords: Enterococcus, hatchability, embryo mortality, pathogen, genetics

SCAD I

M44 The efficacy of inactivated vaccine against H5 clade 2.3.4.4b highly pathogenic influenza virus in turkeys Jiho Lee*¹, Scott Lee¹, Erica Spackman¹, Chang-Won Lee¹ ¹USDA, SERPL, Athens, Georgia, United States

The unprecedented outbreak of clade 2.3.4.4b H5N1 highly pathogenic avian influenza virus (HPAIV) in United States has caused tremendous damage to the poultry industry. Because turkeys have the highest number of affected individual flocks among commercial poultry, evaluating vaccine efficacy and characterizing antibody response of turkeys is crucial. An in-house reverse-genetics generated H5N9 inactivated vaccine (SEP-22-N9) was evaluated for its vaccine efficacy in turkeys against current North American clade 2.3.4.4b H5 isolate, A/turkey/Indiana/22-003707-003/2022 H5N1. Vaccination groups were divided into 3 groups, which were vaccinated at different time points (3, 7, or 9 weeks). All vaccinated groups showed 100% survival rate and significantly reduced viral shedding after the challenge when compared to the sham vaccinated group. The enzyme linked lectin assay-neuraminidase inhibition (ELLA-NI) was applied to assess the capability of detecting birds that have been infected after the vaccination (DIVA-VI). Overall, the SEP-22-N9 vaccine provided fast onset of immunity to protect turkeys from mortality, reduced viral shedding, and was able to apply ELLA-NI assay for DIVA-VI, demonstrating that the vaccine could be used against currently circulating clade 2.3.4.4b H5 HPAIVs.

Keywords: Highly pathogenic avian influenza, Turkey, Inactivated vaccine, Differentiating infected from vaccinated animals (DIVA)

M45 Immunomodulatory effects of essential oils on the in vivo acute inflammatory responses initiated by intradermal injection of *Salmonella* lipopolysaccharide in broiler chickens under heat stress conditions Santiago Uribe-Diaz*¹, Jossie M. Santamaria¹, Chrysta N. Beck¹, Andressa S. Stein¹, Raul Marcon¹, Alvaro Uribe-Serrano², Jaime A. Angel-Isaza², Guillermo Tellez-Isaías¹, Billy Hargis¹, Gisela F. Erf¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ²Promitec Santander SAS, Unidad de Innovación Y Desarrollo Biotecnológico, Bucaramanga, Colombia

Global warming is a growing challenge to the poultry industry as heat stress reduces productivity and weakens immune function. Essential oil (EO) supplementation has shown immunomodulatory activities in chickens. The objective of the study was to determine the effect of dietary supplementation of three EOs on the acute inflammatory responses to intradermal (i.d) injection of lipopolysaccharide (LPS) in chickens under heat stress (HS). One-day-old COBB 500 broiler chicks were randomly assigned to one

of five treatment groups (thermoneutral (TN), HS, HS-EO1, HS-EO2, or HS-EO3) reared in environmental chambers. Cyclic HS was applied from 4d onwards. At 32d of age, 12 growing feathers (GFs) of 7 chickens/group were i.d.-injected with 1µg of LPS. GFs and heparinized blood were collected before (0h) and at 6 and 24h post-pulp injection (p.i.) to determine leukocyte profiles. GF-pulp- and whole blood-cell suspensions were immunofluorescently stained to identify monocytes/macrophages, heterophils, B- and T-cells by flow cytometry. Data were analyzed by two-way repeated measures ANOVA to determine the effects of treatment, time, and their interactions. Tukey's HSD multiple means comparisons were used as needed; significance was set at $P < 0.1$. Heterophil levels in GFs and blood were elevated at 6h p.i., then returned to pre-injection levels at 24h for all treatments. There was no effect of treatment on macrophage, and T- and B-cell levels in LPS-injected GF-pulps. In the blood, few treatment effects were observed post-LPS injection; i.e., the concentrations of leukocytes (WBCs) were lower ($P < 0.1$) in HS compared to TN broilers at 24h and those of heterophils were lower ($P < 0.1$) in HS-EO1 compared to HS-EO2 at 6h. For all treatments, i.d. LPS injection decreased blood concentrations of T- and B-cells at 6h p.i. Interestingly, before LPS injections, the concentration of circulating WBC and T cells was higher in TN than in HS groups, a HS effect partially restored with EOs supplementation. In summary, essential oils supplementation may help mitigate some of the negative effects of heat stress on immune system development and function of chickens, particularly in maintaining white blood cell concentrations and ability to respond to bacterial inflammatory stimuli.

Keywords: LPS, Immune response, Essential oils, Heat stress, Chicken

M46 Protection efficacy of Infectious laryngotracheitis virus (ILTV) DNA plasmid vaccine formulation expressing viral glycoproteins B, D, and I delivered via eye drop Roshan Paudel*¹, Ana C. Zamora¹, April L. Skipper¹, David J. Hurley², Lorraine H. Keller³, Maricarmen García¹ ¹University of Georgia, Department of Population Health, Athens, Georgia, United States; ²University of Georgia, Department of Infectious Diseases, Athens, Georgia, United States; ³MBF Therapeutics Inc., Ambler, Pennsylvania, United States

Novel vaccination strategies to improve protection against ILTV while ensuring safety are being explored. This study aimed to assess the protective efficacy of MBFT-308, a DNA plasmid vaccine expressing ILTV glycoproteins gB, gD, and gI, delivered on polyethyleneimine-derivatized calcium phosphate nanoparticles and administered via eye drop in a prime/boost strategy by itself or to chickens vaccinated in ovo with a recombinant LT vaccine expressing glycoproteins D and I. Briefly,

four of six groups of specific pathogen free (SPF) chickens (n=133) were vaccinated while two remained non-vaccinated (NVx). One group received the Innovax ND-ILT® (Merck Animal Health) at 19 days of embryonation (doe) (HVT-LT), a second group received ILTV plasmids at 3 and 17 days of age (doa) (LT-PL), the third group was vaccinated with the HVT-LT at 19 doe followed by LT-PL via eye drop at 3 and 17 doa (HVT-LT + LT-PL), and the fourth group received the live chicken embryo origin (CEO) LaryngoVac® (Zoetis Animal Health) at 9 doa. At 35 doa, four vaccinated groups and one NVx group were challenged with an ILTV virulent strain. The CEO vaccinated group showed 100% survival after the challenge with significantly lower ($P < 0.05$) clinical signs scores (CSS) and ILTV genome load at 3 and 5 days post-challenge (dpch) than the NVx/Ch group. The LT-PL and HVT-LT+LT-PL vaccinated groups exhibited survival rates of 91% and 78%, respectively, vs. 80% and 65% survivability for HVT-LT and NVx/Ch groups. No significant reduction in CSS and ILTV genome load were observed for LT-PL and HVT-LT+LT-PL groups compared to NVx/Ch group. Although not statistically significant, trends toward reduction in CSS and ILTV genome load for the LT-PL and HVT-LT+LT-PL groups, respectively, were observed. However, compared to the HVT-LT group, the HVT-LT+LT-PL group had numerically higher CSS and ILTV genome load. Taken together, ILTV plasmids formulation administered via eye drop in a prime/boost strategy avoided mortalities, however it did not mitigate the ILTV replication or reduce clinical signs. Additionally, administration of eye drop prime/boost ILTV plasmids to previously HVT-LT chickens did not improve protection against disease.

Keywords: Infectious laryngotracheitis (ILT), Protection, Recombinant vaccine, ILTV plasmids formulation, ILTV glycoproteins (gB, gD, and gI)

M47 Using animal challenge models to elucidate the pathogenicity of novel APEC serogroups Klao Runcharoon*¹, Julia Ines-Lima¹, Catherine M. Logue¹, Yu Yang Tsai¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

Colibacillosis, caused by Avian Pathogenic *Escherichia coli* (APEC), results in high morbidity and mortality in poultry. Currently, 188 O groups are linked with bacterial antigenicity and pathogenicity. Among APEC, serogroups O1, O2, and O78 are often implicated in disease. However, our APEC collection from Georgia colibacillosis cases identified several emerging serogroups. This study evaluated the pathogenicity of 10 APEC strains, representing nine serogroups, using the embryo lethality assay (ELA), chick, and 3-week-old chicken challenge assay. We hypothesized some strains would exhibit higher virulence than others. In the ELA, each group consisted of 10, 12-day-old SPF eggs inoculated with APEC strains (300-500 CFU/0.1 ml) via the allantoic fluid. Negative control (PBS) and positive control (APEC WT O18) were included. Eggs were candled daily, and deaths recorded for 6 days. Results showed all APEC strains were virulent, with highest mortality (100%) for O152 and O145, while lowest mortality (50%) was noted for O88. In chicks challenge, 10 One-day-old chicks per group were inoculated subcutaneously with 100 μ L (10^8 CFU) of APEC strains. Death times and clinical scores were combined for pathogenicity scores (PS). *E. coli* isolates killing >50%, 10%-50%, and 0%-10% of chicks were classified as virulent, moderately virulent, and avirulent. A one-way ANOVA comparing PS among APEC serogroups showed O15, O91, and O88 had significantly lower ($p < 0.05$) PS than the positive control (APEC O18), while O25, O152, O115, and O45 showed numerically higher PS. In older birds, 3-week-old SPF chickens were challenged with 10^8 CFU/ml of bacteria via the

intratracheal route. Mortality and clinical signs were observed for 5 days, and lesions and bacterial counts evaluated. APEC O91 was highly virulent, causing 80% mortality 1 d.p.i., while O115 and O86 were moderately virulent. These novel APEC serogroups exhibited varying pathogenicity and high virulence in embryos, chicks, and chickens. Interestingly, virulence varied across models, suggesting that the route of infection and immune status influence disease development. Moreover, these serogroups could significantly impact poultry health, especially in younger birds, warranting further investigation and new mitigation strategies.

Keywords: Avian pathogenic *Escherichia coli*, poultry, colibacillosis, in vivo, pathogenicity

M48 of dietary high-flavonoid corn (PennHFD1) on performance and pathogen responses in broilers subjected to a necrotic enteritis model Vinicuis Buiatte*^{1,5}, Mahmoud A. Mahmoud¹, Katarzyna Miska², Monika Proszkowiec-Weglarz², Ana Fonseca^{1,5}, Darby R. Boontarue¹, Tyler Lesko³, Bryan Panek³, Surinder Chopra³, Mark Jenkins⁴, Alberto Gino Lorenzoni¹ ¹Pennsylvania State University, Department of Animal Science, University Park, Pennsylvania, United States; ²Beltsville Agricultural Research Center, USDA, Animal Biosciences & Biotechnology Laboratory, Beltsville, Maryland, United States; ³Pennsylvania State University, Department of Plant Science, University Park, Pennsylvania, United States; ⁴Beltsville Agricultural Research Service, USDA, Animal Parasitic Diseases Laboratory, Beltsville, Maryland, United States; ⁵Pennsylvania State University, One Health Microbiome Center, Huck Institutes of the Life Sciences, University Park, Pennsylvania, United States

The poultry industry's shift towards antibiotic-free production has been associated with higher incidences of gastrointestinal diseases such as necrotic enteritis (NE), caused by *Clostridium perfringens* (CP), impacting poultry's gut health and performance, and hindering productivity for farmers worldwide. Alternatives to AGPs are needed to improve poultry health and production. Flavonoids are plant-derived metabolites with demonstrated health-promoting effects. Previous studies from our group showed that feeding a high-flavonoid corn line (PennHFD1, Penn State University) to broilers subjected to a NE model ameliorated disease impacts. The NE model consisted of co-infection of *Eimeria maxima* (EM) and CP, and a pro-inflammatory diet containing wheat, fishmeal and lard. The purpose of this study was to differentiate the effects of feeding PennHFD1 to birds infected with EM alone vs. birds co-infected with EM and CP as part of a NE model. Using a 2x3 factorial design, 432 chickens were allocated into floor pens, and assigned to one of the six treatments (6 replicates/treatment): 1. Uninfected control + Diet A (Commercial-corn-based diet), 2. Uninfected control + Diet B (PennHFD1-based diet); 3. *E. maxima* infection (EM) + Diet A; 4. EM + Diet B; 5. Co-infection of *E. maxima* and *C. perfringens* (NE) + Diet A; 6. NE + Diet B. Growth performance parameters, NE lesion scores, oocyst shedding and ileal *C. perfringens* quantification (RT-qPCR) were evaluated. Statistical analyses were performed using a general linear model and preplanned contrasts in R Studio. Significance was claimed when $P \leq 0.05$. Birds fed PennHFD1 showed significant BWG improvements ($P < 0.01$) in uninfected and EM-only treatments without affecting oocyst shedding ($P = 0.705$). No differences due to corn type were observed in the incidence ($P = 0.566$) and severity ($P = 0.306$) of NE lesions or *C. perfringens* quantification regardless of infection status. We concluded that PennHFD1 improves health and production in chickens under mild intestinal challenges without directly affecting EM and CP. Ongoing research in our laboratory explores the effects of the PennHFD1-

flavonoids on the immune response and gut integrity in chickens undergoing mild intestinal inflammation.

Keywords: Polyphenols, Gut Health, Inflammation, Phytogetic, Enteritis

SCAD II

M49 Comparison of Vaccine Takes for Combinations of Mass and GA08 Type IBV Vaccines Applied in a Commercial Hatchery Brian Jordan*¹, Po-Hsin Yu¹, Kalen Cookson¹, Daniel Bautista¹ ¹Zoetis, Madison, Georgia, United States

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

Keywords: Infectious Bronchitis Virus, Vaccine, Serotype, Spray Application

M50 Fecal calprotectin for assessing coccidiosis severity in broiler chickens: correlation with oocyst shedding and performance metrics Mahmoud A. Mahmoud*¹, Vinicius Buiatte¹, Craig R. Baumrucker¹, Dervan Bryan¹, Mark Jenkins², Alberto Gino Lorenzoni¹ ¹Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States; ²Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Service, Beltsville, Maryland, United States

Coccidiosis, caused by *Eimeria* parasites, is a major challenge in poultry production. This study evaluated the impact of *E. maxima* infection on broiler performance, oocyst shedding (OS), and fecal calprotectin (CAL) levels, examining CAL's potential as a biomarker for assessing coccidiosis severity. Forty-eight 1-day-old Ross 308 male broilers were individually housed and split into two groups: a positive control (PC; uninfected, pro-inflammatory

diet, n=12) and a Coccidiosis (Cocci; infected with 5×10^3 *E. maxima* oocysts on day 12, pro-inflammatory diet, n=38) group. All birds had ad libitum access to feed and water. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured on days 0, 12, and 19. Feces were collected on days 6, 7, and 8 post-infection (P.I.) for OS and CAL assessments. Oocyst counts were assessed with a modified McMaster protocol, and CAL levels were measured from pooled day 7 P.I. samples of 40 birds using a commercial ELISA. Birds in the Cocci group were classified as high oocyst shedders (HOS), or low oocyst shedders (LOS) based on a quantile-based approach. Statistical analyses in R Studio included a Pearson correlation to explore the relationships between BWG, FI, FCR, CAL, and OS within the Cocci group (n=31). A one-way ANOVA was conducted to compare the PC (n=9), HOS (n=8), and LOS (n=8) groups. Results are reported as means \pm SE. BWG during days 12-19 showed a positive correlation with FI ($r = 0.54$, $P < 0.001$) and fecal weight ($r = 0.52$, $P < 0.001$), and a negative correlation with FCR ($r = -0.4$, $P = 0.027$) and CAL concentrations ($r = -0.5$, $P = 0.007$). CAL positively correlated with FCR ($r = 0.58$, $P = 0.001$). The one-way ANOVA indicated a significant reduction in BWG for HOS (398 ± 14.1 g) compared to LOS (455 ± 21.4 g, $P = 0.040$) and PC (484 ± 14.6 g, $P = 0.004$). FCR was significantly higher in HOS (1.37 ± 0.025) compared to LOS (1.22 ± 0.050 , $P = 0.031$). CAL levels were significantly elevated in HOS (16762 ± 3400 ng/ml) compared to LOS (7900 ± 1598 ng/ml, $P = 0.011$) and PC (1057 ± 273 ng/ml, $P < 0.001$). Calprotectin levels correlate with oocyst shedding and performance metrics, highlighting its potential as a non-invasive biomarker for evaluating the severity of coccidiosis infection in broiler chickens.

Keywords: Calprotectin, Broiler Chickens, Coccidiosis, *Eimeria maxima*, biomarker

M51 Assessment of pagP gene function in avian pathogenic Escherichia coli pathogenicity using a broiler chick challenge model Hailey Fugate*¹, Linan Jia¹, Xin Ye¹, Manhong Wang¹, Christopher Magee², Xue Zhang³, Pratima Adhikari¹, Kelsey Robinson², Li Zhang¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi, United States; ³Mississippi State University, Animal and Dairy Sciences, Mississippi State, Mississippi, United States

Avian pathogenic *E. coli* (APEC) is a significant bacterial pathogen in commercial poultry production, responsible for colibacillosis, a prevalent and economically significant disease worldwide. Previous studies using reverse vaccinology and a host-pathogen interaction model highlighted the immunogenic role of the lipid A palmitoyl transferase (*pagP*) gene, making it a promising vaccine candidate target. This study aims to investigate the function of the *pagP* gene in APEC pathogenicity by utilizing an APEC *pagP*-knockout strain. The experiment was conducted in an Animal Biosafety Level 2 facility using Ross 708 male broiler chicks in a randomized complete block design (RCBD) across four rooms. Each room contained eight pens with twelve birds in each pen (96 birds/room). The chicks were given a week of acclimation then they were subcutaneously injected in the neck with 0.1 mL of bacterial suspension (1×10^8 CFU/mL) or sterile saline. There are four treatment groups that were randomized within each room: a positive control (APEC strain MS1657), the MS1657 Δ *pagP* strain, a non-pathogenic *E. coli* field strain, and a negative

saline control. Chicks were monitored over a 14-day period, with sampling points of 0, 1, 3, 5, and 7 days post-inoculation. Data collected included body weights, organ index measurements, mortality counts, and lesion scores for commonly affected organs. Statistical analyses for body and organ weights were conducted using SAS PROC GLIMMIX, while mortality data was analyzed using a Kaplan-Meier survival model. Lesion scores were assessed using the Kruskal-Wallis test and ordinal logistic regression, with significance set at $P \leq 0.05$, respectively. Results indicate that the MS1657 $\Delta pagP$ strain significantly reduced APEC-associated mortality ($P < 0.001$) and lesion severity ($P < 0.05$) compared to the positive control. Additionally, significant differences were observed in body and organ weights among treatment groups. These findings underscore the *pagP* gene as a critical factor in APEC virulence and infection. By revealing the molecular mechanisms contributing to APEC pathogenicity, this study provides insights that could guide the development of targeted vaccines or antimicrobial strategies to mitigate colibacillosis.

Keywords: Colibacillosis, Lipid A palmitoyl transferase (*pagP*), Reverse vaccinology, Vaccine candidate, Lesion score

M52 *Enterococcus* genus affecting broiler chickens: evolution of an emerging threat Autumn Gregg*¹, Jay Kay Thornton¹, Isabella Hannay¹, Martha Pulido-Landinez¹ ¹*Poultry Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University, Pearl, Mississippi, United States*

For many years *Enterococcus cecorum* (EC) has been associated with its translocation from the intestine to the free thoracic vertebrae in broiler chickens, older than four weeks, causing vertebral osteoarthritis. Currently, the major concern is the presence of this bacterium causing severe systemic disease, lameness and high mortality in chickens younger than three weeks. The Poultry Research and Diagnostic Laboratory (PRDL) of Mississippi State University is constantly evaluating the dynamics of the diseases caused by bacteria from the genus *Enterococcus*. Based on cases registered on the PRDL accession system, an accession list was built including 604 cases received from January 2023 to October 2024. The goals of this study were: 1) to analyze the distribution of *Enterococcus* spp. cases and 2) to compare EC and *Enterococcus faecalis* (EF) age of presentation and presence of lesions. The prevalence of EC in both 2023 and 2024 was lower than EF. However, EC was more commonly isolated from cases with visible lesions, while EF was mostly isolated from young chickens without visible lesions and from hatchery samples with reports of a decrease in hatchability. In 2023, there were 117 cases of EC and 223 cases of EF. EF was isolated from the yolk sac and embryos in 68% of cases, while EC was isolated from the heart and liver in 40% of cases. In 2024, there were 56 EC cases and 186 EF cases. Again, EF was isolated from the yolk sac and embryos in 65% of cases, while EC was isolated from the liver, hock and femur in 40% of cases. Additionally, EF was primarily isolated from birds one week of age or less representing 85% (2023) and 82% (2024) of cases. Along with this, 74% (2023) and 71% (2024) of cases were identified in hatchery samples and pullets submitted for routine chick checks. EC was primarily isolated from birds 3-4 weeks of age, representing 40% (2023) and 46% (2024) of cases. Broilers were the primary type of bird in which EC was isolated from representing 69% of cases in 2023 and 57% of cases in 2024. This data indicates a higher prevalence of pathogenic EC in broilers, with EF primarily being isolated from yolk sac and hatchery samples. Further research needs to be conducted to determine the survival patterns of EC and EF in common poultry rearing conditions.

Keywords: Enterococcus, cecorum, faecalis, disease, bacteria

M53 Comparison of B lymphocyte responses in broiler chickens vaccinated with electron beam or formalin inactivated *Staphylococcus aureus* Ruvindu Perera*¹, Jossie M. Santamaria¹, Chrysta N. Beck¹, Gisela F. Erf¹, Adnan Alrubaye¹, Palmy Jesudhasan² ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*; ²*University of Arkansas, USDA-ARS, Fayetteville, Arkansas, United States*

Chicken meat is a common source of foodborne *Staphylococcus aureus* (SAu) infection and a major cause of bacteremia and infective endocarditis in humans as well as septicemia and arthritis in chickens. Hence, SAu poses significant health hazards and severe economic losses due to meat condemnation. Lethal electron Beam (eBeam) treatment inactivates bacteria by disintegrating nucleic acids while preserving surface epitopes that endow antigenicity and immunogenicity in hosts. Our objective was to compare local and systemic B cell responses in broiler chickens vaccinated with eBeam inactivated (eB)- or formalin-killed (FK)-SAu. Endotoxin-free PBS was the vaccine (sham) control. This study had 6 treatment (trt) groups with 5 chickens/group, where each vaccine trt was divided into two groups (Group A and B; i.e., A-eB, A-FK, A-sham, B-eB, B-FK, B-sham). Group A received the vaccine/sham treatments *in ovo*. At 34d of age, intradermal (i.d.) injections of respective trt into the pulps of growing feathers (GFs) were conducted to elicit booster- and primary-immune responses in groups A and B, respectively. Blood was collected at specific time intervals post-*in ovo* vaccination (Phase 1) from Group A chickens for immunofluorescent (IF) staining and B cell analyses by flow cytometry, and to compare relative levels of plasma SAu-specific antibodies by ELISA. Following i.d. GF injections (Phase 2), blood was similarly sampled and analyzed from both groups. Additionally, in Phase 2, GFs were collected at various times p.i. to assess local; primary and booster B cell presence in pulps by IF staining of pulp cell suspensions and flow cytometry. Two-way ANOVA was conducted to test effects of treatment, time, and their interactions followed by Bonferroni post hoc tests. Statistical significance was set at $P < 0.05$. eB vaccine resulted in higher SAu-specific IgM and IgA levels early in Phase 1, while in Phase 2, all groups had similar circulating levels of SAu-specific IgM, IgG and IgA antibodies and B cells. FK vaccine resulted in higher levels of B cells in GF-pulps at 24 h p.i. in both the primary and booster responses. In conclusion, the higher plasma levels of SAu-specific IgM and IgA after the *in ovo* eBeam SAu vaccination may reflect better protection at mucosal surfaces.

Keywords: Staphylococcus, eBeam, vaccine, B-cells, Chicken

M54 Effect of silane quaternary ammonium and hypochlorous acid treatments on microbial contamination in the hatch cabinet environment, enteric colonization at hatch, and 7-day performance in broiler chickens James Higuera*¹, Aaron Forga¹, Diego Cortes¹, Kasey Matusik¹, Alexandra Mendoza-Reilly², Ivan Alvarado², Justin Welsh², Faizan Aly³, Danielle Graham¹ ¹*University of Arkansas, Fayetteville, Arkansas, United States*; ²*Merck Animal Health, Madison, New Jersey, United States*; ³*Aguaire, Marietta, Georgia, United States*

The purpose of the study was to investigate the impact of treatment with a silane quaternary ammonium (Armatrex™) alone or in combination with hypochlorous acid (Clean Republic™) on the microbial load in the hatch cabinet, the gut microflora at hatch, and 7-day performance in broiler chickens. Groups included: 1) Non-challenged control (NCC), 2) Challenged Control (CC) Treated, 3) CC + Formaldehyde, 4) CC + Armatrex (AT), 5) CC + AT + Clean Republic (CR), 6) CC + CR, 7) CC Untreated. CR was applied as a fog to T1-6 on DOE18 at transfer using a Sanicart™. AT was

applied to T4 and T5 using a handheld electrostatic sprayer following initial CR fogging treatment at DOE18. For T2-7 at DOE19, 100 μ L of the CC challenge (*Escherichia coli*, *Enterococcus faecalis*, *E. cecorum*, and *Aspergillus fumigatus*) was applied to the blunt end of the eggshell. T3 hatch cabinets were fumigated with formaldehyde 10x from DOE19 to 12h prior to hatch pull. For T5-6, CR treatment was fogged into the hatch cabinets 4x from DOE20-DOE21. To assess the microbial load in the hatch cabinet, the open-agar plate method was used 3x at DOE20 and 1x at DOE21. Whole gut samples were collected from 12 chicks/group at hatch to enumerate relevant bacteria. At hatch, chicks were placed in floor pens (8-10 pens/group; 20 chicks/pen). Pen and feeder weights were recorded at d0 and d7 to calculate BWG and FCR. For all data, ANOVA was used to determine significant differences at $P < 0.05$ with means further separated using Student's t test. Gram-negative bacterial recovery from the hatch cabinet environment was lowest in T4 compared to all groups, including T3. *A. fumigatus* recovery was also reduced in T3-6 compared to T2 and T7. *Enterococcus* recovery from the gut was significantly lower in T4 compared to T2 and 7, but markedly higher compared to T3. Gram-negative bacterial recovery was statistically lower in T1 and T3 than all groups. T4 and T5 had significantly higher d0-7 BWG than T3. T5 had significantly lower FCR than T1-3, T6, and T7. These results indicate that CR and AT may reduce microbial contamination in the hatch cabinet environment, shift early gut colonization, and improve early performance. Studies exploring CR and AT as alternatives to formaldehyde in a commercial setting are underway.

Keywords: Clean Republic, hypochlorous acid, Armatrex, silane quaternary ammonium, hatchery

M55 Chicken alloantigen systems MHC-B, D, E, and I haplotypes are associated with resistance to coccidiosis Abhisek Niraula^{*1}, Anna Wolc²³, Robert L. Taylor⁴, Janet E. Fulton², Rami A. Dalloul¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²Hy-Line International, Dallas Center, Iowa, United States; ³Iowa State University, Department of Animal Science, Ames, Iowa, United States; ⁴West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States

Major histocompatibility complex (MHC) genes, along with other blood alloantigens, are associated with the level of disease resistance in poultry, including coccidiosis, a major protozoal disease caused by *Eimeria* species. Pedigreed White Leghorn offspring segregating for the MHC-B region, plus four additional alloantigen systems A (C4BPM), D (CD99), E (FCAMR), and I (RHCE) were tested for differential resistance to coccidiosis in five 26-day (d) trials (N=235). For each trial, chicks were hatched, individually wing-tagged, and group housed until challenged. On d 19, all birds were orally inoculated with *E. acervulina* (25,000), *E. maxima* (5,000), and *E. tenella* (10,000) sporulated oocysts and allocated to individual cages. Birds and feed were weighed on d 19 and d 26 to calculate BWG, FI, and FCR. All birds were euthanized on d 26 followed by scoring for gross and microscopic coccidiosis lesions in the duodenum, jejunum, and ceca. Fecal samples were assessed for oocyst shedding (oocysts per gram, OPG), and blood collected for DNA extraction and single nucleotide polymorphism genotyping of the five blood systems to determine the haplotypes present in each chick. Data from the five trials were combined and analyzed using a linear model, GLM, and Kruskal-Wallis tests. Significant differences ($P \leq 0.05$) between genotypes were evaluated by Tukey's HSD/Steel-Dwass tests. A positive and negative association means an increase and decrease in phenotypic trait respectively, with each additional copy (0, 1 or 2) of a given

haplotype. The CD99-H01 haplotype association was positive with BWG but negative with FCR. Genotype B21B21 had the highest gross lesion scores (GLS) in the jejunum establishing a positive association between MHC B21 and jejunal GLS. Further, the B12B15 genotype had a lower *E. maxima* OPG compared with the B12B21 genotype. The I system RHCE-H01 haplotype had a negative association with jejunal and cecal GLS. Duodenal GLS was lower in FCAMR-H02/H02 compared to the FCAMR-H07/H07 genotype of the E system. Specific haplotypes of the MHC and alloantigen systems D, E, and I differed in resistance traits. Further examination of these haplotypes and their effects on coccidiosis resistance in commercial lines may validate their incorporation in selection programs.

Keywords: Coccidiosis, *Eimeria*, Major histocompatibility complex, Alloantigen systems, Disease resistance

M56 Genomic insights into Type VI Secretion System in Avian Pathogenic *Escherichia coli* Julia Ienes-Lima^{*1}, Klao M. Runcharoon¹, Cierra Dunham¹, Catherine M. Logue² ¹University of Georgia, Population Health, Athens, Georgia, United States; ²University of Georgia, Population Health, Athens, Georgia, United States

Avian pathogenic *Escherichia coli* (APEC) is one of the most common bacterial pathogens of poultry and causes the disease colibacillosis, leading to morbidity and mortality in avian species. Currently, APEC is considered a significant threat to global poultry production and food security. The Type 6 Secretion System (T6SS) plays an essential role in the pathogenesis of several bacterial species. Specific T6SS genes, including *icmF* and *hcp*, are implicated in APEC adherence and invasion of epithelial cells, as well as its survival within macrophages. Three distinguishable and conserved T6SS loci are characterized in APEC strains, and recent studies suggest they are also involved in APEC virulence. This study aims to analyze the frequency of these three T6SS loci in a collection of APEC genomes of strains isolated from production birds of Georgia. Forty-one APEC genomes were analyzed, and the sequence type (ST) and serotype of each isolate were identified using MLST and SeroTypeFinder, respectively. The T6SS loci were identified through BLAST analysis, using the T6SS sequences from *E. coli* strain ED205 as a reference. To establish a positive presence of specific loci, a query cover and percentage identity threshold of 60% and 70% were set, respectively. Among the APEC genomes analyzed, 15 different O groups, 10 different H groups, and 15 different STs were identified. At least one T6SS locus was observed in 23 (56%) genomes. The prevalence of T6SS1, T6SS2, and T6SS3 loci was 41% (n=17), 22% (n=9), and 0% (n=0), respectively. Analyses are ongoing to identify the prevalence of T6SS genes among T6SS-positive genomes and explore the relationship between T6SS, *E. coli* sequence type (ST), and serotype. The presence of T6SS loci, particularly T6SS1 and T6SS2, suggests their potential involvement in APEC virulence. In summary, this study contributes to our understanding of the molecular epidemiology of APEC, especially on the prevalence of T6SS loci and genetic diversity among APEC strains. Further investigations will enhance our ability to develop targeted strategies for the prevention and control of colibacillosis in poultry populations.

Keywords: *E. coli*, APEC, Type VI Secretion System, virulence, poultry

M57 Phylodynamic Analysis of Variant ARV in the US Using Bayesian Markov Chain Monte Carlo (MCMC) and a Relaxed Molecular Clock Model Olatunde Oluwayinka^{*1}, Sonsiray Alvarez-Narvaez², Steven Conrad², Holly

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In the 1960s, ARV was identified as the causative agent of viral arthritis, and modified live vaccines (MLVs) developed in the following decade provided effective protection for commercial flocks broilers from vaccinated breeder flocks. In this study we aimed to investigate the phylodynamics of variant ARV in the US using published heterochronous data and Bayesian MCMC, a method of probabilistic phylogeny estimation and time-tree reconstruction. A Maximum Clade Credibility tree was constructed using 154 nucleotide sequences of the viral attachment protein, Sigma C, primarily comprising 131 US variants, with 12 Asian, 10 European, and 1 Australian isolate included to broaden the temporal and geographical scope of our data. Our results show that the estimated time to most recent common ancestor (TMRCA) of the US genetic cluster (GC)1 variants is between 1972 and 1980, coinciding with the introduction of the first live vaccine, which, like other commercial MLVs, was derived from a GC1 isolate (S1133). Additionally, the TMRCA of the US GC 5 variant isolates is 2012, aligning with the timeline of increased isolation from cases of tenosynovitis. For GC 7 isolates, which emerged in 2017, the common ancestor dates back to 2016. In contrast, GCs 2 and 4 isolates have older common ancestors and show multiple divergent events leading to current isolates, supporting the wide genetic diversity reported within these genetic clusters. Together, our results suggest that the introduction of MLVs may have contributed to the emergence of variant GC 1 isolates. Furthermore, with the exception of GCs 2 and 4, other GCs have more recent common ancestors, highlighting a pattern of ongoing evolution. Our study contributes to understanding key events in the epidemiology of variant ARVs in the US and emphasizes the importance of continued molecular surveillance of this pathogen in poultry flocks.

Keywords: Avian reovirus, σ C protein, epidemiology, phylodynamics, Bayesian MCMC

M58 Effect of *Ligilactobacillus salivarius* UMNPBX2 cell-free extracts on replication of low pathogenic H4N6 influenza virus Amritha Ajayan^{*1}, Dhananjai Muringattu Prabhakaran², Hamza Javaid¹, Maxim Cheeran³, Venkatramana D. Krishna², Anup K. Johny¹ ¹*University of Minnesota, Animal science, Saint Paul, Minnesota, United States;* ²*UNIVERSITY OF MINNESOTA, ANIMAL SCIENCE, Saint Paul, Minnesota, United States;* ³*University of Minnesota, Department of Veterinary Population Medicine, Saint Paul, Minnesota, United States*

Avian influenza virus (AIV), a member of the *Orthomyxoviridae* family, is a highly contagious virus that affects poultry. Avian influenza outbreaks related to high and low pathogenic strains have been reported in the United States and worldwide, resulting in significant economic losses to the poultry industry. This present study evaluated the antiviral potential of *Ligilactobacillus salivarius* UMNPBX2 (*L. salivarius*) cell-free extracts (CFE) on a low pathogenic avian influenza (LPAI) H4N6 virus strain in MDCK (Madin–Darby canine kidney) cells and in 10-day-old embryonated chicken eggs. The treatment groups used were: 1) Negative control (NC; *L. salivarius* CFE/PBS control), 2) Positive control (PC; LPAI H4N6 virus at 10^{6.6} TCID₅₀/ml), 3) Treatment group 1 (T1; H4N6 at 10^{6.6} TCID₅₀/ml + *L. salivarius* CFE from 10⁹ CFU/ml), 4) Treatment group 2 (T2; H4N6 at 10^{6.6} TCID₅₀/ml + *L. salivarius* CFE from a diluent with a concentration equivalent to 10⁷ CFU/ml), and 5) Treatment group 3 (T3; H4N6 at 10^{6.6} TCID₅₀/ml + *L. salivarius* CFE from a

diluent with a concentration equivalent to 10⁵ CFU/ml). In addition, immune gene expression in MDCK cells treated with *L. salivarius* CFE from 10⁹ CFU/ml was determined using qRT-PCR for IFN- γ , IL-1 β , and IL-6 expression normalized to GAPDH as the reference gene. At least six experimental units per treatment group were included in all studies ($n=6$), and data were statistically analyzed using One-way ANOVA and t -test on R ($P<0.05$). A significant dose-dependent reduction in viral replication was observed with increasing concentrations of CFE, with complete inhibition in groups treated with CFE from *L. salivarius* grown to 10⁹ CFU/ml of ($P<0.05$) in MDCK and embryonated chicken eggs. We observed a significant upregulation of IFN- γ expression in the study and downregulation of IL-6 ($P<0.05$). Results indicate that *L. salivarius* UMNPBX2 has a high potential for developing antiviral strategies against AIV (MDA #3005-11027-00102719; MnDRIVE GFV Fellowship).

Keywords: Avian influenza, MDCK cells, *L. salivarius*, embryonated eggs, LPAI

M59 Avian Metapneumovirus (aMPV) Serosurveillance of Broiler Breeder Flocks to Evaluate Exposure and Risk in the State of Mississippi Marcela Arango^{*1,2}, Scott Baughman^{2,1}, Cindy King³, James Watson³, Natalie Manginsay^{2,1} ¹*Mississippi State University, Pearl, Mississippi, United States;* ²*Poultry Research and Diagnostic Laboratory, Pearl, Mississippi, United States;* ³*Mississippi Board of Animal Health, Jackson, Mississippi, United States*

Avian Metapneumovirus (aMPV) is an enveloped RNA virus that affects primarily turkeys and chickens, causing turkey rhinotracheitis (TRT) and swollen head syndrome (SHS), respectively. Since the detection of aMPV subtypes A and B for the first time in chickens and turkeys in the United States during late 2023 and 2024, the virus has spread rapidly across the country, with detections in 30 states. As of November 2024, Mississippi has had no confirmed cases of aMPV. The objective of this study was to perform surveillance to establish the aMPV serological status of commercial broiler breeders in Mississippi. Serum included in this study had been submitted to the Poultry Research and Diagnostic Laboratory for routine NPIP testing. Selected serum had been submitted between July and October 2024 from 25 to 50 week old broiler breeder flocks. Seventy submissions from all six broiler integrators in Mississippi were selected for inclusion in the study, with farms selected according to their geographic distribution in the state. Ten sera per submission were randomly selected and tested using the IDEXX Avian Pneumovirus Antibody Test Kit. Geometric mean titers (GMT) were allocated into three categories following previously established criteria: No seroconversion (<397), equivocal or suspect (397-2000) and seroconversion (>2000). The distribution of aMPV GMTs was as follows: 57% ($n=40$) no seroconversion, 40% ($n=30$) equivocal or suspect and 3% ($n=2$) seroconversion. aMPV GMTs ranged from 4 to 8,572. Suspect GMTs were detected from serum submitted during all months and from all age groups included in the study. The two seropositive flocks belonged to different companies and tested positive in the same month (August 2024). No clinical signs or significant increases in mortality or egg production drops were reported in the seropositive flocks. These results indicate that at least two broiler breeder flocks were likely exposed to aMPV, while a number of others may have been exposed. Clinical signs were not reported in the seropositive flocks, suggesting that aMPV infection may sometimes be subclinical. This study highlights the importance of serologic testing for aMPV and provides evidence for the first time of aMPV seropositivity in Mississippi flocks.

Keywords: Avian metapneumovirus, aMPV, broiler breeder chickens, ELISA serology, Mississippi

M60 Case study: Evaluating the use of chlorine as a treatment during an outbreak in two flocks infected with *Campylobacter hepaticus*, the causative agent of spotty liver disease in layer hens Roel Becerra*¹, Catherine M. Logue² ¹University of Georgia, PDRC, Athens, Georgia, United States; ²University of Georgia, Population Health, Athens, Georgia, United States

Spotty liver disease (SLD) has emerged as an important cause of disease in table egg layers in many parts of the world. In the United States (US), it has been detected in the Midwestern and Southern states. The organism *Campylobacter hepaticus*, has been reported as the causative organism of SLD resulting in multifocal lesions on the livers of infected birds, reduced egg production, reduced egg size, and increased mortality of highly valuable hens. Currently, there are no approved treatments, and no commercial vaccine available for *C. hepaticus*, and research on effective control strategies for the control of this pathogen in organic or antibiotic-free laying hens remains limited. This case reports an outbreak on two flocks in south GA. In a two-house farm of free-range and antibiotic-free brown layers. House 1, at 40 weeks of age reported a 5% increase in mortality and a 5% decrease in egg production. During the field visit, multiple birds looked lethargic

and had high body temperatures. Necropsy performed in 31 birds showed gross spotty liver lesions in all of them. Bacteriological analysis of bile from infected birds showed characteristic colonies of *C. hepaticus* which was further confirmed by PCR. Histopathological analysis showed pericholangitis, hepatocyte necrosis, and bile duct hyperplasia. The affected flock was treated with 10 ppm chlorine in the drinking water for two weeks. House 2 in the same complex was also treated with 10 ppm chlorine in the drinking water for two weeks even though that house was not affected during that time. However, house 2, six weeks later presented a 12% drop in egg production and 1% increase in mortality due to *C. hepaticus* confirmed by gross spotty liver lesions, bacteriology, PCR, and histopathology. Results suggest that using chlorine in the water to treat infected flocks with SLD may help to reduce mortality but does not appear to eliminate the infection. Since there are limited effective treatments or no commercial vaccines currently available for *C. hepaticus*, further research is necessary to determine the most effective treatment and prevention strategies for SLD in laying hens.

Keywords: Spotty liver disease, *Campylobacter hepaticus*, Layer hens, Chlorine, Treatment

Welfare & Behavior I

M61 Effects of carotenoid supplementation on performance, egg quality, and antioxidant response in early-laying hens Maria Alvarenga*¹, Alexis Clark¹, Mireille Arguelles-Ramos¹, Ahmed Ali¹ ¹Clemson University, Animal & Veterinary Science Department, Clemson, South Carolina, United States

Yolk pigmentation is influenced by yellow and red carotenoid concentrations in feed. These carotenoids exhibit immunostimulant and antioxidant properties. This study examined the effects of supplementing canthaxanthin, a red-preferred carotenoid (R), and β -apo-8'-carotenoid acid ethyl ester, a yellow-preferred carotenoid (Y), on performance metrics, blood parameters, and early layer production egg quality. Hy-Line® Brown hens (N=405) at wk 20 were randomly assigned to 15 pens (27 birds/pen). Treatments were allocated to pens within blocks (5 replicates). A basal diet was used as control (CON). Pigment-supplemented diets included basal diet plus pigments R and Y at 5 and 10 mg/kg, respectively (P5-10), or 10 and 20 mg/kg, respectively (P10-20). ADFI, feed: gain (FCR), and hen-day egg production (HDEP) were measured at wk 24, 30, 36, and 40, with 15 eggs/treatment used for egg quality (n=10 for internal/external quality; n=5 for malondialdehyde). Blood samples were taken at wk 24, 36, and 40 for lipid profile and total antioxidant capacity (TAC) analysis. Performance, egg quality, and blood assays were analyzed in R using a generalized linear mixed model with a Poisson family distribution in the 'lme4' package. Mean comparisons were conducted using pairwise comparisons of pigmented treatments with CON, with $P \leq 0.05$ considered significant. No difference between pigmented treatments was observed, except in yolk color for each week ($P \leq 0.05$), where birds fed P10-20 had consistently darker yolk. FCR was more efficient in P5-10 and P10-20 birds at wk 40 compared to CON birds (all $P \leq 0.05$). Triglycerides were lower for pigmented treatments compared to CON at wk 24 and 36 (all $P \leq 0.05$), while no differences were observed at week 40. Levels of TAC were significantly higher in P5-10 and P10-20 compared to CON birds across all weeks (all $P \leq 0.05$). Albumen weight was significantly higher in P5-10, and P10-20 birds compared to CON birds at wk 30, 36 weeks and 40 (all $P \leq 0.05$). Similarly, yolk weight was significantly higher in P5-10, and P10-20 birds compared to CON birds across all weeks (all $P \leq 0.05$). Pigment supplementation in

diets can improve TAC, lipid profile and egg quality in early production layer hens.

Keywords: Layer hen, Carotenoid, egg quality, lipid profile, antioxidant capacity

M62 Early-life psychological stress changes cecal microbiota composition and behavior of laying hens Hari Marasini*¹, Lexie Luna¹, Young M. Kwon², Prafulla Regmi¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States

Early-life stress can have negative consequences for long-term fitness in animals. However, if psychological stress during these formative weeks can shape gut microbiome and influence the chick's behavior is yet to be understood. This study examined how isolation stress during the first two weeks influences gut microbiota and behavior in egg-type chicks. A total of 144 Hy-Line Brown one-day-old chicks were randomly assigned to Control and Stress rooms, with each treatment comprising six replicate pens of 12 birds each. Stress group underwent one-hour daily isolation in anechoic chambers between 7–11 days of age, while Control group were undisturbed. Behavior was continuously recorded using six CCTV cameras. On day 12, three chicks per pen (n=18/treatment) were sacrificed for microbial DNA extraction from pooled luminal content and mucosal scrapings, followed by 16S rRNA sequencing (QIIME2, ver. 2024.2). Behavior was analyzed via scan sampling every 5 minutes across four daily intervals: morning (8–9 am), early afternoon (12–1 pm), late afternoon (3–4 pm), and pre-lights-off (1–2 am). Behaviors recorded included feeding, drinking, exploratory foraging, sitting, sleeping, preening, dust-bathing, and feather-pecking. Statistical analysis was performed using the Kruskal-Wallis test (SciPy, Python 3.11.1), with $P \leq 0.05$ considered significant. At the family level, *Erysipelothrichaceae* and *Peptostreptococcaceae* were enriched in the Stress group ($P < 0.05$). Genus-level shifts included *Erysipelothrix* ($P < 0.001$), *Merdibacter*, and *Clostridioides* ($P < 0.05$). Similarly, at species level, a novel bacterium *Erysipelothrix pisciscarius* ($P < 0.001$), was significantly enriched along with *Merdibacter massiliensis*,

and *Clostridioides difficile* ($P < 0.05$) in stressed chicks. Predicted KEGG pathway analysis (PICRUSt2) revealed that four of the top seven upregulated pathways in the Stress group were linked to branched-chain amino acid (BCAA) biosynthesis (ILEUSYN-PWY, VALSYN-PWY, PWY-5101, PWY-5104; $P < 0.05$). Behaviorally, stressed chicks exhibited increased sleeping & dust-bathing and decreased exploratory foraging ($P < 0.01$). These findings suggest early-life isolation induces microbiota shift towards BCAA biosynthesis and alters expression of maintenance and exploratory behaviors in chicks.

Keywords: isolation stress, cecal microbiome, behavior, *Erysipelothrix piscisicarius*, branched chain amino acids

M63 Isolation in the light, but not dark, reduces brain serotonin activity suggestive of a negative affective state in layer hens Isabella von Seggern^{*1}, Gregory S. Fraley² ¹*Purdue University, Animal Sciences, Columbia City, Indiana, United States*; ²*Purdue University, West Lafayette, Indiana, United States*

An isolation fear test is used to measure 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 our study was to understand how isolation affects central dopamine (DA) and serotonin (5-HT) activity. DA and 5-HT activity are well established to be tightly associated with an animal's affective state. In order to achieve this goal, hens were isolated for 90 minutes in an anechoic chamber outside of their home rooms. This time frame ensures we can observe any potential changes in neurochemistry. 18 W-36 hens approximately 25 weeks of age were used (6 hens/group). Tests occurred during the birds' photophase. Group A was isolated for 90 minutes in the chamber with a light on while Group B was isolated with no light. Controls (Group C) were collected directly from their home enriched cages. Vocalizations were audio recorded and latency and duration of vocalizations obtained for Groups A & B. After 90 minutes, hens were euthanized and brains were collected. The brains were micro-dissected into the caudal (CM) and rostral mesencephalon (RM), diencephalon (DI), and lateral telencephalon (LT). DA and 5-HT turnover were measured using mass-spectrometry. Brain data were analyzed with an ANOVA and vocalization data using a Mann-Whitney U Test. Birds placed in isolation with a light had a shorter duration ($p = 0.0005$) to and percent of time ($p = 0.0039$) vocalizing compared to birds in the dark, in that birds isolated in the dark did not vocalize at all. While there were no significant differences among the groups for DA turnover, we found that hens isolated with a light had significantly higher 5-HT turnover, thus lower synaptic activity, in the CM ($p = 0.0004$), RM ($p = 0.01$), and LT ($p = 0.0002$) compared to Groups B & C. These neurochemical results suggest that controls and birds isolated in dark had similar affective states.

Keywords: Brain chemistry, Stress, Layers, Isolation

M64 Visual-assisted Autonomous Path Planning and Navigation for a Legged Robot Roaming in Cage-free hen houses Aravind Mandiga^{*12}, Guoming Li¹³, Tianming Liu², Venkat U. Bodempudi¹³, Ramviyas Nattanmai Parasuraman²³, Ramana M Pidaparti⁴ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*; ²*University of Georgia, School of Computing, Athens, Georgia, United States*; ³*University of Georgia, Institute of Artificial Intelligence, Athens, Georgia, United States*; ⁴*University of Georgia, College of Engineering, Athens, Georgia, United States*

Indoor navigation is critical for a robot to search for floor eggs in cage-free housing, but the navigation signals can be blocked by

animals and indoor facilities. This problem was solved by guiding the Unitree Go1 Edu robot to follow a 5cm fluorescent yellow path on the ceiling in a rectangular layout. The ceiling was 10ft high, and the dimensions of the layout were 24 ft × 10 ft. An RGB-Depth camera was used to capture real-time videos, which was then processed for color segmentation, contour detection, and bounding box approximation. Erosion and dilation were used to reduce nearby noises after color segmentation. Key points were identified to determine path direction. Angle and distance deviations were calculated relative to the path to ensure the robot's precise alignment along the path. These values were then sent to the legged robot through the POST Application Programming Interface (API). After receiving this information, the robot used its internal motion control APIs to realign and return it to its intended path. A Proportional-Integral-Derivative (PID) controller provided continuous feedback for angle and distance deviation to enhance stability. The experiment evaluated Robot's angle deviation correction, distance deviation correction by running it at 0°, 45°, and 90° to the fluorescent path's orientation for 50 instances. The robot's final orientation with the fluorescent path was analyzed after running the algorithm. We have applied one-way ANOVA, LSD post hoc analysis for overall and pair-wise comparisons respectively. One-way ANOVA demonstrated no significant difference in mean deviations between groups ($F = 1.670$, $p = 0.199$). LSD indicated no significant differences between the pairs ($p > 0.05$). The errors in mean angle deviations for 0°, 45°, and 90° were 5.60%, 6.53%, and 4.25%, respectively, with overall mean error of 5.68%, indicating 94.32% accuracy. After the angle deviation was corrected, we applied distance deviation algorithm. The accuracy was calculated using the expected position and the final position of the robot relative to the fluorescent path, achieving an average distance deviation correction of 87%. The proposed system shows excellent precision in indoor navigation in cage-free houses and supports automatic floor egg collection.

Keywords: path planning, poultry, robotics, autonomous navigation

M65 Validating RFID technology to identify broiler breeder pullets with high and low water use Allison D. Weaver^{*1}, Suzanne M. Leonard², Sara Orłowski³, Lisa Bielke¹, Ramon D. Malheiros¹, Allison N. Pullin¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²*North Carolina State University, Department of Animal Science, Raleigh, North Carolina, United States*; ³*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States*

Broiler breeder drinking behavior can inform genetic selection. Maintaining individuals in a genetic stock that drink less could reduce overall water use of a flock and maintain dryer litter, thus improving sustainability and welfare. We aimed to validate radio frequency identification (RFID) as a tool for tracking individual water use. A total of 960 Cobb 500 pullets were housed in 16 floor pens with a 2x2 factorial design: two feeding regimens (skip-a-day, SAD, and every-day, ED) by two water provision strategies (restriction 3h daily or *ad lib* access; 60 birds/pen). One RFID reader/pen was mounted on the waterline and RFID tags were attached to 10 focal birds/pen. At wk 22, RFID readers and video cameras recorded drinking behavior simultaneously for 7d. From the RFID data, the 2 highest drinkers (HD) and 2 lowest drinkers (LD)/pen were moved to individual cages to monitor 48h water usage (g/d). Data were analyzed using a 3-way ANOVA. HD used more water than LD on the on-feed day ($p < 0.05$; HD: 322.2 ± 21.3g, LD: 257.6 ± 20.7g; mean ± SE). SAD used less water than ED on the off-feed day ($p < 0.05$; SAD: 218.1 ± 16.3g, ED: 271.2 ± 15.3g), but used more water than ED on the on-feed day ($p < 0.05$,

SAD:325.4±21.7g, ED:254.3±20.3g). Water provision treatment did not affect water use ($p>0.05$). Additional validation was performed on 8 random pens (2 pens/treatment) for 45-min from 1 on-feed day. Average drinkers (AD), HD, and LD were classified separately from RFID and video footage and compared using a confusion matrix. The overall classification accuracy of RFID was 0.5 compared to video. Precision was similar across drinker class, while sensitivity was highest for LD (HD:0.50 and 0.50, AD:0.52 and 0.34, LD:0.49 and 0.71, respectively). LD metrics should be interpreted with caution as many RFID values in this group were zero. SAD specifically had higher overall accuracy and HD precision and sensitivity (0.53, 0.58, 0.58) than ED (0.48, 0.42, 0.42, respectively). The 7d RFID data successfully identified HD and LD individuals as demonstrated by their actual water use. RFID is most reliable for classifying HD SAD birds, likely because they drank more consistently and increased the chance of tag reads during on-feed days. Further refinement could improve this tool for genetic selection.

Keywords: broiler breeder, RFID, drinking, water, validation

M66 The Impact of Feeder Removal on the Post-Feeding Behaviors of Feed Restricted Broiler Breeder Pullets at 10 and 15 Weeks of Age Mazette Croom^{*1}, Courtney Daigle², Rosemary Walzem¹ ¹Texas A&M University, Poultry Science, Bryan, Texas, United States; ²Texas A&M University, Animal Science, College Station, Texas, United States

Restricted feeding schedules are commonly implemented for rearing commercial broiler breeder pullets and create a paradigm where the presence of a feeder becomes a secondary motivating cue for feeding. This raises important welfare considerations, as behavioral responses to the removal of these secondary cues may provide insights into how birds cope with feed restriction. For this reason, we examined the behavioral responses of pullets, on a 3:4 and 4:3 skip-a-day feed program at 10 and 15 weeks of age (WOA), respectively, to feeder removal on skip days. Two commercial strains of breeder pullets ($n=200$ birds/strain) were blocked by strain, randomly assigned to one of 16 floor pens (2.32 m², 25 pullets/pen), and their behavior video recorded for 4 days. The proportion of pullets (%) engaged in locomotion, inactivity, maintenance, foraging, and object pecking was decoded from two 30-minute video recordings on both feeding and skip days that commenced four hours post (expected) feeder placement (15:00–15:30 and 16:00–16:30). A Generalized Linear Mixed Model (PROC GLIMMIX) assessed the effects of strain (S1, S2), feeder presence, and their interaction on bird behavior where Δ = Absent% – Present%. At 10 WOA, regardless of strain, when the feeder was absent, fewer birds were observed in locomotion ($\Delta = -3.1\%$, $P<0.0001$) and object pecking ($\Delta = -23.8\%$, $P<0.0001$), while more birds engaged in maintenance ($\Delta = +4.9\%$, $P<0.0001$), inactivity ($\Delta = +10.6\%$, $P<0.0001$), and foraging ($\Delta = +11.3\%$, $P=0.0001$). Strain-specific responses to feeder removal at 10 WOA were object pecking (S1 $\Delta = 0\%$; S2 $\Delta = -44.4\%$, $P < 0.0001$), inactivity (S1 $\Delta = 0\%$; S2 $\Delta = +27.5\%$, $P<0.0001$), and maintenance (S1 $\Delta = +1.9\%$; S2 $\Delta = +8.7\%$, $P<0.0001$). Feeder removal at 15 WOA did not prompt strain-specific responses. Feeder removal at 15 WOA reduced average object pecking ($\Delta = -7.0\%$, $P=0.0007$), with all other behaviors remaining similar. Reduction of hunger stress by 15 WOA was evidenced by a decrease in object pecking (47.7% to 14.7%) and an increase in inactivity (26.9% to 51.7%) compared to 10 WOA. Outcomes suggest that hunger stress was mitigated over time and emphasize the need for age- and strain-tailored management practices.

Keywords: Broiler Breeders, Feed Restriction, Behavior, Hunger Stress

M67 Influence of light wavelength on the activity and tibia morphology of broilers Angela Perretti^{*1}, Chantel Pennicott², Victor Oyeniran¹, Jaelen Cherry¹, Zachary Grider³, Alexander Nelson³, Shawna Weimer¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ²University of Arkansas, Poultry Science, Farmington, Arkansas, United States; ³University of Arkansas, Electrical Engineering and Computer Science, Fayetteville, Arkansas, United States

Lighting plays a critical role in the health of broilers. While research on lighting has focused on flicker, intensity, and duration—key elements for the success of broilers—studies on the impact of lighting wavelength on broiler activity and leg health remain limited. The objective of this research was to examine the effects of blue, green, and white grow-out light on broiler activity levels and tibiotarsus (tibia) bone morphology. Using a completely randomized design, day-of-hatch Cobb-Vantress by-product male broilers ($N=600$) were randomly assigned to 12 pens ($N=50$ /pen) and raised under one of three wavelength treatments (blue [450nm], green [560nm], or white [462nm]). Diet phase changes occurred every two weeks (Starter (S) [D0-D14], Grower (G) [D14-D28], and Finisher (F) [D28-D42]) where body weight (BW), feed consumption, and mortality (mort) were measured for feed conversion ratio (FCR). Activity levels were measured ($N=12$ birds, 1 bird/pen) from D11-D14 and D38-D41 using an accelerometer (AC) secured on their backs with elastic bands. Acceleration was measured using magnitude (M) calculated from the X, Y, and Z coordinates of their movements in the pens. On D41, L and R tibias were collected from the AC birds for bone morphology. A one-way ANOVA analyzed the treatment effects and age in JMP. Production measures (S [BW=0.48g, FCR=1.21], G [BW=1.88g, FCR=1.42], F [BW=3.31g, FCR=1.87], and mort=8.68%) were not significant ($P>0.05$). Higher M was found in green compared to blue and white for D11 (297.43 M vs 140.17 M, $P=0.003$), D12 (281.62 M vs 208.78 M, $P=0.0002$) and D13 (240.71 M vs 197.74 M, $P=0.03$). Birds under white light had lower M on D40 compared to blue (99.3 M vs 176.82 M, $P<0.0001$) and D41 compared to green (97.33 M vs 164.07 M, $P=0.004$). Tibias from birds raised under blue light were longer (105.63 mm, $P=0.0025$) compared to green and white (100.62 mm). Tibias from birds raised under green light (10.69 mm) were wider at the midpoint compared to blue (9.76 mm, $P=0.038$), and the proximal head angle was smaller for green (36.82) birds compared to blue and white (39.20, $P=0.022$). These results suggest that green wavelength treatments may promote activity, but future studies should explore the effects of light on the tibia morphology of broilers.

Keywords: accelerometer, tibiotarsus morphology, lighting, wavelength, activity levels

M68 Enhanced Deep Learning Approaches for Reliable Tracking of Multiple Individual Broilers in Dense Group Settings Sai Akshitha Reddy Kota¹, Guoming Li^{*1}, Tongshuai Liu¹, Chongxiao Chen¹ ¹University of Georgia, Athens, Georgia, United States

Tracking individual broiler activities is crucial for assessing bird health, well-being, and productivity, enabling early detection of issues and timely intervention. Deep learning-based tracking algorithms often face challenges like identity switching due to occlusions, overlaps, and visual similarities in crowded environments. This study optimized computer vision models for accurate and consistent tracking of broilers. Video data were collected using overhead cameras above pens (1.2 m × 3 m) containing 37 Cobb 500 male broilers on pine shavings, recorded at 15 frames per second. The optimization process addressed four critical components: (1) Custom Object Detection: YOLOv8 and

YOLOv11 models were evaluated, and YOLOv11m was selected for its balance of precision (0.967), recall (0.954), and mAP@0.5 (0.985). (2) Model Pruning: A pruning level of 0.09 improved recall (0.957) and mAP@0.5-0.95 (0.799) while maintaining precision. Pruning reduced inference time from 6.7 ms to 6.5 ms per image, with a minor increase in postprocessing time from 11.9 ms to 13.0 ms. (3) Custom Feature Extraction: Eight models, including VGG-19, EfficientNetB7, and Vision Transformer, were tested. Vision Transformer achieved the highest validation accuracy (0.90), and Grad-CAM was applied for visualizing critical features influencing predictions. (4) Tracking: Pruned object detector weights and the custom feature extractor were integrated into ByteTrack, which reduced identity switches by 70% compared to baseline methods. The final model maintained consistent identities, achieving a multi-object tracking accuracy of 0.84 and precision of 0.94, while processing video at 27 frames per second. This optimized approach provides a robust framework for extracting kinematic features such as acceleration and velocity, offering valuable insights for improving broiler genetics, health, and disease management. These results demonstrate the potential of advanced tracking algorithms for enhancing broiler welfare and production in dense, dynamic environments.

Keywords: poultry, broiler tracking, broiler detection, computer vision, artificial intelligence

M69 A user-friendly deep learning-based platform for monitoring animal activity index Mahtab Saeidifar*²³, Guoming Li²³⁴, Lakshmish M. Ramaswamy¹, Chongxiao Chen²⁴, Tongshuai Liu²⁵, Ehsan Asali²¹, Venkat U. Bodempudi²³ ¹University of Georgia, Computer Science, Athens, Georgia, United States; ²University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ³University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States; ⁴University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States; ⁵Henan University of Animal Husbandry and Economy, College of Animal Science & Technology, Zhengzhou, Henan, China

M70 Predicting gait scores of individual broilers in later phases of production based on spatial and temporal kinematic features in early stages Hosna Mohammadilabadi*¹, Guoming Li¹, Tongshuai Liu¹, Venkat U. Bodempudi¹, Oluwadamilola M. Oso¹, Mahtab Saeidifar¹, Ehsan Asali¹, Aravind Mandiga¹, Sai Akshitha Reddy Kota¹ ¹University of Georgia, Athens, Georgia, United States

Predicting broiler gait scores early is crucial for implementation of corrective management strategies to support optimal leg health and welfare in poultry production. This study investigated the potential for early-stage kinematic data to predict broiler gait scores. Broiler chickens were raised in experimental pens (1.2 m wide × 3.0 m long, 37 birds per pen), and overhead videos were recorded continuously and converted into images for further analysis. Ten birds were randomly selected and color-marked for individual evaluations. Fast Segment Anything Model (Fast-SAM) and Track Anything models were first used to identify and consistently track multiple birds at the same time in weeks 2 and 3, and then key spatiotemporal kinematic variables, (velocity, acceleration, movement dynamics, distribution index, and feeding behaviors) were extracted and correlated with three-point gait scores assessed in week 7. The statistics (e.g., minimum, maximum, standard deviation, mean, median, quantiles, IQR, kurtosis, and skewness)

Monitoring the activity index of animals is crucial for assessing their welfare and behavior patterns. However, traditional methods for calculating the activity index, such as frame differencing of entire frames, often suffer from significant interference and noise, leading to inaccurate results. These methods also lack the capability to measure the activity index of individual animals, making it impossible to track the movement of specific animals within a group. Furthermore, there is currently no free and accessible online platform available for non-technical researchers to use for this purpose, creating a gap in tools available for animal welfare studies. To address these limitations, we developed a user-friendly, open-source platform using Streamlit that enabled researchers to calculate the activity index of animals, either individually or in groups, from video footage. Users can easily upload videos of any species (e.g., hens, broilers, cows, and pigs) and select animals for targeted tracking. A general deep learning-based image segmentation model, Segment Anything Model2 (SAM2), was used to segment and track individual animals across frames without the need for extensive training or annotation. The platform ensured consistent and accurate segmentation and tracking, overcoming the challenges posed by noise and interference in traditional methods. Additionally, a segmentation comparison was conducted between different general segmentation models, including SAM2, TrackAnything, You Only Look Once (YOLO) v8, and YOLOv11. The results showed that SAM2 consistently outperformed the other models, achieving the highest segmentation success rate (100%), Intersection over Union (IoU) (92.21% ± 0.012), precision (93.87% ± 0.019), recall (98.15% ± 0.011), and F1 score (95.94% ± 0.006) for segmenting and tracking broilers. These metrics were calculated from 1,157 images. By applying frame subtraction techniques on the segmented masks, the platform automatically calculated and visualized the activity index of animals over time. This user-friendly tool provides researchers with an accessible and efficient way to track and analyze animal behavior patterns, delivering accurate and reliable insights into animal welfare without requiring extensive programming knowledge.

Keywords: animal behavior, deep learning, Streamlit, activity index

Welfare & Behavior II

of these kinematic variables were extracted, resulting in a total of 86 features per bird. Ten birds per gait score category were used for further evaluation, and each analyzed video lasted 20 minutes for each bird. Machine learning classifiers were ensembled to enhance gait score predictions, such as Light Gradient-Boosting Machine (Light-GBM), Categorical Boosting (CatBoost), and Extreme Gradient Boosting (XGBoost) classifiers. This approach achieved a 71% precision and 73% recall. The results also highlighted that features related to speed, acceleration, and unexpected changes in direction were more important predictors than feeding behavior features, particularly quantiles of these metrics. The mean, maximum, and standard deviation of speeds were 0.59, 1.34, and 0.60 m/s, respectively; the mean, maximum, and standard deviation of moving distance were 3.52, 9.47, and 1.99 m, respectively. This approach underscored the potential of advanced tracking algorithms to enable automated kinematic analysis for early gait score prediction, establishing a novel link between early motor characteristics and future gait scores. The methodology offers the poultry industry potential tools to improve leg health of individual birds in an early manner.

Keywords: Gait Score Prediction, Tracking, Fast-SAM, Spatiotemporal Kinematic Variables

M71 A novel three-dimensional deep learning approach for auditing gait scores of individual broiler chickens Ehsan Asali*¹, Guoming Li², Tongshuai Liu³, Chongxiao Chen²⁶, Mahtab Saeidifar⁴²⁵, Venkat U. Bodempudi⁴², Sai Akshitha Reddy Kota²⁵, Oluwadamilola M. Oso², Aravind Mandiga²⁵ ¹University of Georgia, Computer Science, Athens, Georgia, United States; ²University of Georgia, Poultry Science, Athens, Georgia, United States; ³Henan University of Animal Husbandry and Economy, College of Animal Science & Technology, Zhengzhou, China; ⁴University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States; ⁵University of Georgia, School of Computing, Athens, Georgia, United States; ⁶University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States

Manually auditing gait scores of broiler chickens is labor-intensive and subjective, prompting the need for an automated, objective method. This study introduced a deep learning pipeline for three-dimensional (3D) gait scoring to assess walking ability in broilers, predicting gait scores from 0 (good walking ability) to 2 (poor walking ability). A total of 480 chickens, sampled weekly over four weeks (weeks 4 to 7), were recorded traversing a 1.75-meter wooden platform, with gait scores assigned based on movement speed and distance. RGB-Depth data were collected using an Intel RealSense L515 LiDAR camera mounted at a 2.5-meter height, capturing 1920 bag files across all samples. The pipeline began with RGB-Depth frame extraction and synchronization, followed by keypoint detection using a YOLOv11-based chicken pose estimation model trained on 1000 annotated frames, achieving an mAP50(P) of 99.5% and validation pose loss of 0.06 on 200 test frames. Keypoints (head, tail, left wing, right wing) and bounding boxes were projected from 2D to 3D using camera intrinsics and depth data, creating a single, ordered list of 3D points. A frame validation model with convolutional and fully connected layers excluded frames with human interference. Platform orientation was detected using 2D Hough line detection to identify edges, correcting camera tilt by unifying platform boundaries. The Segment Anything Model (SAM) segmented the chicken's body using the pose estimation centroid to extract a 3D point cloud and generate a mesh layer. This mesh was transformed to align the chicken's initial position with the 3D world model origin, enabling precise 3D movement tracking along the platform. Key 3D kinematic features, including velocity, acceleration, and head turn frequency, were extracted from transformed mesh data. These kinematic features served as inputs for a multi-layer perceptron classifier, achieving 95.56% ± 1.24 Precision, 91.16% ± 1.63 Recall, and 93.31% ± 1.10 F1-Score in predicting gait scores of 90 chickens. This novel approach demonstrates the feasibility of automated, objective 3D gait scoring in broilers, potentially reducing manual effort while improving the consistency of leg health assessments and overall scoring reliability across the poultry industry.

Keywords: Artificial intelligence, software development, precision livestock farming, sensor, animal monitoring

M72 Multifactorial Analysis of Broiler Welfare Indicators Correlated with Individual Body Weight, Litter Moisture Content, Diet, and Litter Type Oluwadamilola M. Oso*¹, Guoming Li²³⁶, Chongxiao Chen², Tongshuai Liu², Venkat Umesh Chandra Bodempudi³², Nicolas Mejia-Abaunza², Mahtab Saeidifar³², Sai Kota³², Muhammad Ali⁴, Catherine Fudge⁵, Ehsan Asali⁶², Aravind Mandiga⁶², Woo Kyun Kim², Prafulla Regmi², Jeanna Wilson², Lilong Chai² ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ³University of Georgia, Institute for Artificial Intelligence,

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Evaluating factors contributing to welfare of broiler chickens is crucial for predictive analysis and precision management purposes. The objective of this research was to identify the important factor(s) influencing welfare outcomes in broiler chickens. A total of 1,776 one-day-old Cobb 500 broilers were randomly assigned to 48 pens containing either fresh/used litter and four levels (5, 125, 250, and 500 ppm) of copper supplementation. Ten broilers per pen were randomly selected and color-marked for continuous evaluation at weeks 4-7. Three-point scoring systems were used to determine all welfare indicators, with 0 being best welfare situation and 2 being worse welfare conditions. Individual body weight and litter moisture contents were recorded. Five classifiers, which were Random Forest, Categorical Boosting, Light Gradient Boosting Machine (LightGBM), Extreme Gradient Boosting (XGBoost), were trained to identify key predictive features of each welfare indicator, with the inputs of individual body weight, litter moisture contents, diet, and litter type. LightGBM was selected for the feature importance score due to superior classification accuracy. The result identified individual body weight as the most important factor in influencing footpad dermatitis score, gait score, plumage cleanliness score, hock burn score, and breast blister score. Litter moisture content played the second important role in predicting breast blister and plumage cleanliness. While diet was identified as the second most important feature for footpad dermatitis score, gait score, hock burn score. The correlation analysis revealed that body weight was moderately correlated to gait score ($r=0.33, P<0.001$) and hock burn score ($r=0.35, P<0.001$). While positive, a weak correlation existed between body weight and footpad dermatitis ($r=0.29, P<0.001$). There was a weak but negative correlation between diet and footpad dermatitis ($r=-0.26, P<0.001$) and hock burn score ($r=-0.18, P<0.001$). Litter condition was weakly correlated with hock burn score ($r=0.15, P<0.001$), plumage cleanliness ($r=0.02, P=0.40$), and moisture content ($r=0.025, P<0.001$). In conclusion, individual body weight is an important predictor of welfare indicators in broilers whereas litter condition also plays an important role.

Keywords: Machine learning, microminerals, housing management, welfare, poultry

M73 Evaluating Broiler Activity index, Stretching and Preening behaviors as Affected by Stocking Density Shengyu Zhou*¹, Nasiri Amin¹, Tanner Thornton³, Xiao Yang², Hao Gan¹, Tom Tabler¹, Yang Zhao¹ ¹University of Tennessee, Knoxville, Knoxville, Tennessee, United States; ²China Agricultural University, Beijing, China; ³University of Tennessee, Animal Science, Knoxville, Tennessee, United States

Stocking density (SD) is crucial for profitability and impacts broiler welfare. The activity index, stretching, and preening behaviors in broilers reflect their comfort, welfare, and environmental suitability, offering valuable insights into their welfare. This study investigated the effects of stocking density (SD) (27, 29, 32, and 44 kg/m²) on the activity index (AI), stretching, and preening behaviors in Ross 708 and Cobb 700 broilers on day 28 and 56. A total of 432 birds per strain were used, with 10, 12, 14, and 18 birds per pen (1.1 m x 1.5 m), corresponding to the respective SDs. Each SD treatment had 8 replicates. The desired market body weight of 4 kg and the targeted SD were reached by day 56. Broilers activities were monitored through the computer vision system continuously. Activity index, stretching and preening behavior of broilers at the fourth and

eighth weeks of age were analyzed. Results showed that Ross broilers raised at 44 kg/m² increased their activity index (AI) and stretching behavior at 28 days ($P < 0.01$) but reduced AI at 56 days ($P < 0.01$) likely due to crowding. Stretching ($P = 0.12$) and preening ($P = 0.89$) behavior were unaffected by SD on day 56. Ross broilers exhibited more stretching and preening in morning than afternoon on day 28. For Cobb broilers, increasing stocking density to 44 kg/m² slightly raised AI ($P < 0.01$) but significantly reduced stretching and preening behaviors ($P < 0.01$) on day 28. the AI ($P = 0.06$) and stretching ($P = 0.79$) behavior of Cobb were unaffected by SD on day 56. the AI, stretching and preening patten (by hour) of Cobb were relatively stable throughout the daytime. In conclusion, SD of 44 kg/m² promoted Ross's activity and expression of welfare-related behaviors in the early stage but had a negative impact on Cobb's welfare-related behaviors.

Keywords: broiler, stocking density, activity index, stretching, preening

M74 Deep Learning Methods for Tracking Activities of Male Birds in Cage-Free Flock Bidur Paneru^{*1}, Ramesh B. Bist¹, Xiao Yang¹, Anjan Dhungana¹, Samin Dahal¹, Lilong Chai¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

Roosters' (male poultry) activities are critical for egg fertility and hatchability in broiler and layer breeding houses. Desirable roosters are expected to have strong legs, healthy footpads, and sexual maturity. However, roosters are not always performing as well as what we expect. Low-productive roosters are damaging the broiler or laying hen breeding. Currently, there are lack of an automated system for identifying roosters with low performance or issues. The objectives of this study were to develop a deep learning method for tracking individual roosters and monitor behavior of roosters for assessment of potential wellbeing conditions. The specific features of rooster behavior or activity such as mating and locomotion could be a sign for productivity and welfare. Extracting desirable features of roosters from manual annotations of the recorded videos is a challenging and time-consuming task that might pose limitations such as cognitive bias and fatigue. In addition, we proposed a noble method for detecting chickens with the YOLOv11 model (You Only Look At Once version 11) and tracking any model (TAM) of the male birds raised in a cage-free (CF) housing environment. Six male birds were marked and kept with 200 Lohmann White females in a CF research facility at the University of Georgia, USA. A total of 3,000 images were used, with each image featuring at least one male bird present. The models' performance matrices such as precision, recall, and mAP across YOLOv11n, YOLOv11s, YOLOv11m, & YOLOv11l models were compared using one-way ANOVA at a 5% significance level. YOLOv11l resulted in the highest precision of 89%, recall of 73%, and mAP of 82%. Our method provides a reference for tracking male birds in floor-raised housing systems such as laying hen and broiler breeder houses.

Keywords: Cage-free housing, male birds, detection, tracking activities, precision poultry farming

M75 Weight prediction of Broiler chickens using a deep learning framework Venkat Umesh Chandra Bodempudi^{*12}, Guoming Li²¹³, Tongshuai Liu², Ehsan Asali⁴², Aravind Mandiga⁴², Mahtab Saaidifar¹², Sai Akshitha Reddy Kota⁴², Oluwadamilola M. Oso², Sravan Sai Rahul Nalla⁵ ¹*University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States;* ²*University of Georgia, Department of Poultry Science, Athens, Georgia, United States;* ³*University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States;* ⁴*University of Georgia, Computer Science, Athens,*

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In large-scale broiler production, traditional weighing methods like manual measurement and platform-based electronic scales face challenges due to labor intensity, bird movement, and voluntary participation by broilers. These methods often miss unwilling or abnormal broilers that avoid the scales, resulting in bias in evaluating body weight uniformity and consistency. To address the limitations, a deep learning framework embedded in non-invasive computer vision systems was developed for automatic weight assessment using video footage, enabling accurate weight estimation by capturing data from a wide variety of broilers, including those avoiding platform weighers. A total of 1,776 day-old Cobb 500 male broilers were randomly assigned to 48 experimental pens (1.2 m × 3.0 m). Ten broilers per pen were randomly selected, color-marked, and overhead videos were continuously recorded from weeks 1 to 7. Manually measured live weights from these 480 individual broilers were recorded to train, validate, and test the deep learning models. The methodology for broiler weight estimation began with detecting individual birds within video footage using the You Only Look Once version 11 (YOLOv11) object detection model. Detected birds were then classified as stretching or resting to enable focused segmentation of features using the YOLOv11 segmentation model. During stretching, the wing, shank, and claw were segmented, while the entire body was segmented in resting broilers. The feeder, drinker, and pen dimensions served as reference points to adjust for camera height variations across pens. Bird feature measurements were calculated using these references and correlated with broiler body weight, achieving over 0.8 Pearson correlation coefficient values. A multilayer perceptron framework was trained and processed using these features to predict weights. The results demonstrated lower accuracy from weeks 1 to 3, with a mean absolute error (MAE) of ±125g (22% of actual average weight 573g), but improved from weeks 4 to 7, with an MAE of ±305g (10% of actual average weight 3020g) due to better visibility of bird features as they grew larger.

Keywords: poultry, broiler weight, deep learning framework, segment, multilayer perceptron

M76 Comparison of Drinking Behavior in Water Conversion Birds Natalie K. Johnson^{*1}, Kirsten Shafer¹, Maricela Maqueda¹, Rosemary Whittle¹, Chantel Pennicott¹, Shawna Weimer¹, Sara Orłowski¹ ¹*University of Arkansas, Center of Excellence for Poultry Science, Springdale, Arkansas, United States*

Agriculture accounts for over 75% of freshwater usage therefore finding ways to reduce water usage is imperative for improving sustainability. In 2019, a divergent selection program was implemented to understand the effect of selection for water conversion ratio (WCR= water intake/body weight gain) on broiler performance and water sustainability. The objective of the current study was to evaluate behavioral differences after 3 generations of selection for WCR. Lines included the low water conversion ratio (LWC), high water conversion ratio, (HWC), and a modern random bred (MRB) control population. For generation three, cameras were placed above pens and video was recorded for 24 hours on d 26. Using generational WCR data from 0-4 weeks, pens selected for video evaluation included the best and worst performing pen for LWC and HWC line and 1 pen of MRB for a total of 5 pens. Three times of day were selected for behavior evaluation, including 30 min after lights on (AM), midday (MID), and 30 minutes before lights out (PM). Drinking duration and percentage of birds near the drinker nipple (nipple proximity) was recorded via scan sampling. Linear mixed effects models in R were

used, with line and time of day serving as the main effects. The Best HWCR pen drank for a longer ($P=0.02$) duration (40.5 s) than the Worst LWC (27.3 s). There was an inconsistent time of day effect on the drinking duration within each genetic line. Birds in the Best HWC, Best LWC, and Worst HWC pens drank for longer ($P<0.05$) durations in the AM than the MID and PM, while birds in the Worst LWC and MRB pens drank for similar durations throughout the day. Birds near the nipple of the drinker were greater in the PM (5.97%) than MID (5.11%, $P=0.04$) and did not differ from AM (5.61%, $P=0.63$). The greatest percentage of birds near the drinker were from Worst LWC (7.91%) and differed from Worst HWC (5.58%, $P<0.001$), MRB (4.18% $P<0.001$), and Best LWC (3.48%, $P<0.001$), but didn't differ from Best HWC (6.66%, $P=0.13$). In conclusion, this shows that the birds who drink longer may be fulfilling their thirst more efficiently than the birds who drink for a shorter amount of time and more frequently, and that the more water-efficient line does not exhibit resource guarding.

Keywords: Water Conversion Ratio, Broilers, Genetics, Sustainability

M77 Exploratory analysis of broiler welfare indicators at two U.S. processing plants Samantha Vitek*¹, Leonie Jacobs¹ ¹Virginia Tech, School of Animal Sciences, Blacksburg, Virginia, United States

Routine assessment of animal welfare indicators at the processing plant allows for continued monitoring and subsequent improvement of broiler chicken welfare. However, data on commercial broiler chicken welfare in the United States are scarce. The objective was to explore associations between flock characteristics and routinely collected broiler welfare measures from commercial processing plants. Data from 35 flocks (mean \pm

SD flock size = $145,723 \pm 87,199$) representing 5,100,313 broiler chickens from 33 farms were analyzed. Two plants provided data on flock characteristics, including flock size (n), bird live weight at slaughter (mean \pm SD live weight = 2.90 ± 0.99 kg (6.39 ± 2.19 lbs), age at slaughter (mean \pm SD age = 46 ± 10 days), and on-farm mortality rates (mean \pm SD = $5.64 \pm 2.37\%$ of the flock). Welfare measures included dead on arrivals (DOA; % of flock), wing fractures (% of ≥ 100 birds assessed), and bruising on legs (% of ≥ 100 birds assessed). A wing fracture was recorded if a dislocated or broken wing was observed. A leg bruise was recorded when a bruise was greater than 2.5cm in diameter. This is an exploratory study with preliminary data, so only simple statistical analyses were performed. Associations were explored using linear Pearson correlations in JMP Pro. Data are presented as raw means with standard deviations. DOA rates ($0.422 \pm 0.14\%$) were not correlated with flock characteristics or other welfare measures. The prevalence of wing fractures ($8.17 \pm 11.31\%$) was positively correlated with live weight ($r = 0.618$, $p < 0.001$), age at slaughter ($r = 0.598$, $p < 0.001$), on-farm mortality ($r = 0.615$, $p < 0.001$), and prevalence of bruising on legs ($r = 0.549$, $p = 0.001$). Prevalence of bruising on legs ($2.79 \pm 2.40\%$) was positively correlated with live weight ($r = 0.407$, $p = 0.015$), age at slaughter ($r = 0.452$, $p = 0.006$), and on-farm mortality ($r = 0.518$, $p = 0.002$). The preliminary results show the value of exploring routinely assessed measures for broiler chicken welfare to highlight potential risks that can be mitigated. Further work is needed to thoroughly assess relationships between flock characteristics, welfare measures, and pre-slaughter conditions.

Keywords: animal welfare, broiler chicken, key welfare indicator, routinely collected data

Food Safety I

M78 Antibiotic alternatives result in a differential abundance of lactic acid bacterial populations and fermentation pathways in Salmonella Heidelberg-challenged turkeys, as revealed by shotgun metagenomic sequencing Dhananjai Muringattu Prabhakaran*¹, Divek V.T. Nair¹, Tim Johnson², Anup K. Johnny¹ ¹University of Minnesota, Animal Science, Saint Paul, Minnesota, United States; ²University of Minnesota, Department of Veterinary and Biomedical Sciences, Saint Paul, Minnesota, United States

Antibiotic alternatives (AA) could be practical approaches to mitigate *Salmonella* in turkeys. The restorative effects of AA on the microbiome of turkeys disrupted by the *Salmonella* challenge would identify ways to devise novel microbiome-based strategies to control the pathogen. The effects of two probiotics, *Ligilactobacillus salivarius* UMNPBX2 (LAB) and *Propionibacterium freudenreichii freudenreichii* (PF), and a *Salmonella* Typhimurium vaccine (VAC) on the cecal microbiome of *Salmonella* Heidelberg (SH)-challenged finishing turkeys were determined in this study. Day-old poults were randomly distributed into five groups (n=4/group; 2 studies; N=40): Negative Control (NC; -SH, -AA), Positive Control (PC; +SH, -AA), LAB (+SH, + 10^7 CFU/mL LAB), PF (+SH, + 10^7 CFU/mL PF), and VAC (+SH, +S. Typhimurium vaccine). On the 11th week, groups except NC were challenged with SH (10^8 CFU/turkey) via crop gavage. On the 12th week, cecal samples were collected for DNA extraction. The DNA library prepared from NexteraXT was sequenced on the Illumina NovaSeq 2x150 bp platform. The raw sequences were aligned to the Kraken2 and HUMAnN2 databases for taxonomic and functional profiling of microbiota, respectively. Wilcoxon rank sum test for α and PERMANOVA for β diversity were used as statistical tests. Differential abundance of species was done

using *DESeq2*. MaAsLin2 was used to find differentially abundant pathways in functional profiling. There was no difference in α diversity, but a difference in β diversity in the taxonomic composition was observed between the groups ($P.adj < 0.05$). *DESeq2* found that all AA groups had a differential abundance of multiple lactic acid bacteria species compared to PC ($P.adj < 0.001$). Microbial functional profiling identified multiple pathways differentially abundant in AA groups compared to PC. Probiotic groups had fermentation pathways differentially abundant compared to PC ($P.adj < 0.05$). The study results suggest that AA could improve the gut health of turkeys challenged with SH by increasing the abundance of lactic acid bacteria and pathways associated with probiotic activity. The results provide insights into the mechanism of beneficial microbial modulation exerted by AA in health and disease.

Keywords: Microbiome, Salmonella, Probiotics, Alternatives, Food safety

M79 A farm-to-fork approach for controlling Salmonella Enteritidis in shell eggs using probiotics and postbiotics Ragini Reddyvari*¹, Praveen Kosuri¹, Yuying Ren¹, Eswari Kanike¹, Sulthana Humayoon¹, Mary Anne Amalaradjou¹ ¹University of Connecticut, Mansfield Center, Connecticut, United States

Salmonella Enteritidis (SE) contaminated eggs are a leading cause of foodborne outbreaks. Egg contamination occurs via horizontal transmission from the environment and vertical transmission from SE-colonized hen. Thus, contaminating the outer shell (OS), inner shell (IS) and internal contents (IC) resulting in outbreaks. Hence, there is a need for effective pre- and post-harvest solutions to control SE on eggs. Here we evaluated the efficacy of two novel probiotics, i) *Lactobacillus rhamnosus* NRRL-B-442 (LR)

and *Lactobacillus paracasei* DUP 13076 (LP) as in-feed supplements to reduce SE colonization in layers and ii) their postbiotics as wash treatments to reduce egg contamination. SE colonization studies were set out as a completely randomized design with 19-week-old birds assigned to 4 treatments with replicate pens of 8 birds each for 60 days. Treatments included layer diet without probiotics (Control) and diet supplemented with 9 log CFU/kg of LP, LR, or the cocktail (PR). Birds were challenged with SE (10^{10} log CFU/bird) by crop gavage and eggs tested daily for SE. In the postharvest study, eggs (135, 45/trial) were randomly assigned to treatments including control (water), 200 ppm chlorine (industry control), water with 40% v/v LP postbiotic (LPP) and water with 40% v/v LR postbiotic (LRP). Inoculated eggs (~8 log CFU/egg) were dip washed in different treatments and sampled to enumerate SE populations during refrigerated storage for a week. Data were analyzed using GraphPad and $p \leq 0.05$ was considered significant. In-feed supplementation of LP, LR, and PR significantly reduced SE transmission to eggs. With the OS, only ~45% of the eggs were SE positive in the probiotic groups compared to ~84% SE positivity in the Control. Further, only ~10-15% of IC samples were SE positive in the probiotic groups while >50% SE positivity was detected in the Control. Egg wash with postbiotics also significantly reduced SE populations on OS, IS and IC throughout the study. Moreover, we did not recover any SE from the IC in the postbiotic-washed eggs as compared to all SE-positive samples in the control and chlorine washed eggs. Overall, in-feed supplementation of LP and LR and their postbiotics as post-harvest egg wash offers a farm-to-fork approach to improve egg safety.

Keywords: Salmonella control, probiotics, postbiotics, feed supplementation, egg wash

M80 Prevalence of *Salmonella* and *Campylobacter* from retail poultry in Alabama Maria V. Baca*¹, Abigail McConnell¹, Karla V. Casco¹, Richard Buhr², Dianna Bourassa¹ ¹Auburn University, Auburn, Alabama, United States; ²USDA-ARS, US National Poultry Research Center, Athens, Georgia, United States

Raw broiler meat is often associated with the potential for foodborne illnesses. The objective of this study was to assess the prevalence of *Salmonella* and *Campylobacter* from retail poultry in Alabama. A total of 126 samples were collected from four grocery stores. Sample types were selected based on availability, resulting in breasts (52), wings (29), thighs (7), and tenders (38). Samples were also categorized as conventional (63), antibiotic free (ABF 54), air-chilled (4), and organic (5). Retail package parts were weighed to 1 lb (454 g) sample sizes and rinsed in 150 mL buffered peptone water. To maximize detection rinsates were analyzed for *Salmonella* using bioMérieux GeneUp, 3M MDS, and conventional plating. *Campylobacter* was detected using 3M MDS and conventional plating methods. Prevalence data were analyzed using Fishers Exact Test with significance determined at $P \leq 0.05$. Overall, retail poultry parts were 11.9% positive for *Salmonella* and 4.8% positive for *Campylobacter*. No differences were detected in *Salmonella* prevalence between sample part types ($P \geq 0.5170$) or between production types ($P \geq 0.5892$). *Salmonella* was detected from 10% (5/52) breast fillets, 10% (3/29) wings, 14% (1/7) thighs, and 16% (6/38) tenders. For production type, *Salmonella* was detected from 11% (7/63) conventional, 15% (8/54) ABF, 0% (0/4) air-chilled, and 0% (0/5) organic. Additionally, no differences were detected in *Campylobacter* prevalence between sample part types ($P \geq 0.1556$) or between production types ($P \geq 0.0690$). *Campylobacter* was detected from 6% (3/52) of breast fillets, 7% (2/29) wings, 14% (1/7) thighs, and 0% (0/38) tenders.

For production type, *Campylobacter* was detected from 6% (4/63) conventional, 0% (0/54) ABF, 25% (1/4) air-chilled, and 20% (1/5) organic. Results revealed that although both *Salmonella* and *Campylobacter* were detected in retail broiler chicken samples, part type and production method did not influence prevalence. However, a limitation of this study was the lack of available retail products within the air-chilled and organic production types. These results highlight the need for enhanced food safety protocols in poultry production to reduce the risk of foodborne illness.

Keywords: Salmonella, Campylobacter, retail, production type, broiler

M81 Microbial assessment and antibiotic susceptibility of isolated pathogens on retail chicken Eniola Betiku*¹, Philip G. Crandall², Tomi Obe^{1,2} ¹University of Arkansas System Division of Agriculture, Department of Poultry science, Fayetteville, Arkansas, United States; ²University of Arkansas System Division of Agriculture, Department of Food Science, Center for Food Safety, Fayetteville, Arkansas, United States

Poultry is U.S. Consumers' protein of choice with an annual consumption of nearly 45 kg per person. This increasing demand has required producers to minimize pathogen contamination to protect public health. This study investigated the incidence and microbial load of *Salmonella* and *Campylobacter* in retail chicken products from conventional (CON) and non-conventional (raised without antibiotics, RWA) sources while profiling the antibiotic resistance of selected pathogen isolates. Chicken samples (n=170) were collected from retail stores, focusing on two brands (A & B) based on availability. These included whole carcass: WOG (60; CON=20 & RWA=20 each), parts (80; CON & RWA=20 each), and giblets (30; CON only). *Salmonella* and *Campylobacter* were isolated using the USDA MLG and quantified with the BAX@ system. Aerobic count (AC), Enterobacteriaceae (EB), and Lactic acid bacteria (LAB) were determined using Petrifilms™. Confirmed *Salmonella* isolates were serogrouped. Selected *Salmonella* (22) and *Campylobacter* (24) isolates were tested for antibiotic susceptibility using the Sensititre™ system. Data were analyzed using ANOVA and Tukey's HSD ($p \leq 0.05$) in JMP. *Salmonella* and *Campylobacter* incidence was similar between CON (60% & 50%) and RWA (38% & 45%) for WOG and parts (CON=20% & 13% and RWA=8% & 13%), respectively. Though differences exist between brands. Giblets had a higher incidence of both pathogens (80% & 70%, respectively). For AC counts, RWA-B significantly differed from CON-A for WOG ($p=0.03$) and parts ($p=0.05$) while EB counts were different ($p=0.0008$) between RWA-B and others for parts only. In contrast, LAB counts were similar for both sample types. The most and least abundant *Salmonella* serogroups were C1 (46%) and D2 (1%). Many isolated pathogens possessed resistance to at least one antibiotic, *Salmonella* (90%) and *Campylobacter* (38%) with multidrug resistance in CON=67% & RWA=36% *Salmonella* isolates. The highest resistance was to tetracycline and nalidixic acid for both pathogens and the lowest was antibiotics in the macrolides class. These results highlight the need for robust microbial control from farm to retail levels, as both production practices showed notable contamination and antibiotic resistance, emphasizing the need for retail food safety.

Keywords: Salmonella, Antibiotic resistance, Retail chicken, Food safety, Campylobacter

M82 Use of pre-harvest *Salmonella* status to effectively drive a directed processing approach that reduces *Salmonella* in broilers carcasses Marco Reina*¹, David

Ayala-Velastegui¹, Nikki Shariat¹ *University of Georgia, Population Health, Athens, Georgia, United States*

The broiler industry has reduced *Salmonella* prevalence in parts by over 50% in the last decade. Despite this, there is still pressure to further reduce *Salmonella*. On-farm sampling is a potential tool for evaluating the efficacy of interventions during grow-out and for inferring the microbial load that may enter the processing plant. This study aimed to reduce *Salmonella* prevalence in poultry processing by applying insights from an on-farm monitoring program conducted in commercial broiler houses. For eight weeks, *Salmonella* prevalence, load, and serovars of concern (Enteritidis and Typhimurium (including I 4,[5],12:i:-)) were assessed in an average of ~47 [NS1] flocks (i.e. houses) per week. On average, flocks were 26 days old. Flocks were deemed lower risk if they exhibited no serovars of concern and had lower *Salmonella* prevalence and load compared to other flocks sampled within the same week. At the plant, two strategies were evaluated: 1) directed processing, where a lower-risk flock was slaughtered first after cleaning and sanitation, and 2) non-directed processing, where the order of slaughter was based on standard logistics but not on *Salmonella* status of a flock. Each strategy was assessed in alternating one-week cycles (four weeks per strategy). To guarantee traceability and avoid cross-contamination, only the first flock was sampled at hot rehang (n=5 rinsates/day) and post-chill (n=20 rinsates/day). Differences were established using Chi-squared tests. Flocks that were sampled on directed processing weeks had lower *Salmonella* prevalence at hot rehang compared to non-directed processing weeks ($p < 0.0005$), with prevalence of 37.1% (39/105) and 75.2% (79/105), respectively. However, no differences were observed between samples taken at post-chill ($p = 0.10$), with prevalence of 0.2% (1/420) and 1.2% (5/420) for directed and non-directed weeks, respectively. Serovars of concern were identified only in two post chill samples (2/420), one for Enteritidis and one for Typhimurium, both from non-directed processing flocks. These findings demonstrate that on-farm monitoring for *Salmonella* can provide valuable insights to enhance intervention strategies such as directed processing and can support decision-making to reduce *Salmonella* in poultry products.

Keywords: Salmonella, broilers, pre-harvest monitoring, directed processing

M83 Use of fluorescent spectral imaging system to detect *Listeria monocytogenes* biofilm formation on food processing surfaces

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Listeria monocytogenes is the third leading cause of foodborne illnesses with capacity to grow in low-acidic conditions, at refrigerated temperatures, and ability to develop into unnoticed persistent biofilms creating a hazardous condition for food products. Detection of these biofilms in processing facilities has become more pertinent due to recent outbreaks of *L. monocytogenes* in ready-to-eat meat and poultry products thereby raising the need for innovative safety measures to attack this challenge. The goal of this study is to detect biofilm formation on food processing surfaces at various temperatures using a fluorescent spectral imaging system. Food processing surface coupons (stainless steel, polypropylene, and rubber) were

inoculated with *L. monocytogenes* culture of 10⁵ CFU/ml in Brain Heart Infusion (BHI) broth in 6-well plates. The plates were incubated at 4°C and 20°C and biofilm formation was evaluated every 24 hours over 3 days with multispectral imaging before and after surface staining for growth analysis. Staining of materials was conducted with a 1% crystal violet solution staining for 15 minutes. Images were analyzed using a supervised Convolutional Neural Network (CNN). The unstructured data, collected images, for CNN were split into 70::30 for training (70%), testing (20%), and validating (10%) the model. A threshold for the model's predicted probability for classification was set at 80%. Multispectral imaging system was able to detect *L. monocytogenes* biofilm formation on food processing surface coupons. The CNN models were able to distinguish surfaces with and without biofilm formation at 74.6% accuracy for image classification. The success of multispectral systems for early biofilm detection offers a promising approach to mitigate the risk of *Listeria* contamination in ready-to-eat meat products. These methods could provide a critical tool for sanitation and inspection processes at a plant

Keywords: biofilm detection, spectral imaging

M84 Ferric Uptake Regulator (Fur) plays a significant role in the survival of *Salmonella* Typhimurium on meat

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Food is an important source of *Salmonella* infections, and poultry products are one of the leading causes of disease outbreaks. The success of *Salmonella* Typhimurium as a pathogen lies in its ability to adapt to the changing environment quickly. This adaptability of the bacterium is strictly regulated by numerous DNA-binding transcriptional regulatory factors that respond to signals from the external environment. To identify the factors critical for the survival of *Salmonella* on meat, nine different deletion mutants of transcriptional regulatory factors of *Salmonella* Typhimurium ST 4/74 were grown on chicken meat pieces (breast fillets-1 gram piece each), incubated at 37°C and serially diluted aliquots were plated onto XLD agar plates. The bacterial counts in CFU/mL were plotted against time. Statistical analysis was done using RStudio (version 4.4.2). Of the 9 deletion mutants, ST4/74Δ*fur* (Ferric Uptake Regulator) was highly inhibited compared to the wild type (p-value = 0.0005). Fur protein, during iron-replete conditions, represses the siderophore production gene. On exhaustion of free iron in the environment, Fur protein is released, thereby activating the different siderophore genes, allowing the bacterium to chelate the iron (Fe³⁺) bound to various proteins from the environment. Taking clues from the previous study in milk, inhibition of *fur* deletion mutant could be due to an intracytoplasmic iron accumulation, which could be toxic to the bacterium. To better understand iron regulation, we deleted the gene for enterochelin production, a major siderophore in *Salmonella* (*entC*). ST4/74Δ*entC* grew similar to the wild type (p-value = 0.90), relaxing the inhibition from *fur* deletion. This led to the hypothesis that the deletion of the *entC* siderophore gene blocked the uptake of iron by the bacteria, thereby reducing the iron toxicity in the cytoplasm, which aided in a revival of the growth of the bacterium on the meat surface. Furthermore, the double mutant ST4/74Δ*fur*Δ*entC* also did not show a significant reduction in growth when compared to the wild type (p-value = 0.43). Our data gives preliminary insights into how iron uptake

by *Salmonella* Typhimurium in iron-rich meat is strictly regulated by *fur* and aids in colonization of the food matrix.

Keywords: Salmonella, adaptability, iron-uptake, Fur, siderophores

M85 Synergistic Effects of Polyphenols and Antibiotics Against Multi-Drug Resistant *Salmonella* Typhimurium DT104 Hunter Sheffield^{*1}, Greeshma Bharathan¹, Michelle Hayden¹, Aisha Madi¹, Shabarinath Srikumar¹, Karoll Elizabeth Rodriguez Chinchilla³, Richard Buhr² ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States;* ²*US National Poultry Center, Athens, Georgia, United States;* ³*Zamorano University, Tegucigalpa, Honduras*

The rise of antimicrobial-resistant foodborne pathogens necessitates innovative approaches to enhance the efficacy of existing antibiotics. This study explores the synergistic effects of phyto- polyphenols, specifically hydroxycinnamic acid and itaconic acid, with conventional antibiotics in inhibiting multi-drug resistant *Salmonella enterica* serovar Typhimurium DT104 (DT104). We used DT104 due to its resistance to five antibiotics, offering an opportunity to test multi-antibiotic-polyphenol synergy. Initially, the minimum inhibitory concentrations (MICs) of the polyphenols were determined individually using the standard 96-well plate microdilution protocol. Following this, a checkerboard assay assessed potential synergistic interactions between each polyphenol and various antibiotics. Both hydroxycinnamic acid and itaconic acid exhibited no significant anti-DT104 effects when tested individually. However, in combination with antibiotics, both polyphenols demonstrated excellent anti-DT104 effects, showing synergy with all five antibiotics and reducing their individual MICs. To evaluate practical applications, one-gram portions of boneless, skinless chicken tenderloins were inoculated with DT104. Samples were treated with hydroxycinnamic acid and itaconic acid, while controls received no treatment (PBS). Treated and untreated samples were incubated at 10°C and 37°C, then plated on XLD at 2-hour intervals over 14 hours to assess bacterial growth. Results indicated a significant reduction in DT104 growth in polyphenol-treated samples compared to controls (PBS to Hydroxycinnamic Acid: p-value=0.0007, PBS to Itaconic Acid: p-value:0.0009, and PBS to Both Polyphenols: p-value=0.0009). Statistical analysis was determined using R-Studio version 4.4.2. Importantly, although individual polyphenols showed no anti-DT104 effect in MIC analysis, significant antimicrobial effects were observed in chicken samples. This could be due to the hurdle principle, where meat creates stress environments for DT104 in the presence of polyphenols. These findings suggest polyphenols could enhance antibiotics' effectiveness against multi-drug-resistant pathogens in food matrices, potentially mitigating foodborne illness risks.

Keywords: Polyphenols, Antimicrobial Resistance, Salmonella, Poultry Meat

M86 Protective effects of a killed *Salmonella* ser. Typhimurium vaccine delivered through *In ovo*, Gel or Spray routes in broiler birds Divyashree Ramalingam^{*2}, Ramesh Selvaraj³, Mohan Naidu Korada³, Parimal Sheth¹, Praveen Reddy Kasu³, Waqas Ishaq³ ¹*Endovac Animal Health Inc, Columbia, Missouri, United States;* ²*University of Georgia, Animal Science, Athens, Georgia, United States;* ³*University of Georgia, Poultry Science, Athens, Georgia, United States*

The application of killed vaccines in the USA broiler industry is limited since it is impractical to inject a million birds twice. The objective of this study is to analyze the killed bacterin vaccine efficacy when delivered either as a single dose or two dose through

a mass-vaccination route, during a *Salmonella enterica* Ser. Typhimurium (*S. Typhimurium*) challenge. 450 birds were randomly distributed into the following six treatments in three replications (n = 3). 1. Control, 2. In ovo vaccine, 3. Gel vaccine, 4. Spray vaccine, 5. In ovo + Gel vaccine, and 6. In ovo + Spray vaccine. Birds were challenged with *S. Typhimurium* on D21. The vaccinated birds had 50 to 130 g higher (P < 0.05) D21 body weight gain and 2 to 8 points better feed conversion ratio than the control group. There were no significant differences (P > 0.05) in the mortality percentages between treatment groups on D21. On D7, birds in the all the vaccinated groups had significantly higher antigen-specific lymphocytes when compared to the control group. On D14 and 21, birds in the In ovo + Gel and In ovo + Spray vaccine groups had significantly higher antigen-specific lymphocytes when compared to the control group. On D21, birds that were vaccinated twice had numerically (P > 0.05) higher cecal tonsil CD8+:CD4+ ratio than the other groups. On D21, birds in the In ovo, Gel, and Spray vaccine groups had numerically (P > 0.05) higher anti-*Salmonella* IgG in their serum, compared to D7. On D30, birds in the Spray, In ovo + Gel, and In ovo + Spray vaccine groups had 1.49 to 1.73 log decrease in *Salmonella* loads when compared to the control group. On D30, birds in the In ovo- and Gel- vaccine groups had 1.11 and 0.5 log decrease in *Salmonella* load when compared to the control group. It can be concluded that applying the first dose via In ovo and the second dose of bacterin vaccine through Gel or Spray method can induce cell mediated immune response and protect birds against a homologous *S. Typhimurium* challenge in broiler birds.

Keywords: Salmonella, Bacterin vaccine

M87 Effect of peanut skin as an antimicrobial feed additive against *Salmonella* in broilers Yabaiz Tahir^{*1}, Thien Vu², Justin Lowery¹, Mary Mendoza¹, Krista Chang¹, Katherine Rysdon¹, Demilade Ibiwoye¹, Christina S. Sigmon¹, Ondulla T. Toomer², Lin L. Walker¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*Agricultural Research Service/ USDA, Food Science and Market Quality & Handling Research Unit, Raleigh, North Carolina, United States*

Poultry products are frequently contaminated with *Salmonella*, which is a prevalent foodborne pathogen that causes serious health concerns to consumers. Developing effective strategies to reduce *Salmonella* in poultry is essential. Recent research has shown that peanut skin (PS) extract, rich in polyphenolic compounds, exhibits antibacterial properties. This study evaluated the effect of PS (5% addition) as an antimicrobial agent in broiler chickens against *S. Typhimurium*. One hundred and eight day-old Ross 308 male chicks were randomly divided into four treatments, a full factorial design of diets (PS and conventional) and *Salmonella* challenge (positive and negative), with three pens per treatment. Half of the birds were orally gavaged with a nalidixic acid-resistant *Salmonella* cocktail at 7 log₁₀ CFU/mL on d14. Two random fecal, feed, water, and litter samples per pen were taken on d14 (before challenge), d28, and d44. In addition, on d44, two birds were randomly selected per pen for cecal tonsil and whole bird rinsate samples. Appropriate dilutions of each sample were plated on XLT-4 plates supplemented with 50ppm of nalidixic acid for *Salmonella* enumeration. The original samples in buffer peptone water were also enriched for *Salmonella* detection. Feed intake and body weight gain were recorded weekly, and feed conversion ratio (FCR) was calculated. Data was analyzed in JMP software, using 2-way ANOVA and Tukey's HSD for mean separation for the performance parameters. *Salmonella* counts were analyzed using a *t*-test. Overall FCR of the birds didn't show any significant difference (P > 0.05) across treatment groups.

After *Salmonella* challenge, fecal samples from PS treatment showed lower *Salmonella* counts ($P < 0.05$), whereas, *Salmonella* was rarely noticed in water and feed samples, regardless of the treatment. Litter samples showed a trend where PS treatment had lower counts. No *Salmonella* was recovered from cecal tonsil and whole bird rinsate samples. In conclusion, *Salmonella* counts were lower in the infected birds fed with a PS diet showing a potential for the use of PS diet as a possible feed additive against *Salmonella* infection. Further research is needed to fully understand peanut skin extract antimicrobial efficacy against *Salmonella* in poultry.

Keywords: Peanut skin, Salmonella, Antimicrobials, Broilers, Polyphenols

M88 Effect of organic acid supplementation on *Salmonella* prevalence and growth performance during a challenge model using male broilers Eva G. Guzman^{*1}, Jose I. Vargas¹, Joseph P. Gulizia¹, Jose R. Hernandez¹, Jibely Martinez², Maria J. Brizuela², Matthew Bailey¹, Ruediger Hauck¹³, Dianna Bourassa¹, Kenneth Macklin⁴, Wilmer J. Pacheco¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States; ²Zamorano University, Agricultural and Production Science, Valle de Yeguaré, Francisco Morazan, Honduras; ³Auburn University, Department of Pathobiology, Auburn, Alabama, United States; ⁴Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States

Contaminated poultry meat is a significant source of salmonellosis outbreaks in humans. To mitigate this risk, various pre-harvest strategies have been developed to reduce *Salmonella* levels in poultry meat. This study evaluated the addition of two organic acid (OA) mixtures in drinking water from 1 to 39 d, as a pre-harvest intervention to reduce the prevalence of *Salmonella* in poultry carcasses. Additionally, the study assessed broiler growth performance over the 39-d period. In total, 1,872 d-old male broilers (YPM × Ross 708) were randomly distributed into 72 floor pens (26 chicks/pen) and subjected to three treatments: citric acid; a mixture of lactic, acetic, and propionic acids; or water without OA (control), with 24 replicate pens per treatment. On d 7, birds were inoculated with approximately 1.0×10^7 CFU of a nalidixic acid-resistant *Salmonella* Enteritidis strain. Chick paper and litter boot swab samples were collected at placement. Subsequently, cecal content samples, carcass rinses, and additional litter boot swabs were collected on days 39, 40, and 41 to assess *Salmonella* marker-strain prevalence using XLT4 with nalidixic acid selective media. Feed intake (FI), body weight (BW), and feed conversion ratio (FCR) data were collected at 14, 28, and 39 d of age. Water intake (WI) was measured at d 39. Data was analyzed using a generalized linear model with a Poisson distribution for microbiology data, while analysis of variance was applied to performance data (significance set at $P \leq 0.05$). At placement, 93.3% of chick papers and 12.5% of litter samples tested positive for *Salmonella*. On d 39, the inclusion of citric acid in water reduced FI (4,511 g vs. 4,592 g) and BW (3,230 g vs. 3,293 g) compared to broilers consuming water without OA ($P \leq 0.05$). However, the addition of OA in water did not influence FCR

and water intake ($P > 0.05$). The inclusion of OA in water did not influence the prevalence of *Salmonella* ($P > 0.05$) in ceca content and carcass rinses on d 39. At the conclusion of the experiment, more than 45% of litter boot swab samples tested positive for *Salmonella*. These findings indicate that continuous inclusion of OA in drinking water had limited effectiveness as pre-harvest intervention under the conditions tested in this study.

Keywords: Salmonella, organic acid, broiler, performance, drinking water

M89 Evaluating the effect of feed sanitizer products on feed mill equipment and its impact on feed microbial reduction Emily Magee^{*1}, Jason Sands², Timothy Boltz¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Anitox Corporation, Lawrenceville, Georgia, United States

Feed sanitation is essential to the poultry industry as it contributes to decreasing microbial pathogen loads in feed, increasing bird performance and food safety. Assessing the overall hygiene of the feed mill equipment is critical when evaluating microbial carry-over between batches of feed. Feed manufacturers have different options for products to disinfect both equipment and feed. This study aims to evaluate the efficacy of a synergistic blend of phytochemicals and carboxylic acid (Product 1) and an organic acid blend (Product 2) in reducing microbial pathogens when applied to feed. A corn, wheat, and soybean meal (SBM) diet, meeting the nutritional requirements of broilers during the grower phase, was batch-mixed before treatment with sanitizers. The study was replicated 3 times, with each run consisting of 9 treatments. Depending on the treatment, Product 1 and/or Product 2 were used to treat SBM or feed contaminated with a *Salmonella* *Infantis* inoculum. After each treatment was mixed, feed and swab samples were taken from the mixer. Samples were assessed for *Salmonella* colony enumeration and prevalence. For enumeration, each sample was plated and diluted on Xylose-Lysine-Tergitol 4 (XLT-4) agar. For prevalence, samples were enriched in Tetrathionate broth (TTB) and plated. All data was analyzed using SAS with a one-way ANOVA utilized for enumeration and Chi-square for prevalence data. Significance was set at $\alpha \leq 0.05$. The results of feed sample enumeration indicate that inoculated feed allowed *Salmonella* to remain in the mixer and contaminate future batches of feed. Treatments including Product 1 and/or Product 2 significantly reduced *Salmonella* counts ($P < 0.0001$). From overall feed enumeration results, the product combination treatment demonstrated the greatest reduction with a 1.87 log (~99%) reduction in *Salmonella*. Feed prevalence results showed the promise of carryover from contaminated treatments to non-contaminated treatments ($P < 0.0001$). This study demonstrates that using sanitizers to control feed microbial pathogens can control *Salmonella* on equipment and in feed. Future work will test these products with different strains of *Salmonella*, and diet types.

Keywords: Salmonella Infantis, Feed Microbial Pathogens, Feed Manufacturing, Feed Sanitizer, Feed

Processing & Products

M90 Advantages of using automated feather sexing system to allow for separate sex rearing on performance on the farm and improved saving at the processing plant Ali Matloub^{*2}, Liz Turpin¹ ¹TARGAN, BioProcess, Raleigh, North Carolina, United States; ²TARGAN, Sales & Marketing, Montreal, Quebec, Canada

TARGAN has developed and commercialized an automatic feather sexing system for use in broiler hatcheries. The device has

high accuracy and throughput, further eliminating the need for large crews to process birds. The purpose of this research is to explore the potential outcomes of rearing male and female broilers separately on performance parameters from the hatchery, on the farm and at the processing plant. We conducted a metadata analysis of published articles, data from genetics companies, Agri Stats, internal data and industry experts to evaluate the impacts of

automated feather sexing on the hatchery and the impact of rearing birds separately. The analysis focused on throughput and accuracy at the hatchery, uniformity of body weights, adjusted FCR, impacts on 1st processing, and yield improvements on wing, white meat, and boneless thigh in processing plants with a focus on Ross 708 birds. Ross 708 flocks are significantly different when comparing sex separation to straight run rearing, where both males and females have higher growth rates. Sex separated rearing is significantly different for feed intake, where females groups consume less than a straight run, but males consume significantly more. Uniformity is also improved with individual sexes with much lower CV%'s when compared to straight run rearing. Males outperform feed performance metrics compared to straight run houses. Furthermore, behavior is not significantly different between straight run and sex separated rearing practices. Allowing for little to no change in commercial set-ups to incorporate sex separated rearing. Similar trends are seen with improvements in yields and processing plant parameters with separately raised birds resulting in processing plant cost saving. This has been reported in the literature, matches Aviagen data, and reports from the field resulting in optimized performance in the field with separately raised birds. The analysis concluded that feather sexing automatically can improve hatchery performance, parameters on the farm and in the processing plant. Raising broilers separately can result in better feed efficiency on the farm, reduced variability in live weights, and increase yields in the plant.

Keywords: feather sexing, yeild, FCR

M91 Dietary supplementation of zinc on growth performance, tissue mineral content, skin and carcass quality of broiler chicken A.B.M. Khaleduzzaman*¹, H.M Salim¹, Shariful Haque¹, Zobaidul Kabir², BD Lee³ ¹*Director Production, Department of Livestock Services, Dhaka, Dhaka, Bangladesh;* ²*Ministry of Fisheries and Livestock, Livestock Unit, Dhaka, Bangladesh;* ³*Department of Animal Science and Biotechnology, Animal Science, Chungnam, Korea (the Republic of)*

Dietary zinc is an essential trace mineral for poultry and plays a vital role in skin nucleic acid, keratin and collagen synthesis. A research was conducted to determine the effect of sources and levels of zinc on tissue zinc concentration and the carcass quality of broiler chicken. A total of 6,000 1-day-old unsexed broiler chicks were allotted randomly to four floor pens with 1,500 birds per pen. A corn-wheat-soybean meal basal diet (Control) was formulated, and 40 ppm inorganic zinc (40 IZ), 40 ppm organic zinc (40 OZ), and 80 ppm organic zinc (80 OZ) were added to the basal diet to form four dietary treatments. During the 4-wk experimental period, feed and water were provided ad libitum. At the end of the feeding trial, five birds from each pen were randomly selected as five replicates, slaughtered and carcass evaluation was performed. Results showed that zinc supplementations have no effect on BW gain and zinc content in thigh muscle but a significant increase was found in skins from broilers in 80 OZ (P<0.05). The epidermis thickness of tibia and back skin were not affected by the zinc supplementations. However, significant increases of tibia and back skin dermis thickness were found (P<0.05) by zinc supplementation and organic zinc was more effective than inorganic zinc in this respect (P<0.05). The collagen contents of breast and thigh muscle were not affected by dietary zinc supplementation, but organic zinc increased the collagen contents in the back skin of broiler chicken (P<0.05). Shear force values of back skin and breast muscle were not affected by zinc supplementation. It is concluded that dietary supplementation of organic zinc is more effective in increasing the skin zinc

concentration, and improving skin quality of broiler chicken than inorganic zinc.

Keywords: Growth performance of Broiler, Zinc supplementation, Tissue zinc, Collagen synthesis, Carcass quality

M92 Effects of duck breeds on egg functionality through fourteen days of egg storage Matthew Hughes*¹, Brigid McCrea², Karla V. Casco¹, Javier Garcia³, Dianna Bourassa¹ ¹*Auburn University, Auburn, Alabama, United States;* ²*Alabama Cooperative Extension System, Auburn, Alabama, United States;* ³*USDA-ARS, Egg and Poultry Production Safety Research Unit, Athens, Georgia, United States*

In recent years, duck egg consumption in the US has increased. Duck eggs have been sought out due to their larger size and better baking functionality. The goal of this study was to evaluate how egg functionality differs between duck breeds and how they change during storage. Eggs from 5 breeds (Jumbo Pekin-JP, Rouen-R, Pekin-P, Mallard-M, Khaki Campbell-KC) were obtained from a commercial duck producer, raised on the same farm with the same diet. Eggs were kept at 3°C/93%RH for the duration of the trial. Eggs from each breed were divided into 3 days of sampling (0,7,14). Egg functionality tests performed were angel food cake (volume), custard (weep, height, texture), and mayonnaise (yolk and mayonnaise color (LAB), texture). Statistical analysis was performed using the General Linear Models procedure with means separated by LS Means. JP angel food cake volume was higher than all other breeds (P<0.0001). Volume increased from D0-D7 and stayed the same from D7-D14 (P<0.0001). Custard weep was highest for R followed by M, P, JP, and KC (P=0.0021). Weep increased from D0-D7 and decreased from D7-D14 (P=0.0011). Custard height was not different between breeds (P=0.0687). Height increased from D0-D7 and decreased from D7-D14 (P=0.0003). JP had the firmest custard followed by KC, R, M, and P (P=0.0129). Custard became firmer from D0-D7 and softer from D7-D14 (P=0.0001). M had the lightest yolks followed by JP, P, R, and KC (P=0.0163). JP had the greenest yolks followed by M, P, R, and KC (P<0.0001). R had the yellowest yolks followed by KC, M, JP, and P (P<0.0001). Yolks became lighter and greener over time (P<0.0001, P<0.0001). Yolk b* value did not change over time (P=0.2995). Mayonnaise L* value did not differ by breed or day (P=0.1425, P=0.0700). KC had the greenest mayonnaise followed by R, M, JP, and P (P<0.0001). JP had the yellowest mayonnaise followed by KC, JP, P, and M (P<0.0001). Mayonnaise became less green from D0-D7 and more green from D7-D14 (P=0.0003). Mayonnaise yellowness decreased as eggs aged (P<0.0001). R and P had the firmest mayonnaise followed by KC, JP, and M (P<0.0001). Mayonnaise became softer from D0-D7 and firmer from D7-D14 (P<0.0001). These observed differences between breeds may influence suitability for product preparation purposes.

Keywords: duck, functionality, angel food cake, custard, mayonnaise

M93 Evaluating nozzle types and its effects on water consumption in a poultry processing plant Cameron Smith*¹, Micah T. Black¹, Amit Morey¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*

Water in industrial settings is a commodity with inflated prices being observed across the economy and an increased necessity for safe quality food, and a growing importance for sanitary conditions. Therefore, finding ways to achieve cost savings while furthering process improvement has been a goal for industries internationally. To make achievable cost savings, it has been shown that standardization of nozzle types on high-pressure hoses

make an impact in the efficacy of water usage towards a more sustainable future. Different water hose nozzles that were tested included the following: WaterBoss 750, S151B, RB65, Suttner ST-1000 and an open-end hose. Two trials over two days were conducted where a 6-gallon bucket was filled and timed with a stopwatch during the production and break period. Results from nozzle types showed differences in the volume of gallons used per week (GPW) with minute changes in gallons per minute (GPM). Water volume between the nozzles was compared using Student T-test with significant differences at $p < 0.05$. There were significant differences between the nozzle types but not the two times. The two pressurized pumps offered 650psi at the pump while the hose utilized pressure levels between 550 and 600psi. The psi range affects the nozzle type selection while keep safety a priority due to the pressure rating of the nozzles. The most efficient water range was between 10-12 GPM to achieve cost savings while allowing sanitation technicians the ability to secure a satisfactory sanitation level. With only the sanitation sector, utilizing the standard of water nozzles can generate an increase of hundreds of thousands in savings annually, varying on processing plant size. Standardizing the water nozzles has shown an average reduction by sixty thousand gallons used per sanitation shift.

Keywords: Processing, Water, Sanitation

M94 Effects of broiler genetic strain and diet on growth performance and Wooden Breast incidence Jorge L. Sandoval*¹, J. Wesley Rogers¹, Gerardo A. Abascal-Ponciano¹, Charles W. Starkey², Jessica D. Starkey¹ ¹*Auburn University, Poultry Science Department, Auburn, Alabama, United States;* ²*North American Renderers Association, Scientific and Regulatory Affairs, Alexandria, Virginia, United States*

Understanding of the etiology of the costly broiler chicken meat quality defect, Wooden Breast (WB), is critical to supporting future efforts to develop practical nutritional and/or management interventions to eliminate its prevalence in the broiler industry. To establish a reliable strategy to produce a market-weight broiler flock with normal fillets and 3 WB (mild, moderate, and severe), we found previously that feeding a diet in meal form with a 30% reduction in metabolizable energy, digestible Lys, and digestible Met (R) for the 42-d rearing period did not prevent the development of WB in fast-growing birds (FG), only delayed the timing of its onset compared with those fed a pelleted, corn and soybean meal-based commercial diet (C). Therefore, the inclusion of a "heritage-type," slow-growing broiler strain (SG) as an unaffected control was investigated for feasibility. The objective of this study was to assess the BW, pectoralis major (PM) and biceps femoris (BF) muscle mass, and severity of WB in female SG and FG broilers both fed the C diet (FGC and SGC, respectively) plus the FG broilers fed the R diet (FGR). Birds were reared to 25 d post-hatch in raised-floor pens (4 per pen) bedded with new wood shaving litter. Each day from d 7 to 25, 10 birds per treatment were weighed, euthanized, the WB severity was assessed by manual palpation and the BF and PM muscles were collected and weighed. Data were analyzed as a 1-way ANOVA using SAS (ver. 9.4) PROC GLIMMIX and PDIF for mean separation at $P \leq 0.05$ and FREQ for WB incidence. Bird BW among broiler strains was similar on d 0 ($P = 0.5931$). On d 7 to 10, 12, and 16, FGC bird BW were the heaviest while SGC and FGR were similar ($P < 0.0001$). Though on d 11, 13 to 15, and 17 to 25, FGC birds were the heaviest, SGC were the lightest and FGR intermediate ($P < 0.0001$). From d 7 to 25, FGC birds had the highest and SGC had the lowest PM and BF muscle weights, respectively, with the FGR birds intermediate ($P < 0.0001$). The SGC birds had 100% normal breast fillets from d 0 to 25, while FGC birds were unaffected until d 16 and 100% WB-affected by d

19 ($P < 0.001$). Overall, these results indicate that SGC vs. FGC provides a feasible strategy for producing young broilers for research aimed at understanding WB etiology.

Keywords: Wooden (Woody) Breast, broiler strain, meat quality defect, myopathy model

M95 Advanced oxidation processes effectively attenuated *Campylobacter* species individually and in combination with *Salmonella* Typhimurium in contaminated wastewater Seth O. Adesope*¹, Mark Calson², Nikolay Wooden (Woody) Breast, broiler strain, meat quality defect, myopathy model Barashkov², Casey M. Owens¹, Tomi Obe¹ ¹*University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States;* ²*Micro-Tracers, Inc, San Francisco, California, United States*

Poultry production, a water-intensive sector, generates pathogen-rich wastewater. Conventional disinfection methods, although effective, impose considerable capital and operational costs that burden poultry producers while integrated recycling systems present a promising opportunity for enhancing resources, environmental pollution, and sustainability. This study investigated the use of Advanced Oxidation Processes (AOPs), specifically electrochemical (EC) and photochemical (PC) treatments to reduce *Campylobacter jejuni* (Cj) and *coli* (Cc) individually and combined with *Salmonella* Typhimurium (ST) in contaminated wastewater. In experiment one, Cj and Cc were cultured in Tryptic soy broth (TSB), diluted to 10^6 CFU/mL in 7L contaminated water, and subjected to EC voltage load of 50V and 70V with addition of ammonium sulfate as an electrolyte and ferrous sulfate for AOP enhancement alongside PC using curcumin-coated spheres at concentrations of 0.80% (2X) and 1.6% (4X) for the generation of singlet oxygen and hydrogen peroxide. Samples were collected every 30 min during the disinfection process until 300 min and plated on *Campylobacter* agar that was incubated at 42°C for 48h. In experiment two, Cj was combined with ST, exposed to EC at 70V & PC at 4X, and plated on both *Campylobacter* and XLD agar to assess the combined reduction of pathogens in a mixed population. The results of three replicates were analyzed using ANOVA and Tukey's HSD ($p \leq 0.05$) in JMP. Cj was completely attenuated after 150 min and 120 min of treatments with EC at 50V and 70V, respectively regardless of PC levels ($P > 0.05$, 2X & 4X), indicating a 30-min accelerated attenuation at the higher EC level. Cc was slightly different but followed a similar pattern, with attenuation in 210 min (50V) and 180 min (70V) regardless of the PC levels ($p > 0.05$, 2X & 4X). In a mixed population, Cj and ST exhibited complete attenuation in 120 min, establishing an optimal condition for effectively removing both pathogens in wastewater. Our findings suggest that combined EC and PC treatments effectively killed *Campylobacter* and *Salmonella* in contaminated water, highlighting the potential of AOPs as a sustainable poultry wastewater disinfection strategy, promoting microbial safety, water recycling, and reuse.

Keywords: Campylobacter, Salmonella, Advanced Oxidation Processes (AOPs), Poultry Processing Wastewater, Electrochemical and Photochemical Processes

M96 The effect of silvopasture (vs indoor) environment and broiler breed on meat quality Jean C. Caceres*¹, Camila Hammel Sobreira¹, Afsana Rahaman Munmun², Christina S. Sigmon¹, Allison N. Pullin¹, Jesse L. Grimes¹, Lin L. Walker¹, Yan L. Campbell¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*North Carolina State University, Food, Bioprocessing &*

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Outdoor access promotes movement and potentially better muscle development in broilers, which can influence the quality of the chicken meat produced. This study investigated the effects of 2 broiler production systems, silvopasture (SP) and conventional (CV), and 2 breeds (commercial Ross708 and slow-growing Sasso) on meat quality, including weight, pH, color, and the incidence of woody breast (WB), white stripping (WS) and spaghetti meat (SM) myopathies. A total of 500 birds of each breed were randomly placed in either SP or CV housing, with 5 pens in each system (50 birds/pen). Ross708 (10 birds/pen) were processed at 6 wks and Sasso birds (10 birds/pen) were processed at 8 wks to achieve their targeted weight for each breed. During processing, bird weight, hot carcass weight, cutup weight, pH (15min, 2h, 24h), and color at 24h, as well as WB, WS, and SM myopathies were measured during the day of processing or 24h postmortem. Data were analyzed using 2-way ANOVA and GLM in a 2x2 factorial design by SAS 9.4 with Duncan's multiple range test for mean separation. There were differences ($p < 0.05$) between the production system and breed in weights. In all cases, SP birds

had lower weights compared to CV raised birds (e.g. Live weight: SP=2.70 kg vs. CV=2.84 kg, $p < 0.05$). Also, Sasso birds weighed less than Ross708 birds in both systems ($p < 0.05$). However, SP birds showed a lower pH within 24h of processing (SP pH=5.94, CV pH=6.00, $p < 0.05$). The myopathy scoring showed that meat from Ross708 birds had a greater incidence of WB and WS and a low SM incidence, while meat from Sasso did not have any WB or SM, and very mild WS. Meat from SP system had lighter thigh color compared to CV system for both breeds, and Sasso breasts were darker, redder, less yellow compared to Ross708 ($p < 0.05$). The production system and breed impacted meat quality traits. Meat from the SP system of the Sasso birds showed no WB and SM and very mild WS myopathy conditions, which may favor product quality and consumer acceptance and reduce product defects/downgrades for sustainable broiler meat production. Further investigation should be conducted to evaluate the effects of the housing system and breed for other meat quality attributes such as cook loss, texture, composition, oxidation rate, etc.

Keywords: Rearing Systems, Silvopastoral, Welfare, Broiler, Meat Quality

Metabolism & Nutrition II: General Nutrition

M97 Impact of feed form and supplementary feeding systems on crop fill and early growth performance of broilers Shamar T. Simon*¹, Neketa Hughes², Randy Domer², Doreon Wilson¹, Colwayne Morris³ ¹*University of Guyana, Faculty of Agriculture and Forestry, Georgetown, Guyana;* ²*University of Auburn, Poultry Science Department, Auburn, Alabama, United States;* ³*Zinpro Corporation, North America Poultry Department, Eden Prairie, Minnesota, United States*

The crop serves as a temporary storage organ that influences feed passage to the gizzard. Early crop fill may determine the growth potential of broiler chicks. Two floor pen studies were conducted to evaluate physical feed form and supplementary feeding systems impact on crop fill and early growth performance in broiler chickens during the first 7 Days. A randomized complete block design was employed using 300 Ross 308 broiler chicks, divided into four blocks with two treatments, four replicates per treatment and each replicate consisting of 25 birds. Chicks were randomly allocated into pens with either crumble or mash feed and two different supplemental feeders. The primary objective of the study was to evaluate the impact of physical feed form and supplementary feeding systems on crop fill and early growth performance in broiler chickens during the first 7 days of production. Experiment 1 assessed the impact of Crumble (TRT1) and Mash (TRT2) feeds, while Experiment 2 examined the use of turbo feeders with egg trays (TRT A) versus turbo feeders alone (TRT B). Crop fill percentages and diameters were measured at 12, 24, and 48 hours post-placement, with performance parameters evaluated on d 3 and d 7. Data analysis using one-way ANOVA showed no significant differences ($P > 0.05$) between treatment groups in Experiment 1 for crop fill. In Experiment 2, birds using only turbo feeders recorded higher ($P < 0.05$) crop fill percentages at 48 hours, while birds with both feeding methods showed larger crop diameters at 12 hours. Performance analysis revealed that crumble-fed birds had higher ($P < 0.05$) body weight and daily weight gain on d 3 than those on a mash diet. Feed conversion ratio (FCR) differed in Experiment 2 on d 3, with birds using only turbo feeders being more efficient. In conclusion, while feed form did not impact crop fill, it influenced early growth performance. The type of supplemental feeder affected feed utilization, with turbo feeders alone yielding more efficient FCR. Moreover, feed form impacts early weight gain, and the type of supplemental feeder may influence feed utilization efficiency.

Keywords: Feed form, Crop fill, Broilers, Turbo feeder, Supplementary Feeding

M98 Ileal amino acid digestibility and relative gene expression of ileal nutrient transporter, and pectoralis protein synthesis and degradation in broiler chickens fed reduced-crude protein diets containing soybean meal, canola meal, or corn dried distiller's grains with solubles under coccidiosis vaccination June Hyeok Yoon*¹, Adeleye M. Ajao¹, Revathi Shamugasundaram², Adelumola Oladeinde², Oluayinka Olukosi¹ ¹*University of Georgia, Athens, Georgia, United States;* ²*USDA-ARS, Athens, Georgia, United States*

The objective of the study was to investigate the effect of reduced-crude protein (RCP) diets with soybean meal (SBM), canola meal (CM), or corn-dried distiller's grains with solubles (cDDGS) on ileal amino acid (AA) digestibility and relative gene expression of protein utilization in pectoralis major of broilers under coccidiosis vaccination. A total of 1,296 male chicks were allocated in a 4 × 2 factorial arrangement (diet × vaccination) with 6 replicates. All birds were fed a starter diet from d 0 to 7. Four experimental diets were formulated for the grower (d 7-28) and finisher (d 28-42) phases, namely positive control (PC) corn-SBM-based diet, with 200 or 180 g/kg CP for the grower and finisher phases, negative control (NC) with 40 g/kg CP lower than PC (NC-SBM), an NC diet where 80 g/kg CM replaced 60 g/kg SBM (NC-CM), and an NC diet where 100 g/kg cDDGS replaced 50 g/kg SBM (NC-cDDGS). All diets were isocaloric and formulated to have similar digestible AA concentrations. On d 0, half of the birds were vaccinated with Coccivac B52. Ileal digesta were collected from 2 birds per pen and pooled to generate 6 digesta samples for the non-vaccinated and vaccinated groups on d 7. Ileal digesta on d 15 and 23 were collected from 5 birds per pen. Jejunal tissue and breast muscle were collected on d 23 for relative gene expression of nutrient transporter and protein utilization. On d 7, vaccination numerically decreased the ileal AA digestibility. Ileal digestibility of N, His, Ile, Lys, Phe, and Val in NC-cDDGS was lower ($P < 0.05$) compared to the PC diet on d 15. There was no interaction effect on ileal AA digestibility on d 23; the ileal digestibility of N, His, Ile, Lys, and Phe in NC-CM and NC-cDDGS was lower ($P < 0.05$) than in the PC diet. There were no treatment differences in the relative gene expression of jejunal peptide and AA transporter,

however, protein synthesis gene (EIF4EBP1) expression was lower ($P < 0.05$) in the vaccinated group compared to the non-vaccinated group on d 23. In conclusion, reduced-CP diets with CM and cDDGS may lead to lower ileal AA digestibility compared to the standard-protein diet. However, protein utilization genes in the pectoralis were not affected by the dietary treatments, irrespective of the coccidiosis vaccination.

Keywords: Reduced Protein, Digestibility, Vaccination, Coccidiosis, Broiler

M99 The effect of low-protein soybean meals with graded inclusion levels of soyhull on nutrient utilization, cecal short-chain fatty acid profile, and jejunal gene expression in broiler chickens challenged with mixed *Eimeria* spp. Bhargavi Kasireddy*¹, Iyabo W. Oluseyifunmi¹, Revathi Shamugasundaram², Adelumola Oladeinde², Oluyinka Olukosi¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²USDA-ARS, Athens, Georgia, United States

Water-soluble fiber can act as a substrate for intestinal microbiota to synthesize short-chain fatty acids (SCFA), providing energy to intestinal cells, improving nutrient digestibility and shift the fermentation patterns, improving gut health. This 42-d study investigated the effects of standard soybean meal (SSBM, 464 g/kg crude protein) or low-protein SBM (LPSBM) on ileal nutrient digestibility, SCFA profile, relative mRNA expression of jejunal tight junction and nutrient transporters genes 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. Factors were the four corn-based diets with challenge (CH) or without challenge (NCH). All diets were formulated to have the same levels of metabolizable energy, crude protein and standardized digestible amino acid. Ileal digesta were collected on d 14 and 21 to assess nutrient digestibility before and after 7 d post-*Eimeria* challenge. On d 21, cecal contents were analyzed for SCFA, and jejunal tissue for tight junction proteins and nutrient transporter genes. There were no significant *Eimeria* x diet interactions for nutrient digestibility on d 21 and no treatment effects were observed on d14. *Eimeria* CH significantly reduced ($P < 0.05$) the digestibility of N and amino acids except for Cys and Trp, which were not affected on d 21. The N digestibility was greater ($P < 0.05$) for SSBM and LPSBM2 than LPSBM3. There were no significant interaction or diet main effects for cecal SCFA; however, CH birds had significantly higher ($p < 0.05$) cecal content of propionate, isobutyrate, isovalerate, and valerate. Furthermore, the CH birds had lower ($P < 0.05$) relative expression of Pep T1, BO+AT, EAAT3, GLUT1, and OCLDN genes compared to NCH groups. In conclusion, using LPSBM with 417 g/kg had a comparable effect to SSBM in terms of effects on profile of cecal microbial metabolites and nutrient utilization.

Keywords: *Eimeria*, low protein soybean meal, nutrient digestibility, SCFA, gut health

Metabolism & Nutrition III: General Nutrition

M100 Age-related energy values of canola meal for broiler chickens determined using the regression method Olatunde I. Awonyemi*¹, Olayiwola Adeola¹ ¹Purdue University, Animal Sciences, West Lafayette, Indiana, United States

A study was conducted to determine the ileal digestible energy (IDE), metabolizable energy (ME) and nitrogen-corrected ME (ME_N) contents of canola meal (CM) for broiler chickens on days 8, 14, and 20 post hatching using the regression method. Six hundred and twenty-four male Cobb 500 broilers were fed 3 experimental diets in which canola meal (CM) replaced energy ingredients in a corn-soybean meal-based reference diet at 0, 100, or 200 g/kg. The 3 diets were fed during the last 5 days of each of the 3 post hatching days 8, 14, or 20 in a 3 x 3 factorial arrangement. Chicks were fed with experimental diets, grouped by weight into 8 replicates per treatment in a randomized complete block design. Excreta were collected during the last 3 days of each feeding post hatching age and ileal digesta on the last day of each period. The digestibility of dry matter (DM), nitrogen (N), energy, and IDE of the diet linearly decreased ($P < 0.05$) with increasing dietary levels of CM. Digestibility of DM, N linearly decreased ($P < 0.001$) and a quadratic reduction ($P < 0.05$) in energy and IDE with increasing age. There were both linear and quadratic increase in total tract retention of DM, N, energy, ME and ME_N as the feeding age increased and linear decrease ($P < 0.001$) at increasing levels of CM inclusion. Regression-derived ME_N value of CM as the age of birds increased were 1630, 2240 and 1760 kcal/kg DM with the corresponding IDE values of 2220, 2230 and 2910 kcal/kg DM and ME values of 1810, 2490, 2000 kcal/kg DM for 8, 14 and 20 d, respectively. In conclusion, the study showed that utilization of energy and nitrogen in the CM is influenced by the broiler chicken age and inclusion levels, the energy utilization decreased with increasing inclusion levels of canola meal and increased with age of broiler chickens.

Keywords: broiler, canola meal, age, energy, regression

M101 Effect of feed form, energy level, and conditioning temperature on broiler performance, feed wastage, and nutrient digestibility from 1 to 21 days of age Jose R. Hernandez*¹, Joseph P. Gulizia¹, Jose I. Vargas¹, Eva G. Guzman¹, Samuel J. Rochell¹, Ruediger Hauck¹, Wilmer J. Pacheco¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States

This experiment evaluated the effect of metabolizable energy (ME) and feed form on broiler performance, feed wastage (FW), and nutrient digestibility from 1 to 21 d of age. A total of 648 Ross 708 male broilers were randomly distributed in 72 battery cages (9 birds/cage) and assigned to 6 treatments (12 replicates/treatment). Starter diets were formulated to contain 2 ME levels (2,979 [E2979] and 2,875 kcal/kg [E2875]). Both diets were fed as mash and crumbles conditioned to 85°C and 90°C. Body weight gain (BWG) and feed intake (FI) were determined at 10 and 21 d of age and FCR was adjusted for mortality. On d 10, 15, and 18, the feed spilled was collected from trays placed under each cage to calculate FW. On d 21, ileal digesta (7 birds/cage) was collected to calculate the apparent ileal digestibility (AID) of crude protein (CP) and starch. Data were analyzed as a 2 x 3 factorial arrangement (ME level x feed form) using the GLIMMIX procedure of SAS. Means were separated using Tukey's HSD test and statistical significance was considered at $P < 0.05$. Broilers fed E2979 diets had higher ($P < 0.05$) BWG (868 vs. 809 g) and lower ($P < 0.05$) FCR (1.28 vs. 1.36 g/g) from 1-21 d compared to broilers fed E2875 diets. Broilers fed mash and crumbles conditioned at 85°C with E2979 exhibited higher ($P < 0.05$) BWG on d 21 d compared to broilers fed mash and crumbles conditioned at 85°C with E2875. However, when broilers were fed crumbles conditioned at 90°C, the ME level of the diet did not influence BWG ($P > 0.05$). Additionally, broilers fed mash diets had lower

($P < 0.05$) FI (1049 vs. 1223 and 1215 g) and higher ($P < 0.05$) FCR (1.34 vs. 1.30 and 1.32 g/g) and FW (28.6 vs. 2.3 and 3.0 g/kg) from 1-21 d compared to broilers fed crumbles conditioned to either 85 or 90°C. Broilers fed E2979 diets had improved ($P < 0.05$) AID of CP compared to broilers fed E2875 diets. Broilers fed mash diets had lower AID of CP compared to broilers fed crumbles conditioned to either 85 or 90°C. Starch digestibility was lower ($P < 0.05$) in broilers fed crumbles conditioned to 90°C compared to broilers fed crumbles conditioned to 85°C and mash. Overall, broilers responded to both ME level and feed form with the best performance observed when broilers were fed E2979 crumbles conditioned to 85°C.

Keywords: energy, feed form, broilers, growth performance, feed wastage

M102 The effect of varying pellet diameters and fines inclusion of growth performance of broilers throughout all phases of growth Carter D. Minson^{*1}, Mason Engnell¹, Walter Friesen¹, Diego Lopez¹, Allison Blomme¹, Victoria Wilson¹, Charles Stark¹, Chad Paulk¹, Scott Beyer² ¹*Kansas State University, Grain Science and Industry, Manhattan, Kansas, United States;* ²*Kansas State University, Animal Science and Industry, Manhattan, Kansas, United States*

In the poultry industry, most feed is manufactured in pellet form utilizing a pellet die that produces pellets with approximately a 4 to 4.8 mm diameter. However, limited research has been conducted on how pellet diameters influence subsequent pellet quality and broiler growth performance. Therefore, the objective of this study was to evaluate the effects of varying pellet diameter and percentage of pellet fines on broiler growth performance from 1 to 49 d of age (starter, grower, and finisher). A total of 720 male one-day old broiler chick (Cobb breeder by-product; C500 off-sex males) were allotted into 60 floor pens on day 0. For the starter periods (d 0 to 14), treatments consisted of a common diet pelleted using either a 4.8 mm or 6.4 mm diameter die and then crumbled. For the grower (d 14 to 28) and finisher periods (d 28 to 49), treatments were arranged as a 2 × 3 factorial with factors consisting of pellet diameter (4.8 mm and 6.4 mm) and pellet fines inclusion (0, 30, and 60% fines added to screened pellets). Pellet fines treatments were randomly assigned to pens within previously fed die diameter treatments. All data was analyzed using the GLIMMIX procedure of SAS 9.4. Pen was considered the experimental unit and pen location the random effect. In the starter period, broilers fed diets pelleted using the 4.8 mm diameter die and then crumbled had improved ($P < 0.003$) FI, BWG, and FCR compared to those fed crumbles produced from 6.4 mm diameter die. For the grower and finisher periods (d 14-49) and overall (d 0-49), there was no evidence of pellet diameter × fines interaction and no evidence of main effects for BWG and FI. Broilers fed diets with increasing percentage fines had improved ($P < 0.01$) FCR. In conclusion, feeding crumbles made from a 6.4 mm die during the starter period reduced growth performance of broilers. However, throughout the total grow-out period (d 1-49), die diameter did not influence BWG, FI, or FCR. In addition, increasing the percentage of fines improved FCR. This data demonstrated the potential to increase pellet die diameter to 6.4 mm without negatively impacting growth performance of broilers. Further investigation is needed to determine why feeding increased percentage of fines resulted in improved broiler FCR.

Keywords: Pellet Diameter, Fines, Performance, Broiler

M103 Effect of cumulative nutrient intake on body weight, feed conversion ratio, and mortality of broilers raised to 35 d under commercial tropical conditions Gustavo Quintana^{*1}, Edgar O. Oviedo-Rondón¹, Juan R. Ruiz-Ramirez², Luis C.

Bernal-Arango², Gustavo Martinez-Bernal² ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*Grupo BIOS Inc, Envigado, Colombia*

This data analysis evaluated the impact of energy and nutrient intake (NI) on broiler performance metrics such as BW, feed conversion ratio (FCR), and cumulative mortality under commercial tropical environments, addressing a gap outside controlled research conditions. Weekly performance records from 468 male and 461 female Ross 308 AP broiler flocks, totaling 31.9 million chicks placed between 2018 and 2019, were analyzed to assess cumulative intake (CI) of metabolizable energy (ME), crude protein (CP), digestible lysine (dLys), calcium (Ca), phosphorus (P), and fat across three feeding phases over 35 days. Nutrient intake (NI) by phase and cumulative nutrient intake (CNI) were calculated for each flock and integrated with performance data. Correlation analysis, linear, quadratic, and multiple linear regression (MLR) models were fit using performance indicators as responses to NI. Decision trees were fit to model BW and FCR at 35 d using key management factors and CNI. All data analyses were performed in R and JMP Pro 15 software. No significant correlations or linear effects ($P > 0.05$) were detected between performance variables and NI in the pre-starter and starter phases. However, in the grower phase, positive correlations ($P < 0.05$; $r = 0.44-0.74$) and linear effects ($P < 0.001$) were observed for BW in both sexes with all nutrients except Ca. Correlations for FCR in males were mild ($r < 0.47$), and linear models had low R^2 values, limiting their predictive ability. MLR models indicated positive ME, Ca, and P effects on BW at 35 d for both sexes, while CP showed a negative effect. Single-phase and cumulative NI explained less than 16% of FCR variability. Decision trees indicated that CI of ME, P, dLys, fat, farm altitude, and distance from the hatchery influenced ($P < 0.001$) BW ($R^2 = 0.55-0.71$) and FCR ($R^2 = 0.40-0.48$). In conclusion, BW at 35 d was affected by NI during the grower phase, with ME and dLys as primary nutritional drivers, while early-phase NI showed minimal impact on FCR and BW. Mortality was unaffected ($P > 0.05$) by the nutrients studied. Decision tree analysis underscored the importance of environmental and management factors combined with NI to optimize broiler performance in commercial tropical conditions, offering insights for improved feeding strategies.

Keywords: Data analytics, Nutrition, Environment, Management

M104 Effects of feed form (mash vs. pellets) on turkey hens performance Luis Giron^{*1}, Darby R. Boontarue¹, Mireia Molins¹, Sophia Bianchi¹, John Boney¹ ¹*Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States*

Turkey production plays a critical role in the poultry industry, with a focus on optimizing growth and performance to meet market demands. Recent research has highlighted the impact of feed form on the performance of heavy turkey hens. This study aimed to investigate the effects of two feed forms—mash and pelleted feed—during d 28 to 84 on performance, including body weight (BW), live weight gain (LWG), feed intake (FI), and feed conversion ratio (FCR). A total of 464 Nicholas Select turkey hens were placed in a randomized complete block design across 16 pens containing 29 birds/pen. Both treatments were fed a common crumble starter diet from 0 to 28 d, averaging 845 µm. From 28 to 84 d, birds were divided into two treatment groups: 1) pellets (average % pellet = 57%) and 2) mash (average particle size = 1,030 µm) with identical feed composition. The GLM procedure of SAS was used to perform ANOVA, and the frequency procedure of SAS was used to describe the occurrence of pendulous crop. The results indicated that pelleted diets

significantly improved FCR during d 28–56; ($P=0.009$), d 56–84 ($P=0.005$), and over the entire period of 28–84 d ($P=0.001$) compared to mash feed. Notably, turkeys fed mash demonstrated comparable BW, LWG, and FI to those receiving pelleted feed. However, it is important to note that mash feed increased the incidence of pendulous crops (17% of mash-fed hens), which negatively impacted turkey performance and may pose challenges in the commercial turkey industry. These findings demonstrate that providing pelleted feed enhanced FCR in light hen production from d 28–84 but did not affect BW, LWG, or FI when compared to those fed mash. It is hypothesized that feed form will influence FCR and BW after d 84 due to previous hen turkey feed form work from our lab suggesting that d 85–112 is a critical growth phase, where feed form strongly influences FCR and final BW of heavy hens.

Keywords: Turkey production, Mash, Pellet, Growth, Performance

M105 Impact of dietary fiber types and concentrations on jejunal and cecal gene expression in broilers with a subclinical enteric challenge Rana Waqar Tabish*¹, Yang Lin¹, Samuel J. Rochell¹, Wilmer J. Pacheco¹, Matthew Bailey¹, William A. Dozier¹, Klinton W. McCafferty², Ruediger Hauck^{1,3} ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²USDA-ARS-Poultry Research Unit, Mississippi State, Mississippi, United States; ³Auburn University, Department of Pathobiology, Auburn, Alabama, United States

In poultry diets, insoluble fibers enhance gizzard retention and digestive enzyme activity, while soluble fibers ferment in the ceca, producing short-chain fatty acids that lower pH and inhibit *Clostridium perfringens*. This study examined the impact of different fiber types and concentrations on jejunal and cecal gene expression in broilers experiencing a subclinical enteric challenge. Eight dietary treatments were administered from the day of hatch, consisting of six fiber-supplemented diets and two controls: unchallenged and challenged, both without supplemental fiber. Fiber sources included 3% oat hulls, 3% soy hulls, and combinations of 1.5% soy or oat hulls with either 1.5% wheat middling or sugar beet pulp. Birds in challenged groups received a tenfold dose of a live coccidia vaccine on d 14 and 10^8 CFU of *C. perfringens* on d 18. Jejunal and cecal mucosa scrapings from 9 birds per treatment were collected on d 21 for transcriptome analysis. Differentially expressed genes were analyzed by quasi-likelihood F-test in edgeR (adjusted $p < 0.05$, $\log_{2}FC > 1.5$), with gene ontology, KEGG pathway enrichment, and protein-protein interaction analyses of significant genes. Compared to challenged control, fiber-supplemented treatments showed distinct gene expression, especially in the jejunum, with most treatments inducing unique profiles. The exception was oat hulls, which resembled the challenged control in both tissues. In the jejunum, biological process enrichment highlighted the regulation of lipid metabolism, particularly its localization and storage. In the ceca, a strong defense response to bacteria and immune processes was noted. The KEGG analysis showed regulation of the peroxisome proliferator-activated receptor signaling pathway in the jejunum

across treatments, alongside fatty acid and amino acid metabolism. In the ceca, linolenic and arachidonic acid metabolism and efferocytosis pathways were enriched. Twenty-one genes were consistently differentially expressed in the jejunum across fiber treatments, linking to plasma lipoprotein remodeling and enterocyte cholesterol metabolism. These findings suggest that fiber types influence immune responses and nutrient metabolism, offering a dietary strategy to enhance gut health and disease resistance in poultry.

Keywords: dietary fiber, gene expression, broilers, enteric infection, gut health

M106 Exploration of the antinutrient variability of corn, wheat, and soybean meal Caleb M. Marshall*¹, Virginia Blavillain², Edgar O. Oviedo-Rondón¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²AB Vista, Plantation, Florida, United States

Variability of feed ingredient quality is a challenge for precise nutrition. A commercial dataset of NIRS predictions (AB Vista) containing different sources of corn, wheat, and soybean meal (SBM) was used to analyze the impact of nutrient and antinutrient levels on feed formulation. Data from the United States (USA) and Brazil (BR), obtained from 2019 to 2024, included 54,801 corn ($N_{USA} = 17,390$ and $N_{BR} = 37,411$), 1,430 wheat ($N_{USA} = 564$ and $N_{BR} = 866$), and 60,196 SBM samples ($N_{USA} = 16,170$ and $N_{BR} = 44,026$). All analytical variables for each ingredient were used to partition the data within each country into three clusters of similar nutrient profiles using k-means. One-way ANOVA was used to compare nutrient and antinutrient (Phytic P; PP and Total Non-Starch Polysaccharide; tNSP) content among clusters within each country and ingredient. Mean separation was done using Tukey's HSD. Stepwise differences ($P < 0.05$) were observed for tNSP and PP clusters within each country and ingredient, with few exceptions. Two clusters for BR wheat, comprising 81.80% of the samples, had the same PP content (0.23%), lower ($P < 0.05$) than the highest PP cluster (0.27%). Similarly, two clusters for USA SBM, grouping 92.5% of the samples, had the same mean PP (0.41%), lower ($P < 0.05$) than the highest PP cluster (0.43%). Diets, following Ross 708 recommendations for three feeding phases were formulated. Nutrient values from clusters with the highest PP and tNSP per country were compared with country means. Formulating with the highest PP clusters, representing 8.53 and 49.95% of corn, and 25.69 and 7.50% of SBM samples (BR and USA, respectively), increased feed PP by 0.02% pts to 0.26% for BR and 0.01% pt to 0.26% for USA. For the high tNSP clusters, congregating 37.02 and 15.70% of corn and 29.65 and 7.50% of SBM samples, increased mean tNSP content 1.17% pts to 10.26% for BR and 2.12% pts to 10.76% for USA. In conclusion, clustering ingredients based on nutrient profile can help assess patterns in nutrient variability. Identifying feedstuff cluster characteristics can aid in making better formulation decisions. For example, ensuring accurate PP and tNSP content estimation to decide enzyme inclusion and potentially minimizing gut health issues.

Keywords: NSP, phytate, corn, soybean meal, wheat

Metabolism & Nutrition IV: General Nutrition

M107 Differentiating energy and amino acids nutritional value from soybean meal from various origins fed to broilers Ashir F. Atoó*¹, Crystal Levesque², Jorge Y. Perez-Palencia¹ ¹South Dakota State University, Animal Science, Brookings, South Dakota, United States; ²South Dakota State

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This study aimed to determine energy (Exp. 1) and amino acids (AA) [Exp. 2] nutritional value in soybean meal (SBM) from 7 origins [US: Northwest (NW), Northeast (NE), South, and East; Brazil (Bra), Argentina (Arg), and a reimported US (Re-US)] fed

to broiler chickens. In Exp. 1, a corn-based reference diet and 7 corn-based diets containing the different SBM sources at 30% were formulated. A total 320 one-day-old male broiler chicks (5/cage) were fed a commercial starter diet from d0 to 14; from d14 to 22 each pen received one of the 8 dietary treatments in a completely randomized design (CRD). On d19 to 22, total excreted feces were collected from each cage and, together with the feed samples, analyzed for dry matter (DM), gross energy (GE), crude protein (CP), crude fat (CF), crude fiber (CFi), and titanium (Ti). In Exp. 2, a nitrogen-free diet and 7 corn starch-based diets were formulated with the different SBM sources at 30%. A total of 416 one-day-old male broiler chicks were used (6 or 7 birds/cage). Each cage was assigned to one of the 8 dietary treatments from either d14 to 21 (50% of cages, group 1) or d21 to 28 (remaining 50%, group 2) in a CRD. On d22 and day 29 (group 1 and 2, respectively), birds were euthanized, ileal digesta was collected and analyzed for CP, AA, and Ti. Data were analyzed using MIXED procedure of SAS with cage as the experimental unit. In Exp. 1, there was no difference ($P>0.05$) in feed intake, GE digestibility, apparent metabolizable energy, and apparent total tract digestibility (ATTD) of CP, CF, CFi, NDF, and starch across SBM origins. The SBM from Arg had the highest ($P<0.05$) ATTD of DM (85.98%) while US NE had the lowest (77.43%). In Exp. 2, there were no differences ($P>0.05$) in the standardized ileal digestibility (SID) of CP, Arg, His, Ile, Leu, Met, Phe, or Trp among the different origins. However, SBM from Bra had the lowest ($P<0.05$) SID of Met+Cys (88.1%) and Thr (84.34%) and tended to have the lowest ($P=0.06$) SID Lys (88.58%). Comparing just the US origins, SBM from NW had the lowest ($P<0.05$) SID of Met+Cys and tended ($P>0.05$) to have the lowest SID of Met and Thr. In conclusion, the nutritional value of SBM for broilers was not different across U.S. origins with minor differences compared to Argentina and Brazil.

Keywords: amino acids, apparent metabolizable energy, broilers, digestibility, soybean meal

M108 Soybean Genotype Amino Acid Selection: Impacts on diet linear programming and broiler performance Savannah Wells-Crafton^{*1}, Kenneth B. Nelson¹, Virginia G. Slick¹, Elizabeth S. Greene¹, Michael T. Kidd¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States

This experiment aimed to determine the feeding value of soybean meal (SBM) produced from a variety of soybeans bred for increased amino acid content, compared to soybean meals from conventional soybeans. The nutritionally enhanced variety of soybeans (IP-AA) was processed by Insta-Pro International alongside conventional soybeans sourced by Insta-pro (IP-Conv.) using high shear dry extrusion. Additionally, conventional solvent extracted soybean meal (UA-Conv.) were acquired from University of Arkansas Poultry Feed Mill. Prior to diet formulation, all soy ingredients were submitted for total amino acid and proximate analysis. Three corn and soy-based diets were formulated based on energy value and amino acid profile with each diet containing a different soybean meal source. Diet 1 contained UA-Conv. SBM, Diet 2 contained IP-Conv. SBM, and Diet 3 contained IP-AA SBM. Diets were fed to 540 Cobb 500 male broilers placed in 10 replicate pens of 18 birds and live performance was assessed during the starter (0-14d), grower (14-28d), and finisher (28-45d) phases. At d 45, eight birds from each pen were randomly selected and processed for evaluation of carcass traits and incidence of woody breast. Carcass parameters measured include live weight as well as fat, breast, tender, wing, and leg chilled yields. Additionally, 2 birds per pen were assessed for gut integrity using FITC-D oral gavage methods. All performance data were analyzed using a One-way ANOVA using

JMP Pro 18 software with diet as the fixed effect and block as a random effect with pen as the experimental unit. Statistical significance was considered at $P \leq 0.05$. There were no significances observed between the dietary treatments on bird live performance or processing characteristics. The experimental soybean line was able to be incorporated into the diet without decreasing performance while improving least-cost diet formulation. The reduction in diet cost was largely driven by the increased oil content of extruded soybean meals compared to solvent-extracted soybean meal. Moreover, diets containing the experimental soybean line contained the same protein level and amino acid profile with a lower inclusion level of soybean meal and similar crystalline amino acid supplementation.

Keywords: Soybean Meal, Amino Acid, Broiler, Nutrition, Genetic Selection

M109 Impact of sodium bisulfate feed inclusions on broiler performance and processing metrics Samantha S. Plocher^{*1}, Craig Coufal², Julianna Jespersen², Hudson Thames¹, Xue Zhang³, Kelley Wamsley¹ ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²Jones-Hamilton Co., Rock Island, Illinois, United States; ³Mississippi State University, Animal and Dairy Science Department, Mississippi State, Mississippi, United States

Previous research in our lab has demonstrated benefits to broiler performance and processing yields with sodium bisulfate (SB) feed (SBF) inclusion. However, previous trials also simultaneously utilized SB litter application. To better isolate and quantify the impact of SBF use on broiler performance, processing metrics, and breast muscle characteristics, the current experiment utilized SBF inclusion at 0, 0.2, 0.3 and 0.4% without any SB litter application. Dietary treatments were assigned as a randomized complete block design to 1 of 48 floor pens containing 14 Ross 708 male broilers/pen (0.08 m²/bird; 2x dose of live coccidiosis vaccine at placement) and reused litter. Twelve replicate pens were used per treatment, and data were analyzed using SAS 9.4 and $\alpha \leq 0.05$. From d 0-14, birds fed 0.4% SBF had average daily feed intake (ADFI) greater than birds fed 0.2 and 0.3% SBF, but similar to 0% SBF ($P=0.038$). Body weight gain (BWG) and FCR were not different among the treatments for d 0-14. Day 14-28 ADFI was not significantly different among the treatments ($P=0.273$); although, feeding diets with 0.2, 0.3, and 0.4% SBF reduced FCR approximately 2 pts vs. 0% SBF-fed birds ($P=0.036$). The inclusion of SBF did not impact 28-40 and 0-40 d performance ($P>0.05$), but numerical improvements in 0-40 d FCR were found with 0% SBF having the highest FCR (1.435), followed by 0.2, 0.3, and 0.4% SBF ($P=0.072$). At d 41 processing, birds fed 0.3 and 0.4% SBF had consistently higher carcass and breast weight vs. 0 and 0.2% SBF ($P<0.05$). No differences were observed for breast pH (post-chill) and woody breast scoring (0 to 3 scale) among the treatments ($P>0.05$). Breast meat white striping was scored on a scale of 0 to 3. The 0.4% SBF treatment had the lowest percent 0 scores and highest percent 1 scores vs. 0 and 0.3% SBF ($P<0.05$). No differences were observed between any treatments for white striping scores of 2 or 3 ($P>0.05$). Overall, 0.3 and 0.4% SBF improved performance and processing yields without negative impacts to meat quality. Future research is needed to evaluate the impacts of SBF inclusion under various growing conditions and bird ages at processing.

Keywords: Sodium bisulfate, Feed, Broiler performance, Processing, Muscle quality

M110 Maximizing 17 to 28 d growth performance of broilers with and without a subclinical necrotic enteritis challenge through corn particle size and feed quality Jorge Urrutia^{*1},

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Subclinical necrotic enteritis (SNE) limits growth potential and reduces company profitability. Increasing corn particle size (CPS) and feed quality (FQ) may improve gut health and growth performance. Our previous study found that feeding the combination of 900 µm (Medium; M) CPS (MCPS) and High FQ (HFQ) crumbles (3200 µm) improved 0 to 17 d broiler growth. The current objective was to determine the CPS and FQ combination that maximizes 17 to 28 d male Ross YP x 708 broiler growth within 1) an unchallenged experiment (EXP; EXP1) and 2) SNE-challenged EXP (EXP2). All birds were fed a common diet with MCPS+HFQ crumbles from 0 to 17 d. Each EXP was conducted simultaneously in separate facilities, following a 3 x 3 factorial design 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 x 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 obtain different FQ. Birds were reared in floor pens with used litter (0.05 m²/bird); EXP1 had 10 replications, and EXP2 had 5. 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 ($\alpha \leq 0.05$). In EXP1, birds fed MCPS had improved d 17-28 FCR by ~7pts vs. LCPS and HCPS ($P < 0.0001$); this was likely due to decreased FI ($P = 0.004$) and numerically increased BW gain ($P = 0.102$) for MCPS birds. Feeding HFQ did not impact d 17-28 FCR or FI ($P > 0.05$) but numerically improved BW gain ($P = 0.0669$); no CPSxFQ were observed ($P > 0.05$). In EXP2, d 21 lesion scoring (scores of 0-4, 1 bird/pen) revealed no impact of CPS, FQ, or CPSxFQ ($P > 0.05$) for the duodenum or ceca, though all scores (0-4) were recorded. For the jejunum, birds fed either MFQ or HFQ had a reduced % scored as a "3" vs. LFQ birds ($P = 0.0271$); there were no differences found for CPS or CPSxFQ ($P > 0.05$), and no other differences for FQ ($P > 0.05$). No CPS, FQ, or CPSxFQ differences were found for 17-28 d BW gain, FI, FCR, or mortality ($P > 0.05$). Overall, EXP1 data show a more consistent performance improvement with MCPS+HFQ, with less treatment impact observed in EXP2.

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

Metabolism & Nutrition V: Vitamins & Minerals

M111 Phytase supplementation response in broilers fed different levels of non-phytate phosphorus in the pre-experimental starter period Vitor Santos Haetinger*¹, Olayiwola Adeola¹ ¹Purdue University, Department of Animal Science, West Lafayette, Indiana, United States

Broilers fed low phosphorus (P) diets show higher P digestibility in the following days, which could impact phytase efficacy. A study was conducted to investigate the impact of starter diet non-phytate phosphorus (nPP) level on exogenous phytase efficacy in the subsequent grower period. The experiment consisted of a 2 x 3 split-plot design, with 2 starter diets formulated to contain 3 or 4.5 g/kg of nPP (24 replicate cages with 8 birds per cage), and 3 grower diets that differed on the supplementation of phytase (0, 1,000 or 2,000 FTU/kg) (8 replicate cages with 6 birds per cage). The starter diets were fed from day 0 to 11 post hatching, and the grower diets were fed for the following 11 days, the total Ca: total P ratio was the same for all diets. At the end of the experiment, all birds were euthanized to collect ileal digesta samples, and tibia from one bird per cage. Statistical analysis was conducted as a randomized complete block in a split-plot design, with the starter diets as whole plots and grower diets as subplots. Birds fed the higher level of nPP during the starter period had higher ($P < 0.05$) weight gain and feed intake in the starter and grower periods, higher ($P < 0.05$) tibia weight and breaking strength, as well as lower ($P < 0.05$) dry matter and calcium digestibility, and tended to have lower phosphorus (P) digestibility ($P = 0.088$). The increasing levels of phytase led to an increase ($P < 0.05$) in weight gain, feed intake, feed efficiency, P digestibility, tibia weight, and breaking strength. An interaction ($P < 0.05$) was observed between starter diet nPP level and grower diet phytase supplementation for tibia ash content and concentration, specifically, phytase supplementation of 2,000 FTU/kg led to a greater relative increase in tibia ash in the low nPP starter group (18%) than in the high nPP group (5%), such that the low nPP group supplemented with phytase had similar ash content as the normal nPP group supplemented. In conclusion, starter diet nPP levels did not impact phytase effectiveness during the grower phase for growth, nutrient digestibility, and tibia strength. However, phytase increased tibia

ash concentration in birds fed low nPP to a higher extent than in birds fed normal nPP in the starter period.

Keywords: digestibility, phosphorus, phytase

M112 Additivity of phytate-P utilization in broiler diets containing corn, soybean meal, canola meal, or rice bran in the absence or presence of supplemental phytase Opeadura T. Osunbami*¹, Carrie L. Walk², Olayiwola Adeola¹ ¹Purdue University, Animal sciences, West Lafayette, Indiana, United States; ²AB Vista, Marlborough, Wiltshire, SN8 4AN, United Kingdom

The contribution of phytate-P (PP) to total P utilization in the absence of exogenous phytase is often attributed to endogenous phosphatase. However, it is unclear if the additivity assumption holds when considering the combined activity of endogenous phosphatase and exogenous phytase in releasing PP. A study was designed to determine the additivity of PP utilization in broiler chickens when fed 2 different combinations of mixed diets formulated with corn, soybean meal (SBM), canola meal (CM), or rice bran (RB) without or with exogenous phytase. A total of 960 male broiler chickens were used in the trial from d 18 to 21 post-hatching with 8 replicates per treatment. Dietary treatments were arranged as 6 x 2 + 3 factorial with 6 diets based on corn or SBM or CM or RB as the sole source of PP, or a combination of corn, SBM, and RB (CSR), or corn, CM, and RB (CCR), respectively, all with exogenous phytase at 0 or 750 FTU/kg. The 3 remaining diets were formulated with graded concentrations of monosodium phosphate as the only P source at 0 or 9 or 18 g/kg to standardize digestibility measurement and correct for P contribution from non-phytate-P. The inclusion of phytase improved the apparent and standardized ileal digestibility (AID and SID) of P in all diet types except in the corn diet ($P < 0.001$). Phytase supplementation resulted in the variable disappearance of myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (InsP6) across all diet types, leading to an interaction effect ($P < 0.001$). Regardless of supplemental phytase, the CCR diet resulted in lower SID of total P, PP, and InsP6 disappearance than the CSR diet ($P < 0.001$). The SID of PP was additive in the CSR ($P = 0.257$) and CCR diet ($P =$

0.418) when exogenous phytase was absent. Despite improving the SID of PP, phytase supplementation did not exhibit an additive effect in the CCR diet ($P < 0.05$) and showed a tendency towards non-additivity ($P = 0.056$) in the CSR diet. The results revealed that broiler chickens possess the biological potential to hydrolyze PP in the digestive tract when supplemental phytase is absent. The results suggest that the additive effect of phytase on PP utilization may be diet-specific, highlighting the importance of considering dietary factors when evaluating phytase efficacy.

Keywords: additivity, digestibility, phosphorus, phytate-P, phytase

M113 Evaluation of vitamin D and carotenoid-based nutritional solutions on performance and egg quality in Breeder hens Patrick Tamatey^{*1}, Shelby Corray², Brooke C. Bodle³, John Boney¹, Dervan Bryan¹ ¹*Penn State, Animal Science, State College, Pennsylvania, United States;* ²*DSM-Firmenich Nutritional Products, Plainsboro, New Jersey, United States;* ³*DSM-Firmenich Nutritional Products, Plainsboro, New Jersey, United States*

Over the past five years, broiler breeder hen production efficiency has declined, with issues in ovarian function, reduced egg quantity and quality, decreased fertility persistence, and hatchability. This study hypothesized that dietary inclusion of 25-hydroxyvitamin D3 (HyD), Canthaxanthin (Cx), Copper (Cu), and Gluco-oligosaccharides (GO) could improve egg quality and breeder hen fertility. Using a completely randomized design, 210 breeder hens and 21 breeder males were assigned to one of three treatments: a control diet (C), C with 1 lb/ton MaxiRepro (MR) (MR, containing HyD, Cx, Cu, and GO), or C with 1 lb/ton MaxiChick (MC) (MC, containing HyD and Cx). Birds were placed into floor pens with 7 replicates per treatment (10 females and 1 male per replicate) at 25 weeks (wks) and allowed a 5-week adaptation period. At 30 weeks, diets were provided for 20 weeks, with weekly measures of feed intake, and egg production. Egg quality was assessed at wk 30, 35, 41, 45, and 50, and fertility at weeks 30, 41, and 50. Data was analyzed using one-way ANOVA. Differences were considered significant at ($p \leq 0.05$). MaxiRepro significantly increased egg production at 44 wk and egg weight at 32 and 46 wks, yielding the highest FCR at 36, 40, 41, 44, 45, and 46 wk. At 50 wk, MC produced higher fertility (92.82a) than MR (45.40b) and C (19.54b). MaxiChick and MaxiRepro also improved yolk color throughout the experiment, and shell weight at 45 wk. MaxiRepro increased albumen height and Haugh units at 30 and 35 wk. Findings from this study suggest that MaxiRepro and MaxiChick supplementation can enhance broiler breeder egg quality, performance, and production efficiency.

Keywords: broiler breeders, egg quality, fertility, MaxiRepro, MaxiChick

M114 Niacin and nicotinamide riboside supplementation effects on broiler growth performance and apparent metabolizable energy Victoria Wilson^{*1}, John Gonzalez², Scott Beyer³, Haley Otott¹, Chad Paulk¹, Carter D. Minson¹, Mason Engnell¹, Walter Friesen¹, Diego Lopez¹, Allison Blomme¹ ¹*Kansas State University, Grain Science and Industry, Manhattan, Kansas, United States;* ²*University of Georgia, Animal and Dairy Science, Athens, Georgia, United States;* ³*Kansas State University, Animal Science and Industries, Manhattan, Kansas, United States*

Adequate niacin (NA) levels in broiler diets play a crucial role in enhancing growth performance and efficient utilization of digestible energy and minerals. Nicotinamide riboside (NR), an NA analog, has emerged as a novel feed additive. Because NR may

have a similar or complementary role in animal nutrition, this study aimed to evaluate the effects of supplementing broiler chick diets with varying NA and NR levels on growth performance and apparent metabolizable energy (AME), providing insights into the comparative benefits of these two NAD⁺ precursors. Day-old male COBB by-product breeders ($N = 420$) were used in an 18-d study where five dietary treatments were randomly assigned to 60 cages within 12 locational blocks in 3 Petersime batteries, resulting in 12 replicates per treatment ($N = 7/\text{pen}$). Diets were fed in mash form and consisted of a basal control (28 mg/kg NA, 0 mg/kg NR), and four treatments differentiated by the addition (via hand add) of NA or NR to the basal control at 42 mg/kg or 112 mg/kg, respectively. The experimental design was RCBD, and data were analyzed using GLIMMIX procedure of SAS 9.4, with cage as experimental unit, and cage location as blocking factor. Treatments were evaluated using contrasts: interaction of added NA or NR concentration \times source, NA or NR level linear and quadratic polynomials, and NA vs NR. There was no evidence ($P > 0.05$) of interaction or difference in broilers fed diets containing added NA or NR. There was also no evidence of difference in BWG or FI between treatments ($P > 0.05$). There was a tendency (linear, $P = 0.07$) for improvement in FCR with the addition of NA or NR in the diet. The addition of NA or NR at 42 and 112 mg/kg had no significant impact on AME ($P > 0.05$). In conclusion, adding NA or NR to the diet tended to improve FCR of the broilers up to 18 days of age; however, these improvements were not explained by increases in metabolizable energy.

Keywords: niacin, nicotinamide riboside, apparent metabolizable energy, vitamins

M115 Digestible calcium and digestible phosphorus requirements of broilers from hatch to eight days of age Kean G. Jacobs^{*1,2}, Roselina Angel³, Christine Jansen Van Rensburg², Kirsty Gibbs⁴, Jamie Fourie², Peter W. Plumstead¹, Kyle M. Venter^{1,2}, Nickki Tillman⁵ ¹*Neuro Livestock Research, Brits, South Africa;* ²*University of Pretoria, Department of Animal Science, Pretoria, Gauteng, South Africa;* ³*University of Maryland, Department of Animal and Avian Sciences, College Park, Maryland, United States;* ⁴*IFF, Danisco Animal Nutrition & Health, Oegstgeest, Netherlands;* ⁵*Nutritional Statistics LLC, Buford, Georgia, United States*

The development of digestible Ca (dCa) and P (dP) coefficients has the potential to advance broiler nutrition significantly. There have been major developments that allow for formulating diets based on the digestible fraction of these minerals rather than total amounts. This development offers several advantages in terms of improving the efficiency of mineral utilization, performance, lowering costs, as well as reducing waste and environmental impact. However, research defining the requirements of these nutrients for modern broilers on a digestible basis is still ongoing. This study aimed to determine the dCa and dP requirements of broilers from hatch to d 8. The study followed a RCBD with a 4 \times 4 factorial of dCa (0.35%, 0.50%, 0.65%, 0.80%) and dP (0.30%, 0.42%, 0.54%, 0.66%), yielding 16 TRT combinations with 6 replications per TRT. Ross 308 male broiler chicks ($n=960$) were allocated to 96 battery cages (10 birds per pen). At d 8, growth performance and toe ash as a bone mineralization indicator (% and mg/toe) were determined. Data were analyzed in JMP Pro 17.0, using 2 factor response surface analysis. The coefficient of determination (R^2) was reported for each response to indicate how well the model fits the data. The dCa and dP levels which maximized BW and toe ash, while minimized FCR were determined. The recommended requirements were determined at 95% of the asymptote. Results demonstrated a good fit of the data for all responses. BW, mortality-corrected FCR (mFCR), toe ash

(mg/toe), and toe ash (%) models have an R^2 of 0.93, 0.84, 0.91 and 0.71, respectively. Broilers receiving diets with either high dCa and low dP, or high dP and low dCa led to BW, mFCR, toe ash (mg/toe) and toe ash (%) being affected negatively. The requirements for optimal bone mineralization were 0.55% dCa with 0.53% dP for toe ash (%), and 0.54% dCa with 0.53% dP for toe ash (mg/toe). Requirements for optimal growth performance were 0.49% dCa with 0.45% dP for BW and 0.47% dCa with 0.43% dP for mFCR. Formulating broiler pre-starter diets to bone ash requirements using dCa and dP is critical for supporting optimal bone mineralization and overall growth, especially during the early, rapid phase of skeletal development.

Keywords: broiler pre-starter, digestible calcium, digestible phosphorus, pre-starter requirements, sustainability

M116 Dietary 25-hydroxyvitamin D₃ supplementation improves vitamin D status and protects bone and gut health in broilers challenged with *Eimeria* spp Nicolas Mejia-Abauza*¹, Catherine Fudge¹, Muhammad Ali¹, Huihui Ma¹, Venkata Sessa Reddy Choppa¹, Woo Kyun Kim¹, Cristiano Bortoluzzi², Estefania Perez-Calvo², Chongxiao Chen¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²dsm-firmenich, College Station, Texas, United States

Coccidiosis impacts intestinal and bone health of chickens and consequently growth performance. The objective of this study was to investigate the effects of 25-hydroxyvitamin D₃ (25OHD₃, HyD®, dsm-firmenich, Switzerland) on performance, bone quality, and gut health in coccidiosis-vaccinated chickens challenged with *Eimeria* spp. A total of 900 one-day-old Cobb 500 male chicks vaccinated against coccidiosis 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 x 9 replicates): NCC, Non-challenge control; CC, *Eimeria* challenge control; DD, challenge + 2900 IU/kg vitamin D₃; 25D, challenge + 69 µg/kg of 25OHD₃ (bioequivalent to 2760 IU/kg vitamin D₃). All the basal diets contained 1450 IU/kg of Vitamin D₃. Serum 25OHD was measured on D28 and 42 as vitamin D status biomarker. Growth performance was assessed on D14, 28, and 42. At D28, oocyst shedding, gut lesions score, and permeability were evaluated. Tibia quality was measured at D28 and 42, and whole-body composition was evaluated at D42. Data were analyzed by one-way ANOVA, and Tukey's test ($P < 0.05$). The coccidiosis challenge reduced serum 25OHD₃ compared to the NCC at D28 ($P < 0.05$) but not at D42 ($P > 0.05$). Meanwhile, DD and 25D increased serum 25OHD₃ at D28 and 42, with the group 25D showing the highest value at D42. Coccidiosis challenge significantly impacted ($P < 0.001$) BWG, FI, and FCR at D28, as well as BWG at D42 ($P < 0.001$). Under these conditions, Vitamin D₃ and 25OHD₃ did not improve the performance. For the coccidiosis status, the DD and 25D groups showed lower *E. tenella* lesion scores ($P < 0.001$). At D28, coccidiosis challenge reduced bone-dry weight ($P = 0.005$), fat-free bone-dry weight ($P = 0.001$), and bone ash ($P = 0.001$). Adding 25OHD₃ increased fat-free dry weight ($P = 0.001$) and ash content ($P = 0.001$) in the tibia compared to CC, reaching a similar level as the NCC. In conclusion, the coccidiosis challenge induced gut lesions and negatively affected vitamin D status, bone quality and growth performance 6 days post-challenge. These effects were partially alleviated by the additional inclusion of vitamin D₃, with more evident effects of supplemental 25OHD₃.

Keywords: 25-hydroxyvitamin D₃, broiler, bone health, performance, coccidiosis

M117 Nutrient digestibility response of male broilers subjected to an enteric challenge and fed diets containing varying limestone particle sizes and calcium concentrations Joseph P. Gulizia*¹, Rana Waqar Tabish¹, Jose I. Vargas¹, Jose R. Hernandez², Cristina T. Simões¹, Eva G. Guzman¹, Samuel J. Rochell¹, Ruediger Hauck^{1,3}, Matthew Bailey¹, William A. Dozier¹, Klinton W. McCafferty², Wilmer J. Pacheco¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²USDA-ARS-Poultry Research Unit, Mississippi State, Mississippi, United States; ³Auburn University, Department of Pathobiology, Auburn, Alabama, United States

This study evaluated the effect of limestone particle size (PS) and calcium (Ca) concentration on nutrient digestibility of broilers. In total, 2,100-d-old male broilers (YPM x Ross 708) were distributed into 70 floor pens. Each pen was assigned to 1 of 7 treatments (10 replicates/treatment). This experiment was a 2 x 3 + 1 factorial arrangement including 2 limestone PS (200 and 910 µm) and 3 Ca concentrations (adequate, reduced, and low). For each growth phase, dietary Ca was a two-step, 10-point reduction from adequate Ca concentrations (Starter: 0.95%; Grower: 0.85%; Finisher: 0.75%). The 6 factorial treatments were enterically challenged, while the + 1 treatment served as an unchallenged control with the diet containing 200 µm limestone PS and adequate Ca at each feeding phase. Challenged groups received an oral gavage of a live attenuated trivalent coccidian vaccine on d 14 and 1 x 10⁸ colony-forming units of *Clostridium perfringens* on d 18. Ileal digesta (6 birds/pen) was collected at d 35 to evaluate nutrient digestibility. Data were analyzed as a factorial arrangement using the GLIMMIX procedure of SAS. Means were separated using Tukey-Kramer with significance considered at $P \leq 0.05$. The enterically challenged group exhibited higher Ca digestibility ($P \leq 0.05$) compared to the unchallenged group, while the digestibility of CP, apparent amino acids (AA), fat, phosphorus (P), and apparent ileal digestible energy (AIDE) remained similar between groups ($P > 0.05$). Interactions and limestone PS main effects were not observed for nutrient digestibility on d 35 ($P > 0.05$). Similarly, main effects of Ca concentration were not observed for CP, fat, and apparent AA digestibility ($P > 0.05$). Broilers fed a low Ca diet showed the highest Ca and P digestibility compared to those fed adequate and reduced Ca diets ($P \leq 0.05$). Conversely, AIDE was highest in broilers fed an adequate or reduced Ca diet compared to those fed a low Ca diet ($P \leq 0.05$). This study showed that reducing dietary Ca concentrations by 0.20 percentage points from adequate levels increased Ca and P digestibility but reduced AIDE during an enteric challenge. Although limestone PS did not influence nutrient digestibility on d 35, it should still be considered during feed formulation.

Keywords: limestone particle size, calcium, necrotic enteritis, nutrient digestibility, broiler

M118 The effects of dietary copper and litter condition on broiler growth performance Allison Jun Taguchi Kawaoku*¹, Nicolas Mejia-Abauza¹, Catherine Fudge¹, Muhammad Ali¹, Huihui Ma², Venkat U. Bodempudi¹, Tongshuai Liu³, Guoming Li¹, Jinquan Wang⁴, Chongxiao Chen¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²Henan Academy of Agricultural Sciences, Institute of Animal Husbandry, Zhengzhou, China; ³Henan University of Animal Husbandry and Economy, College of Animal Science & Technology, Zhengzhou, China; ⁴Auburn University, Department of Poultry Science, Auburn, Alabama, United States

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 ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 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 and 15-28d, the interaction effects showed <250ppm Cu resulted in higher BW ($P = 0.003$; $P = 0.0002$) in new litter than used litter, while 500ppm reduced BW and BWG ($P = 0.0039$; $P = 0.0014$) in both litters. From the main effects, 500ppm had higher FCR than 5 and 250ppm from 0-14d ($P = 0.0016$). However, from 15-28d, no differences were observed between 5, 125, and 500ppm, but 250ppm resulted in the lowest

FCR ($P < 0.0001$). From 29 to 42d, the main effects showed 500ppm reduced BW ($P < 0.0001$), BWG ($P < 0.0001$), and FI ($P = 0.0007$) compared to the other treatments. From 43 to 49d, the interactions showed 250ppm in used litter had higher BW than new litter ($P = 0.0067$). 500ppm reduced BW in both litter conditions. From 0 to 49 days, the main effect showed that 500ppm Cu worsened the BWG ($P < 0.0001$) and FCR ($P < 0.0001$). From the broken line model, the margining level to cause adverse effects on BWG is 250ppm for used litter, 200ppm for new litter, FCR is 213ppm for used, and 247ppm for new litter ($P < 0.05$). No significant difference was found in mortality, blood copper levels, or gizzard erosion. In conclusion, copper level interacts with litter conditions; in general the 500ppm showed the worst performance in broilers regardless of litter conditions. The marginal safety level is between 200-250ppm in various responses.

Keywords: Copper Sulphate, Broilers, Growth performance, Gizzard erosion

Metabolism & Nutrition VI: Amino Acids & Feed Additives

M119 Determining the importance of non-essential amino acids, histidine, and phenylalanine + tyrosine in broilers provided reduced protein diets Ruben Kriseldi*¹, Marcelo Silva¹, Alex Corzo¹, Jason Lee², Roshan Adhikari² ¹Aviagen, Huntsville, Alabama, United States; ²CJ Bio America, Downers Grove, Illinois, United States

An experiment was conducted to determine the importance of non-essential amino acids (NEAA), His, and Phe+Tyr in reduced protein diets fed to broilers. Ross × Ross 308 AP male chicks (1,890) were randomly distributed into 126-floor pens receiving 1 of 14 dietary treatments. A positive control (PC) treatment was formulated to follow the 2022 Aviagen nutrition specifications. Using a Plackett-Burman design, 8 factors of Ala, Asp, Glu, Gly, Ser, Pro, His, and Phe+Tyr were evaluated in a reduced CP diet (-1.5% point CP than PC) consisting of 2 levels: base (-1) and enhanced (+1), which resulted in 12 treatments. The negative control (NC) was considered when all factors were at the base level (-1), which contained 3% points lower CP than the PC. Another treatment was added where all factors were at the enhanced level (All+1). Live performances were measured at 12, 25, 35, and 41 d, whereas carcass characteristics were evaluated at 42 d. Data were analyzed as a Plackett-Burman screening design using JMP 17.2 to determine the influence of each factor. One-way ANOVA was used to compare NC, PC, and All+1 treatments. Statistical significance was set at $P \leq 0.10$. Glycine was responsive for improving BW and FCR ($P < 0.05$) at all ages, whereas Ser was responsive in improving ($P = 0.09$) FCR at 41 d of age. Proline enhanced BW ($P = 0.007$) and FCR ($P = 0.003$) during the starter phase only. Across all ages, Glu increased ($P < 0.10$) feed intake. No difference ($P > 0.05$) in BW and FCR were observed between birds provided the PC and NC diets at 41 d. However, birds fed the All+1 treatment had improved BW ($P = 0.001$) and FCR ($P = 0.004$) when compared to birds provided with NC and PC diets. Carcass ($P = 0.001$) and total white meat yield ($P < 0.001$) were enhanced by increasing either His or Gly levels. Birds provided with the All+1 treatment had greater ($P < 0.001$) carcass and total white meat yield compared to those fed the NC and PC treatments. However, birds consuming NC diets had more abdominal fat percentage ($P < 0.001$) than broilers fed the PC and All+1 diets. Lower limiting AA (His) and certain NEAA (Gly, Ser, Pro, and Glu) might play a more noticeable role when reducing dietary protein to maintain performance of broilers.

Keywords: Low Protein Diet, non-essential amino acids, Histidine, Phenylalanine, broilers

M120 Performance and yield response of male and female Cobb 500 broilers with varying levels of amino acid density Jason T. Lee*¹, Rocky Latham², Roshan Adhikari¹, Dan Moore³ ¹CJ Bio America, Downers Grove, Illinois, United States; ²Tyson Foods, Springdale, Arkansas, United States; ³Colorado Quality Research, Wellington, Colorado, United States

Increasing dietary amino acid density can result in improved growth rate and meat yield. However, continuous genetic improvement of commercial broiler lines requires continuous evaluation of dietary requirements to maximize economic return. The objective of this experiment was to evaluate the effect of increasing amino acid density on the performance and yield of Cobb 500 male and female broilers. A total of 6 treatments with 10 replicate pens of male and 10 replicate pens of females Cobb 500 broilers were used in this experiment. The experimental treatments were based on increasing dig Lysine content and fed during all three phases of grow out; Starter (0 to 14 d), Grower (15 to 28 d), and Finisher (29 to 42 d). Each of the dietary treatments were separated by 0.07% dig lysine with ranges for each dietary phase of 1.15% to 1.50% dig lysine for the starter period, 1.03% to 1.37% for the grower period, and 0.91% to 1.26% for the finisher period. The other essential amino acid ratios to lysine were maintained as dig lysine increased. On day 43, all broilers from 4 blocks were processed to determine carcass and breast yield. Statistical analysis included a 6 (lysine) × 2 (sex) factorial ANOVA with main effect means deemed significantly different at $p < 0.05$. Additionally, linear and quadratic regressions were conducted to determine the effect of increasing lysine on male and female performance and yield. The impact of amino acid density was observed as early as 14 days of age with linear improvements in BWG and FCR. At the conclusion of the experiment, all evaluated parameters were highly sensitive to increasing amino acid density resulting in quadratic increases in body weight gain and breast meat yield and linear decreases in feed efficiency. The observed performance difference of evaluated parameters included a range of 177 grams of BW, 13.5 points of feed conversion ratio, 100 grams of breast meat, and 2.4% breast meat yield between the lowest and highest density diets. Males were more sensitive to dietary amino acid density as compared to females. These data can serve as reference data for nutritionists to determine optimal amino acid density to calculate maximum economic return.

Keywords: Amino Acid, Yield, Broiler, Performance, Lysine

M121 of direct fed microbials on the performance and eggshell quality of post-peak laying hens Alyssa M. Lyons^{*1}, Hector Leyva-Jimenez², Brian Dirks², Mike E. Persia¹ ¹*Virginia Tech, School of Animal Sciences, Blacksburg, Virginia, United States;* ²*United Animal Health, Audubon, Pennsylvania, United States*

An experiment was conducted to determine the effects of direct fed microbials (DFM) on 54-74 wk old laying hens using performance, egg quality, and abdominal fat pad weights. A total of 144 Hy-Line W-36 laying hens were fed one of four diets: control with no DFM (C), C + Novela[®] ECL (C+NE), C + Novela[®] (C+N), or C + Amnil[®] (C+A). The experiment was a randomized complete block design with 12 replicate cages of 3 laying hens per treatment. Hen day egg production (HDEP), egg weight (EW), egg mass (EM), feed intake (FI), and feed conversion ratio (FCR) were calculated over two wk periods. Hens were control fed 101 g/d throughout the experiment. Body weights (BW) were measured every four wk and abdominal fat pads were determined at 74 wk as an indicator of energy storage. Eggs were collected every two wk for internal and external egg quality. Performance and egg quality data were analyzed using repeated measures and fat pads using ANOVA in JMP Pro 16.0. Means were separated using Student's t-test ($P \leq 0.05$). There were no differences for HDEP (85.3 to 87.1%; $P = 0.35$) or FI ($P = 0.26$). The C+NE and C+A increased EW ($P \leq 0.01$) resulting in a 4% increase in EM ($P \leq 0.01$). The increased EM without increased FI resulted in a 7- and 9-point improvement in FCR for C+NE and C+A, respectively ($P \leq 0.01$). It is interesting to note that although C+N was not able to directly increase performance, it was able to increase BW (1,589 g) v. C (1,553 g; $P \leq 0.01$). Following a similar pattern, the C+N resulted in a 26% increase in abdominal fat pad weight compared to the C ($P = 0.01$). The larger egg size associated with the C+NE and C+A resulted in reduced specific gravity (1.079) v. C (1.080; $P \leq 0.01$). When eggshell breaking force was considered, C (3.54 kg f) was similar to C+NE (3.55 kg f) and both C+A (3.70 kg f) and C+N (3.89 kg f) increased eggshell breaking force ($P \leq 0.01$). The C+NE and C+A were able to improve the performance of aged laying hens. On the other hand, C+N was not able to increase performance, but was able to alter nutrient utilization as it increased fat storage. Egg shell quality data were mixed as the larger egg size of the C+NE and C+A reduced egg specific gravity, but either increased or did not alter actual eggshell breaking force.

Keywords: direct fed microbial, laying hen, energy, egg quality

M122 Effect of dietary supplementation of spray-dried plasma on performance and semen quality in aging broiler breeders Mário Henrique S. Lopes^{*1}, Carlos A. Granghelli¹, Giovana M. Longhini¹, Isabela E. Silva¹, Melany Y. Lovon¹, Raimundo G. Netto¹, Luís Rangel², Joy Campbell², Joe Crenshaw², Cristiane S. Araujo¹, Lucio F. Araujo¹ ¹*University of São Paulo, Department of Animal Science, Pirassununga, São Paulo, Brazil;* ²*APC, Ankeny, Iowa, United States*

Spray-dried plasma (SDP) is a protein-rich compound that contains substances such as growth factors and immunoglobulins, being extensively used in post-wean piglet diets as a way to aid piglets overcome such a challenging phase. Therefore, the present study aimed to assess the effect of SPD dietary supplementation for broiler breeder hens and roosters on semen quality and hatchability, as well as progeny quality from three different hatches throughout their lifetime. A total of 216 Cobb 500 broiler breeder hens were randomly distributed across two treatments consisting of diets supplemented with 0 or 1% SDP, resulting in 27 replications per treatment and four birds per pen. In addition, 36 roosters, housed in individual pens, were allocated to the same treatments, each bird being considered a replicate. Experimental

diets were fed from 26 weeks until 65 weeks of age. Semen quality, embryonic mortality and quality of newly hatched chicks were evaluated at 29, 45, and 63 weeks. Hens were inseminated with fresh semen, and eggs were incubated following standard procedures. Data was analyzed considering a 3×2 factorial arrangement between age and SDP inclusion as main effects, being all model factors considered nominal variables, while chick quality data was analyzed using the Glimmix procedure. If ANOVA was significant, LSMEANS were separated using a t-test with the significance level set at 0.05. Semen from 63-week-old roosters had higher sperm concentration than other age groups, while 29-week-old rooster semen exhibited higher progressive motility and vigor than semen from older roosters ($P < 0.001$). 45-week-old roosters had the lowest values for semen quality parameters. Additionally, sperm from 45-week-old roosters, regardless of SDP supplementation, had fewer total morphological defects than 63-week-old roosters. However, 1% SDP in the diet reduced total sperm cell defects at 63 weeks of age, as well as total sperm head and tail defects ($P < 0.05$) compared to unsupplemented birds. Dietary SDP reduced late embryonic death in hatches from 63-week-old breeders ($P < 0.05$). The results demonstrate that 1% SDP in breeder diets can reduce late embryonic mortality and sperm cell defects, mainly in aged birds, enhancing the reproductive performance of broiler breeders.

Keywords: breeder age, Chick quality, embryonic mortality, Sperm cell kinetics, Sperm morphology

M123 Throughput agents alleviate concerns of manufacturing feed with larger pellet die thicknesses Lucas E. Knarr^{*1}, Kristina M. Bowen¹, Elizabeth A. Lynch¹, Emily B. Estanich¹, Alexis Renner¹, Jon Ferrel², Joseph S. Moritz¹ ¹*West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States;* ²*Azomite Mineral Products, Nephi, Utah, United States*

Low-moisture corn, due to late harvesting and/or improper handling and storage, may negatively impact pellet production rate (PPR) and pellet durability index (PDI). This is likely due to less lubrication and particle agglomeration associated with the lower water content. Recently, Azomite[®] (AZM) was tested in combination with 32-, 38-, and 45-mm pellet die thicknesses (PDT), which showed that an inclusion of 0.25% AZM increased PPR and practically maintained PDI relative to the 0.0% AZM control within each PDT. Additionally, past research has shown that mixer-added water (MAW) can increase PPR and PDI. The objective of this experiment was to determine the influence of PDT, AZM, and MAW, on PPR, PDI tested for 30 sec (PDI-30), PDI tested for 60 sec (PDI-60), and hot pellet temperature (HPT) of a broiler grower diet manufactured with low-moisture corn. A factorial arrangement of 3 (PDT; 32, 38, or 45mm) x 2 (AZM; 0.0 or 0.25%) x 2 (MAW; 0.0% or 1.5%) was used to create experimental treatments, and manufacture was conducted in a randomized complete block design. All multiple comparisons used Tukey's HSD with a significance level set at $\alpha = 0.05$. Two- and three-way interactions of PDT, AZM, and/or MAW were not observed ($P > 0.05$). Increased PDT resulted in decreased PPR, wherein changing PDT from 32- to 38-mm and from 38- to 45-mm caused a 22.1% and 6.9% decrease in PPR respectively ($P < 0.05$). Including 0.25% AZM increased PPR by 4.8% ($P = 0.014$) and including 1.5% MAW increased PPR by 4.8% compared to their respective controls ($P = 0.014$). Increasing PDT from 32- to 45-mm increased HPT by 1.79°C ($P = 0.038$). Increasing PDT from 32-mm to the average of the 38- and 45-mm levels increased PDI-30 and PDI-60 by 5.14 percentage points (%pts; $P < 0.05$) and 13.66 %pts respectively ($P < 0.05$). The incorporation of MAW increased PDI-30 and PDI-60 by 1.19 %pts ($P = 0.005$) and 3.11

%pts respectively ($P = 0.003$). Based on this data, it could be concluded that AZM and MAW are effective feed additives to increase feed manufacturing efficiency and/or feed quality. However, the observed incidences of plugs and slips at the pellet die associated with the 1.5% MAW inclusion should be considered, as these may cause an overall decrease in high-quality feed production.

Keywords: Pellet Die Thickness, Azomite, Mixer-added Water, Pellet Production Rate, Pellet Durability Index

M124 Evaluation of botanical feed additives rich in saponins and polyphenols in broilers experiencing immunological and environmental stressors Laney Froebel*¹, Mike J. Rincker², Ryan Dilger¹ ¹*University of Illinois, Urbana, Illinois, United States;* ²*DPI Global, Porterville, California, United States*

In commercial production settings, broilers are exposed to numerous environmental and immunological stressors. In this study, two commercial feed additives (Micro-Aid® Feed Grade Concentrate and Micro-Shield™; DPI Global, Porterville, CA) were evaluated for their effects on growth performance, antioxidant defenses, and intestinal health. A total of 420 male Ross 708 chicks at 2 d post-hatch were assigned to 1 of 5 treatment groups with 7 birds allotted to each of the 12 replicate cages. The 42-d study (starter d 0-14, grower d 14-28, finisher d 28-42) was conducted with treatments including 1) control diet fed to birds not exposed to stressors (NC), 2) control diet fed birds exposed to stressors (PC), 3) PC + 500 mg of Micro-Shield/kg of diet in the starter phase only (MS), 4) PC + 500 mg of Micro-Shield/kg diet in the starter, grower, and finisher phases (MSall), and 5) PC + 500 mg of Micro-Shield/kg diet in the starter phase + 250 mg of Micro-Aid/kg diet in the grower and finisher phases (MS+MA). To achieve a multiple mild stressors challenge, birds underwent a 12-h feed withdrawal prior to a coccidiosis vaccine challenge on d 15 of the study, which was designated as 0 d post-inoculation (DPI). Additionally, birds were exposed to cyclic elevated brooder temperatures during the grower phase. All data were subjected to a 1-way ANOVA using the MIXED procedure of SAS with results considered significant when $P < 0.05$. Birds exposed to stressors had lower BWG, lower FI, and a poorer FCR during the grower phase compared with birds in the NC group ($P < 0.05$). On DPI 6, birds in the MS+MA treatment had the highest ($P < 0.05$) levels of catalase in blood serum, potentially indicating higher antioxidant capacity. On DPI 13, IL-1 β and IL-10 gene expression was highest ($P < 0.05$) in the MS+MA and MSall treatment groups, respectively, indicating that Micro-Aid and Micro-Shield may have immunomodulatory effects in coccidiosis-infected birds. While birds in the NC treatment had the highest gene expression of ZO-1 in jejunal mucosa tissue on DPI 6 ($P < 0.05$), MS+MA birds had similar ZO-1 expression compared with NC birds. Overall, these results suggest Micro-Aid and Micro-Shield may confer health benefits onto birds experiencing environmental and immunological stressors.

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

M125 Evaluation of a direct-fed microbial on growth performance in birds subjected to ingredient-based dietary stress Addison Elstner*¹, Rosana Hirai¹, Austin O. Silva², Daniel De Leon², Hector Leyva-Jimenez³, Brian Dirks⁴, Yemi Burden³, Katherine McCormick³, Christopher Bailey¹, Audrey McElroy² ¹*Texas A&M University, Poultry Science, College Station, Texas, United States;* ²*Texas A&M University, Poultry Science, Bryan, Texas, United States;* ³*United Animal Health, Sheridan, Indiana, United States;* ⁴*United Animal Health, Audubon, Pennsylvania, United States*

This experiment evaluated the impact of feeding a *Bacillus*-based direct-fed microbial (DFM) with varying dietary ingredients as a potential model to test feed-additive efficacy. Cobb by-product male chicks were assigned to 64-floor pens using a complete randomized block design (25 birds/pen; 0.067^2m). Two feeding programs (8 pens/treatment) were used: (1) a corn-soybean meal (SBM) basal diet (CS) with distiller's dried grains (DDGS) and/or meat & bone meal; (2) a variable diet (V) including corn, SBM, DDGS, wheat, cottonseed meal, corn gluten, and sorghum at various levels to induce a nutritional challenge. The trial was a 2×2 factorial with main effects being diet (CS or V) and DFM (0 or 500 ppm). Performance measurements included feed conversion ratio (FCR), feed intake, body weight (BW) and BW gain (BWG) at d14, 28, and 42. On d42, 10 birds/pen were analyzed for footpad dermatitis (FPD), gait score (GS), and skin pigmentation. On d43, 5 fresh fecal samples/pen were categorized. Data were analyzed using PROC GLM SAS 9.4 with 2-way ANOVA, and means were separated using Fisher's LSD test ($P \leq 0.05$). GS and FPD were also evaluated with the Kruskal-Wallis test. In the starter phase, DFM group birds had lower FCR ($P \leq 0.05$) when compared to control. For starter and finisher phases, diet comparison showed that CS diets improved FCR and BWG compared to the V diet. Overall, the CS diet resulted in more efficient FCR compared to the V diet ($P \leq 0.05$). Birds without DFM supplementation had a higher incidence of leg issues from d29-42 and a worse average d42 GS ($P < 0.05$). Additionally, birds fed diets without DFM had less FPD compared to the DFM group ($P < 0.05$). For the Diet \times DFM interaction, birds fed a CS diet without DFM had a higher % of normal excreta compared to those on the V diet. However, addition of DFM to the V diet slightly improved fecal quality. When comparing diet modifications, disruption by the V diet was successful, indicated by the CS diet resulting in improved performance when compared to the V diet. Further research is needed using this model of diet ingredient modification with higher enteric stress to further evaluate the effectiveness of DFM strategies to support growth performance under challenge conditions.

Keywords: dietary stress, broiler performance, direct-fed microbial, gut health

M126 Effects of dietary energy and emulsifier on the performance and energy storage of pullets and young laying hens Jennifer Blair*¹, Mike E. Persia¹ ¹*Virginia Tech, Blacksburg, Virginia, United States*

An experiment was conducted to determine the effects of an emulsifier when fed at two dietary energy levels to 15 to 45-week-old laying hens using performance, egg quality, and abdominal fat pads. A total of 288 Hy-Line W-36 laying hens were fed one of six diets as part of a 2×3 factorial arrangement including a positive control (PC) fed industry relevant energy concentrations and a negative control (PC - 40 kcal/kg) in the presence of 0, 250 and 500 ppm emulsifier. The experiment used 16 replicates cages of 3 laying hens per treatment. Egg production (EP), egg weight (EW), egg mass (EM), feed intake (FI), and feed conversion ratio (FCR) were calculated over two wk periods. Egg quality and body weights (BW) were determined every four wk. Abdominal fat pads (AFP) were collected and weighed at 45 wk. All performance, egg quality and body weight data were analyzed using repeated measures and abdominal fat pads were analyzed using ANOVA in JMP Pro 16.0. There were main effects for dietary energy from 24 to 45 weeks including EW, EM, FI, FCR and BW ($P \leq 0.05$). As expected, increased dietary energy increased egg weight (59.3 v. 59.0 g), and EM, but decreased both FCR (1.728 v. 1.760) and FI. Performance was generally not altered by treatment with the emulsifier. Differences in relative shell weight and specific gravity

may suggest an interaction between dietary oil and Ca digestibility and should be further researched. Differences in energy utilization with the emulsifier were noted in both the interactions of energy and emulsifier for BW and AFP weights. This interaction occurred as the PC without the emulsifier resulted in increased BW and AFP weights compared to the NC, but when the emulsifier was added to the NC diets at 250 ppm both BW and AFP weights returned to the PC. The NC with 500 ppm did not result in any response and the emulsifier fed to the PC did not alter responses. Overall, 40 kcal/kg energy reduced laying hen performance and at least 250ppm of the emulsifier was able to release some of the 40 kcal of energy as demonstrated by the body weight and abdominal fat pad responses. Questions remain about the concentration of emulsifier as 500ppm was not effective and how best to manage dietary energy levels as effectiveness in the PC was not noted.

Keywords: emulsifier, laying hen, energy, egg quality, fat pads

M127 Isolation of probiotic candidates from broiler chicken ceca using culturomics approach Manhong Wang^{*1}, Xin Ye¹, Hailey Fugate¹, Chuan-Yu Hsu², Xue Zhang³, Peixin Fan³, Katie E. Elliott⁴, Pratima Adhikari¹, Kenneth Macklin¹, Li Zhang¹ ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²Mississippi State University, Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States; ³Mississippi State University, Department of Animal and Dairy Science, Mississippi State, Mississippi, United States; ⁴USDA, Agriculture Research Service, Poultry Research Unit, Mississippi State, Mississippi, United States

Maintaining gut microbiome homeostasis plays a pivotal role in optimizing immune function, nutrient utilization, and bird growth in poultry production. Probiotic supplementation is a widely applied strategy, while conventional single- or multi-strain formulations fail to replicate the complex microbial ecosystem of the native gut microbiome, leading to inconsistent efficacy *in vivo*. To address these limitations, culturomics, an innovative methodology that integrates diverse culture conditions with high-throughput sequencing technologies, that enables comprehensive identification and isolation of beneficial microorganisms. Previously we used culturomics to isolate microbial communities from the ceca of broiler chickens and five media (MSA, MRS, BBE, CNAB, CNA) had demonstrated efficacy in capturing microbial diversity. This study utilized these five media under both aerobic (AE) and anaerobic (AN) conditions to enrich the cecal microbiota from six broiler chickens. Five morphologically distinct single colonies were isolated from each condition and subcultured, resulting in 251 viable isolates with 108 from AE and 143 from AN condition. Genomic DNA was extracted from these isolates and subjected to full-length *16S rRNA* gene amplicon sequencing using Oxford Nanopore Technology. Results revealed that 60% (n=150) of the isolates were dominated by a single species (>95% relative abundance), while 40% (n=101) exhibited as multispecies cultures. Among the 150 single-species isolates, 14 different species were identified, including 10 with documented or potential probiotic properties. Statistical analysis using Chi Square Test indicated no significant differences in species isolation efficiency between oxygen conditions ($P > 0.05$). However, certain species were isolated only under specific AE or AN condition, indicating the need for both oxygen condition to achieve comprehensive bacterial isolation. This study demonstrated the efficacy of culturomics in isolating diverse beneficial bacteria from broiler chicken ceca. Future investigations will focus on optimizing culture parameters and increasing sample size to enhance the isolation and characterization of probiotic candidates that better represent the native poultry gut microbiota.

Keywords: Culturomics, Gut microbiome, Culture Condition, Beneficial isolates, Nanopore Technology

M128 Assessing the role of natural feed additives in shaping the broiler environmental microbiome Ana Fonseca^{*1,5}, Sophia Kenney¹, Stephanie Bierly², Jennine Lection³, Samantha Seibel⁴, Joshua Wolff⁶, John Boney¹, Erika Ganda¹⁴ ¹Pennsylvania State University, Animal Science, University Park, Pennsylvania, United States; ²Novagene, Centre Hall, Pennsylvania, United States; ³North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina, United States; ⁴Pennsylvania State University, Huck Life Sciences, University Park, Pennsylvania, United States; ⁵Huck Life Sciences, Microbiome Sciences, University Park, Pennsylvania, United States; ⁶Drexel University, Philadelphia, Pennsylvania, United States

Antimicrobial supplementation in broiler diets has been a common practice to enhance feed efficiency, growth rate, and intestinal health. However, growing consumer concerns and strict governmental regulations regarding antimicrobial resistance have driven the industry towards antimicrobial-free systems. In this context, natural additives have emerged as promising replacements for antimicrobial growth promoters, offering a sustainable solution to support poultry health and productivity. The goal of our study was to characterize the effects of a probiotic, an essential oil, a glycan mixture, and a prebiotic on broilers' environmental microbiota. 3072 Cobb 500 1-day-old chicks were randomly allocated in 96 pens with 8 replicates of 32 broilers per pen and were raised until day 43. Treatments consisted of a basal diet (negative control), a basal diet mixed with an antibiotic (BMD), a basal diet mixed with an essential oil, a basal diet mixed with a probiotic (*Bacillus subtilis*), a basal diet mixed with prebiotic (Yucca and Quillaja saponins), and a basal diet mixed with a complex glycan mixture. Sterile boot socks were used to weekly collect broilers' environmental microbiota, which were obtained by targeted sequencing the V4 region of the 16S gene using an Illumina platform. To assess the effect of the feed additives on broilers' environmental microbiota, statistical comparisons for alpha diversity (Kruskal-Wallis), and beta diversity (PERMANOVA) were done. The most abundant phyla were Firmicutes, Actinobacteriota, and Proteobacteria, and the most abundant genera were *Lactobacillus*, *Staphylococcus*, and *Weisella*. There were no differences between treatments for alpha or beta diversity. Time was the major factor of environmental microbiome succession as differences in beta diversity were detected over the weeks ($P = 0.01$) and feeding phases ($P = 0.003$). The study demonstrated that while natural additives did not significantly alter the alpha or beta diversity of the broilers' environmental microbiota compared to the control and antibiotic treatments, they still hold potential as sustainable alternatives to antimicrobial growth promoters. Additionally, the observed temporal changes in the microbiota composition highlight the dynamic nature of microbial communities.

Keywords: Feed Additives, Environmental Microbiota, Broilers

M129 Effect of protected organic acids supplementation on growth performance, gut Integrity, and immune response in broilers challenged with necrotic enteritis Hanseo Ko^{*1}, Jihwan Lee¹, Hemanth Reddy Katha¹, Ishwari Gyawali¹, Kwang Young Lee², Woo Kyun Kim¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Morningbio, Cheonan, Chungnam, Korea (the Republic of)

The aim of this study was to evaluate the effect of protected organic acids (PO) supplementation on growth performance, gut integrity, and immune response in broilers under necrotic enteritis (NE) challenge conditions. A total of 420 broilers (as hatched,

male Cobb 500) were allocated into five treatments with six replicate cages (14 birds per cage) for 28 d. The treatments consisted of non-challenged control (NC) and four NE-challenged groups with different supplementation levels of dietary PO (0, 0.1, 0.2, and 0.4 %). To induce subclinical NE, all birds in NE-challenged groups were orally challenged with 1 mL of *Eimeria maxima* (10,000 oocysts per bird) on d 14 (0-day post-inoculation, dpi) and 1 mL of *Clostridium perfringens* (CP, 10^9 CFU/mL per bird) on 4 and 5 dpi. Body weight (BW), BW gain (BWG), feed intake (FI), and mortality were measured daily or weekly. The CP loads in small intestinal digesta and total ceca were evaluated on 7 and 14 dpi. Gut permeability, NE lesion scoring, and gene expression in the jejunal tissue were investigated on 7 dpi. One-way ANOVA with Tukey's post hoc test was used for all data analyses, and the orthogonal polynomial contrast was used to evaluate the PO dose effect in the NE-challenged groups from 0 to 14 dpi. Dietary PO supplementation levels linearly improved BWG and FI from 0 to 14 dpi (L, $P < 0.05$). Dietary PO levels linearly improved FCR from 0 to 7 dpi (L, $P = 0.001$). Increasing dietary PO level linearly reduced CP loads in the jejunal and cecal contents on 7 dpi (L, $P < 0.01$), whereas it linearly decreased NE lesion score and gut permeability on 7 dpi (L, $P < 0.05$). Dietary PO levels linearly upregulated gene expression of claudin 2 and zonula occludens 1 in the jejunal tissue on 7 dpi but downregulated the expression of claudin 1 (L, $P < 0.05$). Dietary PO levels linearly downregulated the expression of interferon-gamma and interleukin 10 in the jejunal tissue on 7 dpi (L, $P < 0.01$). In conclusion, the results revealed that the dietary PO supplementation had dose-dependent effects that modulated the expression of genes related to intestinal integrity and inflammation, suppressed the CP load of intestinal contents, and mitigated the reduction in growth performance of the broiler under the NE condition.

Keywords: Protected organic acids, Necrotic enteritis, Broiler, Gut health, Feed additive

M130 Evaluation of different Cu sources and dietary inclusion levels on growth performance and Cu absorption in broilers challenged with coccidiosis Austin O. Silva^{*1}, Rosana Hirai¹, Addison Elstner¹, Kasey E. Young¹, Abigail Flores¹, Audrey McElroy¹, Bob Buresh², Melanie Roux² *Texas A&M University, Poultry Science, College Station, Texas, United*

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Copper (Cu) supplemented above requirements has improved intestinal health and performance, making it essential to determine appropriate Cu levels in broiler diets, especially during enteric stress. The study evaluated Cu sources and levels on performance and Cu absorption. 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 pens across 6 dietary treatments (trts): negative control (NC, 0 ppm), Cu sulfate (CuSO₄, 125 ppm), TBCC (TBCC125, 125 ppm), MHAC (MHAC30, 30 ppm), TBCC step-down (TBCC, 125 ppm d0-14; 100 ppm d15-28; 75 ppm d29-43; 0 ppm d44-56), and MHAC step-down, (MHAC, 30 ppm d0-14; 24 ppm d15-28; 18 ppm d29-43; 0 ppm d44-56). Broilers were housed in floor pens with used litter and administered a 5X dose of coccidiosis vaccine on d0. Unexpected heat stress challenge occurred from d29 to end of the trial. Performance was recorded on d14, 28, 43, and 56. Cu concentrations were measured in d14 liver and d28 digesta (jejunum and ileum). Data were analyzed using PROC GLM in SAS and Tukey HSD test was used to separate significant means ($P \leq 0.05$). For d0-28, MHAC, TBCC, TBCC125 or NC improved FCR in contrast to MHAC30, while CuSO₄ had an intermediate FCR ($P = 0.0015$). TBCC125 had higher BW than MHAC30 with MHAC and remaining trts having intermediate BW ($P = 0.0084$). For d0-43, MHAC and NC had lower FCR compared to MHAC30, while CuSO₄ and TBCC sources had intermediate FCR ($P = 0.0167$). No differences in performance were found from d0-56, but mortality was reduced in MHAC compared to NC or TBCC125 ($P = 0.0135$). Liver Cu amount was higher in birds fed MHAC30 or TBCC than in NC or MHAC ($P = 0.0195$). MHAC30 (183 ppm) and MHAC (157 ppm) trts showed lower levels of Cu in ileal digesta ($P < 0.0001$). CuSO₄ and TBCC125 had the highest levels (400 and 435 ppm, respectively; $P < 0.0001$). The same response was observed for Cu in jejunum digesta ($P < 0.0001$). Liver and digesta analysis suggested that MHAC trts presented higher absorption compared to inorganic forms. In birds subjected to both challenges, no significant differences in performance were observed among Cu sources and levels beyond d28, though lower mortality rates in MHAC sources indicated improved recovery.

Keywords: Sustainability, Copper, Coccidiosis, Performance

Metabolism & Nutrition VII: Vitamins & Minerals

M131 Comparing traditional forms of supplemental copper to a monovalent copper on bacteria control Alessandra Rigo Monteiro^{*1}, Wilfried Vahjen², Yron Manaig¹, Ian Cockshott¹ *1Animine Precision Minerals, Anney, France; 2Freie Universität Berlin, Berlin, Germany*

Enterococcus cecorum is an opportunistic pathogen found in the gastrointestinal tract of chickens, being a growing concern in the broiler industry, as it causes locomotion disorders and septicemia. Maintaining optimum gut integrity can help to avoid *E. cecorum* leakage, especially during the first week of the broiler's life. The antimicrobial effect of copper (Cu) is well recognized and its supplementation can control the growth of bacteria. It can be sourced from divalent forms (sulfates, chlorides, carbonates) or from a monovalent source (CoRouge®, CR). The objective of this study was to compare different copper sources on *E. cecorum* growth. The sources of Cu compared *in vitro* in this trial were copper sulfate (CuS; Manica Cobre S.L., Spain), chelated Cu (CuAA; AvailaCu®, Zinpro, USA), tribasic Cu chloride (TBCC; IntelliBondC®, Selco, USA), and monovalent Cu (CR; Animine, France). The *in vitro* study used the strain *Enterococcus cecorum* DSM 20682. BHI medium was used for preparatory and

growth media during the test. The medium was saturated (10g/L) with the Cu products by mixing. After centrifugation, supernatants were autoclaved and used as growth media. Microtiter plates were sealed with gas tight plastic film (PCR grade) and incubated anaerobically at 37 °C in triplicate. Growth was monitored by turbidity measurements every 5 min. Minimal inhibitory concentrations (MIC) were determined after 24h incubation. Resulting growth data were examined for start of growth according to moving average determination. A one factorial ANOVA with Bonferroni post hoc test was used to determine significant differences between products. The results of MIC showed that 64 µg of CoRouge® per mL are enough to stop *E. cecorum* growth, while 128 µg of AvailaCu® and more than 256 µg of IntelliBond® or CuSO₄ are necessary. The growth kinetics was measured below lethal concentration (32 µg/mL) and was observed that *E. cecorum* starts to grow after 6h in the media with divalent sources, while it starts to growth after 11h in the media with monovalent Cu source. In conclusion, monovalent copper showed stronger ability to inhibit the growth of *Enterococcus cecorum* compared to all other Cu sources, being a tool to reduce the risk of *Enterococci* related disorders in broilers.

Keywords: bacteria, broiler, copper, health

M132 Evaluating the bioavailability of Zinc Glycinate and Zinc Amino Acid hydrate organic trace minerals relative to zinc sulfate based on weight gain and Zinc content in tibia ash of 22-day-old broilers fed semi-purified diets Mohamad Mortada*¹, Andrea Hanson¹, Jose Charal¹, Daniel Jones¹, Matthew Deeker¹, Kelsey Frank¹, Sierra Kolan¹, Rachel Proctor¹, Taylor Thoele¹, Safat Shoeb¹, Dawn Buscheck¹, Jason Hickman¹, Catherine Ionescu² ¹ADM Animal Nutrition, Decatur, Illinois, United States; ²ADM International Sarl, Rolle, Vaud, Switzerland

A depletion-repletion model was used to evaluate relative bioavailability (RBV) of Zn Glycinate (ZnGLY) and Zn Amino Acid Hydrate (ZnAAH) compared to ZnSO₄. Day old chicks were fed a Zn-adequate corn-SBM diet from D0 to 4 and a Zn-deficient diet from D4 to 8. Feed was withdrawn from D8 (PM) to D9 (AM). On D9, 819 birds were randomly distributed to cages and fed one of 13 semi-purified diets (including corn starch, dextrose, and soy protein concentrate) with either no supplemental zinc or zinc supplemented via ZnSO₄, ZnGLY, or ZnAAH, at 5, 10, 15, or 20 ppm added Zn until D22 (n = 9 replicate cages with 7 birds/cage). On D22, 2 birds/cage were euthanized, and right tibias were collected for Zn analysis. Measured response variables included BWG, FI, FCR, and tibia Zn concentration. Cage was the experimental unit. Data were analyzed to evaluate the effect of Zn source, Zn level, and the interactions using mixed models with tier and room as random effects. The RBV was estimated using multiple linear regression and slope-ratio techniques. Zinc level, but not Zn source, effected BWG, FI, and FCR (P<0.05). However, tibia Zn was affected by Zn level (P<0.05) and source (P=0.1), and tibia Zn concentration was greater for birds fed ZnGLY and ZnAAH vs. ZnSO₄ (P=0.03). The BWG increased from 507 to 703 g for birds fed 0 and 20 ppm added Zn respectively (linear and quadratic, P<0.05). The FI and FCR also improved (linear and quadratic, P<0.05) with increasing Zn. Tibia Zn increased linearly (P<0.01) with increasing Zn from 126 to 264 ppm for 0 and 20 ppm levels, respectively. Across sources BWG, plateaued at 22 mg of zinc which equates to 12ppm (66.2% of the data fit the broken-line model). The BWG plateaued at 11.1, 11.9, and 11.8 ppm of added Zn for diets with ZnGLY, ZnAAH and ZnSO₄, respectively. Only 0, 5, and 10 ppm treatments were used to determine RBV for BWG because the slope was linear. Slope ratios revealed RBV of 114.8 and 103.1% for ZnGLY and ZnAAH relative to ZnSO₄. Tibia Zn increased linearly with added Zn, and slope ratios revealed RBV of 145.7 and 138.9% for ZnGLY and ZnAAH relative to ZnSO₄. In conclusion, organic Zn sources have higher RBV than ZnSO₄ based on tibia Zn accumulation, and performance was affected by dietary level of zinc.

Keywords: Zinc Glycinate, Zinc Amino Acid Hydrate, Zinc Sulfate, Zinc bioavailability, Broilers

M133 Effect of feeding calcium pidolate (Calpid®) on productive performance, economic evaluations and litter quality in broilers fed reduced calcium and phosphorus diets AbdelRahman Y. Abdelhady*^{1,2}, Abdelhacib Kihal¹, Salah A. El-Safty², Ahmed Radwan¹, Sergio Merinero³, M. Puyalto¹, J.J. Mallo¹ ¹Norel S.A., Madrid, Spain; ²Faculty of Agriculture, Ain Shams University, Poultry Production Department, Cairo, Egypt; ³Nuvet Agriservices S.L.U., Madrid, Spain

The aim of this study was to evaluate the effect of including calcium pidolate (Calpid®, Norel SA) in broiler feed with reduced Calcium (Ca) and available phosphorus (aP). A total of 800 day-old mixed-sex broiler chicks were randomly assigned to 5 dietary treatments with 8 replicates each: control diet (CONT) meeting FEDNA (2021) requirements; 15% reduced Ca and aP diet (N15);

N15 supplemented with 300 g/ton Calpid® (CP15); 30% reduced Ca and aP diet (N30); and N30 with 300 g/ton Calpid® (CP30). The study lasted 35 days, divided into three feeding phases with pelleted feeds. Live body weight (LBW), feed conversion ratio (FCR), feed intake (FI), mortality rates (MR), European Production Efficiency Factor (EPEF), economic evaluations, and litter quality were analyzed using one-way ANOVA. Results indicated that at day 35, broilers fed the CP15 diet had the highest LBW (2,083 g), significantly higher than N15 (1,920 g), N30 (1,876 g), and CP30 (1,925 g), while being significantly similar to CONT (2,013 g) (P<0.05). The CP30 group also showed improved LBW compared to N30 (P < 0.001). CP15 had the best FCR (1.62), outperforming N15 (1.70) and N30 (1.72) (P<0.01), while being similar to CONT (1.63) and CP30 (1.66) (P>0.05). Total FI was highest in CP15 (3,306g), followed by CONT (3,216g), N15 (3,194g), CP30 (3,130g), and N30 (3,160 g) (P < 0.01). EPEF peaked in CP15 (342), followed by CONT (330), CP30 (310), N15 (304), and N30 (287). MR had no significant differences among treatments. Economically, CP15 had the lowest feed cost per kg LBW (€0.783) compared to CONT (€0.791), N15 (€0.814), N30 (€0.815), and CP30 (€0.793), yielding cost savings over the CONT and negative control diets. Litter moisture and pH showed no significant differences. Incorporating Calpid® into broiler diets with a 15% or 30% reduction in Ca and aP significantly enhanced broiler performance compared to negative control groups. The CP15 group achieved results comparable to the CONT group in terms of LBW and FCR, demonstrating improved mineral absorption and utilization. Economic analysis highlighted cost savings with CP15 due to its superior efficiency. Additionally, litter quality remained unaffected, indicating that Calpid® effectively supports a healthy and sustainable rearing environment.

Keywords: Broilers, Calcium pidolate, Growth Performance, Economics, Litter quality

M134 Evaluation of copper source and dose on phytase efficacy in young broilers Kyle M. Venter*¹, Peter W. Plumstead¹, Alessandra R. Monteiro², Jamie Fourie¹ ¹Neuro Livestock Research, Brits, South Africa; ²Animine Precision Minerals, Annecy, France

The use of prophylactic levels of Cu at doses of 150–250 ppm, is used in commercial broilers to provide antimicrobial benefits. However, Cu interactions with phytic acid may impact mineral bioavailability and reduce phytase efficacy. This study assessed the effects of Cu source and dose on growth performance and bone mineralization in broilers when diets contained graded levels of phytase. The trial utilized a 3 x 6 factorial incorporating three Cu trt, copper sulfate (CuS) at 10 and 200 ppm and 200 ppm from monovalent copper (Cu2O) and six dose levels of a novel synthetic 6-phytase per Cu trt (0, 500, 1,000, 1800, 2,500 and 3,500 FTU/kg). Basal diet free of inorganic P was formulated with a low P, and Ca content (1.2 g/kg dP, 3.4 g/kg P and 0.70% Ca) to which Cu and phytase was added to make 18 treatments. Broiler body weight (BW) was determined for the trial period (4-10 days). On day 10, broilers were sampled, the right tibia removed and pooled per pen to determine defatted tibia bone ash (%). Data were analyzed in JMP using the fit model platform to evaluate the significance of linear and quadratic responses to phytase and interactions with Cu, as well as non-linear regression for each Cu trt. Broiler BW and tibia ash% exhibited significant (P<0.05) linear and quadratic responses to incremental doses of phytase. There was no interaction of Cu x phytase for BW with Cu trt exhibited similar asymptote, scale and growth rate for BW. For tibia mineralization, a significant Cu source x phytase x phytase interaction was observed (P < 0.05). The supplementation of diets with 200 ppm from Cu2O attained the asymptote in tibia ash% at

a lower phytase dose compared to 200 ppm and 10 ppm of CuS ($P < 0.05$). For tibia mineralization, a significant nonlinear regression analysis indicated that tibia ash percentage achieved 95% of the asymptote response at 1,550 FTU/kg phytase for CuS and 1,000 FTU/kg for Cu₂O, suggesting that Cu₂O is less susceptible to phytic acid chelation. Results from this *in vivo* study suggest that the Cu source and dose influence bone mineralization, in doing so influencing phytase efficacy in young broilers.

Keywords: Broiler, phytase, copper

M135 A meta-analysis of copper source and level effects on growth performance of broiler chickens Abidemi Adekoya¹, Eric Myers¹, Gabriela Cardoso¹, Frances Yan^{*1} ¹*Novus International Inc., Chesterfield, Missouri, United States*

A meta-analysis was conducted to compare low level of methionine hydroxy analog chelate trace mineral Cu (Cu-MHAC; MINTREX^o Trace Minerals) as a strategy to substitute high levels of tribasic copper chloride (TBCC) on broiler chicken performance. The analysis consisted of six trials conducted from 2012 to 2022. Growth performance data were grouped into 3 phases: 0-17 d, 0-35 d, and 0-49 d. Treatments were analyzed as One-way ANOVA of 3 treatments: Negative Control (NC) with 0, 10, or 16 ppm Cu supplemented from CuSO₄, 125 ppm TBCC, and 30 ppm Cu-MHAC (combined 25 & 30 ppm). Diets used in the trials consisted of corn-SBM and/or wheat-SBM-based diets with or without full-fat soy₂ and the majority of the trials had *Eimeria* and/or *C. perfringens* challenges. Treatments from each trial were between 8 to 16 replicates/treatment and 13 to 25 birds/replicates, which added up to 164 observations in the 0-17 d phase, and 345 and 154 in the 0-35 d and 0-49 d respectively. Data were analyzed using a mixed model, with treatment as the fixed effect, trial as a random effect, and continuous day variable within each phase as a covariate. Least square means were compared using the Student's t procedure, with a significance level at $P \leq 0.05$. No significant differences were observed among Cu sources and levels for body weight, feed intake, and mortality across all phases. From 0-17 d, 125 ppm TBCC improved FCR ($P < 0.05$) over NC, and there was no significant difference in FCR between 30 ppm Cu-MHAC (1.228) and 125 ppm TBCC (1.222). From 0-35 d, FCR was significantly improved ($P < 0.05$) with 30 ppm Cu-MHAC and 125 ppm TBCC compared to NC and there was no difference between 30 ppm Cu-MHAC (1.421) and 125 ppm TBCC (1.421). No significant differences were observed in FCR from 0-49 d among the treatments. In conclusion, the meta-analysis demonstrated that 30 ppm Cu-MHAC can be used to replace 125 ppm TBCC in the diet of broiler chickens to achieve the same growth performance.

Keywords: Copper, Feed efficiency, Broiler, Nutrition, Trace mineral

M136 Effects of varying digestible amino acid densities on the growth performance of Cobb 500 x MX and Ross 708 x YP broiler chickens Tanner Wise^{*1}, Logan Erb¹, Corey A. Johnson¹, Anthony D. Quant¹, Elizabeth J. Miller¹, Nickki Tillman² ¹*Kalmbach Nutritional Services, Nutrition, Upper Sandusky, Ohio, United States;* ²*Nutritional Statistics LLC, Buford, Georgia, United States*

The objective of the following study was to evaluate how varying levels of digestible amino acid (AA) densities affect the growth performance of two commercial strains of broilers. A total of 5,760 straight-run broilers allotted to 96 pens according to a RCBD with a 2 x 6 factorial arrangement of 2 broiler strains (Cobb: Cobb 500 x MX or Ross: Ross 708 x YP) and 6 digestible Lys (dLys) levels, which yielded 12 treatments, 8 replications/TRT and 60 birds/pen. Diets were fed in 3 phases including a starter (D0-13; 1.22-1.47% dLys), grower (D13-27; 1.12-1.37% dLys), and finisher (D27-41; 1.02-1.27% dLys). Birds and feed were weighed on D0, 13, 27, and 41 to calculate feed intake (FI), BW gain (BWG), and mortality corrected FCR (FCRc). Digestible ratios for all other limiting AA were maintained across dietary treatments for each phase, resulting in the concentrations of these AA elevating with dLys. The main effects of genetic strain and dLys density were evaluated along with their interaction. Additionally, orthogonal linear and polynomial contrasts were utilized to evaluate significant linear and quadratic effects of dLys within each strain. Increasing AA density resulted in a linear increase of BWG from D0-27 for both strains ($P < 0.05$); however, BWG was greater for the Cobb birds during this period ($P < 0.05$). Conversely, from D27-41 the BWG was greater in the Ross birds ($P < 0.05$) and there was no linear impact of AA density on the BWG of the Cobb birds ($P > 0.05$); though, these birds had greater BW on D41. Feed intake followed a similar pattern where the Cobb birds consumed more from D0-27 with a reversal to the Ross consuming more during the finisher ($P < 0.05$). Increasing AA density resulted in a linear improvement in FCRc regardless of strain in each phase ($P < 0.05$). The Cobb birds produced the lowest FCRc from D0-27 ($P < 0.05$), but this reversed from D27-41 and from D0-41 where the Ross birds had the lowest FCRc ($P < 0.05$). These data indicate disparate effects of increasing AA densities between Ross and Cobb broilers across dietary phases, with Cobb birds having greater growth and efficiency prior to the finisher phase and the Ross birds performing better later in production with greater sensitivity to AA density.

Keywords: amino acids, broiler, Cobb 500, Ross 708

Metabolism & Nutrition VIII: Amino Acids & Enzymes

M137 The determination of gross energy and total essential amino acid content in soybean meal and soy protein concentrates and isolates by near-infrared reflectance spectroscopy Dylan Moss^{*1}, Thomas Jones², Adam J. Davis¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States;* ²*University of Georgia, Poultry Science, Athens, Georgia, United States*

Soy protein is utilized heavily in human and animal nutrition, but the gross energy (GE) and amino acid (AA) content of soy dietary ingredients can vary based on soybean plant genetics, agronomic conditions and processing procedures. Knowing the AA and energy content of a soy product being used in a diet helps ensure that formulated diets meet nutrient requirements necessary for optimal performance and well-being. As such, the goal of the

current research was to determine if near-infrared reflectance spectroscopy (NIRS) could be used to rapidly determine the GE and essential AA content of different types of soybean meal (SBM), as well as, isolated and concentrated soy protein products. For this research over 250 different soy samples were obtained including full fat SBM, mechanically and solvent extracted SBM, soy protein concentrates, and isolates with 68% of the samples being solvent extracted SBM, the most utilized dietary soy product. Each sample was uniformly ground before obtaining a complete near-infrared spectral analysis using a Bruker MPA: FT-NIR Spectrometer equipped with OPUS software. Approximately half of the samples were used to construct calibration curves to predict the GE and essential AA content of the soy products, while the remaining samples were used to validate the calibration models. The crude protein content of the samples ranged from 36

to 88%. The coefficient of determination (R^2) between the NIRS predicted values and the high performance liquid chromatography (HPLC) determined amino acid values or bomb calorimeter determined GE values were all 0.98 or greater except for tryptophan which had a value of 0.80. Furthermore, the NIRS-predicted values for all samples within the validation curves for the GE and essential AA methods deviated by less than 5% of the laboratory determined values, except for tryptophan. Due to the biochemical properties of tryptophan, the precision of determination via HPLC is reduced, which is reflected in decreased NIRS predictability. Overall, the results indicate that NIRS can be used to accurately predict the GE and total AA content of all the essential AA in SBM and soy protein concentrates and isolates with a comparable margin of error expected from traditional laboratory procedures.

Keywords: diet formulation, soy ingredient quality

M138 Evaluation of interaction effects of various levels of energy and amino acid density in the finisher phase (29-42d) on Ross-708 broiler performance and carcass yield Muhammad Ali*¹, Catherine Fudge¹, Nicolas Mejia-Abauza¹, Alex Corzo², Ruben Kriseldi², Roshan Adhikari³, Chongxiao Chen¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Aviagen, Huntsville, Alabama, United States; ³CJ Bio America, Downers Grove, Illinois, United States

Understanding the interaction of energy and protein in broiler feed is critical for growth optimization. This experiment evaluated Ross-708 broiler response to varied metabolizable energy (ME) and digestible lysine (dig-Lys) levels during finisher phase (29-42 d). Ross-708 broilers were raised under standard conditions until d 28, and 1440 broilers were redistributed in 72 pens (10 males and 10 females/pen). A central composite design was applied with 9 treatments (6 replicates/star point; 24 replicates/central point). Treatments included variable ME (2879, 2943, 3100, 3256, and 3320 kcal/kg) and dig-Lys levels (0.95, 0.98, 1.05, 1.12, and 1.15 %). At d 42, body weight gain (BWG), feed intake (FI), and FCR were calculated, and at d 43, 8 birds per pen (4 birds/sex) were processed. Data was analyzed using JMP Pro 17 by response surface macro-option. For BWG ($R^2=0.35$, $P<0.0001$), FI ($R^2=0.46$, $P<0.0001$), and FCR ($R^2=0.83$, $P<0.0001$), only energy showed linear ($P<0.0001$, $=0.0021$, <0.0001) and quadratic ($P<0.0001$, $=0.0044$, <0.0001) effect, respectively. For processing, cold carcass ($R^2=0.24$, $P=0.0028$), *Pectoralis major* ($R^2=0.44$, $P<0.0001$), and *minor* ($R^2=0.37$, $P<0.0001$) exhibited linear ($P=0.0104$, 0.0004, 0.0017) and quadratic ($P=0.0077$, 0.0013, 0.0017) effects respectively for ME, with *Pectoralis major* showing an interaction effect (dig-Lys * ME; $P=0.0576$). BWG (1.370 kg), FI (2.269 kg/bird), FCR (1.566), cold carcass (2.118 kg), *Pectoralis major* (0.630 kg) and *minor* (0.134 kg) were optimized at same dig-Lys (1.15%; 0.95% for FI) but different ME values (2999, 3039, 3163, 3003, 2899, 2971 kcal/kg) respectively, with performance declining beyond these optimal ME levels. Moreover, model for legs ($P=0.7311$) and wings ($P=0.7971$) was found non-significant. Interestingly, for optimized live performance and breast weight, females showed higher ME than males (3051 vs 2965; 2980 vs 2879 kcal/kg), respectively. Conversely, males showed higher ME values for cold carcass weight optimization than females (3037 vs 2973 kcal/kg). In conclusion, performance and carcass yield were optimized at 1.15% dig-Lys but at relatively lower ME levels used in current experiment. Females exhibited the same dig-Lys but higher ME values than males for optimized live performance and breast yield.

Keywords: Amino Acids, Broilers, Growth Performance, Metabolizable Energy, Processing

M139 Conditioning above 70°C and Pelleting Corn and Soybean Meal-based Diets Containing Under Processed Soybean Meal can Alleviate the Effects of Antinutritional Factors Reuben Adejumo*¹, Kristina M. Bowen², Lucas E. Knarr¹, Elizabeth A. Lynch¹, Emily B. Estanich¹, Alexis Renner¹, Taylor Waugerman¹, Hari B. Krishnan³, Joseph S. Moritz¹ ¹West Virginia University, Division of Animal and Nutritional Sciences, Morgantown, West Virginia, United States; ²Egg innovations, Warsaw, Indiana, United States; ³University of Missouri, Plant Genetics Research Unit, USDA-ARS, Columbia, Missouri, United States

Soybean meal (SBM) is referred to as the “gold” standard of protein; however, SBM also contains antinutritional factors that are known to hinder bird performance. Soybeans are toasted during SBM manufacture, but the optimal temperature and holding time for adequate processing is not well-defined. An initial companion experiment determined that including under-processed SBM in mash diets decreased bird performance. Therefore, diets containing under- and peak- processed SBM conditioned at 70, 80, or 90° for 30 s in addition to an over-processed SBM mash diet were assessed to determine optimal conditioning temperature via live bird performance and amino acid digestibility. The three SBM types (under-, peak-, over-processed) were obtained from a commercial soybean crush plant. Diets were formulated to 85% crude protein and digestible amino acid requirement, differing only in the type of SBM utilized. Diets were fed for 18 days and contrasts were performed to explore differences between treatments. A 2 (SBM type) × 3 (Conditioning temperature) factorial was analyzed for the pelleted diets. The 7 diets were each fed to 12 replicate cages of 9 chicks. On day 18, live performance metrics were measured, and ileal contents were collected. Day 0-18 feed intake (FI) per bird increased with increasing conditioning temperature ($P<0.05$). An interaction between SBM type and conditioning temperature was identified, wherein birds fed the under-processed SBM diet gained more weight with increasing conditioning temperature while live weight gain (LWG) decreased for the peak-processed diet ($P<0.05$). Increasing the conditioning temperature of the under-processed diet to 80° restored LWG to that of the peak-processed 70° diet. Feed conversion ratio (FCR) was increased in the peak-processed diet and decreased by the under-processed diets with increasing conditioning temperature ($P<0.05$). Birds fed the over-processed diet gained the least and had the lowest FI ($P<0.05$). The digestibility of all amino acids increased when the conditioning temperature of the under-processed diet increased from 70° to 80° ($P<0.05$) and did not change for the peak-processed diets. Diets containing under-processed SBM conditioned above 70°C and pelleted mitigated antinutritional performance effects.

Keywords: Optimal temperature, Performance, Amino acid digestibility

M140 Utilization of near-infrared reflectance spectroscopy to predict the digestible essential amino acid content of mechanically and solvent-extracted soybean meal Thomas Jones*¹, Coleman Hatmaker¹, Chongxiao Chen¹, Adam J. Davis¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States

Soybean meal (SBM) is a primary constituent of poultry diets globally due to its protein content and amino acid digestibility. Yet, variations in agronomic conditions, cultivar variety, and processing techniques can alter the digestible amino acid content of SBM. To detect variation in nutritional value, rooster or broiler bioassays with subsequent laboratory analyses can be performed to determine the digestible amino acid content for an individual sample. However, these bioassay determinations are expensive

and time-consuming. In contrast, near-infrared reflectance spectroscopy (NIRS) has the potential to enable rapid ingredient analysis, allowing for real-time assessment of nutrient value prior to incorporation in poultry diets. In the current research, calibration curves were designed to assess if NIRS has the capacity to accurately predict the digestible amino acid content of all essential amino acids for solvent-extracted and mechanically-expelled SBM. The cecectomized rooster bioassay was used to determine the digestible amino acid content of over 200 SBM samples. Each SBM sample was uniformly ground before obtaining a complete near-infrared spectral analysis using a Bruker MPA: FT-NIR Spectrometer equipped with OPUS software. Approximately half of the samples were used to construct each calibration curve, while the remaining samples were used to validate each calibration curve. For the validation samples, the average error between NIRS-predicted and bioassay-determined values, measured as root mean square error of prediction (RMSEP), ranged from 0.010 to 0.059 across the essential amino acid validation sets, indicating no significant statistical differences between the bioassay and NIRS-determined values. Moreover, for all essential amino acids, apart from lysine and tryptophan, 100% of the validation samples predicted within $\pm 5\%$ of their bioassay-determined value. For lysine and tryptophan, 98% and 85% of the predicted values deviated by less than $\pm 5\%$ of their bioassay-determined values, respectively. This research indicates that NIRS can be used to accurately predict the digestible amino acid content of all essential amino acids, with a comparable margin of error expected from the bioassay and laboratory analyses for SBM samples used in poultry diets.

Keywords: Poultry, Feed formulation, Ingredient quality

M141 Varying branched-chain amino acid and tryptophan ratios in corn and soybean meal-based diets influences 0-28d turkey poult performance, blood plasma serotonin concentration, relative immune organ weight, and tibia mineralization Emily B. Estanich*¹, Kristina M. Bowen¹, Lucas E. Knarr¹, Elizabeth A. Lynch¹, Sally Noll³, Arturo Garcia-Morales², Joseph S. Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Morgantown, West Virginia, United States; ²Butterball, LLC, Goldsboro, North Carolina, United States; ³University of Minnesota, Department of Animal Science, St. Paul, Minnesota, United States

Tryptophan and the branched-chain amino acids (BCAA) leucine, isoleucine, and valine are essential amino acids (AA) for poultry. Larger inclusions of concentrated corn proteins can increase Leu levels above requirement, leading to metabolic antagonism and degradation of limiting BCAA in the diet. Excess Leu may also inhibit Trp absorption, transport, and thus, serotonin synthesis. Hence, high-Leu diets could be corrected with supplementary L-Val, L-Ile, and L-Trp to prevent performance reductions associated with imbalanced BCAA/Trp ratios. This study evaluated the effects of varying dietary BCAA/Trp ratios using corn gluten meal, L-Val, L-Ile, and L-Trp on performance, plasma serotonin concentration, immune organ weight, and tibia mineralization in commercial turkey hens from 0-28d of age. A 34.8% corn and 54.0% soybean meal diet served as the Control. Diets were fed to 15 replicate cages of poults utilizing a randomized complete block design. Performance, tibia, and immune organ data were analyzed using a one-way ANOVA followed by Fisher's LSD ($\alpha=0.05$). Plasma serotonin data was calculated using a 4PL regression and analyzed using a one-way ANOVA followed by Tukey's HSD test. Uncorrected high-Leu diets (1.6 Leu:Lys) reduced FI, BW, and LWG relative to the Control at d14 ($P<0.05$). Fully corrected high-Leu diets with additions of L-Val, L-Ile, and L-Trp produced equivalent FI and increased BW and LWG relative to the Control

at d14, 21, and 28 ($P<0.05$). Plasma serotonin concentrations were lower in the uncorrected high-Leu diet and Ile-corrected high-Leu diet than the Control ($P<0.05$). Spleen weight was lower in poults fed the fully corrected high-Leu diets than the Control diet ($P<0.05$), and tibia ash mg/chick was highest in the fully-corrected diet compared to both the Control and high-Leu diets ($P<0.05$). These results suggest that performance reductions from excess Leu may be most important at d14, although high-Leu diets with corrective inclusions of L-Val, L-Ile, and L-Trp can restore or enhance performance throughout a 28d starter phase in turkeys. However, nutritionists should consider the cost of corn and soybean meal-based diets with higher inclusion levels of feed-grade AA and concentrated corn proteins.

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

M142 Effects of high and moderate amino acid density diets in Cobb 500 male broilers Virginia G. Slick*¹, Savannah Wells-Crafton¹, David Reynolds¹, Michael T. Kidd¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States

The majority of research assessing the amino acid responsiveness of the Cobb 500 broiler has been conducted in experimental designs utilizing pens that contain 12 to 25 birds with high replication. This work was conducted to determine Cobb 500 male responses to two diets differing in amino acid density in pens containing 500 birds each. Four-thousand males were weighed and randomly allocated to 8 pens differing only in diet amino acid density fed to birds from 0 to 14, 15 to 28, 29 to 35, and 36 to 42 d of age. Diets consisted of varying digestible Lys across phases and maintaining minimum digestible amino acid ratios to Lys for all other limiting amino acids. The moderate (M) diet was formulated to contain 1.18, 1.10, 1.03, and 0.98% digestible Lys whereas the high (H) diet contained 1.24, 1.16, 1.08, and 1.03%. Both diets met recommended nutrient levels. Bin scales were used to measure pen feed consumption. All birds were weighed in groups of 5 birds to assess pen BW, and subsequent feed conversion. In addition, the group weights were used to establish sample size distribution to predict pen BW for future experiments. All data was analyzed using JMP software to perform a one-way ANOVA and T-test. A half width of 0.02 kg was chosen as the indicator of needed sample size for future studies. H diet had a significantly ($P<0.05$) lower number of birds needed to get within 0.04 kg of the true average BW of each pen, as opposed to M pens, indicating H diet created improved uniformity. No significant differences in BW or livability were found between the two diets at day 42. There was a difference in FCR with the H diet having significantly ($p<0.05$) lower FCR than the M diet. Twenty birds per pen were chosen randomly for processing at 42 d of age. Few differences in carcass yields occurred. However, percentage peritoneal cavity fat was significantly ($P<0.05$) lower and wing yield was higher in birds fed the H diet. No other differences were found. Yield data indicates that feeding higher amino acid density improves wing yield while decreasing peritoneal cavity fat. Along with the processing data results, increasing amino acid density improved both feed conversion and uniformity within the population which could prove useful for processing performance.

Keywords: amino acid, lysine, uniformity

M143 Understanding the branched-chain amino acid interactions on bone morphology of pullets (6-12 weeks of age) Charis A. Waters*¹, Roshan Adhikari², Jason T. Lee², Deependra Paneru³, Woo Kyun Kim³, Ruben Kriseldi⁴, Michael Elliot⁵, Li Zhang¹, Kelley Wamsley¹, Pratima Adhikari¹ ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²CJ Bio

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Branched-chain amino acids (BCAAs) are essential for skeletal development but require balance to prevent excess breakdown and optimize bone health. The objective of this study was to examine BCAA effects on bone morphology of Hy-Line W-36 pullets (6-12 wks of age). A total of 2,160 pullets were housed in 120 pens in a circumscribed central composite design (CCD) with 20 treatments (14 treatments and 6 center points). Each treatment consisted of varying digestible Val:Lys (65 to 95), Ile:Lys (58 to 88), and Leu:Lys (120 to 220) ratios; digestible Lys was formulated at 0.88% (Hy-Line management guide, 2020). At 12 weeks of age, one bird per pen (6 birds per treatment) was randomly selected and euthanized, and the right femur was removed for bone morphology analysis. Data were analyzed as a CCD using the surface response option of JMP v. 15. Due to repeated observance of effects in CCD, means were considered significant at $P < 0.10$. Total bone surface (BS) was significantly increased (1070.76 mm²; $P = 0.0129$; $R^2 = 0.80$) at the lowest Val:Lys (65) and highest Ile:Lys (83) and Leu:Lys (220) ratios. Similarly, cortical BS significantly increased (576.56 mm²; $P = 0.0153$; $R^2 = 0.79$) at Val:Lys (65), Ile:Lys (88), and Leu:Lys (220). Cortical bone mineral density (BMD) significantly increased (0.905 g/cm³; $P = 0.0594$; $R^2 = 0.71$) with the lowest Val:Lys (65) and Leu:Lys (120) and highest Ile:Lys (88) ratios. However, cortical BMD decreased when Ile:Lys was lowered while keeping Val:Lys and Leu:Lys ratios constant. Trabecular BMD significantly increased (0.690 g/cm³; $P = 0.0369$; $R^2 = 0.75$) with Val:Lys (65), Ile:Lys (58), and Leu:Lys (120) ratios. Results indicate that higher Ile:Lys (88) ratios in developing pullets improved total and cortical BS and cortical BMD. Total BS was higher at increased Ile:Lys (88) and Leu:Lys (220) ratios and lower Val:Lys (65) ratios. Lower Val:Lys (65) ratios enhanced BMD and BS in both cortical and trabecular bones. These findings suggest that a lower Val:Lys (65) ratio, combined with a higher Ile:Lys (88) ratio and Leu:Lys (220) ratio in the pullet diet from 6 to 12 weeks of age, may improve bone quality, supporting better skeletal health and growth potential in laying hen pullets.

Keywords: branched-chain amino acids, bone quality, microstructure, pullets, surface response

M144 Dietary arginine responses of Ross 708 broilers subjected to enteric challenge with *Eimeria* spp. and *Clostridium perfringens* Annalise G. Anderson*¹, Jason T. Lee², Roshan Adhikari², Ruediger Hauck¹³, Samuel J. Rochell¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²CJ America, Inc., Downers Grove, Illinois, United States; ³Auburn University, Department of Pathobiology, Auburn, Alabama, United States

The objective of this study was to evaluate the effects of dietary Arg:Lys ratio on performance and processing characteristics of broilers subjected to an enteric challenge with *Eimeria* spp. and *Clostridium perfringens*. A total of 1,800 male Ross 708 broiler chicks were fed a common starter diet (0-11 d) prior to being fed 1 of 7 target digestible Arg:Lys ratios (81, 93, 105, 117, 129, 141, and 153) from 11-29 d and a common finisher diet (29-39 d). Birds were reared in floor pens and treatments were replicated by 8 pens of 25 birds. At 15 d, birds were orally gavaged with a 10-fold dose of a live coccidiosis vaccine followed by 10⁷ CFU *C. perfringens* at 19 and 20 d. Unchallenged birds in control groups (105 and 129 Arg:Lys only) received a sham gavage. At 39 d, 12 birds per pen from 4 treatments (105 or 129 Arg:Lys × unchallenged or challenged) were processed and

deboned to determine carcass and parts weights and yields. Data were analyzed by ANOVA with pen location as a random blocking variable, and pre-planned contrasts were used to evaluate the main effects of Arg:Lys ratio (105 vs 129), enteric challenge, and their interaction. Polynomial contrasts were also used to determine Arg responses across all titration levels within the challenged group. Statistical significance was determined at $P \leq 0.05$. The challenge model impaired body weight gain (BWG) and feed conversion ratio (FCR) during the grower phase ($P < 0.05$), as well as cumulative BWG ($P = 0.003$). Feeding 129 Arg:Lys improved FCR compared to those fed 105 Arg:Lys during the grower phase, primarily due to its effect during the challenge recovery period (22-29 d). During this period, FCR of challenged birds improved linearly with increasing Arg:Lys ($P < 0.001$). Furthermore, increasing Arg:Lys ratio quadratically improved BWG and FCR of challenged birds during the grower phase and cumulatively ($P < 0.05$). For processing characteristics, feeding 129 Arg:Lys improved chilled carcass yield ($P = 0.031$) compared to a 105 ratio. Overall, results of this study indicate that increased Arg:Lys can improve broiler performance during enteric challenge, particularly during recovery periods. This may be due to the diverse roles of Arg in supporting gastrointestinal tract health and immunity.

Keywords: Arginine, Enteric challenge, Broiler, Processing characteristics, Necrotic enteritis

M145 Commercial phytase products varied in efficacy in 0.22% non-phytate phosphorus corn and soybean meal starter diets but were ineffective in 0.13% non-phytate phosphorous diets

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The inclusion of inorganic phosphorus (P), in the form of finite rock phosphate sources, can lead to increased feed costs and environmental impacts. However, the addition of the exogenous enzyme phytase can alleviate these issues. The objective of this study was to evaluate corn/soybean meal-based starter diets that either exclude inorganic P or contain low non-phytate phosphorous (nPP) with commercial phytase supplementation on bird performance, bone mineralization, and the effect on dicalcium phosphate (DCP) equivalency. Birds were weighed and separated into 121 to create uniform initial pen weights. Treatments were assigned using a randomized complete block design consisting of eleven blocks, with one pen of 8 broilers as the experimental unit. A PC and four NC treatments with graded levels of nPP were used to create a standard curve. Phytase A was added to the NC1 (no DCP, nPP= 0.13%) at 1,500 FTU/kg or 2,000FTU/kg. Phytase A, B, and C were added to the NC2 (nPP=0.22%) at 1,500FTU/kg. Data were analyzed using GLM procedure of SAS 9.4, considering pen location within the room as the blocking criterion. Significance was set at $P < 0.05$. Significant Analysis of Variance results were further analyzed using the Fisher's LSD test. Linear and quadratic regressions using SAS PROC REG were conducted to determine impacts of increasing levels of non-phytate phosphorus using data from the standard curve diets. The NC1 and NC2 nPP level in a broiler starter mash diet decreased bird performance, tibia mineralization, and bone breaking strength relative to a similar Ca:nPP ratio of the PC diet (0.95:0.5) ($P < 0.05$). Diets without DCP did not demonstrate improved performance, tibia mineralization, or bone breaking strength with the addition of phytase ($P > 0.05$). A 1,500 FTU/kg inclusion of phytase A increased LWG, IBW, Bird FI, Pen FI, and tibia

mineralization when compared to a 0.22% nPP NC2 diet or NC2 diet containing either phytase B or C ($P<0.05$). Mortality for this study was not significant despite the low nPP level ($P>0.05$). The addition of commercial phytase products were not able to overcome nutrient detriment in inorganic phosphate free, corn/soybean meal-based, mash diets but varied in effectiveness in 0.22% nPP diets.

Keywords: Phytase, Inorganic Phosphate, Dicalcium Phosphate, Broiler Performance, Tibia Mineralization

M146 Reduction of ribonucleotide reductase subunit RRM2 potentially impairs gut function in broilers exhibiting the woody breast condition Majid Shakeri^{*1}, Elizabeth Ziabchenko¹, Janghan Choi¹, Caitlin Harris¹, Richard Buhr¹, Trevor Mitchell¹, Byungwhi Kong¹, Hong Zhuang¹, Brian Bowker¹ ¹USDA-ARS, U.S. National Poultry Research Center, Athens, Georgia, United States

The woody breast (WB) condition impairs the texture and appearance of chicken breast meat causing a financial loss for the poultry industry. Recent studies showed that nutritional treatments reduce WB and suggest that gut health may play a role in WB. However, there is limited information available on gut health and the WB condition. Ribonucleotide reductase, subunit RRM2, is involved in DNA synthesis and mitochondria function. Inhibition of RRM2 increases gastrointestinal disturbances and liver toxicity by disturbing inflammatory mediators and mitochondrial homeostasis. This study aimed to investigate links between RRM2 and gut health in broilers exhibiting WB. Samples were collected 15min post-mortem from 15 severe WB and 15 normal (N) birds. Gene expression was performed by qPCR using SYBR reagents, while biochemical kits were conducted to collect data. Data were analyzed using Prism V.9 and t-test. Results indicated that WB breast muscles exhibited higher pH ($P=0.03$), drip loss ($P=0.03$), compression force ($P=0.0005$), redness ($P=0.02$), and hemorrhages scores ($P=0.004$) vs N samples at 24h post-mortem. RRM2 expression was reduced for WB (duodenum and liver, $P=0.0001$ and $P=0.01$). Bcl-2 ($P=0.03$ and NS), HSP70 ($P=0.002$ and NS), MDA (both $P=0.01$) and DNA damage ($P=0.004$ and NS) increased for WB, indicating greater oxidative damage in the tissues. Expression of genes related to mitochondria function were reduced for WB; ATP-6 ($P=0.07$ and $P=0.08$), CytB ($P=0.06$ and NS), mtDNA ($P=0.05$ and NS), tolemer ($P=0.05$ and NS) and UCP3 (NS and $P=0.01$). WB had shorter villi length ($P=0.004$) and thicker crypt ($P=0.07$), consequently lowered villi/crypt ratio ($P=0.004$). Expression of claudin1 ($P=0.003$ and NS), occludin ($P=0.02$ and NS) and SGLT-1 ($P=0.005$ and $P=0.002$) reduced for WB indicating impaired gut barrier function. Amylase (measured in the duodenum, $P=0.04$) and citrulline ($P=0.07$ and $P=0.04$) were reduced for WB. ATP concentrations were reduced for WB (both $P=0.07$). In conclusion, reduced RRM2 expression impaired mitochondria function leading to increased inflammation and altered the gut morphology and enzyme activities, suggest that RRM2 may play an essential role in gut health and potentially the WB myopathy.

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

M147 Growth performance and immune response of young tom turkeys to diets containing different doses of an endo-1,4-β-D-mannanase Adebayo Sokale^{*1}, M Baxter², Sami Dridi³, A. Fickler⁴ ¹BASF Corporation, Florham Park, New Jersey, United States; ²Ozark Avian Research, Fayetteville, Arkansas, United States; ³University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ⁴BASF SE, Ludwigshafen, Germany

This study aimed to investigate the effects of increasing doses of a β-mannanase (Natupulse[®] TS) on growth performance, cellular stress, and immune response of turkey toms fed an all-vegetable diet containing low protein soybean meal. A total of 1920 male Hybrid Converters turkeys (40 birds × 12 pens/treatment) were randomly allotted to 4 dietary treatments (T; T1= 0, T2=400, T3=800, and T4=1200 TMU/kg feed) in a randomized complete block design. A corn-soybean meal (45% CP) based diet was formulated and fed from 0-5 weeks. Feed intake and body weight data were collected at 3 and 5 weeks. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. On d 21, ileal samples from 5 birds per treatment were used for gene expression analysis. Genes associated with markers of oxidative stress, gut barrier function and inflammation were evaluated. The experimental unit was the pen, and data were analyzed using mixed model of JMP[®]. Although, quadratic polynomial (QP) model was used to fit the titrated levels, no significant Goodness of fit ($P<0.05$) was observed. Therefore, a One-Way ANOVA was performed and reported. There was a significant effect ($P<0.05$) of treatment on the Heat shock proteins (HSP) mRNA expression. The relative expression of HSP60, HSP70, and HSP90 mRNA were significantly lower in T4 in comparison to T1. Similarly, there was a significant treatment effect on barrier function genes, CLDN1 and JAM3. There was a tendency effect ($P<0.10$) of treatment on the relative expression of CAT1 and SOD mRNA. The expression of inflammatory cytokines (IL-6, TNFα, IL-4, and IL-10) and C-reactive protein (CRP) were significantly lower in the mannanase groups in comparison to the control group. There was no significant effect of mannanase on BWG from 0-5 weeks. However, FCR tended to decrease in a dose dependent manner during this period. Overall, the trial showed a benefit of β-mannanase (Natupulse[®] TS) in young turkeys from 0-5 weeks fed a diet containing low protein soybean meal. The positive impact on feed efficiency may be associated with reduction of inflammation, oxidative stress, and modulation of jejunum integrity and barrier function potentially improving nutrient digestibility, and better protein and energy utilization.

Keywords: gene expression, ileum, performance, turkeys, β-mannanase

M148 The effects of mannanase inclusion in corn-soybean meal-based diets on the performance of laying hens Rosana Maia^{*1}, Michele Bernardino², Edney P. da Silva², Rita P. Brito Vieira², Rodrigo K. Messias¹, A. Fickler¹ ¹BASF, Campinas, SP, Brazil; ²UNESP, Jaboticabal, Brazil

A trial was conducted at the UNESP-Jaboticabal research station to evaluate the effects of mannanase inclusion in corn-soybean meal-based diets on the performance of laying hens. A total of 216 Hisex White hens, aged 35 weeks at the start of the trial, were housed in cages. The experimental design was completely randomized, comprising three treatments with 12 replicates of six hens per cage over a 16-week experimental period divided into four cycles (28 days each). The experimental treatments included: 1) Positive Control (PC) – following the nutritional recommendations by Rostagno (2017) containing phytase at 300 FTU/kg; 2) Negative Control (NC) – PC with a reduction of 100 kcal/kg; and 3) NC + Mannanase at 800 TMU/kg. The evaluated parameters included feed intake (FI), egg production (EP%), egg weight (EW), egg mass (EM), feed conversion ratio (kg/kg) (FCRkg), and feed conversion ratio (kg/dozen) (FCRdz). Statistical analysis was performed using Minitab software, considering treatments and cycles, with a significance level set at 5% ($P<0.05$). When ANOVA indicated significance, means were further analyzed using the Tukey test. The interaction between treatments and cycles was significant for EP%, FCRkg, and

FCRdz. For EP%, there was no statistical difference among treatments during periods 1 and 2. However, NC + Mannanase demonstrated superior performance compared to NC and similar performance to PC in periods 3 and 4. In terms of FCRkg and FCRdz, NC + Mannanase was on the same level as the PC group during periods 1, 2, and 3, but exhibited significant differences in period 4. In contrast, NC-fed hens showed significant differences compared to PC in all periods. For FI, EW, and EM, no interaction was observed between treatment and cycle; however, FI was lower

($P < 0.05$) for PC compared to NC and NC + Mannanase. For both EW and EM, NC + Mannanase was significantly different compared to NC and showed similar performance to PC. In conclusion, the application of mannanase in the diets of laying hens on top of phytase improved performance parameters (EW, EM, FCRkg, and FCRdz) compared to the negative control.

Keywords: Laying Hens, Mannanase, Performance

Metabolism & Nutrition IX: Feed Additives

T149 Effect of organic acids in drinking water on gastrointestinal pH and mesophilic aerobic counts in broiler chickens Mariana Ornaghi^{*1}, Caio Tellini¹, Carla Caliarì², Carlos Benito², Jhessica Lang¹, Jovanir Fernandes² ¹*Safeeds, Research and Development, Cascavel, Paraná, Brazil;* ²*Universidade Federal do Paraná, Palotina, Brazil*

Organic acids are commonly used as acidifiers in poultry nutrition to improve gut health by reducing pathogenic bacteria contamination. These additives can decrease the pH of drinking water, potentially enhancing digestion and nutrient absorption while promoting a favourable environment for beneficial microbiota. This study aimed to evaluate the effects of a blend of organic acids (pH2O®) at two concentrations on the pH and the counts of strict and facultative mesophilic aerobes (MA) in the crop, gizzard, duodenum and ileum of broiler chickens. The experiment was conducted at the Animal Science Experimental Center of the Federal University of Paraná, using 24 Ross 308 male broiler chickens, 43 days old. The birds were randomly assigned to three treatments: water without additive (control), water with 0.02% acidifier, and 0.20% acidifier, with 8 replicates, that were fasted for 12 hours and received the water with the treatments during this period, samples were collected from the gastrointestinal segments for evaluation. Data were analyzed using one-way ANOVA in SAS 9.4, and means were separated using the Tukey test. Results were considered significant at $P \leq 0.05$. The results showed that the acidifier significantly reduced the water pH from 6.48 (control) to 3.11 and 3.05 at the 0.02% and 0.20% concentrations, respectively. The MA count in the water decreased from 7.2×10^5 CFU/ml (control) to 2.6×10^1 CFU/ml with the 0.02% acidifier, and < 1 CFU/ml at the 0.20% concentration. In the gastrointestinal segments, pH values varied slightly between treatments, ranging from 5.49 (control) to 5.25 in the crop, from 2.69 to 2.72 in the gizzard, from 5.74 to 5.61 in the duodenum, and from 6.54 to 7.02 in the ileum ($P > 0.05$). No significant differences in MA counts were observed in the crop, gizzard, or ileum across treatments ($P > 0.05$). However, in the duodenum, the MA count was significantly reduced by 0.20% treatment (1.15×10^5 CFU/ml) compared to the control (6.41×10^6 CFU/ml). These findings indicate that using an acidifier added to drinking water can reduce the microbial load in the duodenum of broiler chickens without significantly altering the pH in the gastrointestinal segments. It could potentially benefit the poultry sanitary and production aspects.

Keywords: gut health, pathogenic bacteria, microbiota

T150 Efficacy of double-buffered sodium butyrate supplementation on broiler performance, foot pad dermatitis and intestinal histomorphology Bernardo F. Iglesias^{3,4}, Maria Viviana Charriere³, Virginia Fain Binda³, Silvina Pinto⁵, Clemence Marecaille², Julian E. Melo^{*1,2} ¹*Universidad Nacional de Luján, Tecnología, Escobar, Buenos Aires, Argentina;* ²*Dietaxion SAS, Le Loroux-Bottereau, France;* ³*Instituto Nacional de Tecnología Agropecuaria (INTA), Sección Avicultura, EEA Pergamino, Pergamino, Buenos Aires, Argentina;* ⁴*UNNOBA,*

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Uncoated double buffered synthetic sodium butyrate (DBSB) can have an effect on the upper part of the gastrointestinal tract of the broilers and could improve feed digestibility and performance. The objective of the present study was to evaluate the effect of dietary supplementation of DBSB on the performance, intestinal histomorphology and foot pad dermatitis of broilers. A total of 324 one-day-old male broiler chicks (Cobb-500) were allocated in floor pens in a completely randomized block design (9 replicates/treatment). Starter (1-7 d), grower (8-21 d) and finisher (22-33 d) diets were used. Control diets (CTRL) were formulated according to requirements used by the local industry. DBSB (54% SB; BUTYLin® 54, Dietaxion) was included on-top from 1 to 33 days at 600 ppm in the treated diets. Birds and feed were weighed weekly individually and by pen, respectively, and the mortality recorded daily (0-33d). At the end of the trial all chickens from each pen were examined for foot pad dermatitis following the classification of the Welfare Quality (W. Quality®, 2009). Also one bird per pen was selected by average weight and euthanized. The duodenum and jejunum were fixed in formalin 10% for histomorphological examination: Villus height (VH) and crypt depth (CD). Parametric data were analyzed using ANOVA and non-parametric data through the Chi-square Test. There were significant differences at 33d for live weight (+3.9%) and FCR (-3.1%, $P < 0.05$), but not for feed intake ($P > 0.05$). Histomorphological traits VH, CD and VH/CD were not different between treatments for duodenum ($P > 0.05$), but for jejunum supplemented broilers obtained a higher VH/CD and a lower CD than the CTRL ones ($P < 0.05$). The addition of DBSB to these diets improved foot pad quality, increasing the number of type B claws and decreasing type C claws compared to CTRL ($p \leq 0.05$). In conclusion, the supplementation of 600 ppm of DBSB in broilers diets enables to get a better FCR, live weight, VH/CD of jejunum and foot pad quality compared to birds fed a common field diet, which means that DBSB optimize better the feed and is then a good strategy to save resources and would avoid excess of excretions in the environment.

Keywords: chickens, feed efficiency, feed additive, jejunum histomorphology, claw quality

T151 Use of fructooligosaccharides as functional carbohydrates in the modulation of microbiota and intestinal health of broiler chickens Jaime A. Angel-Isaza^{*1,2}, Alvaro Uribe-Serrano¹, Blanca C. Martínez¹, Loufrantz Parra-Mendez¹, Jaime E. Parra-Suescun² ¹*Promitec Santander SAS, Unidad De Innovación Y Desarrollo Biotecnológico, Bucaramanga, Colombia;* ²*Universidad Nacional de Colombia Sede Medellín, Medellín, Colombia*

This study evaluated the effects of dietary supplementation with short-chain fructo-oligosaccharides (scFOS) on fecal microbial communities in broiler chickens and its relationship with intestinal health markers. Non-digestible carbohydrates in animal feed, have

received limited attention, likely due to insufficient understanding of their effects. However, advancements in sequencing techniques have renewed interest in their ability to modulate intestinal microbial communities. Thus, the present study aimed to evaluate how scFOS interacts with the microbiota to develop more effective strategies for improving intestinal health and productivity. The study was conducted at the San Pablo Agricultural Station of the National University of Colombia, Medellín campus, using 216 one-day-old mixed ROSS AP chickens in a randomized complete block design. Three treatments were tested: D1 (Basal Diet without additives), D2 (Basal Diet + 150 ppm avilamycin), and D3 (Basal Diet + 150 ppm avilamycin + 700 ppm scFOS). On day 42, productive variables were measured, jejunum samples were analyzed for barrier and enzymatic protein expression via RT-qPCR, and fecal microbiota was assessed using 16S rRNA sequencing. Statistical analyses were performed using ANOVA for productive variables and gene expression, and the "Phyloseq" package for microbial community analysis. Results showed significant differences ($p < 0.05$) between treatments, with D3 yielding higher final weight compared to D2 and D1. D3 also exhibited a significant increase ($p < 0.05$) in the relative expression of barrier and enzymatic proteins in the jejunum. In fecal microbiota, D1 showed an increase in the Proteobacteria phylum, particularly *Escherichia*, which was notably low in birds receiving D2. In contrast, D3 birds displayed increased Bacteroidetes, with a greater abundance of *Prevotella*, *Alistipes*, and *Butyrivibrio* (from the Firmicutes phylum). D2 showed a significant reduction ($p < 0.05$) in the Shannon index compared to D1 and D3, which did not differ significantly. The use of 700 ppm scFOS modulated the microbiota and improved intestinal health, leading to enhanced productivity. Functional carbohydrates like scFOS are becoming key in cutting-edge nutrition, as they stimulate intestinal microbial communities

Keywords: poultry, prebiotic, eubiosis, productivity, fiber

T152 The effect of natural compounds on growth performance and necrotic enteritis challenge mitigation in 1-to-28-day old broiler chicks Theresia Lavergne¹, Carl Jones¹, Charlie Elrod¹ *Natural Biologics, Inc., Newfield, New York, United States*

Naverde Plus (Natural Biologics, Newfield, NY) is an all-natural blend of yeast complex carbohydrates, nucleotides, and peptides derived from *Saccharomyces cerevisiae* and saponins from *Quillaja* and *Yucca*. This study was conducted to determine the effect of Naverde+ on broiler growth performance and intestinal lesion scores during a necrotic enteritis challenge. A total of 384 male Ross 308 x Hubbard M99 broilers were allotted to dietary treatments: 1. Non-challenged control (NCC), 2. Challenged negative control (CNC), 3. Challenged positive control (CPC, Coban 90 at 110 g/ton, Elanco) or 4. Naverde + (180 g/ton). This was a randomized complete block design with 12 pens per treatment and 8 birds per pen. Broilers were fed a standard starter and grower diet from day 1 to 14 and day 15 to 28, respectively. On day 14, birds in the challenged treatment groups were administered a 10X concentration of a live *Eimeria* spp. vaccine (Advent, Huvepharma) via oral gavage. Non-challenged birds were administered distilled water via oral gavage. On days 16 and 17, feed was contaminated with *Clostridium perfringens* in the challenged treatment groups. Birds and feed were weighed on days 1, 14, and 28 to calculate body weight gain (gain), feed intake (FI), and feed:gain (FG). On day 28, gross lesion scores were evaluated for one randomly selected bird per pen. From day 1 to 14, there were no treatment differences ($P > 0.05$) for gain, FI, or FG. Broilers in Naverde+, NCC, or CPC treatment groups had higher ($P < 0.05$) gain and better ($P < 0.05$) FG than chicks in the CNC

treatment group from day 15 to 28. From day 1 to 28, broilers in NCC, CPC, or Naverde+ treatment groups had better ($P < 0.05$) FG than broilers in CNC treatment group. There were no differences in duodenal or cecal lesion scores across treatments. Jejunal and ileal lesion scores were similar for broilers in NCC, CPC, or Naverde+ treatment groups, and were higher ($P < 0.05$) for broilers in CNC treatment group. Naverde+ can be utilized to improve growth performance and reduce the severity of intestinal lesions in broilers raised under a necrotic enteritis challenge. Thus, Naverde+ can help mitigate the negative effects of a necrotic enteritis challenge in broilers without the use of antibiotics.

Keywords: prebiotics, necrotic enteritis, *Saccharomyces cerevisiae*, saponins, broilers

T153 Mixtures of short- and medium-chain fatty acid-based products improve the performance of Enterococcus-challenged broilers via distinct mechanisms Nicholas P. Evans¹, Ariel Bergeron¹, Olga Lemale³, Tim Goossens², Rob Shirley¹ *¹Adisseo, Alpharetta, Georgia, United States; ²Adisseo, Sint-Niklaas, Belgium; ³Adisseo, Raamsdonksveer, Netherlands*

To evaluate the effect of dietary supplementation of two mixtures (GB and GP) of short- and medium-chain fatty acid (SCFA-, MCFA-) glycerides on the performance of broilers challenged with a natural *Enterococcus* infection. Each pen contained 40 male Cobb500 birds and 12 replicate pens per treatment were used. Apart from the control group, GB or GP was added to starter, grower and finisher diet at 2.0, 1.5, and 1.0 lbs/ton, respectively. Body weight and feed intake were measured at the end of every dietary stage. During the trial, 59 presumptive *Enterococcus*-related culls were necropsied, and femoral head swabs were cultured for bacterial identification. On day 42, the caecal contents of 12 birds per treatment were analyzed for *E. cecorum* and Avian Pathogenic *E. coli* (APEC) counts, and for IgA levels. Results were processed statistically by ANOVA and post-hoc Student's t-test to compare control with treatment groups. The level of significance was set at $p < 0.05$. At day 42, birds in the GB group were heavier than the animals from the control group (6.085 lbs versus 5.926 lbs, $p < 0.05$) and had a lower FCR (1.696 versus 1.735, $p < 0.05$). Interestingly, GP-fed birds were the heaviest (6.148 lbs, $p < 0.05$), while having the lowest FCR (1.682, $p < 0.05$), but mortality was reduced to a greater extent in the GB group (5.62% versus 8.95%, $p = 0.010$) than in the GP group (7.50%, $p > 0.05$). In 80% of femoral head cultures, *Enterococcus* spp. could be identified. In the majority of these cultures, *E. cecorum* could be detected in 74%. While no significant differences in average *E. cecorum* counts could be found, the level of APEC tended to be lower in the GB-treated broilers ($p = 0.08$). In the GP-supplemented animals, on the other hand, the caecal IgA levels were significantly increased ($p = 0.027$). GB and GP improved the performance of broilers that were naturally infected with *Enterococcus*. Both products contain different ratios of SCFA- and MCFA-glycerides. As these components seem to have distinct gut microbial and immune modulatory mechanisms, it would be interesting to further investigate their modes of action during the first two weeks of broiler production, when intestinal *Enterococci* translocate to other tissues.

Keywords: medium chain fatty acid glycerides, *Enterococcus cecorum*, broilers

T154 Effect of a triple-strain Bacillus-based probiotic on performance of commercial broilers challenged with *Enterococcus cecorum* Antoine Meuter¹, Steve Lerner², Meghan Schwartz², Nima Emami² *¹Nononesis, Lyngby, Denmark; ²Novonesis, Milwaukee, Wisconsin, United States*

While traditionally considered part of the normal intestinal flora, certain pathogenic strains of *Enterococcus cecorum* (EC) have emerged as significant causes of diseases in broiler chickens. Infected birds often develop osteomyelitis and spondylitis, resulting in conditions like vertebral osteoarthritis. Flocks infected with EC commonly experience impaired nutrient absorption and reduced feed intake, leading to a negative impact on feed efficiency and growth. The morbidity and mortality from EC infection can reach up to 35 % and 15%, respectively (Jung et al., 2018). The aim of the study was to assess the potential of a commercial triple-strain Bacillus-based probiotic to mitigate the impact of a naturally occurring EC challenge on performance and livability of broiler chickens. The trial took place in a commercial farm facing recurrent and consistent EC infections. 40 male Cobb birds were placed per pen utilizing a randomized block design procedure with 12 replications per treatment group. The control group (NC) received a standard feed until 42 days while the probiotic birds (PRO) were fed with the same diet supplemented with the probiotic at 2 lbs per ton from d 0 to 14 and 1 lbs per ton from d 8 to 42. Data were analyzed using GraphPad Prism 10.0.2 (GraphPad Software LLC, San Diego, CA) and Fisher's LSD test was used for means comparison. All presumptive EC culls or mortality were necropsied and cultured for laboratory confirmation. The results confirmed a significant presence of pathogenic Enterococci during the trial. At day 42, the body weight of PRO was significantly ($p < 0.05$) higher with a difference of 107g with NC. The feed conversion ratio was also better ($p < 0.05$) in the PRO group (1.99) compared to NC (2.33). However, there were no significant differences in overall livability and EC-related mortality between treatment groups. In conclusion, the commercial triple-strain Bacillus-based probiotic improved the performance of commercial broilers facing an EC challenge. Further investigation is warranted to better understand the probiotic's mode of action and its potential for mitigating the negative impacts of EC infections on bird productivity.

Keywords: *Enterococcus cecorum*, probiotic, Bacillus, Feed efficiency, growth

T155 The effects of Microsaf[®] on the growth performance of Ross 308 x Hubbard M99 straight-run broilers in a 43-day production period Maria T. Terra-Long^{*1}, Alain Riggi¹, Joe Loughmiller¹, Logan Erb² ¹*Phileo by Lesaffre, Opelika, Alabama, United States*; ²*Kalmbach Nutritional Services, Upper Sandusky, Ohio, United States*

The dietary inclusion of direct-fed microbials (DFM) is becoming more common in commercial diets as antimicrobial use has diminished. The most effective DFM's provide performance uplift in challenged and non-challenged environments. Therefore, performance responses to Microsaf[®] (MS) were investigated using 2,400 Ross 308 x Hubbard M99 straight-run broilers. Microsaf[®] is a DFM with *B. pumilus*, *B. amyloliquefaciens*, and *B. licheniformis* and contains proprietary GO+ Technology[®] that can increase the *Bacillus* activity. Diets were formulated to meet or exceed Aviagen nutritional recommendations for broilers from d1-43 and were provided in a three-phase feeding program. Diets containing the DFM were formulated for a final concentration in feed of 1.0×10^5 CFU/g. Broiler chicks were randomly assigned to 3 groups across 48 pens. Group 1 (T1) was provided a control diet devoid of gut health additives. Group 2 (T2) was fed a diet containing a *Saccharomyces cerevisiae* live yeast DFM from d1-15 (500 g/ton) and MS (500 g/ton) from d16-43. Group 3 (T3) was fed a diet containing MS from d1-43 (500 g/ton). The three treatments were arranged in a randomized complete block design and birds were raised on used litter. Outliers were removed by box plot procedure and data was analyzed using one-way ANOVA

mixed models procedure of JMP Pro 17. Means were separated using a Student's T-test post-hoc analysis and $p < 0.05$ was considered significant. Birds fed T2 tended to improve d16-29 mFCR by 1.2-points when compared to those fed the control diet, while birds consuming T3 diet were intermediate ($p = 0.059$), but this did not persist throughout the finisher phase. Overall performance results indicate that birds provided diets containing MS throughout the duration of the trial significantly improved d1-43 mFCR by 2.0-points when compared to birds fed the control diet. Birds provided diets containing MS only in the grower and finisher phases were intermediate ($p = 0.02$). There were no significant differences observed for d43 BW or d1-43 BWG, or FI ($p > 0.05$). The average mortality in this study was 4.12% with no differences between groups ($p = 0.15$). These data suggest that Microsaf[®] supplementation elicits a performance improvement even in a non-challenged environment.

Keywords: Bacillus, DFM, Probiotic, Broilers, Performance

T156 Deoxynivalenol affects gut morphology and liver oxidative stress in broilers: the effect of an algae-clay detoxifier Marie Gallissot¹, Maria Angeles Rodriguez^{*1}, Regiane Santos² ¹*OLMIX S.A., Brehan, France*; ²*Schothorst Feed Research, Lelystad, Netherlands*

Chronic exposure to deoxynivalenol (DON) is a constant challenge in broiler chicken production due to its potential to impair digestive health and growing performance. Particularly, these negative impacts may manifest even at DON concentrations below the FDA advisory limits of 5 ppm. The aim of this study was to evaluate the effects of naturally DON-contaminated feed on growing performance, digestive health and liver oxidative stress in broiler chickens, and to assess the effectiveness of an algae-clay-based detoxifier in alleviating these impacts. One-day-old male chickens ($n=600$; Ross 308) were divided into three treatments with ten replicates (20 birds/rep). The chickens were fed a control diet (CON) with negligible levels of DON (~0.2 ppm), or a diet naturally contaminated with significant levels of DON (~3 ppm), either supplemented (2 g/kg diet; ALG) or not with the detoxifier (DON). Growing performance was measured. Samples of jejunum from one bird per pen at d14, d28, and d37 were collected and fixed for histological analysis (NanoZoomer scan). A liver sample was collected from the same bird and submitted to RNA isolation (SV Total RNA Isolation System), and total RNA was quantified by spectrophotometer. ANOVA analysis was performed comparing means with Tukey test, pen was the experimental unit. No differences were observed in growing performance. Jejunum villus damage was observed on d28 ($P=0.04$), followed by villus shortening on d37 in chickens fed the DON diet (CON=1187 μm vs DON=884 μm). This negative effect was not observed when the diet was supplemented with the ALG detoxifier (1030 μm ; $P=0.01$). On d37, 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, DON exposure had a negative impact on chicken gut health and liver oxidative stress. When the algae-clay detoxifier was added, the harm caused by DON was no longer observed.

Keywords: DON, mycotoxin, detoxification

T157 Use of Diatom Nat for preventing intestinal challenges in broiler chickens David Díez^{*1}, Anna Tesouro¹, Connie Gallardo², Roger Palacin¹ ¹*Biovet S.A., Constanti, Tarragona, Spain*; ²*Universidad Científica del Sur, Lima, Peru*

Intestinal health is the key for nutrient digestion and absorption. Deficiencies in intestinal health are associated with alteration of

the mucosal barrier, rapid transit, inflammation and/or shifts in the gut microbiota composition (dysbiosis). Activated diatoms, pronutrients and phenolic antimicrobial molecules are natural compounds used to control these gut challenges. Activated diatoms are intended to slow down intestinal transit and slightly acidify the digestive environment; pronutrients promote the regeneration of the digestive mucosa and boost the gut local immunity and nutrient absorption; and phenolic antimicrobial molecules are natural compounds that control the gut pathogens. Diatom Nat (DN) is a product that combines these three compounds. A trial was conducted for 42 days to evaluate DN effectiveness to prevent gut challenges, in which 400 broilers distributed into 4 treatments groups with 5 replicate pens each. Treatments were a standard basal diet (SBD) as a Negative Control (NC); a SBD challenged with soybean 3% (SBDC) as a Positive Control (PC); a SBDC, challenged and with DN at 0.5 Kg/t (SBDC+); a SBDC, challenged and with Halquinol at 100 g/t (SBDC-). The challenge consisted of the inclusion of 3% of raw soybean, with high content in ANFs that may alter the gut environment. Intestinal retention time was higher in SBDC+ ($P < 0.05$) group of 20 minutes and 24 minutes on average compared to PC and SBDC- groups, respectively. SBDC+ group showed a slightly increase in the digestibility of protein (+ 2%) ($P < 0.05$) compared to PC. Body weight (BW), body weight gain (BWG) and feed conversion rate (FCR) were significantly better in SBDC+ group than PC and SBDC- ($P < 0.05$). The mortality was significantly lower in SBDC+ group (0%) ($P < 0.0001$) compared to the others. Humidity of litter was significantly higher in SBDC+ group ($P < 0.05$) than PC and SBDC- and dirty cloaca was lower in SBDC+ than the others ($P < 0.0001$). Levels of hepatic enzymes (GGT, FA) were lower ($P < 0.0001$) in SBDC+. In conclusion, DN is effective to prevent gut challenges in broilers intestine improving productive performance and reducing mortality.

Keywords: pronutrients, intestinal health, dysbiosis, phytochemicals, rapid transit

T158 Effects of a proprietary mycotoxin binder, on body weight, feed conversion, and mortality of Ross 708 and Cobb 500 broilers in the absence of increased prevalence of mycotoxins Cody Flores^{*1}, Shameer Rasheed¹, Mark LaVorgna¹, Mahmoud Masadeh¹, Mike Blair¹, Gary Reznik¹, Kristy Dorton¹ ¹*Devenish Nutrition, Fairmont, Minnesota, United States*

Two studies were conducted to evaluate a proprietary mycotoxin binder (MS; MultiSHIELD, Devenish Nutrition, Fairmont, MN) on performance (body weight, weight gain, feed intake, and feed conversion) and mortality in Ross 708 or Cobb 500 broilers. Day-old broiler chicks (as hatched; n = 1800 Ross 708 in Study 1 and 2,800 Cobb 500 in Study 2) were randomly allocated to floor pens (12 or 14 reps/treatment; 50 birds/pen; 1.08 or 0.88 ft²/bird). Treatments included were Control (CON; no MS) or MS at 2 or 4 lb/ton in study 1 and CON or MS at 2, 3 or 4 lb/ton in study 2. Broilers were fed a corn/soy based pelleted diet that had the inclusion of distiller's grains at 5% in the starter, 10% in the grower, and 15% in the finisher and withdrawal diets. A coccidiostat (113.5 g Zoalene/ton feed) was included in all diets, except for the withdrawal diet in study 1. Diets were analyzed for mycotoxin concentrations at the end of the study. Body weight (BW), feed intake, and mortality were measured and feed conversion ratio adjusted for mortality and to a common body weight (FCR_{M+BW}) was calculated. Data were analyzed as a one-way ANOVA using the Mixed procedure of SAS with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$. Diet analysis showed little to no mycotoxin contamination. Broilers

supplemented with MS at 2 and 4 lb in Study 1 and 2 and 3 lb in Study 2 had higher BW ($P < 0.0001$) than CON. Similarly, FCR_{M+BW} was better ($P < 0.0001$) for broilers supplemented with MS at all inclusion rates in Study 1 and 2 compared to CON. Mortality was also reduced ($P < 0.0001$) in broilers supplemented with MS in Study 1. As a result of better feed conversion, estimated feed cost savings ($\$/lb$ live weight) increased with supplementation of MS. This could result in significant feed cost savings over the course of the year. Overall, the results showed that supplementing MultiSHIELD to Ross 708 (big bird program) or Cobb 500 (small bird program) broilers improve growth, feed conversion, feed cost savings, and minimized mortality even when mycotoxins levels were low or not present in the diets.

Keywords: mycotoxin binder, broiler, feed conversion, mortality

T159 Effect of Fytera Perform on Performance and Health of Commercial Broilers with and without an Ionophore Program Karlinton Flores^{*1}, Dan Moore², Marcos Rostagno¹ ¹*Selko, Raleigh, North Carolina, United States*; ²*Colorado Quality Research, Wellington, Colorado, United States*

The poultry industry is reintroducing ionophores usage in certain poultry feed and production. For many years, phytochemicals have been used in antibiotic-free production to fill the void in one or more capacities that antibiotics left. Both tools have their use in production but often are not used together to maximize their strengths. Thus, this experiment aimed to test a novel phytochemical feed additive (Fytera Perform, Selko-US, Indianapolis, IN) that could complement an ionophore program. Fytera Perform contains a full array of target actives from three natural essential oils. Instead of directly affecting the gut microbiota, essential oils target the host through interaction with receptors located on the cells lining the intestinal tract. Maxiban 72 (M72) (Elanco, Indianapolis, IN) was used as a reference anticoccidial product containing an ionophore and a chemical. The total of five treatments included combinations of M72 fed in starter (0-14 days) and grower (14-28 days) or starter, grower, and finisher (28-42 days) diets, followed with or without Fytera Perform during the withdrawal period (42-56 days). The fifth treatment consisted of Fytera Perform, fed throughout the starter, grower, and finisher diet without any added anticoccidial. A total of 960 male birds were housed within an environmentally controlled facility. Upon receipt, birds were vaccinated for coccidiosis (Live vaccine, Coccivac B52, 3X dose). Birds were placed in pens containing re-used wood shavings to induce a sanitary challenge with a 5-8% bird mortality target. Data were analyzed using a one-way ANOVA or a per-feed phase additive inclusion contrast in JMP 17 (SAS, Cary, NC). In the starter and grower feed phases, birds fed M72 had no body weight (BW) differences ($P > 0.05$) but tended ($p \leq 0.16$) to have lower feed intake, and thus, an improved Feed Conversion Ratio corrected for Mortality (MFCR) compared to birds fed Fytera Perform (p -value ≤ 0.002). At 56 days of age, birds fed Fytera Perform overall improved BW by 0.23lb ($p = 0.01$) and MFCR by 2.3 points ($p = 0.003$) compared under contrast to birds fed only M72 through the starter or finisher. These results suggest that Fytera Perform has value in enhancing performance after birds were fed M72 during the starter and grower phases.

Keywords: Ionophores, Challenge, Program, Phytochemical

T160 Preliminary evaluations of a combination of postbiotics and phytochemicals to support mitigating the impact of histomoniasis in turkeys Megan Koppen^{*1}, Manuel Da Costa¹, Maci Oelschlaeger¹, Danielle Graham², Aaron Forga², Benjamin W. Parsons², Lisa Bielke³, Philip Yeboah³, Thaciane Amaral³, Demilade Ibiwoye³ ¹*Cargill Animal Nutrition, Rockingham,*

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Histomoniasis, or blackhead as it is most commonly referred to, is a disease caused by *Histomonas meleagridis* (a protozoan parasite) infecting gallinaceous birds. In 2015, the US FDA withdrew approval of nitarsone, leaving no approved prophylactics available. Several alternative products have been tested focusing on prevention rather than mitigation. The objective of the present studies targeted evaluating a combination of postbiotics and phytochemicals (CPP) fed at 1.6 lb/ton as an intervention to support mitigation of histomoniasis in poult under varying challenge models measuring infection rate, cecal and liver lesions, and performance parameters (pre-, post-challenge and overall trial) in a completely randomized design. In study 1 (S1) 2 out of 8 poult/cage were intraoocally challenged with 10^5 *H. meleagridis* cells/mL at d13. Two dietary treatments groups were evaluated: 1) control (C) and 2) CPP, with 10 replicates/treatment and 10 (pre-challenge)/8 (post-challenge) birds/cage from 0-33 days. In study 2 (S2) all poult were directly inoculated with 10^5 (L) or 2×10^5 (H) histomonads/bird at d10. Four treatment groups were evaluated: 1) L + no CPP, 2) H + no CPP, 3) L + CPP, 4) H + CPP with 6 replicates/treatment and 6 birds/cage from 0-30 days. Birds were fed corn and soybean meal (SBM) based basal diets formulated to meet nutrient requirements (S2) or switched to a lower nutrient SBM diet at d6 (S1). Data for the two trials were analyzed independently using dietary treatments as main factors. S1 CPP contact birds exhibited a significantly lower horizontal transmission rate and incidence of cecal lesion scores than C ($p < 0.05$). S1 CPP seeders exhibited numerically lower mortality as compared to C (-20%). Additionally, S1 CPP's body weight was significantly heavier throughout the trial. In S2, both liver and cecal lesion scores were reduced numerically in birds fed CPP, with lower reported incidences of severity compared to their respective non-CPP counterparts. In S2, post-challenge BW trended heavier and FI was significantly higher ($p < 0.05$) in CPP birds versus non-CPP. Based on these trial results, the dietary supplementation of CPP was supportive in minimizing clinical signs and production performance impact in poult challenged with histomoniasis.

Keywords: histomoniasis, blackhead, feed additives, turkeys

T161 Effect of combining phytochemical feed solutions with free and mono-, di- and triglycerides of short and medium-chain fatty acids on production performance in broilers Manu De Laet^{*1}, Manuel Da Costa¹, Caroline Donaldson¹, Yann Fournis¹, Roberto Montanhini Neto¹ ¹Cargill, Cargill Animal Nutrition, Mechelen, Belgium

An experiment to evaluate the effect of combining phytochemical feed solutions (PFS) with a combination of free and mono-, di- and triglycerides of short and medium chain fatty acids (SMCFA) was conducted. The goal was to determine if the duration of treatment application can impact production performance and necrotic enteritis (NE) lesions in broiler chickens. A total of 2,000 male Cobb 500 broilers were randomly allotted to five dietary treatments, each with 8 repetitions and 50 birds per repetition. Birds were fed a corn/soy-based diet ad libitum. Treatments were T1: infected and not-treated (INT) and 4 groups of infected and treated (IT); with 20 mg/kg Virginiamycin fed day 0-49 (T2), 750 mg/kg PFS+SMCFA fed day 0-21 (T3), as T3 but fed day 0-28 (T4) and as T3 but fed day 0-35 (T5). T3, T4 and T5 were then fed only PFS (150 mg/kg) until harvest at day 49. All birds were vaccinated against coccidiosis on day 1 (Coccivac-B) and challenged with a culture of *Clostridium perfringens* on days 19,

20, and 21. On day 21, five birds per pen were necropsied for NE lesion scoring. Results on day 49 showed that birds in all IT groups had significantly better FCR compared to birds of the control ($P < 0.05$). T2 showed the significantly lowest FCR at day 49 compared to all other treatments ($P < 0.05$). FCR between T3, T4, and T5 did not differ significantly. However, with more extended application of the combination of PFS and SMCFA, the FCR numerically improved. Those birds fed either Virginiamycin or a combination of PFS and SMCFA tended to have a higher body weight compared to control ($P < 0.10$). On day 21, NE lesions were significantly reduced in those birds fed PFS - SMCFA or Virginiamycin ($P < 0.01$). It can be concluded that applying this combination of PFS and SMCFA, followed by PFS until harvest, significantly improved production performance and reduced NE-related gut lesions.

Keywords: Necrotic enteritis, Health, Performance, Broilers, Phytochemical Feed Solutions

T162 Anti-inflammatory and pro-growth immunometabolic response to phytochemical feed additive in the small intestine of the broiler chicken Ryan Arsenault^{*1}, Famatta Perry², Casey Johnson¹, Meghan Schwartz³, Ellen Davis³, Stacie Crowder³ ¹USDA ARS, Food and Feed Safety Research Unit, College Station, Texas, United States; ²University of Delaware, Animal and Food Sciences, Newark, Delaware, United States; ³Fortiva, Arden Hills, Minnesota, United States

This study was conducted to evaluate the effects of a phytochemical feed additive on broiler performance with used litter challenges. Trial was a complete randomized block design where block was based on pen location within the research facility. Groups were Control and SupplTM D, a blend of phytochemical plant compounds at 150 mg/kg (Fortiva, Arden Hills, MN). Jejunum, ileum and cecum tissues were collected and assayed for kinase activity on days 14, 28 and 42 post-hatch. Samples of each tissue were taken from 5 birds for biological replication. Differential phosphorylation was determined by Students T-test on each array of peptides between groups ($P < 0.05$) (PIIKA 2.5 pipeline). Pathway significance was determined utilizing the significant peptides and correcting for multiple comparisons using False Discovery Rate correction ($FDR < 0.05$) (STRINGdb pipeline). Results showed that the greatest impact of the additive in the jejunum was on d28 (70 proteins, 40.2% of total) and d14 (56 proteins, 32.2% of total). The most proteins were affected in the cecum at d28 (57 proteins, 36.8% of total) and d14 (43 proteins, 27.7% of total). The ileum was impacted to a lesser extent, d14 (49 proteins, 37.1% of total) and d42 (45 proteins, 34.1% of total) showed the most changes. A detailed analysis of the individual proteins in the jejunum showed activation of responses that impact both immunity and metabolism. Metabolic responses specifically show a pro-growth phenotype. In the jejunum we looked at the immune pathway Toll-Like Receptor signaling pathway, which was highly represented in the pathway data at d14 ($FDR 2.05^{-10}$). A detailed functional analysis of the phosphorylation events in this pathway showed a general deactivation of the proteins within the pathway. This indicates that the pathway is less active with product than in the control birds, likely resulting in a less inflammatory phenotype. The result of less inflammation and more growth would have a positive impact on performance and may improve disease resistance. Here we describe an intracellular mechanism elicited by the feed additive to explain improved performance and disease resistance in birds fed the product.

Keywords: broiler, phytochemical, immunometabolism, kinome

T163 Effect of a blend of phytochemical compounds on productivity and environmental impact of chicken meat in the

US: meta-analysis and life cycle assessment Clémentine Oguey*¹, Janjoris Van Diepen² ¹*ADM international sarl, Rolle, Switzerland;* ²*Mérieux NutriSciences | Blonk, Washington, District of Columbia, United States*

There is an increasing awareness of the need to minimize environmental footprint of the animal food sector. Meanwhile one of the main challenges of the actors along the chain is to maintain animal productivity and financial profitability. Supporting efficient use of feed by livestock such as broilers through the use of feed additives could facilitate compatibility between those two concerns. The intent of this study was therefore to evaluate the effect of a standardized protected blend of carvacrol, cinnamaldehyde and capsicum oleoresin (XT) on productive and environmental performance of broilers on farm and at slaughter. First a systematic review and a meta-analysis of XT on productive performance of broilers was performed. All experiments included were randomized trials reporting side-by-side comparisons of a control (CT) and CT with the inclusion of XT at 100 g/t. The effects of XT added to broiler diet were evaluated using raw and standardized mean differences and effect size calculations. Analysis included 22 trials for on-farm performance and 8 trials for slaughter performance. Results obtained were then used to perform a life cycle assessment (LCA) to evaluate the environmental performance of broilers produced with and without XT in the US. System boundaries were set from cradle to farm gate, and cradle-to-slaughterhouse gate. The environmental impact was calculated using EF3.0 impact methodology. Supplementation of XT had no effect on FI per slaughtered bird ($P > 0.05$). Compared to CT, XT increased ADG (+1 g/d, $P < 0.001$) and reduced FCR (-1.4%, $P < 0.001$). At market age, birds fed XT had greater final weight (+33 g, $P < 0.001$) and carcass yield (+0.65 points, $P < 0.05$) compared to CT. In optimal conditions, XT did not affect mortality of birds (mean 3.16%, $P = 0.99$), whereas in standard conditions (mortality $> 3.5\%$), XT reduced this outcome by 27% ($P < 0.01$). LCA results show that XT addition to broiler feed could reduce climate change by 2.3% and 2.9% per unit weight of live weight and of broiler meat respectively. No trade-offs were identified in the other environmental impact categories. Overall these results confirm that use of XT can be a tool to support environmental and economical sustainability efforts of the poultry meat sector.

Keywords: broiler production, environmental impact, performance, phytogetic compounds

T164 Combination of a postbiotic and phytogetic supports broiler performance across all feeding phases Vivek Kuttappan*¹, Yann Fournis¹, S. Ali Naqvi¹, Courtney Clemons¹ ¹*Cargill, Inc., Wayzata, Minnesota, United States*

Research have shown that postbiotics and phytogetics can exert beneficial effects on gut microflora, nutrient digestion as well as improved performance in animals. Previous studies have shown that Biostrong® Dual, a unique combination of *Saccharomyces cerevisiae* fermentation postbiotic product and a blend of essential oil compounds, has consistent benefits across studies in improving broiler performance at market age (42d). Analysis of gut microbiome from these birds showed that the dietary inclusion of Biostrong® Dual can support accelerated maturation of gut microbiome to a more resilient population. The present study was intended to evaluate the benefits of the product in broilers across different feeding phases. Eleven independent studies were conducted comparing the same two treatments, control (CON) or test (DUAL), both receiving the same basal diet, with DUAL receiving the on-top inclusion of 0.4kg/MT of Biostrong® Dual. All the studies used commercial broiler strains with 12 to 48 replicates/treatment and 216 to 960 birds/treatment. Body weight, feed intake, and adjusted cumulative feed conversion ratio (cFCR) were evaluated at 0, 14, 28, 35, 42d. All the data were analyzed using PROC MIXED model with block nested in study as random effect, and significant difference determined at $P < 0.05$. Birds in DUAL showed higher ($P < 0.05$) body weight and lower ($P < 0.05$) cFCR at 14, 28, 35, and 42 days when compared to CON. The body weight improvement was 2.7, 2.9, 2.8, and 3.5% at 14, 28, 35, and 42d in DUAL, respectively. Furthermore, DUAL birds had 7, 5, 4, and 5 points improvement in cFCR at 14, 28, 35, and 42 days, respectively. Under the experimental conditions of these studies, the dietary inclusion of Biostrong® Dual helped in supporting broiler performance through the different phases of production such as starter, grower, and finisher. The benefit of Biostrong® Dual in improving performance could be attributed to the accelerated maturation of gut microbiome.

Keywords: Postbiotics, essential oil compounds, broilers, performance, breast meat yield

SCAD III

T165 Development and characterization of *in vitro* chicken lung organoids and modeling of H5 avian influenza virus infection Hannah Nicholson*¹, Christopher Zdyrski¹, Michael Catucci¹, Bryan J. Melvin¹, Lisa J. Stabler², Mohamed Elbadway¹, Megan P. Corbett¹, Eugene Douglass³, Rebecca L. Poulson², Jonathan P. Mochel¹, Karin Allenspach¹, Silvia Carnaccini⁴ ¹*Precision One Health, CVM, Athens, Georgia, United States;* ²*Population Health, CVM, Athens, Georgia, United States;* ³*Pharmaceutical and Biomedical Sciences, Institute of Bioinformatics, Athens, Georgia, United States;* ⁴*University of Georgia, Population Health, Athens, Georgia, United States*

Highly pathogenic avian influenza (HPAI) viruses present a significant global threat to both wildlife and domestic animals, particularly poultry. Of these, HPAI H5N1 viruses are of heightened concern for public health due to their frequent spillover into domestic mammals and humans. The current lack of physiologically relevant *in vitro* models limits the ability to screen the rapidly evolving viral landscape, which has recently expanded to include species previously considered resilient to clinical disease. Organoids, which are three-dimensional, miniaturized

versions of organs produced *in vitro*, recapitulate key functional, structural, and biological features of their respective *in vivo* tissues. Here, we describe the development of the first adult stem cell-derived lung organoids from specific-pathogen-free White Leghorn chickens. The organoids' cellular heterogeneity was confirmed using single-nuclei RNA sequencing, and their transcriptomic profiles were characterized via bulk RNA sequencing. Basic morphological features were examined using light microscopy, immunofluorescence, and transmission electron microscopy. The chicken lung organoids successfully developed into three-dimensional structures containing luminal formations (presumptive air capillaries) lined with a variety of cell types, including ciliated and non-ciliated squamous, cuboidal, and columnar epithelial cells indicative of different epithelial regions of the avian respiratory system. We successfully infected the organoids in liquid suspension with an H5 low pathogenic avian influenza (LPAIV) isolate from a wild mallard duck, as well as an H5 LPAIV poultry isolate generated through reverse genetics. Virus growth kinetics were assessed using the tissue culture infectious dose 50 (TCID50) and RT-qPCR at 0, 12, 24-, 48-, 72-, and 96-hours post-inoculation, across multiplicity of infection

(MOI) levels of 0.01, 0.1, 1, and 10. The poultry isolate exhibited higher growth compared to the wild duck isolate, potentially indicating greater adaptation to chicken lung cells. In conclusion, we have developed a platform capable of modeling avian influenza virus pathogenesis in chicken lung organoids, which could facilitate the prediction of future viral evolution and accelerate discovery of new targets.

Keywords: chicken lung, organoids, modeling, H5 Avian Influenza

T166 The growing feather pulp as a dermal test site in turkeys: Insights from the early acute inflammatory response to lipopolysaccharide Delaney F. Roberts*¹, Jossie M. Santamaria¹, Aaron Forga¹, Chrysta N. Beck¹, Danielle Graham¹, Gisela F. Erf¹ ¹University of Arkansas, Center of Excellence for Poultry Science, University of Arkansas System Division of Agriculture, Fayetteville, Arkansas, United States

In chickens, the growing feather (GF) pulp has been established as a cutaneous bioassay to monitor and assess tissue/cellular immune activities in response to intradermal (i.d.) injections of test materials. The objective of this pilot study was to determine the suitability of the GF bioassay in turkeys by examining the acute inflammatory responses initiated by i.d. GF-pulp injections of lipopolysaccharide (LPS). For this, three, 5-wk-old turkey poults were used. Two poults had 8 GFs injected (10 µL/GF) with low (L)-LPS (0.1 µg/GF) and 8 GFs with high (H)-LPS (1 µg/GF), while a third poult had 8 GFs injected with endotoxin-free PBS and 9 GFs with H-LPS. Heparinized blood and GFs were collected before (0h) and at 3h and 6h post GF-pulp injection. Blood and pulp cell suspensions were stained with a panel of turkey leukocyte-specific fluorescence-conjugated antibodies to identify total leukocytes, heterophils, MHC II+ cells, B cells, and T cell subsets by flow cytometry analysis. Two-way ANOVA was used to test the effects of LPS dose, time, and their interactions on leukocyte profiles in GFs, and one-way repeated measures ANOVA to test the effect of LPS on blood leukocyte (WBC) profiles over time. Tukey's HSD test was conducted when appropriate. Significance was set at $P \leq 0.05$. In GFs, both L-LPS and H-LPS stimulated leukocyte-infiltration, primarily heterophils, by 3h p.i., reaching maximal levels (% pulp cells) at 6h ($P \leq 0.05$), but did not affect levels of MHCII+, CD4+, CD8+, or Bu1+-cells. However, L-LPS triggered a marginally higher $\alpha\beta$ T cell recruitment ($P=0.052$) than H-LPS. PBS also recruited heterophils but at lower levels than LPS. In the blood, i.d. LPS injection resulted in a doubling ($P \leq 0.05$) of heterophil concentrations by 6h p.i., caused a transient drop in concentrations of WBC, CD4+ T cells and $\alpha\beta$ T cells at 3h ($P \leq 0.05$), but did not affect those of CD8+ T cells and B cells. This pilot study highlights the similarities in the inflammatory responses initiated by LPS in turkeys and chickens and demonstrates the applicability of the two-window (GF and blood) approach to study local and systemic immune system responses in turkeys. A study is underway to further assess the inflammatory response to LPS from initiation to resolution in turkeys.

Keywords: Turkeys, Lipopolysaccharide, Inflammatory Response, Leukocytes, Cutaneous Bioassay

T167 Vaccine-based approach to control *Campylobacter hepaticus* in a challenge study Catherine M. Logue*¹, Roel Becerra¹, Julia Ines-Lima¹, Klao M. Runcharoon¹, Yu Yang Tsai¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

Poultry health is critical for the nations' layer and breeder layer stocks. The emergence of spotty liver disease (SLD) caused

by *Campylobacter hepaticus* continues to pose a significant threat to the health and welfare of laying birds, leading to reduced egg production, high morbidity, and sudden mortality. The emergence of *C. hepaticus* may be linked with changes in bird-raising practices to cage-free, pasture-raised, or organic approaches, where birds are vulnerable to pathogen exposure. The implementation of the veterinary feed directive and consumer demand for no antibiotics ever (NAE), antibiotic free (ABF) and organic birds significantly limited approaches to the control of SLD. Moreover, the absence of a commercial vaccine adds to the challenge. Here, we assessed the use of vaccine strategies to control *C. hepaticus* infection in a challenge model. Groups of birds (n=22) at 16 weeks of age (woa) were vaccinated with either live or killed strain of *C. hepaticus* and boosted at 19 woa. At 24 woa when birds were in production, they were challenged with *C. hepaticus* and monitored for up to 25 days post challenge. At days 11, 20 and 25 days post challenge birds were euthanized and examined for liver lesions and cultured for *C. hepaticus*. Lesion scores were found to be lower for the killed vaccine strain ($P < 0.05$) and combination of live and killed vaccine ($P < 0.05$) compared to the naïve challenged birds. In addition, culture analysis failed to detect the challenge strain in killed vaccine dosed birds and the detection rate was lower (prevalence 30%) in birds vaccinated with the live and killed vaccine strain combination or two doses of the killed vaccine (30% prevalence) compared to the naïve challenged birds. These vaccine approaches appear to offer an opportunity to control *C. hepaticus* in laying hens thereby significantly reducing SLD and mortality and enhancing bird health and welfare.

Keywords: *Campylobacter hepaticus*, spotty liver disease, layers, poultry, health

T168 *Enterococcus cecorum* Litter Challenge Model with or without a Litter Acidifier Matthew K. Jones*¹, Charles Hofacre¹, Virginia Baxter¹, M M. Suyemoto², Roy Berghaus³, Juan Suarez⁴, Craig Coufal⁴ ¹Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States; ²North Carolina State University, Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, North Carolina, United States; ³University of Georgia, Department of Population Health, College of Veterinary Medicine, Athens, Georgia, United States; ⁴Jones-Hamilton Co., Walbridge, Ohio, United States

Pathogenic *Enterococcus cecorum* (EC) represents a growing concern amongst broiler producers in the United States. This pathogen infects the broilers and circulates systemically causing sepsis in the first few weeks of life. Subsequently, birds begin exhibiting lameness from infection in the free thoracic vertebra (FTV) and femoral head. Often, broiler litter is re-used from flock to flock which may present a means of exposure to pathogenic EC. Acidification of litter can be an effective strategy to reduce pathogens in broiler houses. As such, sodium bisulfate (SBS; 150 lbs/1000ft²[CC1]) was applied on contaminated litter prior to day old-chick placement (d 0) and compared to a non-treated group for efficacy against pathogenic EC. Birds were placed on litter from a flock that had been challenged with ECstrain SA3. Each treatment consisted of 8 replicate floor pens of 25 male Ross broiler chicks. Coccidia vaccine was applied via coarse spray to all chicks prior to placement. Litter and boot sock samples were collected on d -1, 0, and 2 to evaluate EC in the environment. Sepsis was evaluated on d 14 with spleen sampling. On day 40, 100 representative FTV and spleen samples were collected from each treatment to assess EC colonization. Birds were weighed on d 0, 14, 28, and 40 to calculate performance metrics. EC prevalence data were analyzed with Fisher's exact test and GEE logistic regression. EC boot sock prevalence was 100% positive in

both groups on d-1 and d 2. On day 0, the SBS treatment had lower prevalence (38%) than the control (100%). Litter sample prevalence tracked in a similar manner to boot sock prevalence, but the outcomes were not significant. Trends in body weight gain were observed at 28 d indicating the birds in SBS-treated pens were heavier at this timepoint ($P=0.052$). There were no differences in performance on d 40. A single spleen was EC positive on d 14 from a control pen. By d 40, splenic prevalence increased to 13% in both treatments. There were no differences in FTV prevalence between groups. This trial demonstrated horizontal transmission of pathogenic *Enterococcus cecorum* from flock to flock via the litter can occur and that acidification of the litter may support broiler performance outcomes despite this challenge.

Keywords: Enterococcus, Kinkyback, Spondylitis, Re-used Litter, Litter Acidification

T169 Did Raised Without Antibiotics programs change field levels of Ionophore anticoccidial resistance? Greg Mathis*¹, Brett Lumpkins¹ ¹*Southern Poultry Feed & Research, Inc., Athens, Georgia, United States*

The recent concern of antibiotic resistance in poultry has resulted in Raised Without Antibiotics (RWA) program, which also excludes the use of the anticoccidials; polyether ionophorous antibiotics. The question arises does this lack of Ionophore (IONO) usage with RWA allow for a reduction in field levels of IONO anticoccidial resistance. To examine that question, three anticoccidial sensitivity tests (AST) were conducted. Coccidia was isolated from 3 sources: farms that never used IONOs (Never), farms that continued to use IONOs (Cont.), and RWA farms (no IONO usage for 5-8 years). Each AST consisted of 42 cages with 8 chicks. The treatments were replicated in 3 randomized blocks. Statistical analysis employed the General Linear Models procedure with $P < 0.05$ to determine differences. Treatments were: No Additive, challenged (NMI) and nonchallenged (NMU); Chemical Class (CHEM): Zoalene, Amprolium, Nicarbazine, Robenidene, Coyden, Diclazuril, and Decoquinate; IONO Class: Monensin, Salinomycin, Narasin, and Lasalocid. 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 post challenge), all birds were coccidia lesion scored (LES). Across all 3 ASTs, NMU grew similarly (avg. FCR 1.429). NMI across all three ASTs produce a similar moderate challenge with all species (avg. FCR 2.829 and LES 2.7). Both Chemical and IONO significantly controlled all coccidia challenges. For the Never AST, the avg. IONOs control (FCR 1.639 and LES 1.0) was significantly better than Chemical (FCR 2.077 and LES 1.3). For the Cont. AST, the avg. Chemical control (FCR 1.753 and LES 1.4) was significantly better than IONO (FCR 1.953 and LES 1.9). For the RAW, the avg. IONOs control (FCR 1.664 and LES 0.9) was significantly better than Chemical (FCR 1.992 and LES 1.3). Results showed that the never using IONOs coccidia isolate was very sensitive (low resistance); the continuous usage has led to moderate resistance; and RWA which "rested" the IONO may have resulted in less IONO resistant coccidia on those farms. This information will be useful as more poultry purchasers are able to source birds fed IONOs.

Keywords: Coccidia, Eimeria, Anticoccidial, Ionophore, RWA

T170 Prevention of Necrotic Enteritis using Bacteriophage Administered in Pelleted Broiler Feed Charles Hofacre*¹, Matthew K. Jones¹, Joseph Brown², Joseph Edwards², Ellen Denness² ¹*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States;* ²*Aparon Ltd., Lincoln, United Kingdom*

Bacteriophage (phage) are naturally occurring viruses that only lyse specific bacteria. The mixture of several bacteriophage in the product, Sustavian, used in this study were selected for their ability to lyse *Clostridium perfringens*. Sustavian is a bacteriophage cocktail that has been heat protected allowing it to be used as a feed additive to reduce *C. perfringens* in broiler feed. The phage was mixed into the pre-pelleted feed at 5×10^3 PFU/g, 5×10^4 PFU/g, 5×10^5 PFU/g, 5×10^6 PFU/g, and 5×10^8 PFU/g. The positive control was the antibiotic BMD (55 g/mTon). The study was performed in Petersime style battery cages with 6 replicate cages per treatment and 10 Ross 708 male broilers per cage. The birds were orally gavaged at 14 days with 1000 *E. maxima* sporulated oocysts/bird. Then to simulate *C. perfringens* contaminated feed, the birds were orally gavaged into the crop with 5.0×10^8 CFU/bird on day 19. Statistical analysis was performed using ANOVA with comparison of means using LSD (t-test) at $P \leq 0.05$. Birds and feed were weighed days 0, 14, 22, 28. The N.E. challenge control had 35%^A necrotic enteritis mortality, the 10^6 PFU had the lowest N.E. mortality at 3.33%^{CD} and BMD was at 16.6%^B. N.E. lesion scores on day 22 was highest in the challenge control, 1.17^A. At peak of *C. perfringens* challenge (DOT 0-22), all phage feed treatments had significantly lower non-adjusted FCR (control – 1.784^A; 10^3 – 1.500^B; 10^4 – 1.388^{CD}; 10^5 – 1.432^{BC}; 10^6 – 1.423^{BC}; 10^8 – 1.495^B), while BMD had FCR of 1.519^B. On day 28, intestinal content samples revealed DNA presence of the phage but no viable phage. However, intact phage were recovered from the feed and crop samples on day 28. This study demonstrated no negative effect of the phage for broiler performance. Use of the phage, Sustavian, in the pelleted feed of broilers reduced the incidence of *Clostridium perfringens* and had a derived benefit on the broiler performance.

Keywords: Bacteriophage, Clostridium perfringens, necrotic enteritis

T171 Study investigating impact of AL2 challenge at different ages on the antibody response to a killed SE vaccination and the sparing effect of rHVT-IBD vaccine on antibody titer suppression Kalen Cookson*¹, John Dickson¹, Jon Schaeffer¹ ¹*Zoetis, Lawrenceville, Georgia, United States*

Last year we demonstrated that a 3-week IBDV challenge (AL2) significantly reduced day of hatch spray infectious bronchitis (IB) vaccinal protection in broilers against a 25-day DMV/1639 IBV challenge. The aim of this study was to compare the active antibody response of leghorns challenged with AL2 at different ages and whether a recombinant IBD vaccine (rHVT-IBD) could lessen any immunosuppressive (IS) effects. Study Design: 630 SPF leghorns were placed into 18 Horsfall isolators at hatch and divided into 6 treatments: T01 (NC), T02 (D11 AL2), T03 (D21 AL2), T04 (D28 AL2), T05 (D0 IBD vax; D21 AL2) and T06 (D0 IBD vax; D28 AL2). All groups were vaccinated at 4 weeks of age with a commercial *Salmonella enteritidis* (SE) bacterin also containing Newcastle disease (ND) and IB antigens. Bursas were measured and weights taken on Days 21-28-35-56. ND and Sal Group B and D ELISA titers were measured on D55. All hypotheses were conducted at the $p \leq 0.05$ level of significance with the Shaffer simulated method used to adjust for multiple comparisons. All three AL2 challenge ages resulted in significant atrophy that lasted all the way out to study termination. The D11 AL2 challenge significantly reduced ELISA GMT response to ND (50%), Sal-B (90%) and Sal-D (85%). The D21 AL2 challenge reduced Sal-B (80%) and Sal-D (40%) GMTs and the D28 AL2 challenge reduced ND GMTs by 55%. The rHVT-IBD vaccine protected birds from bursal atrophy and against the depressed titer responses caused by D21 and D28 AL2 challenges. This study showed that bursal atrophy lasted at least 4 weeks when leghorns

were IBDV challenged at D28 and over 6 weeks when challenged at D11. The D11 challenge caused the most profound and consistent depression in titer response to a 4-week killed SE-ND-IB vaccination. While the D21 challenge caused more depression of Sal titers than the D28 challenge, it's curious that the D28 but not the D21 challenge depressed the ND response. Unlike the Sal antibody response, perhaps the timing of temporary IS from the 4-week IBDV challenge was more critical to the productive response to NDV than the earlier 3-week challenge. Regardless, the rHVT-IBD vaccine spared both challenge groups against IS as measured by NDV and Salmonella antibody titer responses.

Keywords: IBDV, vaccine, AL2, Newcastle, Salmonella

T172 Comparative Genomics to Identify *Mycoplasma synoviae* pathotypes Eniope B. Oluwayinka*¹, Naola Ferguson-Noel¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

There is considerable variability among *Mycoplasma synoviae* (MS) isolates in their ability to produce disease and currently only a few virulence factors have been identified. Changes in genes involved in transmissibility, colonization and immunogenicity may alter the virulence of MS strains. The identification of mutations (single nucleotide polymorphisms (SNPs), insertions or deletions) that are linked to increased pathogenicity of MS strains is crucial to the development of more stable and effective vaccines and will also be valuable in predicting the clinical impact of field outbreaks. In this research, *in vivo* studies were carried out to validate the pathogenicity status of MS isolates from commercial poultry farms in USA suspected to be mildly, moderately and very virulent based on *in silico* analysis of three proteins (ObgE, OppF and GAPDH) previously linked to the pathogenicity of MS strains. Groups of chickens were inoculated with the MS isolates and air sac and footpad lesions were scored at two weeks post-challenge. Genetic variation analysis of the MS genomes was carried out to identify changes linked to the MS strains determined to be very virulent. Using Illumina technology, full genome libraries were generated following genomic DNA extraction from the MS isolates; contigs for each strain were annotated using a fully annotated *M. synoviae* reference genome. BWA-mem was used as aligner, FreeBayes for SNP calling and non-virulent MS was used as the target genome for the variation analyses. Different pathotypes of the MS isolates (moderate, mildly and very virulent) were confirmed by the *in vivo* studies. Seven isolates caused footpad lesions, and five isolates caused airsacculitis, two of which caused severe airsacculitis. A nucleotide change (G to A) in an intergenic space distinguished the two MS isolates that caused severe airsacculitis from three that caused moderate airsacculitis, while an SNP (Ser161Ile) in a mobile element protein with a moderate SNP effect was common in the MS isolates that caused airsacculitis. This study indicates that the pathogenicity of MS isolates may be predicted through *in silico* analysis of MS genomes.

Keywords: *Mycoplasma synoviae*, Poultry, Pathotypes, Genomes, Comparative

T173 Individual, targeted vaccination at the hatchery to improve vaccine take and decrease reliance on spread on the farm Liz Turpin*¹ TARGAN, BioProcess, Raleigh, North Carolina, United States

The purpose of this research is to explore methods to improve vaccination of chicks at day of hatch. TARGAN has developed an individualized vaccination system that detects an individual chick and deliver a vaccine to the eye of the chick as it passes through

the hatchery. The device is designed to match current hatchery throughput while improving vaccination. We vaccinated day of hatch chicks with a commercial cocci vaccine with the TARGAN system or with traditional spray cabinets. Chicks were allowed to preen under lights for 30 minutes and placed on feed and water within two hours of vaccination. On day 7, the entire intestinal tuck was collected for individual chicks. The intentional and cecal content was squeezed out into a tube, and the content resuspended in a small volume of McIlvaine buffer. Individual bird samples were counted for large, medium, and small oocysts using a McMaster's chamber and the percent of positive birds and the oocysts output per bird calculated. Results with statistics and stats analysis methods: Multiple studies were conducted with day of hatch chicks vaccinated at TARGAN and at commercial hatcheries. Spray cabinet vaccinated birds resulted in 30-50% of chicks with detectable oocysts of all sized detected on day 7, with low output. The TARGAN vaccinated chicks resulted in over 95% of chicks with detectable oocysts of all sizes by day 7 with high output detected in the birds. This is a statistically significant improvement over vaccine take on day 7 by spray cabinet when compared to untreated and TARGAN vaccinated birds by ANOVA analysis. Individual, targeted vaccination results in a higher percentage of birds vaccinated by day 7 and higher output of oocysts, including *E. maxima* by day 7.

Keywords: cocci, vaccine take, day 7

T174 Secondary immune response profiles to electron beam- or formalin-inactivated *Salmonella* vaccines in egg-type pullets: immunogenic differences Jossie M. Santamaria*¹, Chrysta N. Beck¹, Ruvindu Perera¹, Palmy Jesudhasan², Gisela F. Erf¹ ¹University of Arkansas System Division of Agriculture, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States; ²USDA, Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas, United States

Previously, a first intradermal (i.d.) vaccination with an electron beam (eBeam)-inactivated *Salmonella* Enteritidis (SE) vaccine initiated a greater leukocyte response in the growing feather (GF)-pulp (vaccination site) and a more robust plasma SE-specific antibody response than with i.d. vaccination with formalin-killed (FK) SE. This study assessed the effects of a second i.d. vaccination with eBeam-SE or FK-SE vaccines (10^8 CFU/mL) on local leukocyte responses and SE-specific antibody levels in plasma. For this, 14-wk-old layer pullets were vaccinated with either eBeam-SE, FK-SE (5 birds/vaccine), or PBS (3 birds) in the breast muscle (500 μ L/bird), and at 18-wks by i.d. GF-pulp injection (10 μ L/GF; 200 μ L/bird) of the respective vaccines. GFs were collected before (0d) and at 6h, 1-, 2-, 3-, 5-, and 7-d post-pulp-injection (p.i.) for leukocyte population analyses, and blood was sampled at 0-, 3-, 5-, 7-, 10-, 14-, 21-, and 28-d p.i. to measure relative plasma levels of SE-specific IgM, IgG, and IgA by ELISA. Two-way ANOVA was used for GFs, and 2-way repeated measures ANOVA for blood to assess the effects of treatment, time, and their interactions. Tukey's HSD test was applied as needed, and significance was set at $P \leq 0.05$. In GF, infiltration of heterophils peaked ($P \leq 0.001$) at 6h and declined to near 0d levels (% pulp cells) by 2d; whereby at 6h, levels were higher ($P \leq 0.001$) with eBeam-SE than with FK-SE and PBS. Macrophage levels increased ($P \leq 0.001$) at 6h, remained elevated at 1d, and declined to near 0d levels by 2d p.i. At 1d, macrophage levels were higher with eBeam-SE than with FK-SE and PBS. For both vaccines, CD8+ T cell levels peaked ($P < 0.05$) at 2d, while total lymphocytes and B cells peaked at 3d. Recruitment of B cells and all T cell subsets were higher ($P < 0.05$) with FK-SE than with eBeam-SE and PBS. SE-specific IgM in plasma peaked ($P \leq 0.001$) at 5d, while SE-IgG ($P \leq 0.001$) and SE-IgA ($P \leq 0.001$) peaked by 7d p.i. with

both vaccines. While both the eBeam-SE and FK-SE vaccines generated a similar SE-specific humoral response, the rapid and greater heterophil recruitment to GF-pulps in response to the secondary i.d. eBeam-SE vaccination suggests a form of trained innate immunity that may be beneficial in the elimination of *Salmonella* infection.

Keywords: Salmonella Vaccines, Electron Beam Inactivation, Cellular Immune Response, Antibody Response, Pullets

T175 Effect of a Postbiotic Containing Saponin on the Severity of Avian Pathogenic *Escherichia coli* (APEC) Serotype O78 Infection in a 10 Week Layer Pullet Challenge Model Charles Hofacre*¹, Evan Chaney², Manuel Da Costa², Jean Oliveria⁴, Vivek Kuttappan³, Maci Oelschlager⁵, Matthew K. Jones⁶ ¹*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States;* ²*Cargill Micronutrition & Health Solutions, Wayzata, Minnesota, United States;* ³*Cargill, Inc., Wayzata, Minnesota, United States;* ⁴*Cargill R&D Centre Europe, Vilvoorde, Belgium;* ⁵*Cargill Animal Nutrition, Savoy, Illinois, United States;* ⁶*University of Georgia, Nicholson, Georgia, United States*

The poultry industry remains economically burdened from the adverse health and production impacts caused by Avian Pathogenic *Escherichia coli* (APEC). Recent reports have demonstrated the functionality of postbiotic and phytogetic blends to support poultry health under acute APEC challenge. This study evaluated the effects of a postbiotic and phytogetic blend (SCFP+; Diamond V) to ameliorate the severity of acute, clinical colibacillosis in layer pullets intratracheally challenged with APEC serotype O78. Two-hundred-eighteen day-of-age (D0) Hyline W-36 pullets were floor reared for 8 weeks in pens (Basal Diet or Basal Diet 1.15 lb./ U.S. Ton SCFP+) fed *ad libitum* until study termination at 10 weeks (W10). Within each group, 26 birds were randomly selected, tagged for body weight (BW) and cloacal swab samplings at D0, W3, W9 and W10. At 8W of age, birds were placed into individual cages and given APEC O78 challenge doses 10⁸ or 10¹⁰ CFU/bird W9. Final BW, necropsy and lesion scoring at W10 (n=48 birds/dose/treatment). W10 mean lesion scores for air sacculitis were significantly reduced in birds receiving the dietary SCFP+ treatment as compared to the challenge control (0.61 vs. 0.94; P=0.010). SCFP+ treatment numerically improved the cumulative severity score (0.94 vs. 1.42; P=0.055) while observed means were lower for perihepatitis (0.13 vs. 0.18; P=0.451) and pericarditis (0.20 vs. 0.30; P=0.284) lesions. Within treatments and between APEC doses, observed mean lesion scores were lower for SCFP+ and significantly lower for airsacculitis in SCFP+ birds challenged at 10⁸ CFU (P=0.010). Clinical prevalence (% of birds with a non-zero lesion score), remained lower for SCFP+ birds than challenged controls, approaching significance for pericarditis (P=0.058). Day 0, W3 and W9 bodyweights were not significantly different (P=0.197), however, observed mean BW gain during acute APEC challenge

was higher for SCFP+ birds (+0.07 kg; P=0.162). Samples for microbiome analyses were collected and these results will be presented. These data indicate that SCFP+ may support layer pullet health during acute clinical APEC O78 challenge, reducing the severity of APEC induced lesions and supporting overall bodyweight maintenance under clinical stress.

Keywords: Colibacillosis, postbiotic, saponin, *Escherichia coli*, layer

T176 Bacterial phenotypic plasticity could explain adaptation of *Enterococcus cecorum* to broiler chickens and the rising of a severe systemic disease Martha Pulido-Landinez*¹ ¹*Mississippi State University, PRDL, Pearl, Mississippi, United States*

Enterococcus cecorum (EC) is responsible for an emerging disease characterized by septicemic lesions and mortality in broiler chickens older than 2 weeks. Bacterial phenotypic plasticity refers to the ability of bacteria to adapt to environmental changes by producing different phenotypes in different environments. This is a key mechanism for biological adaptability. This study aimed to genetically evaluate 40 EC isolates recovered from chickens and classified as pathogenic or commensal strains based on the identification of the *cpsO* gene (Borst et al, 2015). To identify genetic markers associated with pathogenicity and adaptability, genome sequencing was performed in 40 EC isolates. A SNP tree was built using as controls pathogenic EC SA3 strain, commensal EC CE1 strain, and *E. faecalis* references. The gene annotation and comparison were done between phylogenetic clades. The 40 evaluated EC isolates formed 5 distinct clades that clearly separated commensal from pathogenic isolates. A total of 421 types of genes and genetic elements were identified as differentials between the evaluated EC isolates. A total of 84 genes and genetic elements were shared among all EC pathogenic isolates (4 clades). These isolates shared a capsular polysaccharide, LPS biosynthesis, and three histidine biosynthesis genes that were absent in the commensal EC strains in this study. A group of 19 EC isolates formed one single clade which is characterized by containing 30 genetic features that are not detected in the EC strains more closely related to the pathogenic EC SA3. On the contrast, this clade does not carry any of the CRISPR-associated proteins previously linked to pathogenic and commensal EC isolates. In addition, cadmium resistance genes were absent or with less than 50% nucleotide sequence similarity in this clade compared to the commensal EC and some pathogenic strains in this analysis. Overall, significant findings linked to absence or possibly acquisition of genes relevant to pathogenesis differing from the SA3 reference were detected in the EC pathogenic emergent isolates, suggesting adaptation of EC to the chicken.

Keywords: *Enterococcus cecorum*, WGS, Bacterial adaptation

Metabolism & Nutrition X: Feed Additives

T177 Black soldier fly as an alternative protein source in broiler chicken diet for mitigating feed cost Md Sazedul Karim Sarker*^{1,2}, US Ety^{1,2}, MT Hossain^{1,2}, NN Lisa^{1,2}, MA Rashid^{1,2}, A Islam^{1,2}, MF Sharmin³, SS Zihan², S Faruque¹ ¹*Bangladesh Livestock Research Institute, Poultry Research Center, Savar, Dhaka, Bangladesh;* ²*BLRI, Strengthening of Poultry Research and Development Project, Savar, Dhaka, Bangladesh;* ³*Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka, Bangladesh*

The primary obstacle to poultry production's expansion, sustainability, profitability, and growth has been the high feed cost. Meal from black soldier fly larvae (BSFL) is one of the world's most promising and widely recognized alternative protein sources. Bangladesh imports almost 68% of the raw materials used in the poultry industry. So, this study was carried out to use black soldier fly as an alternative protein source in broiler chicken diet to mitigate the production cost. Three hundred and sixty-day-old chicks of Arbor Acres were considered to ascertain the appropriate inclusion level in diet and their impact on the growth performance and meat quality attributes. For five weeks, there were six different

dietary treatments (0, 2, 4, 6, 8, 10%) for Black Soldier Fly (*Hermetia illucens*) (BSFM), each with four replications and fifteen chicks per replication. The birds were kept under the usual management conditions on the floor pans. They were given the recommended amount of nutrients, vaccinated properly and biosecurity protocols were observed. Body weight was taken into record every week. At 35 days, 48 birds were slaughtered according to standard method. Carcass characteristics, physiochemical parameters, lipid oxidation (TBARS) and proximate composition of meat were evaluated. The experimental design was completely randomized design (CRD) and data were analyzed by the SPSS 20 version. When compared to other dietary regimens, T₃ (6% BSFM) had the highest body weight of broiler chicken (P<0.05). The broiler chicken dressing output was considerably higher (P<0.05%) in T₃ with breast and thigh meat. Meat color, drip loss (%), cooking loss (%), pH, and water holding capacity (%) were all unaffected (P>0.05) by the addition of BSFM for both breast and thigh broiler meat quality characteristics. Following meat preservation at 7, 14, 21, and 28 days, the T₃ group's breast meat has significantly (P>0.05%) less fat and a lower thiobarbituric acid value (TBARS value) than the thigh meat. The crude protein percentage of meat under T₄ and T₃ were significantly (P<0.01) higher than other treatments. In conclusion, BSFM levels can be used up to 6% in broiler chicken diets and as a substitute for protein sources to reduce feed costs.

Keywords: Black Soldier Fly, Dietary levels, Broiler chicken, Alternative protein, Meat quality

T178 Evaluation of fenugreek seed extract fed to 0 to 21 d old broiler chickens on performance and oocyst shedding Samantha Huffer*¹, Alyssa M. Lyons², Nathan Sudom³, Mike E. Persia¹ ¹Virginia Tech, School of Animal Sciences, Christiansburg, Virginia, United States; ²Virginia Tech, School of Animal Sciences, Blacksburg, Virginia, United States; ³Emerald Seed Products, Avonlea, Saskatchewan, Canada

An experiment was conducted to determine the effects of various doses of fenugreek seed extract (FSE) fed with and without microencapsulation on broiler performance, intestinal permeability and oocyst shedding. A total of 1,512 d old male Ross 708 chicks were housed in floor pens and assigned to six treatments with 12 replicate pens of 21 chicks. Treatments were positive control (PC) using clean pine shaving litter and no feed additive, negative control (NC) with used pine shaving litter seeded with fresh coccidial oocysts and no feed additive, and treatment diets where birds were grown on the same used litter with 900 and 1,300 (g/MT) Nutrifin® (900FSE and 1300FSE) or microencapsulated Nutrifin® (900MFSE and 1300MFSE). The NC litter was generated by providing a 10x CocciVac[®] B52 and allowing these birds to shed oocysts into the litter for 21 d. Body weight (BW), feed intake (FI), and mortality corrected feed conversion ratio (FCRm) were calculated over the 0 to 21 d period. On D 5 to 7, 11 to 13, and 17 to 19, three composite fecal samples were collected per pen to determine oocyst shedding. On D21, litter dry matter was determined, and serum FITC-Dextrin was used to determine intestinal permeability. Data were analyzed using ANOVA in JMP Pro 16.0, and means were separated using Student's T-test. The model was validated as the NC broilers (890 g) had reduced BW compared to the 917 g PC (P ≤ 0.05). The 900FSE, 1300FSE and 1300MFSE treatments resulted in a response intermediate to the PC and NC (P > 0.05). There were no differences in FCRm between the PC and NC, although, 900MFSE and 1300 MFSE reduced the FCRm in comparison to the PC (P ≤ 0.05). Oocyst shedding increased over time and the PC was numerically reduced in comparison to other treatments, but no differences were noted with the seeded litter regardless of

treatment. Litter dry matter ranged from 63.8 – 70.4% (P = 0.40) and intestinal permeability based on FITC-Dextrin was not different (130.7 to 133.5 ng/dl; P = 0.87). Overall, both concentrations of FSE and the 1300MFSE were able to at least partially ameliorate the negative effects of the seeded litter on BW and the 1300MFSE was able to improve the FCRm in comparison to the PC, although further elucidation of the MOA was not able to be determined.

Keywords: fenugreek seed extract, broiler, coccidiosis, oocyst shedding

T179 Effects of supplementing broiler diets with mango peel and seed kernel extracts on growth performance, carcass characteristics, and gut health Zafar Hayat*^{2,1}, Tuba Riaz¹, Sana Ullah¹ ¹University of Veterinary and Animal Sciences, IDRC Project Lab., Lahore, Pakistan; ²College of Agriculture, University of Sargodha, Department of Animal Science, Sargodha, Pakistan

The escalating global concern over antibiotic resistance has prompted a search for sustainable and natural alternatives in poultry production. Mango fruit waste is a rich source of phytochemicals such as gallic acid, which has been used to enhance broiler growth and gut health. This study investigated the potential of mango seed kernel (MSK) and mango peel (MP) extracts, obtained via an optimized ultrasound-assisted extraction system, as dietary supplements for broilers. One-day-old, 560 male chicks (Ross 308) were divided into four treatments, with seven replicates of each treatment containing 20 chicks each by a completely randomized design. The experimental diets were prepared as follows: basal diet with antibiotics (Positive Control), basal diet without antibiotics (Negative Control), basal diet without antibiotics + 25 mL/kg MSK extract (MSK), and basal diet without antibiotics + 25 mL/kg MP extract (MP). As a result, the extracts did not significantly influence the body weight gain, feed consumption, and feed conversion ratio, demonstrating similar performance to both the control groups across all phases. MSK extract improved body weight gain and FCR insignificantly (p > 0.05) compared to other treatments. MP and MSK extracts showed a non-significant influence on carcass characteristics except leg quarter yield and abdominal fat. The intestinal morphology, poultry welfare parameters, and gene expression were unaffected. MSK significantly reduced the T-cell abundance, total bacterial count, and *Escherichia coli* levels, suggesting a potential immunomodulatory and antimicrobial effect. These findings indicated that MSK extract may offer a viable strategy to replace antibiotics in broiler production without compromising growth performance or posing any adverse effects. Further research is warranted to elucidate the underlying mechanisms and optimize MSK supplementation for maximum optimal benefits.

Keywords: Broiler growth, alternative to antibiotics, growth performance, gut health, microbiota count

T180 Yolk coloring efficacy of natural xanthophyll based products in relation to commercial standard C30-ester product Yauheni Shastak¹, Wolf Pelletier¹, Alvaro Gordillo², Kevin Matter*³, Christian Scharch⁴ ¹BASF SE, Ludwigshafen am Rhein, Germany; ²BASF Espanola S.L., Barcelona, Spain; ³BASF Corp., Florham Park, New Jersey, United States; ⁴Feedtest, Wettin-Löbejün, Germany

This study aimed to evaluate the yolk coloring efficacy of natural xanthophyll-based products compared to the commercial C30-ester product, Lucantin[®] Yellow 10% NXT (LY). C-30 ester from LY was supplemented at 1.25 and 2.50 mg/kg in a wheat-barley-soybean meal-based diet. Additionally, two natural xanthophyll

products (Product A and Product B) were included to provide 3.75 and 7.50 mg of xanthophylls per kg, maintaining a 1:3 substitution ratio of synthetic C-30 ester to natural xanthophylls. The basal diet was formulated without the inclusion of carotenoid supplements. The study involved 672 Lohmann laying hens housed in floor pens, which were fed seven experimental diets with eight replicates per treatment for six weeks. Yolk color was assessed using the BASF color fan and L*, a*, b* values were obtained from a Konica Minolta Spectro-Photometer CM-700d, which measures lightness (L), redness (a), and yellowness (b). The relative coloring efficacy of each carotenoid source was calculated using general linear models in SAS software. Separate linear regressions of yolk color parameters were conducted based on the supplemented levels of the corresponding carotenoids for xanthophyll-based products and the BASF product. The slopes of these regressions were then compared to determine the effective substitution ratio of C30-ester to natural xanthophylls. The study found that the substitution ratios of Products A and B compared to LY varied depending on the color parameter measured. For lightness (L), the substitution ratios were 5.4 for Product A and 3.2 for Product B. For redness (a), the ratios were higher, at 6.3 for Product A and 4.1 for Product B. For yellowness (b), the ratios were 3.2 for Product A and 2.2 for Product B. According to the BASF color fan, the substitution ratios were 6.9 for Product A and 4.2 for Product B. In conclusion, the coloring efficacy of natural xanthophyll products compared to LY may exceed the traditionally accepted 3:1 substitution ratio, with significant variation depending on the specific natural carotenoid product and the color dimension assessed. Therefore, careful considerations and assessments are important when choosing between different natural sources, as well as between natural and synthetic options, for yolk coloration.

Keywords: C-30 ester, xanthophylls, laying hen, yolk coloring, comparison

T181 Dietary *Chlorella vulgaris* and cinnamon oil enhance growth performance and intestinal health in broiler chickens Ahmed Kewan*^{1,2}, AbdElrazeq M. Shehata¹, Mahmoud Madkour³, Abdelkawy A. El-Ghoul¹, R. A. Abo-Salem¹, Waleed Abdelmoez¹, Ahmed Ramzy¹ ¹Faculty of Agriculture, Al-Azhar University, Department of Animal Production, Nasr City, Cairo, Egypt; ²University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ³National Research Center, Animal Production Department, Dokki, Giza, Egypt

A study was conducted to investigate the impact of dietary supplementation of dried *Chlorella vulgaris* and cinnamon oil, individually and in combination, on growth performance, intestinal morphology, digestive enzyme activity, and gene expression of nutrient transporters and inflammatory markers in broiler chickens. A total of 120 one-day-old male broiler chicks were randomly assigned to four dietary treatments: a control diet (CTRL), a diet supplemented with *Chlorella vulgaris* (DCV), a diet supplemented with cinnamon oil (CO), and a diet supplemented with both *Chlorella vulgaris* and cinnamon oil (DCV&CO). Results revealed that dietary supplementation with *Chlorella vulgaris* significantly ($p < 0.05$) improved body weight gain and feed conversion ratio. Moreover, both *Chlorella vulgaris* and the combination of *Chlorella vulgaris* and cinnamon oil significantly ($p < 0.05$) enhanced jejunal villus height. *Chlorella vulgaris* alone led to significant ($p < 0.05$) increases in villus: crypt ratio and villus area. Dietary supplementation with all three treatments significantly ($p < 0.05$) stimulated duodenal lipase activity. *Chlorella vulgaris* significantly ($p < 0.05$) increased duodenal amylase activity, while the combination of *Chlorella vulgaris* and cinnamon oil significantly ($p < 0.05$) increased duodenal protease

activity. Additionally, all three treatments significantly ($p < 0.05$) upregulated the mRNA expression of jejunal nutrient transporters, including excitatory amino acid transporter, peptide transporter-1, glucose transporter type 1, cationic amino acid transporter-1, and sodium-glucose linked transporter. Furthermore, all treatments significantly ($p < 0.05$) increased the mRNA expression of jejunal tight junction proteins (claudin-1, zonula occludens-2) and mucin-2. While all treatments significantly ($p < 0.05$) decreased the mRNA expression of tumor necrosis factor-alpha, the combination of *Chlorella vulgaris* and cinnamon oil significantly ($p < 0.05$) increased the mRNA expression of interleukin-8. In conclusion, dietary supplementation with *Chlorella vulgaris* and cinnamon oil has the potential to enhance growth performance, intestinal morphology, digestive enzyme activity, and immune function in broiler chickens.

Keywords: *Chlorella vulgaris*, Cinnamon oil, Intestinal morphology, Nutrient transporters, Broiler chickens

T182 Production performance of laying hens supplemented with essential oils of cinnamon, clove and oregano Midian Nascimento dos Santos*¹, Stephanie Torrey¹ ¹Trouw Nutrition, Puslinch, Ontario, Canada

Essential oils (EO), such as cinnamon, clove, and oregano, have been shown to enhance broiler performance in previous studies. However, the effect of these EO on laying hens' performance remains unclear. This study aimed to evaluate the effects of a product containing EO of cinnamon, clove, and oregano (CCO) on laying performance of layer hens on a commercially relevant scale. A total of 12,288 19-week-old Dekalb White hens were placed in a commercial layer house and randomly assigned to 2 treatments, with 6 tiers per treatment. From each tier, 42-44 enriched cages (24 hens per cage) were tested. Cages were equipped with perches, scratch pads, and a segregated nest area. The treatments included a standard corn-soy diet representative of commercial diet used as a Control (CON; 6,120 hens) and a standard diet supplemented with CCO, included at 250 mg/kg (CCO; 6,168 hens) offered from 19-30 and 50-70 weeks of age. From 31-49 weeks of age, all hens received the control diet. Supplementation was not continuous to minimize costs associated with the addition of CCO. Antibiotics were not used in either treatment in any phase. Egg production, mortality, feed intake, and feed conversions were recorded and calculated based on periods of four consecutive wks (28 days). Egg weights were recorded at 28-day intervals from a pair of cages located on each side of the tiers. Data were analyzed as a completely randomized design with repeated measures using the Glimmix procedure in SAS. Differences were considered significant at $P < 0.05$. The cumulative results indicated that mortality was unaffected by treatment (CON: 0.63%, CCO: 1.01%; $P=0.17$). Hens fed CCO exhibited improved/lower feed conversion ratio per kg of egg (CON: 2.08, CCO: 1.98; $P < 0.0001$) and per dozen eggs (CON: 1.47, CCO: 1.41; $P=0.007$) compared to the control group, without a difference in egg weight per hen (CON: 51.7 g, CCO: 53.2 g; $P=0.11$) or average daily feed intake (CON: 107.7 g/d, CCO: 105.8 g/d; $P=0.26$). Although not statistically significant, CCO resulted in higher daily egg production per hen compared to the control group (CON: 0.873, CCO: 0.896; $P=0.14$). These findings indicate that CCO can enhance the performance of laying hens, even when the supplementation of these EO is not continuous.

Keywords: Essential oils, laying hens, productive performance, feed efficiency

T183 Evaluating the effect of dietary supplementation of NeutraPath® and tribasic copper chloride on the growth performance of Ross 308 broilers exposed to a necrotic

enteritis challenge Anthony D. Quant*¹, Corey A. Johnson¹, Logan Erb¹, Elizabeth J. Miller¹, Jay Hughes², Aldo Rossi² ¹*Kalmbach Nutritional Services, Upper Sandusky, Ohio, United States*; ²*Amlan International, LLC, Chicago, Illinois, United States*

The objective of this study was to evaluate the effects of feeding different feed additives on the performance of broiler chickens exposed to a necrotic enteritis (NE) challenge. A total of 4,320 straight-run broiler chicks (Ross 308 x Hubbard M99) were allotted to 72 floor pens according to a RCBD with a 2x3 factorial arrangement of NE challenge (NC: non-challenged; CC: challenged) and additive treatments (3). Diets were fed in 3 phases including a starter (D1-15; crumbles), grower (D16-27; pellets), and finisher (D28-43; pellets). The dietary treatments included a control (CTL; devoid of gut health additives), NeutraPath® (NTP; Amlan® International) at 1 kg/MT in all phases, and tribasic copper chloride (TBCC) added at 200, 150, and 135 ppm in the starter, grower, and finisher feeds, respectively. Birds and feed were weighed on D1, 15, 27, and 43 to calculate feed intake (FI), BW gain (BWG), and mortality corrected FCR (FCRc). The NE model involved administering the birds a 10x dose of live coccidia vaccine (Advent®, Huvepharma®) via supplemental feed at D15, followed by a *C. perfringens* insult at D19 and D20 via supplemental feed. Main effects were observed for the NE challenge and additive treatments, however limited interactive effects were observed. Prior to the challenge, D15 BW was lower for CTL (435.4g) than NTP (453.8g) and TBCC (455.2g; $P < 0.01$). FCRc from D1-15 was improved by NTP (1.190) and TBCC (1.187), vs. CTL (1.210; $P < 0.01$). The NE challenge increased D1-43 mortality 4.6-fold for CC (19.94%) vs. NC (4.26%; $P < 0.01$). Additionally, CC reduced D43 BW ($P < 0.01$) by 146.9g and increased D43 FCRc ($P < 0.01$) by 3.5 points vs. NC. Main effects of additive treatments existed for BW, as NTP and TBCC increased BW vs. CTL at D15, D27 and D43 ($P < 0.01$). Furthermore, D1-27 FCRc was lower for NTP and TBCC vs. CTL. Income over feed cost was lowest for CTL (\$1.667/bird) vs. NTP (\$1.719/bird) and TBCC (\$1.734/bird; $P < 0.01$). Interactive effects were observed for FCRc from D1-27, as the CC-NTP treatment had the lowest FCRc (1.414) of the CC group, significantly better than CC-TBCC (1.447) and CC-CTL (1.473; $P < 0.01$). These data indicate that feeding NTP or TBCC can improve broiler performance and positively impact FCRc through D27 following a NE challenge.

Keywords: Necrotic Enteritis, Broiler, Ross 308

T184 A microencapsulated blend of botanicals can enhance growth performance and support gut health of broilers in commercial conditions under a conventional anticoccidial program Benedetta Tugnoli*¹, Antonino Messina¹, Giulia Giovagnoni¹, Andrea Piva¹, Ester Grilli^{2,3} ¹*Vetagro, Reggio Emilia, Italy*; ²*University of Bologna, Bologna, Italy*; ³*Vetagro Inc, Chicago, Illinois, United States*

Botanicals are widely used in poultry nutrition for their multiple properties (antimicrobial, anti-oxidant, anti-inflammatory) that can help to support growth and health of birds. The aim of the study was to evaluate the effects of a botanicals-based feed additive, microencapsulated in a lipid matrix, on growth performance and gut health of broilers in commercial conditions under a conventional anticoccidial program. The trial was conducted in a commercial farm with day-old chicks (Ross 308) divided in two houses (19,000 birds/house) assigned to one of two groups: CON group, fed a basal diet treated with Maxiban® (d0-21) and Monteban® (d22-49) or BOT group, fed the same basal diet treated with Maxiban® (d0-21) and Monteban® (d22-49) + the microencapsulated blend of botanicals at 200 g/MT (d0-49).

Necropsies were performed at day 21, 28 and 35 (5 birds/group/time-point) for gut health and footpad lesions scoring. Data were analyzed with T test and differences considered significant at $P < 0.05$. Growth performance were recorded until birds collection for slaughtering around day 49. Regarding growth performance, mortality and final body weight were the same between groups (3% and 3.35 kg) but BOT reached it 2.4 days earlier than CON (46.4 days vs 48.8 days total cycle). Also FCR was improved in BOT compared to CON (1.66 vs 1.76). Consequently, the European Production Efficiency Factor [EPEF = (Final body weight*liveability*100)/(days*FCR)] was improved in BOT compared to CON (415 vs 383). Gut health lesions scores were similar between the two groups throughout the trial, with generally good intestinal scores and low values for *Eimeria* lesions. Footpad lesion scores were similar between the two groups at day 21 but then the lesions were significantly lower in BOT compared to CON at day 28 and 35 (at both time-points score 1 vs 1.6, $P = 0.04$). In this trial, the botanicals-based feed additive was used in combination with Maxiban®/Monteban® shuttle program, allowing a strong improvement of feed efficiency, good intestinal health scores and reduced risk of foot pad lesions. To conclude, this alternative anticoccidial product is compatible with a conventional anticoccidial programs and has the potential to further enhance growth performance, while supporting gut health.

Keywords: botanicals, microencapsulation, anticoccidial, gut health

T185 Gut-released bioactive compounds to improve animal performance Caio Tellini*¹, Mariana Ornaghi¹, Ricardo Castilho¹, Rafaela Berto¹, Cátia Miquilino¹ ¹*Safeeds, Research and Development, Cascavel, Paraná, Brazil*

The increasing use of natural compounds in livestock feed reflects the growing consumer demand for animal products sourced from healthy and sustainable systems. Plant-derived compounds, such as essential oils, offer antimicrobial, antioxidant, and anti-inflammatory benefits, enhancing animal health, immunity, and performance. When combined with organic acids, these compounds demonstrate increased activity due to synergistic effects. This study aimed to evaluate the effect of the additive Avinatus® M300, a gut-targeted blend of essential oils and organic acids, on the diet of broilers challenged with *Clostridium perfringens*. A total of 1,440 male broilers were assigned to five treatments: three dosages of Avinatus® M300 (150, 300, and 450 g/t; A15, A30, and A45), a treatment with Enramycin at 10 ppm (PC), and a control diet without additives or challenge (NC). Except for the NC group, birds were challenged with *Eimeria* vaccination, followed by oral inoculation (0.5 ml) of *C. perfringens* (10^8 CFU/ml) on days 12, 13, 14, and 15. Data were analyzed using one-way ANOVA, with means separated by Tukey's test ($P \leq 0.05$). During the early phases (days 7, 14, and 21), no significant differences were observed among treatments ($P > 0.05$), except for the NC group. However, by day 28, birds receiving the A30 dosage showed higher feed intake and weight gain, with performance comparable to the NC group ($P < 0.05$). By the end of the experiment (42 days), treatments with the gut-released blend (A15, A30, and A45) and Enramycin achieved better feed conversion rates than the NC group ($P < 0.05$). These findings suggest that the gut-released blend of essential oils and organic acids at a 300 g/t dosage can improve performance and feed efficiency in broilers challenged with *C. perfringens*, demonstrating effects comparable to enramycin treatment. This blend offers a promising alternative to antibiotics, supporting sustainable poultry production. Bioactive compounds in certain plant extracts may effectively boost animal health and productivity by strengthening immunity and enhancing the gut's response to

pathogens and toxins. As a result, nutrient utilization and overall animal performance are improved.

Keywords: Essential oils, microbiota, poultry, organic acids

T186 A blend of *Castanea sativa* polyphenols and coated short-chain fatty acids improves the performance and gut health of broilers David Harrington^{*1}, Yann Lever², Jean Francois Le Roux², Nemanja Todorovic¹ ¹*nuance Biotechnology, Lausanne, Switzerland;* ²*Global Nutrition International, Fougères, Veuillez, France*

Butyrate, a short-chain fatty acid (SCFA), has shown beneficial effects on animal performance when used as a dietary supplement. *Castanea sativa* polyphenols are recognized for their antibacterial and antioxidant properties. Optimizing growth and feed efficiency in broilers is critical for poultry production. The objective of the study was to evaluate the effects of protected calcium butyrate supplementation, alone and in combination with *C. sativa* polyphenols, on the performance of broilers. A total of 960 as-hatched Ross 308 broilers were allocated to 3 treatments across 24 pens (40 birds/pen; 8 pens/treatment) in a commercial poultry house: CON: control (basal diet, no additives); CAB: basal diet + coated calcium butyrate (385 g/t); CABP: basal diet + calcium butyrate/polyphenols (750 g/t). Diets contained a natural anticoccidial. Body weight gain (BWG) was measured on days 11, 21, and 32 and FCR determined for days 0-11, 0-21 and 0-32. Fecal quality (score 0-5; best to worst) was assessed on days 16, 19, 23 and 27. Data were analyzed via ANOVA and statistical significance declared at $P < 0.05$. Results indicated that BWG in both CAB and CAPB was higher ($P < 0.05$) than CON on day 11 (370, 375g and 359g, respectively) and day 21 (979, 1043 and 908g, respectively). By day 32, CAPB was heavier ($P < 0.05$) than both CON and CAB (2114, 1902 and 1996g, respectively). FCR differed ($P < 0.05$) between all groups on days 0-11 and 0-21; FCR in CAPB was lower than CAB, which was lower than CON. Final FCR was lower ($P < 0.05$) in CAPB and CAB than CON (1.49, 1.55 and 1.62, respectively). Fecal scores differed ($P < 0.05$) on day 16, where CAB had a lower score than both CON and CAPB (1.14, 1.25 and 1.25, respectively). On day 27, fecal score was numerically lower in CAPB than CON and CAB (2.75, 3.13, and 3.42, respectively) ($P = 0.056$). In conclusion, protected calcium butyrate supplementation enhanced early growth performance, feed efficiency, and feces quality. The addition of *Castanea sativa* polyphenols was synergistic with calcium butyrate, leading to further improved overall performance, feed efficiency and gut health. These findings suggest that a combination of protected SCFAs and polyphenols can be an effective strategy to improve broiler productivity and gut health.

Keywords: calcium butyrate, polyphenols, broilers, FCR, performance

T187 Quantifying the effect of an encapsulated blend of essential oils and organic acid on performance, environmental impacts and feeding costs of broilers: a meta-analytical approach William Lambert¹, Clément Guele¹, Romain Moreau¹, Clarisse Techer^{*1} ¹*miXscience, Bruz, France*

Optimizing feed efficiency to minimize feeding costs and environmental impacts of modern broilers is the primary concern of the industry. Essential oils and organic acid have been shown to stimulate digestive secretion, promote gut acidification and therefore improve nutrient utilization. In a recent US experiment, feeding broilers with a proprietary blend of encapsulated essential oils and organic acid (Lumigard TCB, Mixscience, France) has shown a significant reduction of feed conversion ratio (FCR) by 6pts in 0-32d Cobb broilers compared to a control with a reduced

nutrient density diet. As a follow-up, a meta-analysis was conducted by exhaustively compiling all the broiler feeding trials that were performed on this solution. A database was created by integrating all the raw pen data of 10 broiler experiments, corresponding to 20 treatments (10 with and 10 without Lumigard TCB), 76 pens and 2,446 broilers. Trials were performed in France, USA or Brazil and included either Ross 308 or Cobb 500 broilers. Statistical analysis was performed using generalized linear mixed model with the trial as a fixed effect and trial as a random effect. Each trial was weighed in the model according to its number of broilers x number of replicates (R statistical software). Analysis was done for the whole experimental trial and per feeding phase for all growth parameters, carbon footprint and feeding costs. Over the whole experimental period, the meta-analysis showed that Lumigard TCB significantly improved average daily gain and cFCR by 0.75g/d and -0.03 pts respectively ($P = 0.014$ and $P < 0.001$), increased European Production Efficiency Factor by 18.3 pts ($P < 0.001$), reduced carbon footprint by 29.9 kgCO₂/kg of live weight broiler ($P < 0.001$), and reduced feeding costs by 6.64 USD/kg of live weight ($P < 0.001$). The effect on growth and FCR was observed significantly in all phases of the experimental periods: starter, grower and finisher with similar response across periods (0.5 to 1.6g/d for ADG, -0.015 to 0.058 for FCR). Based on the raw data of 10 trials, it can be concluded that Lumigard TCB is demonstrating robust and consistent benefits to broiler production by improving growth, feed efficiency and environmental performance in a cost-effective and repeatable manner.

Keywords: Encapsulated blend of essential oils and organic acid, Feed efficiency, Environmental performance, Meta-analysis, Broiler growth

T188 A synergistic blend of organic acids administered in water improves the productive traits of broilers Lane Pineda², Giseli Heim^{*1} ¹*Selko, Amersfoort, Netherlands;* ²*Trouw Nutrition, R&D, Amersfoort, Netherlands*

With the poultry industry moving toward antibiotic-free production practices, the search for alternatives to traditional antibiotic growth promoters (AGP) is increasing. Organic acid (OA)- based additives have emerged as a promising option due to their beneficial effect on intestinal health and animal performance. These additives not only compensate for gastric acidification but also enhance nutrient absorption and inhibit pathogenic bacteria, leading to improved growth performance and feed efficiency. To validate the efficacy of OA, a meta-analysis was conducted, combining data from two Asian studies on the effects of a synergistic blend of free and buffered short-chain fatty acids (Selko®-AlpHa, Selko water acidifier) administered via water on the growth performance and health of broilers. In both studies, a total of 2068 oneday-old broiler chicks were assigned to one of two treatments: (1) a basal diet (NC) and (2) a basal diet plus 1L Selko®-AlpHa/1000L water (SWA). The birds were raised for 35 days in an AGPfree, corn-soy-based diet following a two-phase feeding program. The raw data of individual studies were integrated and the effect of treatment on overall performance was analyzed using mixed models in SAS considering the within-study and between-study variations. Throughout the production period, SWA supplementation significantly improved the body weight (BW), average daily gain (ADG), and feed conversion ratio (FCR) of the birds, without adversely affecting the feed intake (81.29 vs 81.25 g/bird/d, $P = 0.97$) and water intake (165.4 vs 163.9 mL/bird/d, $P = 0.72$). Compared to the NC group, SWA increased the BW by 4.1% and ADG by 3%; and reduced the FCR by 5%. The mortality rate was unaffected by the treatments; however, it was numerically lower in the SWA group (5.8 vs 7.1%, $P = 0.32$).

In conclusion, these findings suggest that SWA enhances broiler production performance and could serve as an effective growth-promoting strategy in an AGP-free production system.

Keywords: water acidifier, growth performance, organic acids

T189 The power of data in DFM assessment - waterline application Sasha van der Klein*¹, Niels Christensen¹, Kirsty Gibbs¹ ¹*Danisco Animal Nutrition & Health, Oegstgeest, Netherlands*

Waterline DFM are gaining traction due to their flexibility of application, but questions arise regarding the number of application days needed. This study investigated the effect of a dual strain waterline DFM on performance results over four trials. Each trial consisted of a non-challenged control (NC), challenged control (CC), CC receiving a dual strain DFM in the waterline (1E8 CFU/bird of *Lactobacillus acidophilus* AG01 and *Bifidobacterium animalis* subsp. *Lactis* AG02) through 1 to 5 different application strategies per trial. Strategies consisted of consecutive application from d 4 to 6, consecutive application from d 12 to 21, intermittent application from d 4 to 6, 12 to 14, and 18 to 21, alternate day application from d 1 to 28, alternate day application from d 1 to 42, consecutive application from d 1 to 28, consecutive application from d 1 to 42. Except the NC, all treatments received a 10x dose COCCIVAC®-B52 (Merck Animal Health) on d 14, followed by 1E8 CFU *Clostridium perfringens* (*netB*⁺) at d 16-20. In all trials, diets were corn/soy based and fed *ad libitum* in three phases; starter (0-14 d), grower (15-28 d), and finisher (29-42 d). Birds were placed on reused litter. Parameters related to performance [body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI)] and health (liveability, challenge induction level, average NE score) were evaluated. The statistical analysis was conducted as a meta-analysis for DFM treatments vs CC or NC comparisons based on the cumulative outcomes (performance and health) across all phases. Earlier individual reports on these studies highlighted significant positive effects of the DFM application on both performance and health indicators. In the current analysis, BWG observed a positive effect across all DFM treatments compared to CC at an average of 6.2% and ranging from 3.1% to 9.9% ($P < 0.05$). The level of challenge induction was reduced across all DFM treatments compared to CC at an average of -37.4% and ranging from -16.3% to -55.8%. Increased application length enhanced the effect ($P < 0.05$). In conclusion, the current multi-study analysis confirms that both performance and health indicators are important to determine the efficacy and best practise application of waterline DFM technologies.

Keywords: probiotic, necrotic enteritis, broiler, meta-analysis, direct fed microbial

T190 Effect of dietary inclusion of *Saccharomyces cerevisiae* fermentation product (Original XPC™) and stocking density on production performance and egg quality in brown egg strain laying hens Kelly E. Brannan*¹, Becca Wysocky², Kenneth Anderson² ¹*NC DACS PRS, Midrand, South Africa*; ²*North Carolina State University, Raleigh, North Carolina, United States*

The transition away from conventional management practices in the table egg industry has resulted in an increased focus on alternative dietary supplements as well as decreased stocking densities to promote hen health and welfare. Yet the impact of these more holistic management practices on hen performance and egg quality has not been fully established. The influence of dietary inclusion of a *Saccharomyces cerevisiae* fermentation product (SCFP) and stocking density on hen production performance and

egg quality were evaluated in commercial brown egg layers. Dietary treatments compared a standard commercial diet (CN) against one supplemented with SCFP (Original XPC™ included at 0.68 kg/MT, SC). Stocking density treatments were designated as either high (471 cm² per bird, HD) or low (497 cm² per bird, LD). A total of 3,690 hens were housed in a commercially relevant trial facility, with 45 replicates for each of the 4 treatments. Data were collected every 4 wk from 17 to 41 wk of lay and analyzed in a 2 x 2 factorial design using stocking density and diet as the main effects. Improvements in egg production were observed for both the SC and LD treatments, concurrent with an increase in feed intake for the LD hens. Increased egg weights were also exhibited in the SC treatment, arising from a greater percentage of extra-large eggs. Higher shell strength and percentage of solids were also found in the SC group. The percentage of USDA grades and other egg quality variables remained similar between treatments. Both SC and LD were shown to support enhanced performance for brown egg strains, with additional improvements in egg size and quality exhibited in SC hens. While the improvements in performance for the LD hens are favorable, producers must also consider the economic impact of the increased feed intake noted for this group. The dietary inclusion of SC supports enhanced performance and egg size without negatively impacting feed intake, regardless of the stocking density evaluated in this study, which can benefit markets driven by egg size.

Keywords: *S. Cerevisiae*, stocking density, brown egg, production performance, egg quality

T191 Yeast Fermentate Supplementation May Enhance Early Cecal Microbiota Maturation in Chicks Elena G. Olson*¹, Ashley A. Tarcin², Lindsey A. Wythe⁴, Abe Scheaffer³, Steve Ricke¹ ¹*University of Wisconsin, Madison, Wisconsin, United States*; ²*University of Wisconsin, Animal and Dairy Sciences, Madison, Wisconsin, United States*; ³*Harvest Fuel Inc, Walhalla, North Dakota, United States*; ⁴*Texas A&M University, College Station, Texas, United States*

In this study, we evaluated the impact of varying yeast fermentate (YF) concentrations on the early development of cecal and fecal microbiota in chicks. 400 Ross 308x308 chicks were housed in battery cages and fed a commercial diet supplemented with four YF concentrations: 0%, 0.2%, 0.5%, and 0.75%. Cecal and fecal droppings and feed samples were collected on days 1, 3, and 5 ($n=10$ /treatment). The V4 region of the 16S rRNA gene was amplified using custom dual-indexed primers and sequenced on an Illumina MiSeq platform. Sequencing data were analyzed in QIIME2-2024.5, with alpha and beta diversity assessed using Kruskal-Wallis and ANOSIM tests, while taxonomic differences were analyzed using ANCOM-BC. Main and pairwise effects were considered significant at $P \leq 0.05$ and $Q \leq 0.05$. Our results indicated no significant microbial composition changes in the 0% YF group across days or cecal and fecal samples ($P > 0.05$). However, across treatments, cecal and fecal microbiota were similar on days 1 and 3, but cecal populations showed a significant increase in richness and evenness by day 5, resulting in phylogenetic differences ($P < 0.05$). Additionally, fecal and cecal profiles differed significantly from feed microbiota ($P < 0.05$). Interestingly, feed microbial populations were not affected by treatment but differed in richness, abundance, and phylogenetic composition on days 3 and 5 compared to day 1 ($P < 0.05$). While the 0.5% YF treatment did not significantly alter microbial diversity across days, the 0.2% YF group exhibited increased microbial richness, evenness, and phylogenetic diversity by day 3 ($P < 0.05$). Similarly, the 0.75% YF group showed comparable changes between days 1 and 3, with additional trends toward increased microbial abundance between days 1 and 5 (Unweighted

UniFrac, $P = 0.05$, $Q = 0.08$) and significant shifts between days 3 and 5 ($P = 0.03$, $Q = 0.05$). On days 3 and 5, 0.75% treatment enriched several taxa, including *Subdoligranulum*, *VadinBE97*, *Mucispirillum*, and *Microbacteriaceae*, commonly associated with lower GIT and improved bird performance. These findings suggest that supplementing poultry feed with 0.75% YF may accelerate cecal microbiota maturation and promote beneficial microbiota during early post-hatch development.

Keywords: yeast fermentate, feed additive, cecal microbiota, chicks, broilers

T192 Effects of a *Saccharomyces* yeast postbiotic and reduced protein and amino acid diet on broiler performance Mathew A. Vaughn^{*1}, John Gonzalez², Edgar Miller¹, James McNaughton³ ¹*Puretein Bioscience, Research and Development, Maxwell, Iowa, United States*; ²*University of Georgia, Athens, Georgia, United States*; ³*AHPharma, Hebron, Maryland, United States*

The purpose was to investigate effects of a *Saccharomyces* yeast postbiotic (celluTEIN) on broiler growth performance when fed reduced protein and amino acids. Broilers ($N=5,616$) were feather sexed, randomly assigned to 108 pens ($N=26$ males and females), and pens were assigned to 1 of 9 dietary treatments ($n=12$) fed 42 d in 3 phases. Treatments included a negative control (NC:basal diet/clean litter) and other treatments were housed on dirty litter: positive control (PC:basal diet), basal diet containing *Saccharomyces* yeast postbiotic (SYP:300 ppm

starter/50 ppm grow-finish), PC and SYP with 3 grades of reduced protein and amino acids (RCA, RCB, RCC and RSYPA, RSYPB, RSYPC). Body weight (BW), mortality, and feed conversion ratio (FCR) were collected at d-14, 35, and 42. Data were analyzed as a completely randomized design. There was no treatment effect ($P=0.527$) on d-0 BW, but treatment did affect all remaining days' measures ($P<0.001$). At d-14, 35, and 42, SYP broilers weighed more than PC and NC broilers ($P<0.001$), except NC did not differ ($P\geq 0.401$) on d-42. Addition of SYP to all RC diets (RSYPA, RSYPB, RSYPC) increased BW and ADG on all days ($P<0.001$). The SYP broilers did not differ in feed consumption compared to NC and PC broilers during all periods ($P<0.001$) except periods 0-42 and 0-14 ($P=0.280$), respectively. Addition of SYP to RC diets increased feed consumption compared to all RC diets during period 0-14 and RSYPB and RSYPC diets on periods 0-42 ($P\leq 0.027$). During all periods, SYP broilers had smaller FCR than NC and PC diets ($P<0.001$), and SYP addition to RC diets reduced FCR ($P<0.001$), except for RSYPC during period 0-14 ($P=0.515$). During all time periods, SYP did not differ in mortality or uniformity compared to NC ($P\geq 0.077$), but both had less than PC ($P\leq 0.001$). Addition of SYP to all RC diets reduced mortality during period 0-14 and for the RSYPA diet during period 0-42 ($P<0.001$). Addition of SYP to RC diets increased uniformity for all diets at all periods ($P\leq 0.003$) except RSYPA and RSYPB diets during period 0-35 ($P\geq 0.132$). These data suggest supplementing celluTEIN can positively affect all measures during a challenge even when fed reduced protein and amino acids.

Keywords: Broiler, postbiotics, saccharomyces

Welfare & Behavior III

T193 Current and future tools to monitor laying hen welfare in the transition to cage-free systems T. Bas Rodenburg^{*1} ¹*Utrecht University, Utrecht, Netherlands*

Worldwide, a transition can be seen from cage systems to cage-free systems for laying hens. Compared with cage systems, cage-free systems provide the hens with more space and more opportunities to meet their behavioral needs (such as foraging, dust bathing, perching and nesting behavior). At the same time, cage-free systems are more challenging to manage compared to cage systems and birds are more at risk of behavioral problems, such as damaging pecking behavior and piling behavior, and injuries such as keel bone fractures. The purpose of this study is to provide an overview of the current and future tools to monitor and safeguard laying hen welfare in the transition to cage-free systems. In The Netherlands, we conducted a large study in white and brown layer flocks to investigate risk factors for feather damage at 40 weeks of age. A group of 47 commercial cage-free layer flocks was monitored from parent stock to rearing and laying farm. Flocks were visited at 1, 5, 10, 15 and 40 weeks of age. First, analysis was conducted by a two-way ANOVA to assess separate factors to influence feather damage at 40 weeks of age. Hereafter, the final GLM for predicting feather damage at 40 weeks of age included only variables which had $P < 0.1$ in the two-way ANOVA. Significant risk factors for feather damage at 40 weeks of age were high levels of severe feather pecking and high fear of humans at 5 weeks of age (explained variance 29% and 5.3%, resp.). Modified management during the laying period, such as use of a radio in the house, pecking blocks, or roughage supply helped to reduce the risk of feather damage (explained variance: 26%). This illustrates that prevention of feather pecking during rearing is important for a laying period with minimal feather damage. It also underlines the importance of modified management, including the supply of pecking enrichments, to prevent feather pecking. In the project Best Practice Hens, these and other best practices are shared with

the industry to optimize management of cage-free flocks. Practical tools proposed by the project include a practical welfare assessment protocol that farmers can use to monitor their flock. Furthermore, best practices were formulated for successfully keeping pullets and laying hens in cage-free systems.

Keywords: laying hen, housing, welfare, behavior, cage-free

T194 Golda: a genetic model to eliminate male chick culling in the egg industry Enbal Ben-Tal Cohen¹, Olga Genin², Michael Pfann¹³, Michal Maimon¹, Izel Cohen¹³, Tatyana Kushnir¹, Amit Haron¹, Shanthi Dharanivasan¹, Eva Lamed², Dmitry Dima Shinder², Shelly Druyan¹, Yuval Cinnamon^{*12} ¹*Institute of Animal Sciences, Agricultural Research Organization - The Volcani Institute, Department of Poultry and Aquaculture, Rishon LeTsiyon 7505101, Israel*; ²*NextHen, Bnei Brak, Israel*; ³*The Hebrew University of Jerusalem, Rehovot, Israel*

Unwanted by the egg and meat industries, approximately 7 billion male chicks are culled worldwide each year using inhumane and costly methods. This practice imposes a significant economic burden on farmers and raises serious animal welfare concerns. This study aimed to develop a genetically based solution to eliminate male chick culling by halting male embryogenesis through a controlled, optogenetic approach, ensuring only female chicks hatch. These females are genetically identical to the layers currently used in table egg production. Our goal was to create a system that is 100% effective in preventing male development without affecting female viability, allowing for seamless integration into the poultry industry. The experimental design involved generating the GOLDA chicken breed, a transgenic line with a sex-linked, optogenetically regulated trait integrated on the maternal Z chromosome, which segregates only to male embryos. This trait, which halts embryogenesis at an early stage, remains inactive without blue light illumination; however, illuminating the

eggs before incubation activates the trait, preventing male hatching. The eggs were then incubated, and hatchling sex was verified and confirmed to be exclusively female. Control hatch rates of males (71) and females (82) followed the expected ratio ($p=0.419$), while the experimental group yielded 1,200 female hatchlings with no males ($p=1.57 \times 10^{-362}$). Further illumination experiments confirmed that male embryo mortality increased consistently with the illumination dose, providing data for reliability verification of conditions for preventing male hatching. Importantly, the hatched females reached sexual maturity and produced standard table eggs free of genetically modified material, meeting non-GMO classification requirements in many countries. This study provides a practical, humane, and compassionate solution to the male chick culling issue, with potential for global adoption. By utilizing a sex-linked, optogenetically controlled genetic trait, the GOLDA breed eliminates the need for male chick sorting and culling, setting a new standard in animal welfare for poultry production. This innovation also supports sustainability by reducing resource use in hatcheries, conserving energy, space, and labor.

Keywords: Animal Welfare, Male Chick Culling, Transgenic Chickens, Poultry Genetics, Sustainable Agriculture

T195 Effect of humidity during the hatching phase of incubation on broiler chick quality, tibia bone morphology, and behavior Chantel Pennicott¹, Angela Perretti², Victor Oyeniran¹, Sara Orłowski¹, Rosemary Whittle¹, Jaelen Cherry¹, Shawna Weimer¹ ¹University of Arkansas, Poultry Science, Farmington, Arkansas, United States; ²University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States

The incubation environment affects broiler embryonic development, health, and welfare. This study aimed to determine the effects of late-stage incubation humidity on broiler health and behavior. Fertile broiler eggs (N=695) of two lines (A, B) were incubated in a multi-stage incubator from embryonic day (ED) 0 to 17. In a completely randomized experimental design, ED18 viable eggs were distributed between three commercial hatcheries set to a constant temperature of 36.7°C with: 1) low relative humidity (LRH=65%; N=240), 2) high relative humidity (HRH=80%; N=240) or 3) Control (CRH=65%; N=215). On day-of-hatch, chicks were weighed and quality scored (CQ, unhealed navel and leg issues) and placed into 1 pen per line. Focal birds (N=75) were subjected to the isolation test on D19, and the number of escape attempts was recorded. On D42, birds were sexed and the right and left tibiotarsus bones (Tibia) were collected to measure bone morphology (mm) and ash content (%). Data were analyzed using a three-way ANOVA to determine the effects of treatment, line, and sex. For line A, chick hatch weight was lower for LRH (38.0g) compared to HRH (39.3g) and CRH (39.1g; $P=0.04$), and leg issues tended to be higher for LRH (47.6%) and HRH (40.3%) compared to CRH (25.5%; $P=0.1$). For line B, more chicks had leg issues for HRH (14.4%) compared to LRH (9.1%) and CRH (3.06%; $P=0.02$). Line A attempted to escape the isolation test fewer (1.33) times than B (2.44; $P=0.04$) during the isolation test. Line A tibia bone ash was greater in females (45.5%) than in males (43.5%; $P=0.004$). Line A had longer tibias (101.4mm) with smaller proximal head (PH) widths (24.6mm) compared to B (98.3mm, 26.0mm; $P \leq 0.03$). Line A had deeper lateral notch depths (ND) (1.98mm) and medial ND (2.01mm) compared to B (1.60mm, 1.55mm; $P < 0.0001$). Line B had deeper distal ND (5.41mm) and greater PH angle (43.5) than A (5.31mm, 40.3; $P < 0.0001$). LRH had wider tibias at 90% (18.7mm) and 75% (12.1mm) of the length than CRH (17.5mm, 11.2mm) and HRH (17.3mm, 11.3mm; $P \leq 0.02$). These results suggest that genetics and sex affected the leg health and behavior of broilers more than

incubation relative humidity, indicating that multiple factors may have a cascade effect on welfare and performance later in the growing stages.

Keywords: incubation, relative humidity, chick quality, bone morphology, behavior

T196 Evaluation of mechanical cervical dislocation methods for euthanasia in chicks Aaliyah Gore^{*1}, Laya Silva¹, Nathan Nelson¹, Sara Reichelt⁴, Monique Parris-Garcia¹, Rocio Crespo², Allison N. Pullin³ ¹North Carolina State University, Population Health and Pathobiology, Raleigh, North Carolina, United States; ²North Carolina State University, Population Health and Pathobiology, Raleigh, North Carolina, United States; ³North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ⁴Aviagen, Huntsville, Alabama, United States

In the poultry industry, cervical dislocation (CD) is a common euthanasia method, particularly on farms. Most existing studies on euthanasia have focused on older birds, with limited research on euthanasia methods for chicks under one week of age. Challenges with CD in chicks can present welfare and personnel concerns, especially the emotional stress associated with decapitation. The goal of this study was to evaluate the efficacy of Iris (I) and Kelly (K) scissors for cervical dislocation in 1-day-old and 7-day-old chicks and compare it to manual CD. Parameters used to assess insensibility and death included colonic convulsions, cardiac arrest, gasping frequency, feather erection, and pupillary light reflex. Other variables measured included degree of laceration and hemorrhage, site of dislocation, and type of fracture. Data were analyzed in RStudio (Version 4.2.2), using Generalized Linear Models, with age (1 and 7 days) and euthanasia method (ICD, KCD, and CD) as explanatory variables. Multiple comparisons were performed using post hoc Tukey tests. Statistical significance was set at 5%. All three euthanasia methods were effective at rendering chicks unconscious, and shortly, followed by death. However 1-day-old chicks euthanized with ICD or KCD endured a longer duration before experiencing cardiac arrest. Seven-day-old chicks in the CD group displayed significantly shorter clonic episodes, gasping frequency, time to cardiac arrest, and duration of feather erection compared to ICD and KCD. Fractures were less common with CD, and KCD had the highest frequency for cervical vertebral fractures. In conclusion, CD resulted in faster death to the chick but may be more traumatic to the handler because of the larger incidence of decapitation. While the time needed to verify the death of the chick was longer with the ICD, this method can be more acceptable for chicks 1-7 days of age, because there are minimal numbers of fractures, and can be less stressful for the handler. Based on our observations the use of KCD should be avoided as it can cause suffering of the bird due to a higher incidence of fractures and crushing of the vertebra.

Keywords: euthanasia, cervical dislocation, chicks

T197 A Semi-Supervised Auto-Labeling Approach to Enhance Artificial Intelligence in Poultry Farming Ramesh B. Bist^{*1}, Hannah Atungulu², Lilong Chai³, Shawna Weimer⁴, Chantel Pennicott⁵, Xiao Yang⁶, Sachin Subedi³, Yang Tian¹, Dongyi Wang¹⁷ ¹University of Arkansas, Biological and Agricultural Engineering, Fayetteville, Arkansas, United States; ²Haas Hall Academy, Springdale, Arkansas, United States; ³University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ⁴University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ⁵University of Arkansas, Poultry Science, Farmington, Arkansas, United States; ⁶University of Georgia, Athens, Georgia,

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The rapid growth of artificial intelligence (AI) in agriculture, particularly in poultry farming, has highlighted challenges in annotating large and diverse datasets essential for training AI models. Manual labeling is time-consuming, costly, and impractical for continuous data generation. This study explores semi-supervised auto-labeling methods, which combine a small amount of labeled data with a large volume of unlabeled data to enhance model accuracy and reduce annotation efforts. While unsupervised models often struggle with accuracy due to noisy labels, semi-supervised approaches have shown promise by iteratively refining labels with minimal human intervention. The research investigates the effectiveness of semi-supervised auto-labeling for poultry data, encompassing varied breeds, behaviors, and environmental conditions. The study utilizes broilers and laying hens datasets, with video data collected from the University of Georgia and the University of Arkansas facilities. The video footage includes laying hens (spanning 0 to 85 weeks, captured from four rooms housing 200 hens each) and broilers (up to 45

days old, recorded from 12 pens, each containing 50 broilers). From these videos, images were extracted, and 500 images per breed were manually annotated. These labeled images were then used to train different models, including Grounding DINO (GDINO) and other models (YOLOv9n, YOLOv10n, YOLOv11n, FasterRCNN, and ResNet with GDINO attached). The trained models were then used to auto-label 10,000 images each. The model results were analyzed using ANOVA ($P \leq 0.05$) to assess their effectiveness. The results indicate a significant difference between the results obtained with GDINO and different models with GDINO attached ($P < 0.05$). The semi-supervised methods achieved auto-labeling accuracy exceeding 86%. These results suggest that semi-supervised auto-labeling can significantly improve model precision, providing a practical solution for the poultry industry to optimize AI-driven systems while minimizing manual labor. Further research is needed to refine these methods and improve accuracy beyond current levels.

Keywords: Poultry farming, Behaviors, Auto-label, Machine learning, Artificial Intelligence

Metabolism & Nutrition XI: General Nutrition

T198 Impact of Dried Blood - Rumen Content Mixture as a Replacement for Sesame Seed Cake on Broiler Chick Performance Michaele Y. Berhe*¹ ¹Aksum University, Animal Science, Shire, Tigray, Ethiopia

The effects of feeding dried blood and rumen content mixtures on the performance of broiler chicks was evaluated using 225 unsexed day old broiler chicks which were randomly distributed to five dietary treatment groups in a complete randomized design (CRD). Each treatment was replicated thrice with 15 birds per replicate. The experimental diet were formulated to contain 100% sesame seed cake (SSC) + 0% dried blood rumen content mixture (DBRCM) (T1), 75% sesame seed cake (SSC) + 25% DBRCM (T2), 50% sesame seed cake (SSC) + 50% DBRCM (T3), 25% sesame seed cake (SSC) + 75% DBRCM (T4) and 0% sesame seed cake (SSC) + 100% DBRCM (T5) during the starter phase (1-28 days) and finisher phase (29-56 days) of growth. The data were analyzed using the SPSS software version 20. The result of the feeding trial revealed that the daily DMI ranged from 75.8 to 80.4 g /bird during the entire experimental period. Birds in the T1 had lower ($P < 0.05$) DMI compared with T3 (79.0g), T4 (78.9g) and T5 (80.4g). The mean daily body weight gain of birds ranged from 11.74g to 15.37g and a significance difference ($P < 0.05$) was detected among treatment groups. The feed conversion ratio (FCR) ranged from 3.32 to 4.18 and did not differ ($P \geq 0.05$) among treatment. The edible carcass weight and its dressing percentage ranged from 907.95-1184.95g and 63.96-77.78%, respectively, with significant difference ($P \geq 0.05$) observed among the treatment group. The nonedible offal components was similar ($P \geq 0.05$) among were as the edible offal was not significant difference ($P < 0.05$) among treatments. The highest ($P < 0.05$) and the lowest feed cost ($P < 0.05$) total feed cost was observed for birds in T1 and T5, respectively. Birds in T3 and T4 showed the highest ($P < 0.05$) economic efficiency and relative economic efficiency compared to T1. Based on the result obtained it could be conclude that using DBRCM as a replacement for sesame seed cake at 50% - 75% during the starter and finisher phase in SassoC44 broiler production can reduce the cost of feed required to raise broilers to market age and maximizes the economic efficiency due to local availability and cost effectiveness without affecting the total body weight gain, feed conversion ratio, performance index and carcass characteristics of birds.

Keywords: chicken, dried blood, rumen content, carcass characteristics, performance

T199 Bootstrapping Analysis to Uncover Treatment Mechanisms in Performance Trials: Effects of Heat Stress and Functional Oils on Feed Efficiency and Growth Joan Torrent*¹, Leandro Kuritza² ¹Innovad USA, Durham, North Carolina, United States; ²Solene, Cascavel, Paraná, Brazil

This study employs a bootstrapping technique to deepen the understanding of performance trials, building upon the meta-analysis by Pastorelli et al. (2012) that quantified feed intake and growth responses following various challenges. The bootstrapping method involves resampling raw data to generate simulated samples, which are subsequently used in regression analysis to investigate the effects of different treatments on average daily gain and feed efficiency. The primary objective is not to identify significant differences among treatments, but to elucidate the modes of action of products or challenges. Data from 4 previously published studies, including those investigating heat stress and the efficacy of two functional oils (castor oil and technical cashew nut shell liquid) versus antibiotic additives under different challenges, were analyzed using this bootstrapping technique. The data sets were resampled 100,000 times using a program written in Python, and the various treatments were regressed against either the positive or negative controls via Monte Carlo methods. Results indicated that the effects of treatments were context-dependent, with models exhibiting stronger R^2 values under more severe challenges (e.g., coccidiosis challenges) compared to milder challenges (e.g., heat stress or no challenge). Notably, the analysis revealed that while antibiotics improved feed efficiency by reducing microbial load, functional oils enhanced immune responses, resulting in differences in nutrition planes and feed efficiencies between antibiotics and functional oils. Additionally, the analysis highlighted that the absence of challenges led to weak R^2 values, suggesting minimal treatment effects on feed efficiency. Overall, the bootstrapping technique demonstrated that different treatment yielded different modes of action, emphasizing that functional oils may serve as an effective tool in high-coccidiosis scenarios. This approach offers insights beyond conventional statistical analyses, revealing distinct mechanisms of action for both products and challenges.

Keywords: Bootstrapping, Performance trials, Functional oils

T200 Development and validation of a transcriptomics-based index to assess the intestinal functions of chickens Luis F. Romero*¹, Laura M. Payling¹, Krutik Patel¹, Maria C. Walsh¹ ¹BIOFRACTAL, Loule, Portugal

Assessment of intestinal health in chickens typically involves lesion scoring, histology, microbiota profiling, and selected biomarkers, but lacks a standardized functional evaluation method. A novel approach was developed using tissue transcriptomics to assess intestinal function. This method quantified mRNA via poly-A selection (>20 M reads/sample) targeting >5,000 genes in 200 pathways grouped into seven intestinal functions: digestion and absorption, nutrient metabolism, immune function, oxidative balance, microbial metabolites, and gut-brain axis. A topology-based pathway activation algorithm (BIOFRACTAL, Portugal) analyzed signatures of significantly activated pathways in treatments relative to their respective controls. Significance was evaluated at adjP < 0.05. A standardized Gut System Index (GSI; Gut SAVVY™, BIOFRACTAL, Portugal) was calculated using a similarity score comparing treatment signatures with a benchmark acute enteritis population, where -100% matched enteritis and +100% indicated the opposite profile. The GSI was validated with four datasets: 1) jejunum samples from *E. maxima* challenged Leghorns at d21 versus controls (n=512), 2) ileum samples from *E. maxima* challenged vs. non-challenged Ross 708 broilers at 6 DPI (n=20), 3) jejunum samples from low vs. high FCR farms in a U.S. complex at d27 (n=64), and 4) ileum samples from chickens fed Avilamycin vs. healthy controls at d35 (n=20). In dataset 1, GSI values for challenged chickens were -30%, -57%, -99%, and -73% (adjP < 0.05) at 2, 4, 6, and 8 DPI, respectively, with digestion and absorption showing early impact (-50% at 2 DPI) vs. controls. Dataset 2 showed a GSI of -94% (adjP < 0.05) in broiler ileum at 6 DPI. Dataset 3 indicated a +58% GSI (adjP < 0.05) for low-FCR farms compared to high-FCR farms, correlating with a 7-point FCR difference by d63; digestion and absorption peaked at +81% in low-FCR farms. Dataset 4 revealed a +47% GSI (adjP < 0.05) with Avilamycin, highlighting changes in immune modulation (+55%) and oxidative balance (+55%). This transcriptomics-based method effectively quantified changes in seven key intestinal functions, offering a functional index for both detrimental and beneficial impacts in independent datasets.

Keywords: intestinal health, transcriptomics, gene expression, health index, chicken

T201 New approach on mycotoxin risk management in poultry Ricardo H. Rauber*¹, Alexandro Marchioro², Arthur D. Massei² ¹Vetivova - Strategic Animal Health, Curitiba, PR, Brazil; ²Agromed Patent Co Brazil, Londrina, PR, Brazil

This study aimed to develop and validate a novel approach to managing mycotoxin risk in poultry. Current methods typically rely on average contamination levels in raw materials or feed, guided by set thresholds for action. However, this often leads to inadequate responses that fall short of protecting the animals. To address this, an epidemiological risk management model was designed to provide a clearer indicator of the actual risk to animal groups. The model uses average mycotoxin contamination (µg/kg) and its prevalence (%) over time to calculate the Mycotoxin Risk (MR) as follows: MR = average contamination x prevalence^{1,3}. A patent application for this model is underway in Brazil. To validate it, a 122-week dataset (from week 14 of 2022 to week 30 of 2024) on mycotoxin contamination in a feed mill was used, assessing aflatoxin (AR: 1,196 samples), fumonisin (FR: 1,183 samples), and deoxynivalenol (DR: 1,197 samples) risks. The weekly MR values were then matched with fertility and hatchability data from 13 broiler breeder flocks (ages 25-70 weeks) that consumed feed from the same mill. Linear regression analysis included models

that tested each mycotoxin's MR individually and together, adjusting for flock age and farm location. Results were deemed significant at P≤0.10. Significant effects were observed across models (P=0.00 for both combined and individual MR models). In the combined model, FR and DR reduced fertility (R²=70%), while AR, FR, and DR negatively impacted hatchability (R²=66%). In individual models, AR significantly affected hatchability (R²=66%), and DR impacted both fertility (R²=70%) and hatchability (R²=66%), while no significant effect of FR was found. In conclusion, the model effectively highlights the impact of specific mycotoxins on broiler breeders production parameters, offering a more targeted approach to mycotoxin risk management in the poultry industry.

Keywords: Mycotoxins, Risk Management, Epidemiology, Broiler Breeders

T202 Comparison of Protein Turnover and Lipid Turnover Effects on Body Composition and Broiler Breeder Performance When Dietary Energy is Altered Cole Umberson*¹, Diego A. Martinez¹, Joshua Wilkes¹, Craig Coon¹ ¹University of Arkansas, Fayetteville, Arkansas, United States

A study was conducted to compare body weight gain curve (BWGC), feed allocation, energy level, and energy source on protein and lipid turnover, and breeder performance. A completely randomized design with a factorial arrangement 2 x 5 (2 studies and 5 feeding phases) was used. Lipid turnover was measured via intraperitoneal infusion of deuterium oxide (99%) H₂O achieving 2.5% atom percent excess (APE), 5% deuterium water was supplemented for drinking water to maintain 2.5% APE. Breeders 4/age/treatment (trt) were euthanized by CO₂ asphyxiation, fat pad was sampled 24 hours post-infusion for synthesis and 1 bird was sampled 7 days post infusion for triglyceride degradation. Protein turnover was measured by infusion of isotopic ¹⁵N phenylalanine in the brachial vein, 4 breeders/age/trt were infused and euthanized by CO₂ asphyxiation. Synthesis was determined by ¹⁵N PHE incorporation in mixed skeletal protein, protein degradation was determined by loss of 3-methylhistidine. Body composition was measured using dual energy x-ray absorptiometry (DEXA); body crude protein, ash, and fat content/trt was determined by scanning 10 birds/trt/age. Low BWGC trts peaked higher in %egg production at 29 WOA, while high BWGC trts produced higher %hen-day and total egg production for 20-week production period (p<0.05). Low BWGC trts, at 29 WOA, required higher protein fractional breakdown rates (FBR) to achieve peak egg production but dropped significantly at 35 WOA not supporting post-peak production (p<0.05). High BWGC trts peaked in protein FBR at 35 WOA, as they did not require increased energy to achieve peak egg production, instead utilized protein FBR for post-peak egg production (p<0.05). Lipid degradation acts as an energy buffering system for protein turnover supporting growth and egg production mitigating protein skeletal muscle degradation. Medium BWGC trts utilized lipid degradation efficiently supporting pullet development and breeder egg production resulting in higher production as body conformation was not sacrificed (p<0.05). Body composition at 29 and 35 WOA was observed having significantly higher amounts of fat in high BWGC trts regardless of energy level or source (p<0.05). Protein and lipid turnover can improve breeder performance when utilized efficiently.

Keywords: Broiler Breeders, Lipid Turnover, Protein Turnover, Body composition, Energy

T203 Performance, Processing Yield, and Economics of Broilers Fed Diets Formulated with Productive Energy Diego A. Martinez*¹, Cole Umberson¹, Ehsan Sheikhsamani¹, Sarah

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A study was conducted to assess the effect of formulating broiler diets with productive energy (PE). 90 floor pens were assigned to five feeding phases and treated as independent experiments (before starting the phase, all birds were fed standard diets). Starter, Grower, and Finisher 1, 2 and 3 pens hold 30, 23, 18, 16 and 16 pens, 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) to obtain a linear response. BW, BW gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality (MO) were determined by phase. BWG was fitted to Gompertz curve first 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. 9 additional pens were fed the treatment diets continuously 1-56 days, and 148 birds were processed at 56 d to determine carcass (CAR), leg quarters (LQU), wings (WIN), and breast meat + wing (BPW) yields and woody breast (WB) and

white stripping indexes (WS). Feeding cost was calculated per bird (FCB), per kg BW (FCW), and per kg carcass (FCC). Market value (MKV, \$/bird) was determined based on each cut-up piece weight and its market price, gross profit (GPR, \$/bird) as MKV – FCB, and ROI as GPR/FCB. All data was adjusted to processing weights. 100% PE requirement response (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. Actual continuous feeding of PE diets produced higher BW ($P=0.01$) than those calculated based on independent phases, suggesting an improved protein synthesis rate the higher the diet PE. Increasing PE (T2 to T3) produced linear positive effects on cFCR, EBI, CAR, LQU, WIN, FCC, and ROI ($P\leq 0.05$). T4 reported better cBW, cBWG, cFCR, EBI, CAR, LQU, FCC, MKV ($P<0.001$), GPR ($P=0.002$), and ROI ($P<0.001$) than T1. In conclusion, PE shows potential to improve production efficiency and economics of broiler production compared to AMEn.

Keywords: Productive energy, Formulation, Processing yield, Meat quality, Economics

Metabolism & Nutrition XII: Feed Additives

T204 Saponin-rich plants mixture (Norponin® XO2) supports coccidiosis vaccination program in slow-growing broiler chickens Hoa Bui¹, Weishan Wang², Mohammed el Amine Benarbia*¹ ¹Nor-Feed, R&D, Angers, France; ²Nor-Feed, Beaucauzé, France

Vaccines have gained considerable attention as effective prevention to avian coccidiosis. However, few adverse effects from vaccination seem unavoidable, such as the negative impact on growth, poor protection period during the establishment of specific immunity, and/or the lack of cross-protection from other intestinal infections. Therefore, the supplementation of feed additives combined with a vaccine program could be a holistic approach to gut integrity and animal performance. Our previous study on fast-growing broiler chickens showed an interest in combining NPXO2 with vaccination. This study investigated the association between cocci vaccination and a saponin-based feed additive (Norponin® XO2) as a coccidiosis-preventive program in slow-growing broiler chickens. 900-day-old male broiler chickens were distributed in 12 experimental units, with 75 birds each. Chickens were randomly divided into 2 groups: VAC – birds received cocci vaccine at 1 day old; VAC-NPXO – birds received cocci vaccine plus saponins-based feed additive (NPXO) in feed at 250 g/ton from 1 day old. Growth performances including live weight, feed intake, feed conversion rate (FCR) and mortality were monitored. Statistical analyses were performed by student test using SPSS (25) software. The results showed that birds in the VAC-NPXO2 group had significantly lower FCR ($p<0.05$) compared to birds in the VAC group, with 2.30 compared to 2.34 respectively. The average final body live weight is numerically higher in VAC-NPXO group (1.85 kg) compared to VAC group (1.79 kg). Compared to VAC birds, VAC-NPXO birds had higher average daily gain (29.2 g vs 30.3 g) and better average daily feed intake (68.4 g vs 69.8 g), although no statistical difference was found. No difference in mortality was observed between the 2 groups. This study evidenced that Norponin® XO2 supplementation is a complementary tool to support cocci vaccination programs to maintain animal performance.

Keywords: Saponins-based additive, Avian coccidiosis, Standardisation, Characterization, Saponins-based anticoccidial solution

T205 Effects of Multistrain *Bacillus* based probiotics on growth performance of broilers during a Coccidiosis challenge and the ability to minimize pathogens in-vitro John Schleifer*¹, Shameer Rasheed¹, Charles Hofacre², Jennie Baxter², Mark LaVorgna¹, Mahmoud Masadeh¹, Gary Reznik¹, Kristy Dorton¹ ¹Devenish Nutrition, Fairmont, Minnesota, United States; ²Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States

Four studies were conducted to determine the effects of *Bacillus* based probiotics on growth performance and mortality of Cobb 500 (Study 1, 2, and 3) or Ross 708 (Study 4) broilers challenged with a 20x dose of Coccivac B-52 (Merck Animal Health) on d 14, 4, 4, or 3, respectively. Day-old broiler chicks (as hatched; $n = 900$ to 1,980) were randomly allocated to floor pens (9 to 13 reps/treatment; 50 to 60 birds/pen; 0.73 to 1.08 ft²/bird). Treatments in Study 1, 2, and 3 were: Control (CON; no probiotics), ValuPro-SSA (VP, triple strain probiotic; 1.5×10^6 CFU/g of feed; Devenish Nutrition) and Probiotic A (ProA; 1.6×10^6 CFU/g of feed). Study 4 treatments were CON and VP. Broilers were fed corn/soy based pelleted diets. Body weight, feed intake, feed conversion, and mortality were measured. Data were analyzed as a one-way ANOVA using the Mixed procedure of SAS with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$. Supplementation of VP significantly reduced mortality by 53% in Study 2 ($P = 0.002$) and tended to reduce mortality by 20% in Study 1 ($P = 0.09$), 46% in Study 3 ($P = 0.06$), and 64% in Study 4 ($P = 0.06$). Results were statistically similar between ProA and VP. Two additional studies were conducted at Southern Poultry Research Group to evaluate the ability of VP to minimize *S. Enteritidis* (SE), Avian Pathogenic *E. coli* (APEC), and *C. perfringens* (CP) in vitro using an optical density model. Besides Non-Challenge Control (sterile media only) and Challenge Control (pathogen at 1.0×10^6 CFU/mL and sterile media), solubilized VP (equivalent to 1.5×10^6 CFU/g of feed) was added to wells containing pathogen and cell free supernatant. Optical density (OD) was measured using a microplate reader at 630 nm and 37°C every 2 h through 8 h and at 24 h. Test plates for CP were measured only at 18 h. Results were expressed as the average OD of three wells. A lower OD value indicated a lower concentration of pathogen. Compared to the challenged control, VP was able to minimize the growth of SE, APEC, and CP in vitro

as indicated by lower OD values. Results indicate that VP minimized mortality in broilers and pathogens in vitro.

Keywords: Probiotics, broiler, pathogen reduction, mortality

T206 Effect of dietary probiotic superdose during starter phase on growth performance, fiber digestibility, and bone mineralization in broiler chickens Keonjah P. Martin*¹ ¹*Florida A&M University, Agricultural Science- Animal Science, Tallahassee, Florida, United States*

Probiotics are widely recognized for improving gut health and immunity in poultry. However, most studies have investigated probiotic supplementation through feed and primarily focused on nutrient utilization and gut health. The current study was designed to explore an alternative approach by administering probiotics via water during the starter phase and examining the impact of superdosing on bone health in addition to growth performance and nutrient digestibility, providing insights into their potential benefits for bone mineralization and overall poultry management. Three treatments were evaluated: a basal diet with no probiotics (BD), BD + 1x probiotics (BD1x), and BD + 3x probiotic (BD3x). A total of 180 day-old male broiler chickens (Ross 708) were assigned to the dietary treatments, each with 6 replicate cages, and 10 birds per replicate in a randomized complete block design. Birds were fed a standard corn-soybean meal-based diet from d 1 – 21. The probiotic used was a commercial brand added to drinking water daily, only from d 1 – 21. Six birds per cage were selected for necropsy on day 21, the remaining 4 birds were transitioned into schoolers and raised on pasture until 45. The results indicated no difference in d 1 – 21 BW, BW gain across treatments. There was a tendency for early treatment effect on FCR from d 1 -7 ($P = 0.084$). However, birds on BD3x had 21.3% increase in BW gain on d 21 – 45 compared to BD. Apparent digestibility of dry matter, neutral/acid detergent fiber or digesta viscosity were unaffected by probiotic supplementation from d 1 – 21. Tibia ash (%) and breaking strength (kgf) were unaffected by treatment at d 21, however both metrics were improved in BD3x relative to BD (12 – 27% improvement, $P < 0.05$) and BD3x relative to BD1x (5 – 18% improvement, $P < 0.05$) at d 45 for both left and right tibias. These results suggest that water-based probiotic superdosing may enhance growth and bone quality in pasture-reared broilers, particularly in later growth stages, though fiber digestibility effects were minimal. Follow-up analyses are being conducted on bone morphology at day 45 using scanning electron microscopy to visualize microstructural changes to further elucidate the effects of probiotic supplementation on bone quality.

Keywords: bone, broiler, pasture, probiotic, superdose

T207 The effect of protease and non-starch polysaccharide (NSP) degrading enzyme supplementation on growth performance, nutrient utilization, and gut morphology of broiler chickens fed corn-soybean meal-based diets Waqas Ahmed*¹, Sara Mubeen¹, Akhtar Nawaz¹, Uzair Hassan¹, Zubair Ali², Mehran Asjad² ¹*University of Agriculture Faisalabad Punjab Pakistan, Institute of Animal and Dairy Sciences, Rawalpindi, Pakistan;* ²*University of Agriculture Faisalabad, Sub-Campus Toba Tek Singh Punjab Pakistan, Toba Tek Singh, Pakistan*

The present study was conducted to check the impact of protease and non-starch polysaccharide (NSP) degrading enzyme supplementation on growth performance, nutrient utilization, and gut morphology of broiler chickens fed corn-soybean meal-based diets. To execute the trial, 320-day-old broiler birds were randomly classified into 4 dietary treatments having eight replicates and each replicate contained 10 birds per replicate. The whole trial was conducted for 35 days and treatments (N1 = 80

mg/kg NSP + protease; N2 = 120 mg/kg NSP + protease; N3 = 80 mg/kg NSP; N4 = 120 mg/kg NSP) were offered to the birds. All the findings were analyzed statistically using the analysis of variance (ANOVA) technique under a completely randomized design and means were compared using Tukey's test at a 5% significance level. The data on feed intake, body weight gain, and feed conversion ratio were recorded weekly. Based on the findings, birds fed diets containing both NSP and protease showed significantly lower ($P < 0.05$) feed intake. Birds fed N2 and N3 diets showed significantly higher ($P < 0.05$) weight gain and improved ($P < 0.05$) feed conversion ratio. Birds kept on dietary treatment N3 showed better ($P < 0.05$) results for the digestibility of crude fiber. There was no significant ($P > 0.05$) difference seen in the villus height and crypt depth among all the dietary treatments. Therefore, it is concluded that the supplementation of a broiler diet with NSP and protease enzyme can improve bird's performance and nutrient digestibility.

Keywords: Non-starch polysaccharide, Protease, Growth performance, Nutrient digestibility, Gut morphology

T208 Broiler chickens' response when fed diets formulated with low soybean meal or crystalline amino acids without or with supplementation with multi-component protease Veronica Cheng*¹, Felix Muchiri¹, Anhao Wang², Rob Patterson², Elijah Kiarie¹ ¹*University of Guelph, Animal Biosciences, Guelph, Ontario, Canada;* ²*CBS Bio Platforms Inc., Alberta, Ontario, Canada*

Genetic advances in broiler chickens have created superior growth for lean muscle mass, which altered the rates of protein turnover and increased dietary protein and amino acids. However, over 20% of the crude protein in feedstuffs is undigested, consequently challenging the health of poultry and humans, the environment, and economics of production. This study investigated the effects of supplementation of multi-component protease (MCP) for diets reduced on soybean meal or crystalline amino acids on growth performance, liver and breast weights, and manure attributes in broiler chickens reared on floor pens. A total of 1,350 at-hatch Ross 708 broiler chicks were distributed equally with pen weights balanced in 45 floor pens (30 birds/pen) and randomly assigned to 5 treatments until end of finisher phase. The treatment diets were provided in 4 phases (starter, grower, finisher 1 [F1], and finisher 2 [F2]), and were: positive control (PC, nutrient sufficient, corn-wheat-SBM based), negative control 1 (NC1, isoenergetic with PC with ~5% less SID AA from SBM), NC1+MCP (NC1+250 ppm MCP), NC2 (isoenergetic to PC, with ~5% less SID AA from synthetic AA), and NC2+MCP (NC2+250 ppm MCP). The broilers had ad libitum access to feed and water. Mortality date and individual body weight were recorded for mortality gain. During each phase change, feed intake and pen body weights were recorded. At the end of F1 and F2, two birds on each occasion were sacrificed and dissected for breast yield and liver weight. Litter was collected and analyzed at the end of the trial. The data were analyzed using PROC GLIMMIX procedure of SAS with diet as a fixed effect in the model, and the cage was the experimental unit. Both NC+MCP treatment groups produced performances most similar with the PC group in terms of BW, BWG, FI, and FCR compared with the other NC groups throughout the trial ($P < 0.01$). No significant differences were observed in liver and breast weights among all treatments. Litter parameters were similar amongst the treatments, however, Ammonium-Nitrogen amount (mg/kg wet) in both NC+MCP groups were lower than corresponding NC treatment ($P < 0.05$). In conclusion, supplementing diets with MCPs while reducing protein content enhanced protein digestion and utilization in the chicks.

Keywords: Protease, Broiler, Digestibility, Amino Acid

Environment & Management II

T209 The Effect of a two-way grading on some KPI, physical characteristics, and sertolli cell counts on broiler breeder males Jose J. Bruzual*¹ ¹Aviagen, Dacula, Georgia, United States

Hatchability and male livability have been experiencing a continuous decline in the US in the last 5 years. One issue appears to be poor uniformity of males coming into production leading to poor and uneven skeletal development and poor testicular development. This experiment's focus was to improve the uniformity of the birds by sorting them by Body Weight (BW) categories and feeding them according to their BW category. 5,368 Ross males were subjected to a 2-way grading (sorting) system and divided into groups with an equal number of birds of Small-Medium and Medium-Large BW. The cut-off point between groups was the average BW for the whole flock. Birds were fed to reach standard BW during the grow-out period with the goal of both groups having similar weights at the time of transfer to the breeder house. Grading was done at 4 and 11 weeks of age (WOA). Three groups of 50 birds each of different BW (~ 1lb./small, ~ 1.50 lb./average, and ~2 lbs./heavy; individually wing-banded at 4 WOA) were followed to understand the growth profile at various ages. Birds in the small and average groups were grown on the small-medium bird side and those in the heavy group were grown on the medium-heavy bird side. CV% was calculated at 4 and 11 WOA. The CV% of the whole population decreased from 17.91% to 9.90% in the Small-Medium group and to 11.34% in the Medium-Large group at 4 WOA. The CV% of the entire population decreased from 11.34% to 7.33% in the Small-Medium group and 7.88% in the Medium-Large group at 11 WOA. BW and Shank Length (SL) were recorded at 4, 11, 18, and 27 WOA. A linear regression ($R^2 = 0.281$ to 0.910) between BW and SL was created for each WOA. A Student's t-test ($p < 0.05$) compared weight groups on shack length and body weight at each WOA. In addition, testicular weight and microscopic evaluation of the testicles for Sertoli cell counts from 6-7 birds per group were done at 27 WOA in the stud house before the males were used as spike males. In summary, a 2-way grading system is a tool that helped improve male uniformity with a positive effect on livability in the grow-out period and improved the male quality of the birds coming into production. A follow-up in the breeder house is planned to see the effect on livability, numbers of spikes, and fertility.

Keywords: Grading, Uniformity, Males, Testis, Sertoli

T210 Bed bugs – Re-emerging threat to chickens and workers in the poultry industry Rocio Crespo*¹, Jenny A. Nicholds², Wes Watson³, Ronald Baynes¹, Olivia Petritz⁴, Coby Schal³ ¹NC State University, Population Health and Pathobiology, Raleigh, North Carolina, United States; ²Univeristy of Georgia, PDRC, Athens, Georgia, United States; ³NC State University, Entomology and Plant Pathology, Raleigh, North Carolina, United States; ⁴NC State University, Clinical Sciences, Raleigh, North Carolina, United States

Bed bugs (*Cimex lectularius*) are obligate blood-feeding insects that can affect poultry and people. The prevalence of bed bug infestations has risen sharply in the last two decades with infestations reported in all 50 U.S. states. Bed bugs are one of the most challenging parasitic infestations to treat due to insecticide resistance and their rapid reproduction. The goal of this study was to understand the extent of bed bug infestations in poultry houses, their severity, and impact on health, production and workers. In 2023, we released an anonymous survey through the University of Georgia using Qualtrics^{XM}, a web-based survey tool. The survey links were shared with leaders in the poultry industry and disseminated from these individuals to veterinarians and

producers. Each survey had 3 sections – General information, Pests and pest management and bed bug-specific questions. A total of 52 veterinarians and 46 growers responded to the survey. JMP Pro version 17 was used to analyze the data. Chi-square and T-test were used to compare the veterinarian and grower's categorical and numerical responses respectively. Significance was set at $P < 0.05$. Broiler breeder (39.2%) and table-egg layer (31.1%) flocks were significantly more affected. The flock size did not significantly affect the infestation with bed bugs. Bed bugs were significantly less common than other pests, but the frequency and severity of bed bug infestations in poultry farms increased in the past 5 years and were difficult to control. On the other hand, growers ranked the cost of control of this pest significantly higher than veterinarians (25.6% vs 15.8% respectively). Both growers and veterinarians ranked bed bugs as having strong adverse effects on bird welfare and worker morale. They also recognized the importance of bed bug dispersal between farms and workers' homes, but 42.7% of responders indicated that workers' homes are not treated for bed bugs (55.1% don't know). In conclusion, bed bugs are a re-emerging threat for poultry and people. Bed bugs are more commonly found in poultry houses with long-lived birds (breeders and layers). Infestations are difficult to control. A holistic approach to control these bugs is needed as they can move between the poultry houses and the workers' homes.

Keywords: Bed bug, grower, insect, pest, poultry

T211 Effect of cooling system on broiler housing spatial uniformity Jessica Drewry*², Jonathan Moon³, Matthew Rowland¹, Joseph Purswell¹, Beau Ivy² ¹Agricultural Research Service, United States Department of Agriculture, Mississippi State, Mississippi, United States; ²Mississippi State University, Agricultural and Biological Engineering, Mississippi State, Mississippi, United States; ³Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States

On farm water footprints are gaining scrutiny under a changing climate as cooling water needs can make up a substantial portion of water usage during hot weather. Broilers are predominately cooled via tunnel ventilation in combination with evaporative cooling (EC) systems that reduce air temperature through latent heat exchange with the evaporating water. Sprinklers have been proposed as a supplement to EC to lower the system's water usage. However, the spatial variability of environmental parameters under this management strategy have not been studied. Non-uniform conditions could impact bird uniformity and production under this management strategy. The objective of the study was assessing the spatial uniformity of environmental parameters under EC and evaporative cooling with supplement sprinklers (ECSS). Two commercial houses (122 x 13.4 m) were outfitted with supplemental sprinkler cooling and assessed over 4 flocks in 2023 and 2024. Treatment and control houses were alternated for each flock. Environmental parameters were measured at 18 locations within each house over the course of each flock that required cooling. A repeated measures ANOVA was used to determine the effect of cooling method, location, and sampling date on litter moisture and air temperature and relative humidity. Significance was assessed at $\alpha = 0.05$ using Tukey's Method. When looking at the last four days of the flock when heat stress is likely to be most pronounced, the ECSS treatment led to an increase in air temperature of 1.2 °C ($p = 0.005$) however, the relative humidity was 8 percentage points lower during operation ($p = 0.003$). We failed to detect a significant difference in litter moisture between treatments ($p = 0.95$) during any week. Under both treatments, temperatures were significantly higher in the third of the house near the fans ($p < 0.001$) and no spatial relationship

was found for relative humidity ($p=0.32$). While the data does not suggest the ECSS treatment provides a more uniform environment, it lowers house relative humidity which may impact bird welfare. More data is needed to fully characterize the impacts of supplemental sprinkler cooling on production.

Keywords: broiler, supplemental cooling, heat stress

T212 Consumer Egg Survey for Carotenoid Accumulation and Yolk Fan Score by Production Scheme and Label Claims Kaeden Smith², Sara Cloft^{*1}, Tyler Lawson²³, Torbert Rocheford²³, Darrin Karcher¹, Evan Rocheford³ ¹*Purdue University, Department of Animal Sciences, West Lafayette, Indiana, United States;* ²*Purdue University, Department of Agronomy, West Lafayette, Indiana, United States;* ³*Nutram maize, West Lafayette, Indiana, United States*

Egg brands are establishing standardized yolk color scores as part of their independent brand identity and marketing strategy to customers. Customers frequently associate darker orange yolks with healthier or tastier eggs. We surveyed yolk color of major brands for 1 year. Shell eggs from 17 different brand products were purchased from retail stores in Indiana monthly, September 2023 to August 2024, when available. All egg products were assigned by organic status and production system based on carton labels. Twelve eggs from each were yolk color scored using the Digital YolkFan™ Pro, scores can range from 1 to 16 as a light yellow to darker orange color. Additionally, carotenoid content from 14 products were evaluated using high performance liquid chromatography analysis. Statistical analysis consisted of ANOVA with Tukey mean separation, when $P \leq 0.05$ using JMP Pro 16. Yolk carotenoid contents were influenced by organic status and production system ($P < 0.0249$) but not the interaction of them ($P > 0.16$). Organic and cage-free labeled eggs had lower carotenoid content than non-organic and pasture-raised or free-range labeled eggs. Yolk color scores were influenced by organic status, production system and the interaction of them ($P \leq 0.0045$). Similarly, cage-free eggs had paler, lower scored yolk color than pasture-raised and free-ranged labeled eggs and organic labeled eggs were lower scored than non-organic labeled eggs of each production system. Each egg product produced significantly different yolk scores month-to-month ($P \leq 0.0024$). However, in each month surveyed cage-free labeled eggs had lower scores than other production systems ($P < 0.0001$) and organic labeled eggs had lower scores than non-organic labeled eggs for all but months 2 and 8 ($P \leq 0.0007$; Month 2 $P = 0.1601$; Month 8 $P = 0.2839$). Past research has shown that yolk color is influenced by carotenoid intake and that likely explains the observed differences in this survey. Notably, pasture-raised and free-range systems allow hens outdoor access which may expose them to additional dietary carotenoids. Also, organic standards prohibit the use of highly concentrated pigment supplements from chemical extraction, potentially explaining why organic yolk color scores were lower than non-organic eggs.

Keywords: Egg, Yolk Color, Carotenoids, Pigment, Survey

T213 Quantification of *S. Enteritidis* and *S. Typhimurium* in broiler chickens inoculated with a cocktail of *Salmonella* serovars using novel duplex qPCR assays Xiang Li^{*12}, Adelumola Oladeinde¹², Michael Rothrock¹², Sohyun Cho¹², Samuel Aggrey³, Jodie Lawrence¹² ¹*USDA-ARS, Athens, Georgia, United States;* ²*USNPRC, EPPSRU, Athens, Georgia, United States;* ³*University of Georgia, Poultry Science, Athens, Georgia, United States*

Precisely quantifying pathogen loads is essential for assessing environmental contamination levels and informing public health

interventions. While singleplex quantitative polymerase chain reaction (qPCR) has been widely used due to its high sensitivity and specificity, it cannot identify PCR inhibitors in environmental samples, potentially leading to false-negative results or underestimation of pathogen concentrations. This study aimed to develop and validate a more robust method for quantifying *Salmonella* serovars of concern in environmental samples. We designed a duplex qPCR incorporating an internal amplification control (IAC) to monitor amplification interference, crucial for environmental sample testing. To account for experiment-to-experiment variation, replicate measurement variability, and uncertainties in related steps, we employed a Bayesian Markov chain Monte Carlo (MCMC) approach to generate master calibration curves for each duplex assay, using at least six independent instrumental runs. This method was applied to develop assays for *S. Enteritidis* and *S. Typhimurium*. Calibration curves for *S. Enteritidis* and *S. Typhimurium* assays exhibited R squared values of 0.99, with amplification efficiencies of 1.04 and 0.93, respectively. Statistical analysis using Bayesian confidence intervals (95% BCI) revealed lower limits of quantifications (LLOQs) of 36.85 and 36.02 Cq values for *S. Enteritidis* and *S. Typhimurium*, respectively. To validate the assays under real-world conditions, we tested cecal samples from experimental broiler chickens raised on fresh poultry litter for 7 days post-inoculation. Our qPCR found 6.83–7.55 and 6.90–7.68 Log₁₀ gene copies/g of *S. Enteritidis* and *S. Typhimurium* in positive samples, respectively. In conclusion, our assays demonstrate decent LLOQs and amplification efficiencies, which are crucial parameters for accurate quantification methods. The successful quantification of *S. Enteritidis* and *S. Typhimurium* using Bayesian MCMC models supports the potential application of this approach in environmental settings. This method offers a more reliable and accurate tool for monitoring *Salmonella* contamination in complex environmental samples, potentially improving public health surveillance and risk assessment strategies.

Keywords: Salmonella quantification, duplex qPCR assay, inhibitor, environmental samples, Bayesian MCMC

T214 Investigating the spatiotemporal distribution of *Escherichia coli* and aerobic bacteria in commercial hatching eggs Maryam Mohammadi-Aragh^{*1}, Jessica Drewry¹, Katie E. Elliott² ¹*Mississippi State University, Mississippi State, Mississippi, United States;* ²*United States Department of Agriculture, Mississippi State, Mississippi, United States*

Healthy chicks are essential for poultry to reach their genetic potential. Therefore, understanding the hatchery environment and identifying pathogen risks is critical. Setters may serve as sources of pathogenic *Escherichia coli* contamination; however, *E. coli* spatiotemporal distribution within setters is not well understood. The study aimed to investigate *E. coli* spatiotemporal distribution within a setter environment. A pilot-scale incubation study using commercial hatching eggs was conducted across three trials, arranged in a randomized complete block design. In each trial, broiler hatching eggs were incubated in two incubators (900 eggs/ incubator). Within each incubator, eggs were either non-disinfected, disinfected using 2% hydrogen peroxide to use as negative controls ($n = 120$), or inoculated ($n = 180$) with 0.1 mL of an overnight *E. coli* culture. Eggs were randomly distributed across the 6 levels of each incubator with eggs from each treatment present on levels 1, 3, and 6. Samples were taken on days 0, 6, 12, and 18. Aerobic bacteria were cultured from control eggs sampled in trials 2 and 3 using Aerobic Plate Count Petrifilms. Per rack, 6 inoculated, 4 control, and 4 disinfected fertile eggs were sampled, and eggshell surface bacteria were suspended in 10 mL phosphate-

buffered saline. Rinsates were plated on Chromocult agar and Petrifilms, and *E. coli* and aerobic bacteria colonies were recorded after 24 hours of incubation. The mean *E. coli* count across 3 trials on day 0 was 6.81 log₁₀CFU/g; however, no *E. coli* growth was observed by day 12. Aerobic bacteria significantly decreased over time ($P < .0001$), but the location within the incubator had no statistically significant effect at $P \leq .05$. Day 0 aerobic bacteria were enumerated at 5.40 log₁₀CFU/g, and mean aerobic counts were 4.84, 4.03, and 3.69 log₁₀CFU/g for d 6, d 12, and d 18,

respectively, for all trials. Aerobic bacteria had a mean reduction of 1.72 log₁₀CFU/g over 18 days. Initial high temperatures, combined with limited moisture and nutrients on eggshells, may contribute to rapid *E. coli* reduction. Aerobic bacteria persisted on the eggshell but decreased over 18 days within the incubation environment.

Keywords: hatchery, setter, *E. coli*, chick health

Food Safety II

T215 Antimicrobial Resistance profiling of *Salmonella* recovered from environments of No-Antibiotics-Ever (NAE) and Conventional broiler complexes Yagya Adhikari*¹, Dianna Bourassa¹, Sabin Poudel¹, Matthew Bailey¹, Richard Buhr³, Kenneth Macklin² ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²Mississippi State University, Department of Poultry Science, Starkville, Mississippi, United States; ³USDA ARS Poultry Microbiological Safety and Processing Research Unit, Athens, Georgia, United States

Antimicrobial resistance is a serious global public health threat that could endanger the efficacy of antibiotics used to treat diseases in humans, animals, and plants. The objective of this study was to characterize the antimicrobial resistance (AMR) pattern of *Salmonella* isolated from environmental samples of different stages of both No-Antibiotics-Ever (NAE) and conventional integrated broiler complexes. ResFinder was used to identify AMR genes from assembled genomes while 14 different antibiotics were phenotypically tested using the broth microdilution method. The odds ratio and 95% confidence limits were determined for different complexes and stages of broiler production ($\alpha=0.05$). The AMR genes identified were *aac(6)-Iaa*, *aph(6)-Id*, *aph(3'')-Ib*, *blaCARB-2*, *sull1*, *tet(A)*, *tet(B)*, *tet(G)*, *floR*, *fosA7*, *gyrA*, and *parC*. The phenotypic results showed that 58% (49/85) of isolates were resistant to at least one antibiotic class, 24% (20/85) of isolates were resistant to at least two or more classes of antibiotics and 6% (5/85) of isolates were multi-drug resistant. Among 14 different antibiotics tested, 41%, 39%, 6%, 5%, and 2% of total isolates (n=85) showed resistance to sulfisoxazole, tetracycline, nalidixic acid, ampicillin, and ciprofloxacin respectively. Interestingly, 80% (32/40) of Kentucky isolates showed phenotypic resistance to either tetracycline or sulfisoxazole or both. The odds of *Salmonella* strains detected in the environments of NAE broiler complex having resistance to at least two or more tested antibiotic classes were 7 times (1.37 – 43.45; 95% CL) as likely as that for strains detected in conventional broiler complex ($p=0.0233$). Notably, all 4 Typhimurium and one Rough_O:r:1,5 isolate recovered from the NAE broiler complex environment were multi-drug resistant. In addition, there was 100% consistency in genotypic and phenotypic results for tetracycline, ampicillin, ciprofloxacin, and nalidixic acid while there were high discrepancies for sulfisoxazole and chloramphenicol resistance results. Overall, drug-resistant *Salmonella* were present in the environments of production houses and facilities, which can potentially contaminate the raw chicken product and can lead to complicated antimicrobial therapy during foodborne infections in consumers.

Keywords: *Salmonella*, antimicrobial resistance, no-antibiotic-ever, broiler complex, conventional

T216 Effect of a monoglyceride-based feed ingredient (Biolution™) on *Salmonella* vaccinated broiler chickens Kevin Watkins¹, Rigo Fernando Soler², Stacie Appleton*³, Eric Heskett⁴, Ramesh Selvaraj⁵, Marcos Sanchez-Plata² ¹FoodFirst LLC, Indianapolis, Indiana, United

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Salmonella vaccination and in-feed glycerides are used for preharvest control of *Salmonella*. Since glycerides have antimicrobial and immune modulatory activity, can their use affect *Salmonella* vaccination? A floor-pen trial using a randomized block design with 4 blocks of 2 pens/treatment (total of 8 pens/treatment) was conducted to evaluate the effect of a monoglyceride-based feed ingredient (MG) on *Salmonella* vaccination. Sixty straight-run *Salmonella*-vaccinated (AviPro Megan Vac1, Elanco) day-old chicks/pen were fed either Control feed, 1 lb/ton MG (Alura, Inc.) in all but starter feed, 1 lb/ton MG in all feeds, or 2 lb/ton MG in all feeds for 57 days. *Salmonella* prevalence, enumeration, and type, bile vaccine-specific IgA, and growth performance were measured throughout the trial. Fisher Exact Test was used to evaluate *Salmonella* prevalence. Bacterial counts were log transformed and analyzed using Kruskal-Wallis Test with Wilcoxon mean separation. IgA and growth performance results were analyzed using ANOVA with Tukey mean separation. *Salmonella* prevalence generally decreased over time, but no treatment differences were seen. There were no treatment differences in *Salmonella* counts of boot swab, liver+spleen, or carcass rinse samples. Birds fed 1 lb/ton MG in all but starter feed had higher ($P<0.05$) ceca *Salmonella* counts on day 21 and birds fed 1 lb/ton MG in all feeds had higher ($P<0.05$) ceca counts on day 56, compared with all other treatments. Only 2 carcass rinse samples had counts greater than 10 CFU/ml and both were in the Control group. At 21 and 56 days, *Salmonella* from carcass rinses were presumptively 100% and 50% vaccine strain, respectively, with no treatment differences. While IgA increased ($P<0.05$) from 21 to 56 days, there were no treatment differences. However, on day 21, birds receiving MG collectively had numerically higher ($P=0.08$) IgA compared with Control. At 35 days, birds fed 2 lb/ton MG had increased ($P<0.05$) body weight compared with Control and 1 lb/ton MG in all but starter feed and had numerically better ($P=0.07$) FCR compared with Control. There were no treatment differences in performance on day 57. MG had no effect on vaccine efficacy but may have resulted in earlier immune activation and improved 35-day growth performance.

Keywords: Monoglyceride, *Salmonella*, IgA, Broiler chicken, Food safety

T217 Impact of Organic acid supplementation on Cecal Microbiome of Broiler Chicks inoculated with *Salmonella* Sabin Poudel*¹, Fanny Abigail Contreras Zelaya¹, Eva G. Guzman¹, Wilmer J. Pacheco¹, Ruediger Hauck¹, Dianna Bourassa¹, Kenneth Macklin² ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²Mississippi State University, Department of Poultry Science, Starkville, Mississippi, United States

Non-typhoidal *Salmonella* is a leading cause of foodborne illnesses and reducing *Salmonella* in poultry is needed to reduce human infection. The objective of this study was to evaluate the effect of organic acid (OA) [Lactic/Acetic/Propionic acid (LAPA) and Citric Acid (CA)] on cecal microbiome of broilers challenged with *Salmonella* Enteritidis. A total of 1872-day-old Ross 708 male broilers were randomly distributed to three treatments Control (C), LAPA, and CA; 24 replicates/treatment and 25 birds/pen. All birds were challenged with 10^7 CFU of *Salmonella* at d 7 and supplemented with OA continuously via drinking water. Cecal samples were collected from 12 birds per treatment on d 41. Thus, collected samples were used for 16s rRNA gene VI-V9 sequencing using Minlon (Oxford Nanopore). Reads were base-called and demultiplexed using MinKnow and analyzed using EPI2ME and R-packages. Interestingly, even though birds were challenged we were unable to detect the *Salmonella* in the cecal content via sequencing and qPCR using *invA* gene. The alpha diversity indexes indicate that there was no difference ($p > 0.05$) in richness and evenness in cecal microbiome between treatments. Additionally, there was no significant difference between treatments for beta-diversity Bray-Curtis (adonis $p = 0.273$). While comparing the bacterial species in ceca between the treatments *Lactobacillus crispatus* and *Streptococcus alactolyticus* were two most abundant bacterial species ranging between 8-14% mean abundance. While comparing the number of bacterial species between treatments, CA had the highest unique species number (110) compared to C (82) and LAPA (55). However, while comparing the differentially abundant species there was no concordance between the different abundance methods as ANCOM-BC showed a significant difference in *Limosilactobacillus ruddii* between C vs CA, whereas ALDEx2 showed no significant difference between the treatments. Since we did not detect the *Salmonella* in control and OA-treated birds we were unable to conclude the effectiveness of OA on *Salmonella* control. However, this result indicates that OA were unable to significantly modulate the cecal microbiome of the birds as there was no significant difference in the abundance of bacteria between the treatments.

Keywords: cecal microbiome, *Salmonella*, organic acid, broiler chicks

T218 Vaccination with the USDA BBS 866 *Salmonella* DIVA vaccine reduces intestinal colonization and systemic dissemination of DSC *Salmonella enterica* serovar Enteritidis in chickens Shawn Bearson^{*1}, Bradley Bearson², Samuel Whelan¹, Maya Encinosa¹, Durga Neupane¹² ¹USDA-ARS-NADC-Food Safety & Enteric Pathogens Research Unit, Ames, Iowa, United States; ²USDA-ARS-National Laboratory for Agriculture and the Environment, Ames, Iowa, United States

Salmonella enterica serovar Enteritidis (Enteritidis) is one of the top *Salmonella* serotypes associated with human foodborne illness globally and is frequently linked to poultry. A strain of Enteritidis with decreased susceptibility to ciprofloxacin (DSC), a critical antibiotic prescribed for complicated human infections, has emerged in chickens. The purpose of the current study was to evaluate vaccination efficacy of the live, attenuated USDA BBS 866 DIVA vaccine for reduction of intestinal colonization and systemic dissemination of DSC Enteritidis in broiler chickens. One-day-old, commercial broiler chicks (n=44) housed in an ABLS2 isolation room at NADC were vaccinated via aerosol spray and booster vaccinated at two weeks of age via water administration ($\sim 3 \times 10^8$ colony forming units (CFU)/chick); a second room of 44 chicks was similarly administered PBS as the mock-vaccinated control group. At 5 weeks of age, all chickens were challenged via oral gavage with 1×10^9 CFU of DSC

Enteritidis. At 7- and 14-days post-inoculation (dpi), chickens were randomly selected for euthanasia to collect cecum, spleen, and bone marrow tissues. Statistical analysis of qualitative and quantitative bacteriology for *Salmonella* was determined using Fisher's exact test (prevalence) and an unpaired t test (colonization load), respectively. Prevalence of DSC Enteritidis (i.e. +/-) was significantly decreased in vaccinated chickens for the cecum (14 dpi), spleen (7 and 14 dpi), and bone marrow (7 dpi) compared to mock-vaccinated chickens. For bone marrow, 5 of 13 (7 dpi) and 3 of 13 (14 dpi) mock-vaccinated chickens tested *Salmonella*-positive; bone marrow from all vaccinated chickens tested *Salmonella*-negative. Quantitative bacteriology revealed significant reduction in colonization levels of DSC Enteritidis in the vaccinated chickens at 7 and 14 dpi for the cecum (1.6-2.7 logs) and spleen (1.6-2.4 logs) compared to mock-vaccinated birds. Vaccination with the USDA BBS 866 *Salmonella* vaccine significantly reduced intestinal colonization and splenic dissemination as well as prevented dissemination to the bone marrow by DSC Enteritidis in broiler chickens, thereby providing a prospective intervention to reduce food product contamination.

Keywords: *Salmonella*, food safety, Enteritidis, vaccine, decreased susceptibility to ciprofloxacin

T219 Mitigation of necrotic enteritis in broiler chickens through the actions of black cumin seeds and bacteriophage Vishal Manjunatha^{*2}, Justice-Alucho C.H.³, Brett Lumpkins¹⁴, Yang Gang⁵, Annel K. Greene¹, Jingxue Wang⁵, Xiuping Jiang² ¹Clemson University, Department of Animal and Veterinary Sciences, Clemson, South Carolina, United States; ²Clemson University, Department of Food, Nutrition and Packaging Sciences, Central, South Carolina, United States; ³Clemson University, Department of Biological Sciences, Clemson, South Carolina, United States; ⁴Southern Poultry Feed & Research, Inc, Athens, Georgia, United States; ⁵Ocean University of China, Department of Food Science and Engineering, Qingdao, China

Coccidiosis and necrotic enteritis (NE) are serious poultry diseases that contribute to decreased growth performance, high mortality rates, and substantial economic losses. *Clostridium perfringens* (Cp) is the causative agent of NE. Increasing antibiotic resistance and the growing demand for antibiotic-free poultry products underscore a need for natural alternatives. This study assessed the effectiveness of crushed *Nigella sativa* (black cumin) seeds (CBCS) and Cp-specific bacteriophage, individually or in combination as potential antibiotic alternatives to control coccidiosis and mitigate NE in broilers. *Clostridium* phage CU1, a member of the *Myoviridae* family, consisting of 51,700 bp (34.3% GC content) free from virulence and antibiotic resistance genes, was isolated from environmental samples. An *in vitro* study was conducted to identify a CBCS product with strong anti-Cp activity, followed by an *in vivo* study involving 384 Cobb 500 male broiler chicks in a randomized block design. The animal trial included treatments with 5% CBCS in feed, 1:15 bacteriophage in drinking water (administered on days 19-26), and their combination for birds challenged with coccidia and Cp strain Cp#4. Broiler performance, disease outcomes, and Cp populations were measured. CBCS, bacteriophage, and the combination treatment had no detrimental effect on broiler live performance. The NE scores for CBCS treated and combination groups were significantly ($p < 0.05$) lower than the positive control and similar to the bacitracin methylene disalicylate (50 g/ton) antibiotic control. All treatments reduced NE mortality compared to 12.5% rate observed in the positive control with the combination of CBCS and phage treatment exhibiting zero mortality rate ($p < 0.05$) similar to that of the negative control (0%). Cp spores and vegetative cells

decreased for all treatments with plating results corroborating with qPCR results indicating that the treatments under investigation reduced the Cp vegetative cell counts with the combination treatment having the best results. In conclusion, CBCS and phage effectively reduced *C. perfringens* infection and mortality in chickens without impacting broiler growth performance; the combination treatment is a promising alternative to traditional antibiotic use.

Keywords: Coccidiosis, Necrotic enteritis, Clostridium perfringens, Nigella sativa, Bacteriophage

T220 Molecular characteristics of *Salmonella* spp. from retail broiler meat Karla V. Casco*¹, Maria V. Baca¹, Sabin Poudel², Richard Buhr³, Dianna Bourassa¹ ¹Auburn University, Auburn, Alabama, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ³USDA-ARS, US National Poultry Research Center, Athens, Georgia, United States

Foodborne *Salmonella* infection poses a public health threat, with significant economic losses in the United States. Non-typhoidal *Salmonella* is a leading foodborne illness-causing bacteria, with approx. 23% of salmonellosis cases are attributed to the consumption and handling of contaminated poultry meat. This study aimed to determine the molecular characteristics of *Salmonella* isolated from the retail meat samples from retail markets in Alabama. A total of 126 samples were collected from grocery stores. For *Salmonella* spp. isolation, samples were screened with the bioMérieux Gene-UpOsystem, then *Salmonella* was isolated from positive samples, resulting in 7 isolates from different samples. Bacterial isolates forming black colonies on XLT4 agar were confirmed to be *Salmonella* via PCR using the *invA* gene. Libraries were prepared using a Rapid Barcoding Kit (SQK-RBK114.24) and sequenced in MinIon Flow Cell (Oxford Nanopore). Raw reads were processed using MinKnow for base calling and demultiplexing, and EPI2ME wf-bacterial-genome was used for further processing. The Busco Completeness Score for 7 isolates ranged between 96.0 to 99.2%. The median read quality was approximately 21 for all isolates. Based on *serentica_achtman_2* MLST results, all 6 *Salmonella* Infantis fall under ST-32, whereas Thompson falls under ST-26. Resfinder was used to predict the presence of antimicrobial resistance genes (AMR). *Salmonella* serotype prevalence data were analyzed using Fishers Exact Test with significance $P \leq 0.05$. From the 7 positive samples, *Salmonella* Infantis (6) was more frequently detected than *Salmonella* Thompson (1, $P=0.0291$). All (100%) Infantis isolates had point mutation on *gvrA* and *parC*, and had the *aac(6)-Iaa* gene. AMR genes *aadA1*, *sull*, and *tet(A)* were detected in 66.67% of isolates, whereas *dfrA14*, *aph(3')-Ia*, *blaCTX-M-65*, *aph(4)-LA*, and *aac(3)-IV* genes were detected in only one isolate. In conclusion, *Salmonella* Infantis was the more common serotype found in this study and had multiple antimicrobial resistance genes. This information can be further used to trace the foodborne illness related to *Salmonella* in humans.

Keywords: Salmonella Infantis, serotype, sequencing, antimicrobial resistance

T221 A systematic review and meta-analysis on the efficacy of organic acids to reduce Salmonella colonization in crop and ceca of broilers Jinquan Wang*², Bharath Mallavarapu¹, Pranita Patil¹, Deepak Subedi¹, Harshavardhan Thippareddi¹, Amit K. Singh³, Dianna Bourassa⁴, Sabin Poudel⁵ ¹University of Georgia, Poultry Science Department, Athens, Georgia, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ³Delaware State University,

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A systematic review and meta-analysis were conducted to evaluate the efficacy of organic acid delivery route (water or feed) on the prevalence and levels of Salmonella in the crop and ceca of broilers. A total of 2,290 studies were identified through searches in Google Scholar. After applying exclusion criteria, 29 and 22 studies were included for prevalence and quantitative level analysis, respectively. Data on total sampled birds and positive birds for control and treatment groups were extracted from prevalence studies. Mean values, standard deviations, and replicates for control and treatment groups were extracted to calculate mean difference (log₁₀ CFU) from quantitative levels studies. Data were analyzed using the meta package in R (R Core Team, 2024) with a significance level set at 0.1. All models had a medium or high heterogeneity ($I^2 > 50\%$), therefore, results from the random effect model were reported. Organic acids via feed reduced the odds of Salmonella positives in the crop by 83% compared to the control ($P < 0.01$). However, supplementation through drinking water was less effective compared to the feed, reducing the odds by 45% compared with the control ($P < 0.01$). Both delivery routes reduced the odds of Salmonella positives in the ceca by 73% and 46%, respectively ($P < 0.01$). Organic acid supplementation in the drinking water ($P = 0.012$) and feed ($P < 0.01$) reduced Salmonella level in the crop by 0.69 and 0.92 log₁₀ CFU, respectively. Similarly, both routes effectively reduced Salmonella levels in the ceca by 0.69 and 1.16 log₁₀ CFU, respectively ($P < 0.01$). There was no difference between the two delivery routes in their efficacy against Salmonella level in the crop ($P = 0.68$) or ceca ($P = 0.25$). In summary, supplementing organic acids, regardless of the delivery route (water or feed), reduces the odd of Salmonella prevalence and its level in broiler crop and ceca, with reductions of 71% and 66% in odds as well as 0.81 and 1.03 log₁₀ CFU, respectively. Organic acid supplementation can be a valuable intervention during grow-out or feed withdrawal period to control Salmonella in broilers. However, caution must be taken in adopting a specific organic acid intervention strategy and their efficacy should be evaluated prior to implementation.

Keywords: Salmonella, Intervention, Pre-harvest, Broiler, Food Safety

T222 Effect of dietary fat sources on colonization and dissemination of *Salmonella* in broiler chickens Shijina Manjankattil*², Jinquan Wang¹, Sabin Poudel², Matthew Bailey², Yagya Adhikari³, Karla V. Casco², Samuel J. Rochell², Dianna Bourassa² ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ³Auburn University, Department of Poultry Science, Auburn, Alabama, United States

The study evaluated the efficacy of four different fat sources in broiler feed on *Salmonella* presence in litter, spleen, liver, ceca, and carcasses. In this study, 1152 day-old chicks were allocated into four different treatments randomized among 48 pens. Fat source based treatments included Soy Oil (T1), Poultry Fat (T2), Acidulated Fat (T3), and Palm Oil (T4). All birds were orally challenged on day 7 with 10^8 CFU of a nalidixic acid resistant *S. Enteritidis* and *S. Typhimurium* cocktail. On days 14, 21, 28, 35, and 42, for the recovery of *Salmonella*, boot swabs were collected from each pen, and spleen, liver, and cecal contents were sampled from one bird per pen (12 birds per treatment). On day 43, 16 post-pick whole carcass rinsates per treatment were collected.

Statistical analysis was performed by PROC ANOVA of SAS and Chi square of R. Boot swabs from all pens were *Salmonella* positive throughout all sampling days. On day

Salmonella prevalence decreased in spleen and liver to 0% on day 42 in all treatments except for 1 liver sample from T1. On day 14, cecal contents were 92%, 83%, 83%, and 92% *Salmonella* positive for T1, T2, T3, and T4 with an average of 3.5 to 4.6 log₁₀CFU/g. However, on day 42, *Salmonella* was not detected by direct plating in cecal contents and prevalence decreased to 25%, 36%, 25%, and 46% from T1, T2, T3, and T4, respectively. Post-pick whole carcass rinses were 100%, 100%, 100%, and 81% positive from T1, T2, T3, and T4, respectively. No significant differences in *Salmonella* counts or prevalence were observed among different dietary fat source treatment groups. These results indicate that the clearance pattern of *Salmonella* from internal organs over time post-infection is not influenced by variations in fat sources. Although internal *Salmonella* contamination appeared to have mostly cleared by day 42, the continued presence and survival of *Salmonella* indicated by litter boot swabs may have resulted in post-pick carcass contamination.

Keywords: dietary fat, broiler, *Salmonella*, carcass, dissemination

T223 Comparison of egg quality characteristics of different poultry species of Bangladesh Nushrat N. Lisa*¹, Md Sazedul Karim Sarker¹, Shakila Faruque², Muhammad A. Rashid¹, Umme S. Ety¹, Aminul Islam¹, Sadman S. Zihan³, Farhana Sharmin⁴ ¹Bangladesh Livestock Research Institute, Poultry Research Center, Savar, Dhaka, Bangladesh; ²Bangladesh Livestock Research Institute, Dhaka, Savar, Bangladesh; ³Strengthening of Poultry Research and Development Project, Bangladesh Livestock Research Institute, Dhaka, Savar, Bangladesh; ⁴Bangladesh Jute Research Institute, Dhaka, Bangladesh

Eggs' high protein content and plenty of vitamins and minerals make them a very vital diet. Eggs have been the focus of food safety debates, just like other food categories. Consumers question the usage of intensive production methods that involve several chemical additions. This study was conducted to compare external, internal quality attributes and some chemical parameters of eggs from three different poultry types/species namely, native chicken, guinea fowl (white, lavender, pearl, mixed) and turkey (white, black, bronze and bourbon red) available at BLRI, Bangladesh. The birds were reared in floor pans and provided with a balanced ration (Layer diet: ME 2,900 kcal/kg, CP 17% from 17 week till the end of the laying period). In this study, total 120 freshly laid eggs (40 per species) were randomly collected and taken into consideration for this study which are produced under an intensive farming system. External, internal qualities and cholesterol value of eggs were evaluated according to standard method. Data were statistically analyzed using analysis of variance technique in completely randomized design. Statistical package SAS (version 9.4M7, 2020) was used for statistical analysis at a significance level of 5%. Significant difference was found for all egg quality parameters except Haugh unit. There was significant difference ($P < 0.05$) found in egg weight, albumin percentage, yolk percentage, shell percentage, yolk color, yolk index, albumin index and shell thickness. The highest egg weight (66.31 g) and albumin index (0.117) were found in turkey eggs. The highest yolk percentage (32.21), shell percentage (15.47), shell thickness (0.524

14, *Salmonella* was detected from 16%, 25%, 42%, and 33% of spleen samples and 83%, 58%, 50%, and 75% liver samples from T1, T2, T3, and T4, respectively ($P > 0.05$).

mm) were noted for guinea fowl eggs. On the other hand, highest albumin percentage (61.47) and yolk index (0.473) were observed for chicken eggs. Yolk color of the chicken egg was 7.88 which is significantly higher ($P < 0.05$) than that of other two species. There was no significant difference was found in Haugh unit among three species and all were above 83. Significantly highest cholesterol ($P < 0.05$) was found in turkey eggs (300.36 mg/100 g) and lowest cholesterol in guinea fowl eggs (264.57 mg/100 g). It can be concluded that the type and species of bird have a major impact on some of the characteristics of egg quality.

Keywords: egg quality, cholesterol, native chicken, turkey, guinea fowl

T224 Effect of *Corchorus capsularis* as Functional Feed Additives on Performance, Meat Composition and Oxidative Stability in Turkey Meat F Sharmin*², Md Sazedul Karim Sarker¹, NN Lisa¹, SS Zihan¹, AM Shohail³, US Ety¹, MS Rahman² ¹Bangladesh Livestock Research Institute, Poultry Research Center, Savar, Dhaka, Bangladesh; ²Bangladesh Jute Research Institute (BLRI), Dhaka, Bangladesh; ³Jahangirnagar University, Dhaka, Bangladesh

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, B₂, and B₉) 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 ingredients 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 T₀- control feed; T₁- supplemented with 0.5% and T₂- 1% Jute leaf meal. Turkey poults were reared up to 70 days of age and fed ad libitum. 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 T₂ 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 T₂ groups compared to T₀ group after the fourth week of preservation. Thigh meat cholesterol was also significantly difference in T₂ 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-addition

POSTER ABSTRACTS – STUDENT COMPETITION

Environment and Management

P225 Evaluating the effects of ventilation shutdown plus heat (VSDH), carbon dioxide (VSDCO₂), and nitrogen gas (VSDN₂) on laying hen electroencephalograms (EEGs) Kari Harding*¹, Emmillie Boot¹, Jackson Evans², Sanjay Shah², Ramon Malheiros¹, Kenneth Anderson¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*North Carolina State University, Biological and Agricultural Engineering, Raleigh, North Carolina, United States*

Highly Pathogenic Avian Influenza (HPAI) outbreaks continue to cause shortages of depopulation resources, leading to the need for alternative methods. While most research has focused on speed, there is little research on the impact of depopulation method on laying hen stress. The objective of this study was to compare laying hen electroencephalograms (EEGs) signals when subjected to one of three treatments, namely, ventilation shutdown plus heat (VSDH), ventilation shutdown plus carbon dioxide (VSDCO₂) and ventilation shutdown plus nitrogen gas (VSDN₂). It was hypothesized that VSDN₂ would result in decreased EEG activity compared to the other treatments due to raised nitrogen levels being less noticeable. Twelve late cycle laying hens were used with four per treatment. Hens were placed individually into one of four chambers. The EEG electrodes were subcutaneously inserted in each hen's head to monitor brain activity. Data was analyzed with JMP Pro 15 using GLM with full factorial effects for all three treatments that were fit to each of the response variables. If significant ($P \leq 0.05$), Tukey's HSD was used to separate the means. Transformed EEG analysis utilized the integral area under the curve calculated using the Trapezoidal method with an NPARM analysis. Time of death (TOD) for the VSDH hens was significantly longer ($P < 0.0001$) than VSDCO₂ and VSDN₂ which were not significantly different from one another. Final hen core body temperature was significantly greater ($P < 0.0001$) in the VSDH group. There was no significant difference between percent EEG time within each millivolt (mV) range analyzed between treatments. However, there was a significantly greater ($P < 0.0312$) integral area under the curve for the VSDN₂ method compared to VSDCO₂ treatments. However, these treatments were not different from VSDH. While both VSDCO₂ and VSDN₂ were significantly quicker to TOD compared to VSDH, VSDN₂ had significantly greater brain activity. Even though VSDN₂ was quicker, it may not be an optimal alternative method due to the EEG levels from this study potentially indicating a greater hen stress response. Further research is needed to fully evaluate laying hen stress using these methods.

Keywords: Laying Hen, Nitrogen, Depopulation, Highly Pathogenic Avian Influenza, Electroencephalogram (EEG)

P226 Impact of environmental enrichments on litter moisture Chelsea S. Castillo Najera*¹, Katy J. Tarrant¹ ¹*California State University, Fresno, Animal Sciences and Agricultural Education, Fresno, California, United States*

Litter moisture is a critical component of broiler management, as litter quality is integral to bird welfare and productivity. Welfare issues may arise from litter that is too dry and friable or moist, resulting in wet litter. Dry litter may cause increased dust levels within broiler facilities reducing air quality, meanwhile, wet poultry litter is associated with the development of footpad dermatitis and lesions in broilers. Within poultry facilities, the use of structures such as platforms has grown in recent times to enhance welfare by promoting species-specific behaviors through environmental enrichment. Thus, it is essential to consider how the

placement and design of enrichment structures impact litter moisture. In this pilot trial, we evaluated the litter moisture content in the surrounding environment of two structural enrichment types and a control. The enrichments included an a-frame design consisting of a plastic perforated triangular structure birds can go underneath, and a wooden box platform birds can be on top of. Six replicants of each enrichment type and four controls were conducted. Litter samples were collected by hand at distances of 0cm, 50cm, and 100cm from the north and south ends, and 0cm, 40cm, and 80cm from the east and west sides of each enrichment. Samples were kept frozen until the time of the experiment. Three replicants of each litter sample (5.00g) were dried in a convection oven (170°F) to constant mass. % moisture content was calculated as dried litter mass divided by initial litter mass. Data were evaluated using Tukey's HSD. Samples were pooled for the pilot analysis. Mean mass loss as a result of drying was significantly different amongst sample enrichments at 0.279 ± 0.003 , 0.242 ± 0.003 , and 0.223 ± 0.003 , for the wooden box, a-frame, and control samples, respectively ($P < 0.001$). These findings highlight the importance of understanding how different enrichments impact moisture levels in addition to factors such as stocking density, wind speed, and humidity. Evaluating these differences is vital to addressing the feasibility and effectiveness of enrichment practices. For the broiler industry, such data is critical to inform producers considering the implementation of environmental enrichments in commercial facilities.

Keywords: litter, broiler, enrichment

P227 Effect of different levels of phenolic essential oils on broiler water intake and growth performance Reagan Barnett*^{1,2}, Carson Scharlau², Timothy Broderick², Audrey McElroy¹ ¹*Texas A&M University, Poultry Science, College Station, Texas, United States;* ²*Ralco Nutrition Inc., Marshall, Minnesota, United States*

Essential oils (EO) are used in poultry production for their gut health benefits. Phenolic compounds in EO are responsible for antimicrobial and antioxidant properties; however, high concentrations of EO have been observed to affect water intake. Water intake is correlated with feed intake, therefore a change in water intake will affect feed intake. In this study, the effect of phenolic EO administration through drinking water on water intake and growth performance was evaluated. For this trial, 396 15-day old Cornish Rock females were divided into 22 1.22m x 1.22m pens. All pens had statistically similar starting weights. Birds were fed basal diets and administered one of 4 water treatments, expressed in mL EO/L: 0 mL (6 pens), 0.25 mL (5 pens), 0.50 mL (6 pens), and 0.75 mL (5 pens). Birds were evaluated over 10 days with 3 data collection periods: 2 days pre-treatment (d-1- d0), 6 days on treatment (d1 - d6), and 2 days post-treatment (d+1- d+2). Average daily gain (ADG), average daily water intake (ADWI), and average daily feed intake (ADFI) were analyzed using SAS 9.4 software and correlated using Pearson's r. Means were evaluated using Tukey's LSD and separated at $P < 0.05$. Initial EO administration (d1) decreased ADWI in all treatment groups relative to the untreated control ($P < 0.01$) with 0.75mL having significantly lower intake than birds administered 0.25 or 0.50mL. ADFI significantly decreased in birds administered 0.50mL and 0.75mL ($P < 0.01$) at d1 relative to untreated birds. During the 6-day treatment period, cumulative water intake was decreased in birds receiving 0.75mL ($P = 0.04$). Cumulative feed intake tended to be lower in birds administered 0.75mL ($P = 0.06$). No differences in daily feed or water intake

were observed before or after EO supplementation. Significant positive correlations were observed between ADFI and ADWI ($r = 0.82$, $P < 0.01$), ADWI and ADG ($r = 0.82$, $P < 0.01$) and ADFI and ADG ($r = 0.91$, $P < 0.01$). Results suggest high levels of phenolic EO may lead to a decrease in water intake, with significant differences observed upon initial EO addition to the water. These products may be administered in moderation to promote gut health; however, excessive concentrations of phenolic EO may have negative effects on flock performance.

Keywords: phenolic compound, essential oil, water intake, broiler performance

P228 Spatial Analysis of Microbial Contamination Sources in Poultry Litter: Influence of Feed, Water, and Environmental Factors on Bacterial Counts Maxim A. Peckenschneider^{*1}, Elena G. Olson¹, Steve Ricke¹, Megan McCutchen¹, Jake Byrnes², Casey McGinley² ¹University of Wisconsin-Madison, Animal Science, Madison, Wisconsin, United States; ²BarnwellBio, San Diego, California, United States

Understanding the spatial distribution of microbiota in poultry litter is crucial for optimizing biosecurity and improving the health and productivity of laying hens. This study assessed microbial counts in poultry litter in relation to potential sources, including water, feed, and the surrounding environment, across two pens housing laying hens. Litter samples were systematically collected at 32 points arranged in a square grid with 1.5-foot spacing. Each sample was divided into three technical replicates, each dot-plated in duplicate on three different media types. Aerobic bacteria were quantified using tryptic soy agar (TSA), lactic acid bacteria using De Man–Rogosa–Sharpe (MRS), and Enterobacteriaceae counts using MacConkey agars. The samples were diluted, dot-plated onto appropriate media, and incubated at 37°C for 24 hours. The average microbial counts from replicates were log-transformed and analyzed with a linear regression model. Since microbial counts did not differ significantly between the two pens (ANOVA, $P > 0.05$), data from both pens were combined to examine the relationship between microbial communities and distance to sources, we applied a regression model for each plate type, using average microbial count as the response variable and distances to feed, water, and the external environment as predictors. Results revealed a significant linear association for aerobic bacterial counts, where feed and the interaction between feed and water significantly influenced microbial abundance ($P < 0.05$). A positive coefficient of 0.78 for feed suggested higher counts at locations farther from the feed source, while a small negative coefficient of -0.09 was noted for the interaction. Lactic acid bacterial counts did not exhibit significant associations with distance to feed, water, or the external environment ($P > 0.05$). Enterobacteriaceae counts were weakly negatively associated with distance from the external environment ($P = 0.05$), indicating a decline in counts with increasing distance from external sources. These findings highlight the influence of feed, water, and environmental interactions on microbial dynamics in poultry litter, offering crucial insights for improving management practices to control contamination and promote the health of laying hens.

Keywords: Poultry Litter, Microbiota, Feed, Water, Environment

P229 Predictive performance of light modeling software in commercial broiler environments Joshua A. Etherton^{*1}, John Linhoss¹, Jeremiah D. Davis², Joseph Purswell³, Anna Linhoss¹, Jessica D. Starkey⁴ ¹Auburn University, Biosystems Engineering, Auburn, Alabama, United States; ²Auburn University, National Poultry Technology Center, Auburn, Alabama, United States; ³USDA-ARS, Poultry Research Unit, Starkville, Alabama,

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Light plays a crucial role in the successful management of broilers and can influence their performance and welfare. However, no research is available that evaluates the use of commercially available lighting software to model the lighting environment inside broiler houses. The purpose of the study was to evaluate the usefulness of AGI32 (Lighting Analyst, LLC, Jacksonville, FL) software at modeling light intensities in two 18.3 × 186 m broiler houses in Alabama providing natural light via different window configurations. One house had twenty-nine 0.6 × 1 m windows installed on both the north and south sidewalls [two-sided window (2SW)] as well as two additional windows of the same size on the west end wall (brooding end). The other house had twenty-three 1.1 × 1.4 m windows installed only on the north sidewall [one-sided window (1SW)]. Photometric light sensors were used to measure light intensity at 750 locations per house (2 replicates; 1,500 total measurements per house). The 2SW dataset was used for calibration of the model parameters, while the 1SW dataset was used to validate the model. A handheld colorimeter was used to measure the reflectance of all surfaces and equipment inside the house as well as transmissivity of the windows. A sensitivity analysis showed that window transmittance and reflectance of the ceilings, sidewalls, window shutters, and fans strongly influenced model outputs. Model performance was calculated via the coefficient of determination (r^2), RMSE-observations standard deviation ratio (RSR), and Nash-Sutcliffe efficiency coefficient (NSE). Performance metrics for the 1SW calibration dataset were $r^2 = 0.81$, RSR = 0.58, and NSE = 0.66. Validation performance metrics for the 2SW dataset were $r^2 = 0.75$, RSR = 0.58, and NSE = 0.67. Based on the performance results, it was concluded that AGI32 can effectively model the lighting environment of commercial broiler houses providing natural light.

Keywords: Light intensity, Broilers, Natural light, AGI32, Poultry

P230 Feed raking behavior may not relate to excessive feed wastage of week old broiler chicks in commercial barns Taylor Waugerman^{*1}, McKenzie Setler¹, Joseph S. Moritz¹ ¹West Virginia University, School of Agriculture and Food Systems, Harpers Ferry, West Virginia, United States

In commercial broiler houses, raking feed out of pans and onto litter is a common chick behavior observed when feed pans are set to flood. Raking may also be attributed to selective feeding of crumbs over fine particles. Raking behavior can be perceived as an economical loss due to excessive feed wastage. Two experiments were conducted using a randomized complete block design to determine the interaction and main effects of time and pan location on raking behavior of week old broiler chicks in commercial broiler houses, and to determine feed consumption in pans with low and high observed raking. Experiment 1 utilized three pan locations spread equally apart from the feed hopper to the end of the feed line, with each location being a treatment. Measurements were taken 2, 4, and 6 hours post-feed augering. Treatments were replicated three times using three commercial barns. Experiment 2 utilized the same three barns with three treatments consisting of one pan location of an observed high degree of raking, one pan location of an observed low degree of raking, and one pan location being completely emptied in the vicinity of the pan to mimic a high degree of raking. Measurements were taken 2, 4, and 6 hours post-augering feed. Each barn measured 19m x 214m and housed 65,000 Hubbard x Ross 308 week old broiler chicks. Factorial analysis in experiment 1 showed no interaction between time post-augering and location ($P > 0.05$). The middle pan location within the feed line had the most raked feed ($P < 0.0001$). Contrast analyses in experiment 2 showed feed

consumption from pans with observed high raking did not differ from the observed low raking feed consumption ($P > 0.05$). Feed consumption of the emptied pan, to mimic a high degree of raking, had higher feed consumption than both observed raking pans ($P < 0.0001$). Results for these pans suggest raking occurred in the middle pan location, likely due to having the greatest mixture of crumbs and fines. Pans with varying degrees of observed raking did not differ in feed consumption. However, when the pan was emptied, 88% of the feed was consumed from litter. Therefore, the perceived relationship between feed raking and excessive feed wastage are not supported by these data.

Keywords: Raking Behavior, Feed Wastage, Crumbled Feed, Feed Raking

P231 Computational fluid dynamics model of a poultry incubator and its environmental spatiotemporal behaviors Melvy Fernandes*¹, Shanti Bhushan¹, Greg W. Burgreen¹, Jessica Drewry¹, Joseph Purswell² ¹Mississippi State University, Mississippi State, Mississippi, United States; ²USDA, Starkville, Mississippi, United States

Environmental conditions of a poultry incubator impact chick embryo development. The current practices of monitoring and control based on limited probe locations inside an incubator gives incomplete knowledge of the overall spatiotemporal distributions of important environmental factors such as temperature, humidity, and carbon dioxide. Detailed knowledge of these factors can

potentially provide insights into reducing pathogen spread. To better understand the environmental conditions inside an incubator, we have developed a computational fluid dynamics (CFD) model to predict air ventilation patterns and scalar transport in a lab-scale incubator with two egg racks (1260 eggs total) over an incubation period of 18 days. Various CFD modeling approaches were investigated to identify a cost-effective numerical model to efficiently perform long time duration simulations that generate reliable environmental data distributions. The experimental design reflects that of any newly developed CFD digital twin with a universal hypothesis that computational predictions should positively correspond to the experimental data. This project was a proof-of-concept effort to develop a CFD model of a poultry incubator, and thus only qualitative comparison was made to unreplicated data from a single incubator with data collected at 4 interior locations measuring temperature, relative humidity, and carbon dioxide. The CFD model showed reasonable qualitative agreement with experimental trend lines at 1.5, 30.0, and 50.0 mean percent errors in the temperature, relative humidity, and carbon dioxide, respectively, over the 18-day incubation period. Parametric studies of particle transport provided insights into potential pathogen spread. This research demonstrates the potential of CFD to accurately predict complex biological spatiotemporal gradients of relevant environmental factors in a poultry incubator.

Keywords: computational fluid dynamics, incubator

Food Safety

P232 Survival and translocation dynamics of Salmonella Typhimurium on eggshells and internal egg components incubated under hatching environmental conditions Colin P. Wallrich*¹, Elena Olson¹, Emily Matiak¹, James Corban², Srivatsan Raman^{2,3}, Steve Ricke¹ ¹University of Wisconsin-Madison, Animal and Dairy Science, Madison, Wisconsin, United States; ²University of Wisconsin-Madison, Biochemistry, Madison, Wisconsin, United States; ³University of Wisconsin-Madison, Bacteriology, Madison, Wisconsin, United States

This study examined Salmonella Typhimurium translocation from eggshells to internal components of eggs while mimicking hatching conditions, 37°C and 60% humidity, over 24 hours. A total of 30 eggs were collected from an aviary system; 20 eggs were surface inoculated with a nalidixic acid (NA) resistant strain of S. Typhimurium, and 10 eggs were used to monitor changes of the indigenous microbial community. 5 eggs were sampled at 1, 4, 8, and 24 hours for the inoculated group, and non-inoculated eggs at 1 and 24 h. Inoculation involved rinsing intact egg in 10⁸ CFU/mL S. Typhimurium in 10 mL buffered peptone water (nBPW) for 1 minute. Non-inoculated eggs were rinsed in nBPW without Salmonella. After incubation, eggshell S. Typhimurium levels were assessed by rinsing each egg in nBPW, serial diluting, and plating on XLD agar with 64 ng/μL NA. To assess Salmonella and local microbiota within the eggshells and contents, eggs were decontaminated with 70% ethanol, air-dried, and cracked. The shells were macerated in nBPW, serially diluted, and plated for Salmonella. The egg contents were mixed, diluted 1:10, and plated for Salmonella. Non-inoculated samples were plated on aerobic plate count (APC) petri films. Bacterial counts were log-transformed and analyzed using ANOVA and Tukey's HSD, with significance $P < 0.05$. Attachment of Salmonella and penetration was assessed after 1 h of incubation. Salmonella counts were 5.97 log₁₀ CFU/ml on eggshell exterior, 5.02 log₁₀ CFU/ml inside shell, and 2.67 log₁₀ CFU/ml in contents. S. Typhimurium counts on surface increased by over 1 log within 8 h compared to 1 h,

resulting in 7.5 log₁₀ CFU/mL at 24 h ($P < 0.05$). Within eggshell, Salmonella levels increased by over 2 logs by 8 h compared to 1 h, remaining stable through 24 h ($P < 0.05$). Notably, Salmonella levels in egg contents increased by over 2 logs within 1 h but declined to undetectable levels at 4 and 8 h, then increased again to 3 logs by 24 h ($P < 0.05$). In the non-inoculated group, APC counts were 3 log₁₀ CFU/mL at 1 h and showed a significant decrease by 24 h across all egg compartments ($P < 0.05$). These findings provide insights into Salmonella kinetics in hatching environments for targeting interventions, such as bacteriophage that depend on host multiplication.

Keywords: Eggs, Salmonella, Translocation

P233 Impact of Iron Availability on the Growth Kinetics of Campylobacter jejuni Emily Matiak*¹, Elena G. Olson¹, Joshua Jendza², Eric Neeno-Eckwall³, Jessica Hite³, Steve Ricke¹ ¹University of Wisconsin-Madison, Animal and Dairy Science, Madison, Wisconsin, United States; ²Qualitech Incorporated, Chaska, Minnesota, United States; ³School of Veterinary Medicine, Department of Pathobiological Sciences, Madison, Wisconsin, United States

Campylobacter thrives in the gastrointestinal tract (GIT) of poultry where iron is readily available. Studies have shown that reducing iron from the GIT environment has the potential to limit growth of enteric pathogens. This study investigated the impact of different iron treatments—free iron (FeSO₄*H₂O), encapsulated iron with PolyTransport technology (SQM, Qualitech®), and SQM without iron—on the growth kinetics of *Campylobacter jejuni*. Treatments were administered based on equimolar iron content to account for differences in iron concentration across sources. Using a 96-well plate, treatments were prepared at varying concentrations (10, 20, and 50 ppm) and combined with chelated Mueller-Hinton broth. Each well was inoculated with 10² colony-forming units (CFU) of *C. jejuni* and incubated for 48 h at 42°C under microaerophilic conditions. Optical density (OD) was measured in 20 min intervals

at 600nm. OD data was analyzed in R Studio using the growth_rates package to calculate key growth parameters such as carrying capacity (K), initial growth (y_0), maximum growth rate (μ_{\max}), and model fit (r^2) for each treatment. Significant effects of treatments were analyzed using ANOVA, with pairwise differences evaluated through Tukey's HSD. Statistical significance was determined at $P < 0.05$. Results indicated that at the highest concentration (50 ppm), both K and μ_{\max} were significantly impacted by the treatments ($P < 0.05$). K varied significantly across all treatment groups ($P < 0.05$), while μ_{\max} differed notably between the free iron and SQM treatments compared to the SQM without iron ($P < 0.05$). Except for the SQM without iron group, the r^2 values were high across all treatments and concentrations (e.g., $r^2 = 0.9344$ at 50 ppm), indicating high variability in *C. jejuni*'s response to SQM without iron across concentrations. Free iron treatment resulted in the highest K and μ_{\max} values, while the SQM without iron group showed significantly lower y_0 , K, and μ_{\max} as treatment concentrations increased ($P < 0.05$). These findings underscore the importance of iron as an essential nutrient for *C. jejuni*. Ultimately, encapsulating iron in SQM appears to negatively affect *C. jejuni* growth by reducing carrying capacity, particularly at higher iron concentrations.

Keywords: Campylobacter jejuni, iron encapsulation, growth kinetics, poultry

P234 Reduction of microbial spoilage on chicken breast fillets using air plasma-generating device Katherine Sierra*¹, Micah T. Black¹, Amit Morey¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States

Microbial spoilage of highly perishable products such as raw poultry contributes to food waste and loss. Reducing spoilage microorganisms can potentially extend shelf life, lowering costs and reducing food waste. Room-temperature plasma, a novel non-thermal, non-chemical technology, generates ions and reactive oxygen and nitrogen species (RONS) that effectively damage bacteria in food products without negative effects on product quality. Companies have now developed air plasma-generating devices that can be used to reduce spoilage microorganisms in food. Air plasma-generating devices are user-friendly and cost-effective for industrial applications. They operate at low temperatures, avoiding potential heat damage to food products. This study aimed to evaluate the efficacy of an air plasma-generating device and plasma-activated hydrogen peroxide (H₂O₂) in reducing microbial load on fresh, raw chicken breast fillets. Two trials were conducted with six different treatments and three repetitions each (n=36): a control, H₂O₂ alone, 5 and 10 minute direct plasma exposure, and 5 and 10 minute plasma-activated H₂O₂. Chicken samples (2.5 cm²) were exposed to the treatments, diluted in 9 mL of buffer peptone water (BPW), plated on trypticase soy agar (TSA), and incubated for 24 hours at 35°C. Viable colonies were then counted for each treatment. Data was analyzed using analysis of variance (ANOVA) ($P \leq 0.05$) to determine statistical differences between treatments. In the first trial, H₂O₂ alone achieved a 0.42 log CFU/mL reduction, while 5 and 10 minute direct plasma exposure reduced counts by 0.98 and 1.29 log CFU/mL, respectively. Plasma-activated H₂O₂ for 5 and 10 minutes reduced counts by 0.63 and 1.4 log CFU/mL, respectively ($P \leq 0.05$). The second trial showed greater efficacy, with H₂O₂ achieving a 0.67 log CFU/mL reduction. Five and 10 minute direct plasma exposure reduced counts by 0.63 and 1.19 log CFU/mL, while plasma-activated H₂O₂ reduced counts by 1.6 and 2.85 log CFU/mL ($P \leq 0.05$). These findings suggest that the air plasma-generating device is effective reducing microbial levels and has the potential to extend the shelf life of chicken products

while reducing chemical usage and water waste in processing plants—both of which are also concerned to consumers.

Keywords: Microbial spoilage, Air plasma generating-device, Chicken breast, Hydrogen peroxide

P235 Salmonella load and shedding in broiler live production varies temporally Emily E. Cason*¹, Nikki Shariat¹ ¹University of Georgia, Population Health, Athens, Georgia, United States

As ~85% of broiler houses in the United States are *Salmonella* positive, understanding *Salmonella* persistence and live-production changes is critical to improving food safety. Our objective was to evaluate temporal changes in *Salmonella* prevalence and load in commercial broiler houses then correlate that with prevalence seen at processing. Eight houses from four farms over two complexes were sampled via chicktray swabs, pooled feces, bootsocks, and bootsock-rollers at 0 and 3 days then weekly post-placement. Samples were evaluated for prevalence and quantification by culture and molecular assays. At the plant, 10 intact pre-exsanguination broiler carcasses rinses were collected and cultured from each flock. All incoming chicktray samples were *Salmonella* culture positive (n=8), while pre-placement bootsocks and bootsock-rollers found 63% and 50% of houses were culture and qPCR positive, respectively. Post-placement, all houses were *Salmonella* positive at all time points. In bootsocks ($p=0.020$), bootsock-rollers ($p=0.002$), and feces ($p=0.046$), qPCR outperformed culture *Salmonella* detection (Chi² McNemar's test). Prevalence in feces was highest by both culture and qPCR. Quantification ranged from 31.8 - 50.5 Ct and <0.3 - >110000 MPN/mL. Molecular load peaked on day 29, when Ct values of bootsocks, bootsock-rollers, and feces averaged 40.0, 40.1, and 40.6, respectively. *Salmonella* prevalence remained high while overall load decreased as flocks approached market-age, suggesting reduced *Salmonella* shedding. At processing, 86% of carcasses sampled were *Salmonella* positive. Pre-exsanguination there were no *Salmonella* prevalence differences between complexes (two-sample test of proportions, $p=0.105$). Ultimately, *Salmonella* shedding varied with flock age and detection method. On day 29, *Salmonella* prevalence and load peaked in 5/8 and 4/8 houses, respectively, suggesting an optimal time for sample collection to determine *Salmonella* status of flocks. High prevalence at the farm correlated with high pre-exsanguination *Salmonella* prevalence at the processing facility. Improving understanding of live-production *Salmonella* and how it relates to what is detected at processing can guide targeted intervention application and determine when to collect surveillance samples.

Keywords: Broiler, Salmonella, Surveillance sampling

P236 A multi-antigen subunit vaccine enhances maternal and offspring immunity against Campylobacter jejuni in chickens Mostafa Ahmed*¹, Shreeya Sharma², Abigail Schneider¹, Ari Bragg¹, Khaled Abdelaziz¹ ¹Clemson University, Animal and Veterinary Science, Clemson, South Carolina, United States; ²Clemson University, Animal and Veterinary Sciences, Central, South Carolina, United States

Campylobacter jejuni, the leading cause of bacterial gastroenteritis worldwide, affects 1.5 million people annually in the United States alone. With chickens serving as the primary reservoir and source of infection in humans, effective immunization strategies are crucial for preventing its transmission to humans. This study investigated the immunogenicity of *C. jejuni* outer membrane proteins (OMPs) and Toll-like receptor 21 ligand (CpG ODN) in layer breeders as well as the levels of maternal antibody in their

eggs and offspring. Four groups of layer breeders (n= 10 per group) were subcutaneously vaccinated with 200 µg *C. jejuni* OMPs and 50 µg CpG-ODN, individually or combined, or with PBS as a negative control. Fecal *C. jejuni* counts as well as blood and yolk antibody levels were monitored in laying hens for ten weeks post-vaccination. Offspring were subsequently observed for maternally derived antibodies (MDA) over a period of five weeks. Data analysis using one-way ANOVA with Tukey's post hoc test revealed that hens immunized with the combination of OMPs and CpG ODN exhibited significant reduction in fecal *C. jejuni* counts by 1.02 log₁₀ at week 4 and 1.37 log₁₀ at week 10 (P<0.001) post immunization. This group also showed significantly higher serum and egg yolk IgY and IgM levels compared to the negative control group (P<0.0001), and importantly, the hatched chicks sustained significantly higher serum IgY, IgM, IgA levels up to five-weeks post-hatch (P<0.0001). Overall, these findings suggest that a combination of *C. jejuni* OMPs and CpG ODN could offer a promising vaccine strategy to reduce *Campylobacter* colonization in breeders and to boost and sustain high levels of *C. jejuni*-specific MDA in hatched chicks. Further research is needed to evaluate the protective effects of maternally derived immunity against *Campylobacter* infection in a commercial broiler model.

Keywords: *Campylobacter*, CpG ODN, maternal antibodies, vaccine, outer membrane proteins

P237 Use of an all-natural, organic certified, yeast blend product to mitigate *Salmonella* Enteritidis in laying hens Carlos A. Guardado*¹, Pratima Adhikari¹, Fozol Ovi², Theresia Lavergne², Charlie Elrod² ¹Mississippi State, Poultry Science, Starkville, Mississippi, United States; ²Natural Biologics, Inc., Stephenville, Texas, United States; ³Panthera Vax, Ames, Iowa, United States

This study aimed to evaluate the impact of Naverde (Natural, Biologics, Newfield, NY), an all-natural blend of hydrolyzed yeast and highly concentrated yeast cell wall, on fecal shedding, organ translocation and egg production in hens challenged with *Salmonella* Enteritidis (SE). A total of 54 hens were housed in an Animal Biosafety Level-2 facility and completely randomized into three treatment groups: 1) Negative Control (NC; no SE challenge), 2) Positive Control (PC; SE challenge), and 3) Naverde (SE challenge with Naverde at 100g/ton of complete feed). Following a four-week adaptation, all groups were orally challenged for two consecutive days with approximately 10⁹ colony forming units (CFU) of nalidixic acid resistant SE. Each cage had two birds with an individual feeder and fecal collection tray. Feces were enumerated for *Salmonella* at 0-, 3-, and 7-days post inoculation (dpi). Fecal samples were pre-enriched in buffer peptone water (BPW) at 37°C for 24h. One ml of pre-enriched sample was transferred for enrichment in 10 ml of tetrathionate enrichment (TT) broth. The enriched samples were serially diluted in phosphate buffer saline (PBS) and 100 µl of the sample was further inoculated in xylose lysine tergitol 4 (XLT4) with 200 ppm nalidixic acid and incubated at 37°C for 24h. The data were log-transformed and analyzed using PROC GLM procedure of SAS 9.4. By 7 dpi, fecal SE shedding was reduced (P=0.025) in the Naverde group (0.0760 log CFU/g) compared to the PC group (0.1657 log CFU/g). At 7 dpi, SE colonization in the Naverde group was numerically lower (P>0.10) in liver, ovary, and spleen compared with the PC group. Although not significant (P>0.10) cecal SE was reduced by 1.016 log CFU/g in the Naverde group (1.597 CFU/g) compared to the PC group (2.613 log CFU/g). In addition, during the post-challenge, the egg production was 90.4% in the Naverde group, 89.3 % in the NC group and 92.5% in the PC group. These findings suggest that supplementing

a yeast product in laying hens can enhance flock health and help sustain egg production, even during periods of pathogenic stress. This approach may help mitigate economic losses in production units by maintaining steady productivity during challenging conditions.

Keywords: Laying hens, *Salmonella* Enteritidis, *Saccharomyces cerevisiae*, prebiotics, complex carbohydrates

P238 Evaluating the presence of humoral immune response to *Campylobacter* colonization in broiler chickens Tanmaie Kalapala*¹, Anna L. F. V. Assumpcao¹, Geetha Philips¹, Tomi Obe¹, Palmy Jesudhasan², Annie Donoghue², Komala Arsi² ¹University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ²Poultry Production and Product Safety Research, USDA-ARS, Fayetteville, Arkansas, United States

Campylobacter jejuni (Cj) is a leading cause of foodborne illness, globally, often linked to consumption of poultry. Unfortunately, current strategies including vaccines to control Cj in poultry have had limited success. In this regard, understanding the humoral immune response to Cj colonization in broiler chickens is essential for developing effective vaccines. Ten day-of-hatch chickens were used at the beginning of the study. Cecal and yolk sac contents along with blood were used to assess maternal immunity and the cecal contents were further assessed for the presence of *C. jejuni*. For the study, 56 day-of-hatch chicks were randomly divided into eight treatment groups (n=7 birds/ treatment), including a negative control (NC), and allocated to their respective pens. On day 7, birds were orally gavaged with 0.25 mL of vehicle (NC) or wild strains K1, K5, K6, S1, S3, S4, or 6-strains cocktail for respective groups (~1×10⁷ CFU/mL). On day 14, birds were euthanized, samples were collected for microbiological (cecal contents) and immunological analyses (blood and cecal contents). Cecal contents were diluted and plated on *Campylobacter* line agar plates to enumerate Cj. Blood, yolk sac, and cecal contents were processed to extract serum and mucus. Indirect ELISA was performed to determine anti-Cj IgY, IgM, and IgA levels. *Campylobacter* counts were log₁₀ transformed for statistical analysis. All the data were analyzed using one-way ANOVA followed by Dunnett's post hoc comparison, using GraphPad Prism 10 Software, and a P-value<0.05 was considered significant. Microbiology analysis confirmed the presence of Cj in all the groups except the negative control. Immunological analysis showed high titers of anti-Cj IgY in the yolk sac and blood serum, and anti-Cj IgA in the cecal mucous of day-of-hatch birds, strongly suggesting maternal immunity transference against Cj. Additionally, IgM levels increased from the day-of-hatch to day 14 in all the groups, with a significant increase in all the K strains and the cocktail group, suggesting the development of an anti-Cj humoral immune response. In conclusion, our study found that Cj induces a humoral immune response appropriate for breeders to transfer immunity vertically, providing the basis for further Cj vaccine studies.

Keywords: *Campylobacter jejuni*, Maternal immunity, Broiler chickens, ELISA, Vaccines

P239 Changes in the microbiota of treated and untreated litter of broilers given organic acids in the drinking water Jolie A. Stumpff*¹, Eva G. Guzman², Andrea Pietruska³, Rana Waqar Tabish², Wilmer J. Pacheco², Dianna Bourassa², Kenneth Macklin⁴, Ruediger Hauck²³ ¹Auburn University, Department of Animal Science, Auburn, Alabama, United States; ²Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ³Auburn University, Department of Pathobiology, Auburn, Alabama, United States; ⁴Mississippi State University,

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Salmonella is a globally leading cause of food-borne illness and poultry products are frequently associated with *Salmonella* outbreaks. This study evaluated the effect of organic acids (OA) in drinking water and litter treatments as pre-harvest strategies to control *Salmonella* transmission. Here, the effects of the treatments on the litter microbiota will be presented. The study was conducted in floor pens with *Salmonella* infected broilers. Broilers underwent 3 water treatments: citric acid (CA), a mixture of lactic, acetic, and propionic acids (AA), or water without OA, with 24 pens/treatment. Litter from each water treatment group was either composted (CP), treated with formic acid salt (FAS), or left untreated. Samples were collected the day after bird removal (d0) and before the next flock, 7 days later (d7). FAS was applied to the litter 6 days after bird removal, and CP occurred between d0 and d7. DNA was extracted from litter samples and the 16S rRNA V4/5 region was amplified by PCR. The products were sent for Illumina sequencing. The sequences were preprocessed in QIIME2 and analyzed in R. Alpha diversity was assessed by observed amplicon sequence variants, and Shannon index differed between groups on d0 (Kruskal Wallis test, $p=0.025$; $p=0.027$). Beta diversity was assessed by Bray-Curtis distances that showed significant differences between groups on d0 ($p=0.036$). Pairwise comparisons showed differences between CA and AA ($p=0.037$), and CA and control ($p=0.012$). On d7, PERMANOVA of Bray-Curtis distances showed significant differences between litter treatments ($p=0.036$). Pairwise comparisons showed significant differences between the control and CP groups ($p=0.025$). Bacterial families with significantly different relative abundances ($p<0.05$) between treatments on d0 included *Dietziaceae* and *Eggerthellaceae*. On d7, families whose relative abundances significantly changed between water treatments included *Morganellaceae* and *Rhizobiaceae*, while the litter treatments changed relative abundances of *Enterobacteriaceae* and *Bacillaceae*. These findings demonstrated that both treatments influenced the composition of litter microbiota. Further research is needed to confirm the roles of these microbial communities in controlling *Salmonella* in broilers.

Keywords: Poultry, Litter, Microbiota, Salmonella, Acids

P240 Evaluating *Salmonella* sampling methods through broiler processing Amber Richards^{*1}, Caitlin Harris², Elizabeth McMillan², Richard Buhr², Nikki Shariat¹ ¹University of Georgia, Population Health, Athens, Georgia, United States; ²USDA-ARS, Poultry Microbiological Safety and Processing Research Unit, Athens, Georgia, United States

Testing antimicrobial efficacy depends on effective sampling methods. A single carcass rinse for *Salmonella* is problematic as it may not represent a whole flock, and because rinsing does not dislodge all salmonellae that might be adhered to a carcass. This project had two objectives: 1) assess use of MicroTally (MT) mitt swabs as an alternative to rinses; and 2) identify a representative carcass sample that could be sampled on-line. Three broiler plants were visited twice; at each visit, rinses (n=40) and mitts (n=40) were used to sample single carcasses and 4lb wings. In addition, a multi-MT sample was collected from 50 carcasses (on-line) or 100 wings, (n=20). Samples were collected at hot-rehang (n=25), post-chill (n=25), pre-dip wings (n=25), and post-dip wings (n=25; total n=100). At one visit, 83 samples were collected. *Salmonella* prevalence at hot-rehang was 72.7% (109/150), and 0.7% (1/150) at post-chill. Prevalence increased slightly post cut up (5.1%; 7/136) but decreased after the antimicrobial dip (0.7%; 1/147). Considering hot-rehang samples only as these bore positives for both rinses and

MTs, *Salmonella* recovery was higher in rinses (73.3%; 44/60) than single MT swabs (66.7%; 40/60) though a Chi-squared test of independence showed no difference between the two, $X^2(1, N=120) = 0.36, p = 0.55$. Recovery with multi-MT mitts was higher (83.3%; 25/30) than both single carcass sampling methods. Nine positive samples from other processing stages comprised eight rinses and a single MT mitt. Deep serotyping of all *Salmonella*-positive samples showed that 51.7% (61/118) contained multiple serovars. In hot-rehang samples 54.5% (24/44) of rinses and 60% (24/40) of single MT swabs had multiple serovars, with no difference between the two methods ($X^2(1, N=84) = 0.08, p = 0.78$). The multi-MT mitts had lower serovar complexity (48%; 12/25) than the single carcass sampling methods. The two positive post-antimicrobial wings samples had one serovar present. These data show that antimicrobial interventions effectively reduce *Salmonella* prevalence and complexity. Both single MT mitts and rinses were effective for sampling at hot-rehang. Further research is necessary to fully assess the efficacy of MT mitts at the post-antimicrobial stages of processing.

Keywords: Broilers, Salmonella, Deep serotyping, MicroTally, Processing

P241 Effect of novel *Salmonella* bacterin vaccines on immune response to a heterologous challenge in layers Mohan Naidu Korada^{*1}, Ramesh Selvaraj¹, Parimal Sheth³, Praveen Reddy Kasu¹, Waqas Ishaq¹, Divyashree Ramalingam² ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²University of Georgia, Animal Science, Athens, Georgia, United States; ³Endovac Animal Health Inc, Columbia, Missouri, United States

Killed vaccines provide limited cross-immunity against different serovars of *Salmonella*. A mutated strain of *Salmonella* lacking an outer O side chain exposes the inner core polysaccharide, which can induce cross-protection. The objective of this study is to analyze the efficacy of killed *Salmonella* bacterin vaccines on immunological response to a heterologous challenge. 180 *Salmonella*-free Hy-Line W-36 pullets were randomly divided into three treatments in eight replications (n=8). 1. Control; 2. *Salmonella enterica* serovar Typhimurium vaccine; and 3. *Salmonella enterica* serovar Typhimurium + *Klebsiella* vaccine. Vaccines were administered intramuscularly on weeks 13, 17, and 24. Birds were challenged with *Salmonella* Enteritidis on W27. Analyzed internal organ *Salmonella* load, T-lymphocyte recall response, anti-*Salmonella* IgG, and IgA on W28. On W28, birds in vaccinated groups had no significant reduction of *Salmonella* load in the liver and ceca compared to the control ($P>0.05$). On W28, quantitative analysis using the Most Probable Number showed that vaccinated groups had no significant reduction of *Salmonella* load in the liver and ceca compared to the control ($P>0.05$). On W28, birds in vaccinated groups had significantly lower ($P<0.05$) proliferation of antigen-specific T lymphocytes compared to control. On W16 and W20, birds in vaccinated groups had 2 to 4-fold higher ($P<0.05$) anti-*Salmonella* IgG antibodies in serum after the first and second doses of vaccines. Antibody response after the third dose is not significant compared to the control. However, post-challenge, there is a 3-fold increase in anti-*Salmonella* IgG antibodies in vaccinated groups compared to the control ($P<0.05$). On W28, there is no significant difference among treatments for the anti-*Salmonella* IgA antibodies in the bile ($P>0.05$). After a heterologous challenge, vaccinated groups showed significant serum IgG response but no significant bile IgA response. Increasing the dose of vaccine may elevate bile IgA, which could potentially reduce organ *Salmonella* load.

Keywords: Salmonella, Bacterin vaccine

P242 Effects of Short-Temperature Abuse During Less-than-truckload (LTL) Transportation on Spoilage of Raw Chicken Breast during Retail Vianca Tashiguano*¹, Madison Sirmon¹, Payten Leeds¹, Jodie Jones¹, Jakob Doster¹, Cameron Smith¹, Laura Garner¹, Sungeun Cho¹, Amit Morey¹ ¹Auburn University, Poultry Science, Auburn, Alabama, United States

Raw poultry is highly perishable commodity, with a microbiome of spoilage bacteria, both aerobic and anaerobic, affecting spoilage rates. The high moisture and protein content in chicken provides ideal conditions for bacterial growth under favorable temperatures, especially during LTL transportation. This study aimed to evaluate the effects of short-temperature abuse on the microbiome of fresh chicken breast and its impact on retail shelf-life. Freshly processed, boneless, skinless chicken breast trays from a commercial poultry processing plant were transported under refrigeration (4°C) to Auburn University's Department of Poultry Science. A total of 150 trays (n=150) were used for the experiment. The products were subjected to three temperature abuse (TA) regimes: (1) 4°C for 24 hours (control), (2) TA at 30°C, and (3) TA at 37°C. Only two-thirds (100 trays) of the samples were subjected to TA cycles lasting 7.5 hours, with a sequence of 30 minutes at 4°C followed by 1 hour at 30°C or 37°C, repeated over five cycles. After the TA cycles, the samples were stored at 4°C and evaluated for shelf-life analysis at time intervals on days 0, 2, 4, 6, and 8. Each time, 10 trays were randomly selected from each treatment for testing. The middle fillet from each package was subjected to microbiological. Meanwhile, the fillet on the right side of the same tray was used for volatile compound analysis with an Electronic Nose. Data was analyzed using ANOVA (P < 0.05) to determine statistical differences between treatments. TA cycles at 30 °C and 37 °C increased the anaerobic and LAB counts by 1 log, while aerobic counts increased by 1 and 2 log, respectively, compared to the control samples after day 8 of storage. At 37 °C, the shelf-life differences were 5.97, 5.53, and 117.62 days for the anaerobic, aerobic, and LAB results, respectively, compared to the control (P < 0.05). These findings align with the detection of volatiles compounds, where amines, carboxyl acid, esters, ketones and sulfur groups containing compounds emerge as biomarkers of meat spoilage and contribute to the formation of off-odors. The research demonstrates that temperature abuse for a short time can lead to increase in spoilage rate during retail emphasizing the need for better cold-chain management.

Keywords: Short-Temperature Abuse, Microbiome of Raw Chicken Breast, Spoilage bacteria, Shelf-life

P243 Impact of feed withdrawal and bacteriophage SP6 on *Salmonella Typhimurium* reduction and microbial dynamics in a poultry cecal model Haley Tarcin*², Elena G. Olson², Emily Matiak², Colin P. Wallrich², Megan McCutchen², James Corban¹, Srivatsan Raman¹, Steve Ricke² ¹University of Wisconsin-Madison, Madison, Wisconsin, United States; ²University of Wisconsin-Madison, Animal and Dairy Science, Madison, Wisconsin, United States

This study evaluated the impact of *Salmonella*-specific bacteriophage SP6 on reducing *Salmonella Typhimurium* under feed withdrawal (FW) conditions using an *in vitro* poultry cecal model. Phage effects on lactic acid bacteria and phage counts were examined. Four birds were used per group, either with a 12-h FW or no feed withdrawal (NFW) prior to euthanasia. Cecal contents were collected aseptically, pooled, and diluted 1:3000, with five technical replicates per group. Treatments included: (1) cecal contents only, (2) cecal contents with *S. Typhimurium*, (3) cecal contents with *S. Typhimurium* and phage SP6, and (4) cecal contents with phage SP6 alone. *S. Typhimurium* resistant to 64 ng/μL nalidixic acid (NA) and phage SP6 were administered at a

concentration of 10⁶ colony-forming units (CFU) or 10⁶ plaque-forming units (PFU) per mL to the respective treatment groups. Incubations were conducted at 37°C under anaerobic conditions, with sampling at 0, 2, 4, and 6 h. *Salmonella*-inoculated groups were plated on XLD agar with NA, while non-*Salmonella* treatments were plated on MRS and MacConkey agars to evaluate lactic acid bacteria and *Enterobacteriaceae* counts. Phage counts were assessed using plaque assays. Data were analyzed with ANOVA and Tukey's HSD tests, with significance set at P < 0.05. Results showed SP6 significantly reduced *S. Typhimurium* counts by over 1 log CFU/mL in FW groups compared to NFW groups at 4 and 6 h (P < 0.001). *Salmonella* counts were initially higher in FW groups at 2 h but decreased significantly by 4 h (P < 0.001). Lactic acid bacterial populations showed no significant differences between FW and NFW groups over time (P > 0.05), except at 0 h (P < 0.001). *Enterobacteriaceae* counts were initially higher in FW groups at 2 h but were lower by 6 h (P < 0.05). SP6 phage inclusion increased *Enterobacteriaceae* counts in FW by ~1 log CFU/mL at 4 and 6 h, while reducing them in NFW groups by ~1 log CFU/mL at 6 h (P < 0.05). Phage counts were ~1 log PFU/mL throughout time in FW groups compared to NFW inoculated with *Salmonella* (P < 0.05), suggesting FW may enhance phage replication. This indicates that FW can boost phage efficacy and potentially alter microbial dynamics, favoring phage activity without disrupting beneficial lactic acid bacteria.

Keywords: Salmonella, Bacteriophage, Feed withdrawal, Poultry

P244 Evaluation of environmental stresses contributing to the persistence of *Salmonella Infantis* in processing environments Prantho Malakar Dipta*¹, Eniola Betiku¹, Tomi Obe¹ ¹University of Arkansas-Fayetteville, Department of Poultry Science, Fayetteville, Arkansas, United States

Salmonella Infantis (SI) has become an emerging serovar in poultry and research indicates that environmental conditions can influence survival and persistence, posing food safety risks. This study evaluated phenotypic changes in SI in response to various environmental stresses relative to poultry processing. A pre-harvest SI isolate was exposed to gradually increasing concentrations of peracetic acid (PAA) and quaternary ammonium compounds (QAC), starting at 40ppm and 1ppm, respectively until growth ceased, and the minimum inhibitory and bactericidal concentrations (MIC & MBC) were established. The stressed cells underwent two shock treatments at 4°C (60 min) and 55°C (6 min) simulating the chiller and scalding, respectively. This resulted in three variants for each antimicrobial. These variants, PAA/QAC-stressed (SI-P, SI-Q), cold-stressed (SI-P4, SI-Q4), heat-stressed (SI-P55, SI-Q55), and unexposed control (SI); were evaluated for phenotypes like tolerance to PAA and QAC, biofilm formation and recovery post PAA or QAC treatment at MIC and MBC levels for 5 min, and antibiotic susceptibility. The biofilm data was analyzed using ANOVA in JMP. The MBC of SI after daily exposure was 140ppm (PAA) and 8ppm (QAC). After stress, the MIC of the PAA variants was similar to the control (120ppm) while the QAC variants were slightly increased (5ppm to 6-7ppm). Biofilm formation on plastic surfaces was the same across all variants (stressed and control), however, bacterial recovery post PAA and QAC treatments significantly differed (p<.0001). Notably, dual stressed (PAA & temperatures) variants (SI-P4, SI-P-55) exhibited higher recovery of bacterial growth at MIC levels relative to SI-P and SI. Similarly, at MBC levels, all the stressed variants recovered compared to SI, signifying a profound tolerance to PAA treatments. In contrast, all variants and controls recovered similarly in response to QAC treatments. A notable change in antibiotic susceptibility was observed for all stressed variants with

higher tolerance to azithromycin and chloramphenicol and lower for 1 (QAC) and 7 (PAA) antibiotics. This study shows that environmental stress can influence the survival, persistence, and virulence of *Salmonella* Infantis, underscoring the need for further genetic analysis.

Keywords: *Salmonella* Infantis, PAA, QAC, Biofilm, stress-adaptation

P245 Challenges and efficacy of a nanoparticle-based *Salmonella* subunit vaccine in poultry administered through drinking water against *Salmonella* infection Sara Dolatyabi*¹, Philip Yeboah², Demilade I. Ibiwoye², Kate McGovern¹, Jennifer Schrock¹, Ganesh Yadaigiri¹, Ekachai Dumkliang¹, Manisha Mehdi¹, Thaciane Amaral², Veeru Patil¹, Dina Bugybayeva¹, Toni Thea Sullivan², Lisa Bielke², Renukaradhya J. Gourapura¹ ¹*Ohio State University, Department of Animal Science, Wooster, Ohio, United States;* ²*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Salmonella enterica serovar Enteritidis (SE) is a major foodborne bacterium, with poultry being a major source of human infections. Widely used live *Salmonella* vaccines present limitations such as reversion to virulence and regulatory restrictions. Earlier we showed that a novel nanoparticle-based SE subunit vaccine administered through oral gavage reduced the challenge SE load by one log. This study was conducted to determine the efficacy of the vaccine administered through drinking water under commercial-like poultry settings. Sixty *Salmonella*-free broilers were divided into three groups. Each bird vaccine dose was calculated at 25 µg of OMP+FLA antigens entrapped in mannose chitosan nanoparticle administered through drinking water at age 3-day and boosted at 3-week. Birds were challenged at age 5 weeks with 1x10⁸ CFU of *S. Enteritidis*, euthanized 7 days later, and collected blood, cloacal swabs, bile, intestinal wash, and cecum samples. Statistical analysis was performed using the GraphPad Prism 9 and used means ± SEM of 20 birds and significance (P<0.05) calculated by one-way ANOVA and Tukey's post-hoc. Our results showed an increased trend (not significant) in specific IgA production in the intestinal wash and cloacal swab samples of vaccinated birds, indicating a localized immune response at mucosal surfaces. Gene expression analysis revealed an upregulation (non-significant) of *IL-10*, *TNF-β*, *TLR1*, and *TLR5* mRNA in the cecal tonsils of vaccinated birds, suggesting an activation of both anti-inflammatory and pathogen recognition pathway responses. Importantly, we detected a 0.33 log CFU reduction in the cecal SE load in vaccinated challenge birds. These findings suggest that drinking water delivery of nanoparticle-based SE vaccine induced mucosal immune responses and a slight reduction in cecal bacterial load. However, this data was not better than our earlier oral gavage delivered the same vaccine-challenge data in broilers. Suggesting the need for optimization of the drinking water delivery method of

nanoparticle-based SE subunit vaccine dose, frequency of delivery, incorporating additional adjuvants, and supplementing probiotics to enhance mucosal immune responses and efficacy.

Keywords: Nanovaccine, *Salmonella*, Drinking water delivery, mucosal immunity, foodborne disease

P246 Elucidating the Role of Transcriptional factors in the Multiplication of *Salmonella* Typhimurium in Chicken Egg yolk Aisha Madi*¹, Greeshma Bharathan¹, Hunter Sheffield¹, Richard Buhr², Shabarinath Srikumar¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States;* ²*USDA-ARS, Athens, Georgia, United States*

Undercooked egg yolk and their products, consequently, have been associated with *Salmonella* outbreaks. Our study aims to obtain a molecular understanding of how *Salmonella* adapts and proliferates in egg yolk, particularly when the pathogen is exposed to yolk-associated stress and antimicrobial factors. We conducted a comparative growth analysis using 10 *S. Typhimurium* regulatory mutants in pasteurized egg yolk, where inoculated under controlled laboratory conditions. Each mutant was deleted for a gene encoding DNA binding transcription factors (TFs) and the growth kinetics were evaluated using Growth Curve Analysis for 16 hours of post inoculation. Student's t-test was used, and Mutants were compared to wild type and identified at a p-value threshold of < 0.01, 0.05, and 0.001. Particular mutants viz. ST4/74Δ*fnr* (regulating anaerobic stress response), ST4/74Δ*hilD* (regulating SPI1 invasion), and ST4/74Δ*dam* had aberrant growth kinetics. While ST4/74Δ*fnr* and ST4/74Δ*dam* were highly inhibited (p <0.001), ST4/74Δ*hilD* contrarily, was hyper-replicating compared to the wild type (P ≤ 0.05). Inhibition of the ST4/74Δ*fnr* (the *fnr* gene encodes the FNR (fumarate and nitrate reduction) protein, crucial in the bacterial response to anaerobic stress), shows that *Salmonella* is exposed to anaerobic conditions during survival in the yolk. The *dam* gene encodes for the enzyme DNA adenine methyltransferase (Dam), which methylates adenine bases within specific DNA sequences, particularly GATC. Dam is known to affect the transcription of certain genes because methylation of GATC sites affects the binding of RNA polymerase. Reduced proliferation of ST4/74Δ*dam* in egg yolk shows that the Dam regulation is important for *Salmonella* yolk proliferation. *HilD* regulates *Salmonella* virulence by modulating *Salmonella* Pathogenicity Island 1 and bacterial invasion to epithelial cells. Hyper-replication of the ST4/74Δ*hilD* shows that the pathogen downregulates *hilD* expression during yolk survival. The *hilD* downregulation could signal energy conservation by inhibiting the unnecessary activation of SPI1 during yolk survival, though validation will need further study. Overall, our findings provide early insights into the survival mechanisms used by *Salmonella* in egg yolk.

Keywords: *Salmonella*, Transcription factors, Transcription factors, pathogenicity

Processing and Products

P247 Effect of strain and welfare enrichment on turkey meat quality Camila Hammel Sobreira*¹, Christina S. Sigmon¹, Jesse L. Grimes¹, Allison N. Pullin¹, Yan L. Campbell¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

Injurious pecking between turkeys is an animal welfare concern as it can cause painful injuries, culling, and mortality. Environmental enrichment may redirect pecking from flockmates onto an inanimate object, improving welfare and performance. The

objective of this research was to determine the effects of strain and welfare enrichment (E) on turkey meat quality. The study used a 4x2 factorial design, comparing four strains sourced from two commercial breeds (1A, 1B, 2A, and 2B) housed with or without a hanging hemp rope enrichment (3.8 cm in diameter, 30.5 cm in length; control, C, or enrichment, E). Male turkeys were randomly assigned to each treatment (n=6 pens/treatment, 22 birds/pen). Turkeys were reared for 20 weeks (market weight) for processing. One turkey from each pen was selected for yield performance and

meat quality. Bird weight, hot carcass weight, pH (15 min, 2h, 24h post-processing), color, drip loss, and composition were evaluated. Data were analyzed using 2-way ANOVA and GLM in SAS 9.4, with Duncan's multiple range test for mean separation. There was no difference ($P > 0.05$) in bird and hot carcass weights with or without E, but strain 1A and 1B had greater bird and hot carcass weights than the other 2 strains in breed 2 ($P < 0.05$). Strain 2BE had greater pH at 15min than 1AC, 2BC, 1BE, 2AE, while 1BE had greater pH at 24h than 1AE ($P < 0.05$). 1A was redder and yellower than the other 3 strains at 2h, while 1AC was redder and yellower at 2h than other treatments, except for 1AE ($P < 0.05$). Drip loss rates were not different between strain and E ($P > 0.05$). Strain 1AE was lighter in breast color compared to 2AC and 2BC at 24h ($P < 0.05$). There was no effect of E on the fat, protein, moisture, collagen, and ash ($P > 0.05$). Strain 1A had less protein compared to the other three strains ($P < 0.05$), but no differences were detected in fat, moisture, collagen, and ash for strains ($P > 0.05$). In conclusion, the strain had a greater impact on weight and composition than E, with both strains from breed 1 having the most favorable weights. Strain and E impacted color and pH slightly and did not impact drip loss, or composition. More research is needed to investigate the relationship between E and injurious pecking behavior, but E does not negatively impact meat quality.

Keywords: Turkey, Breed, Strain, Welfare enrichment, Meat quality

P248 The effect of peanut skin supplemented corn/soy diet on broiler meat quality Afsana Rahaman Munnun^{*1,2}, Jean C. Caceres³, Christina S. Sigmon², Yabaiz Tahir², Thien Vu⁵, Ondulla T. Toomer⁴, Lin L. Walker², Yan L. Campbell² ¹North Carolina State University, Food, Bioprocessing & Nutrition Sciences Department, Raleigh, North Carolina, United States; ²North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ³North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United States; ⁴United States Department of Agriculture, Agricultural Research Service, Raleigh, North Carolina, United States; ⁵United States Department of Agriculture, Raleigh, North Carolina, United States

Metabolism and Nutrition: Amino Acids and Enzymes

P249 Effects of graded levels of methionine on immune organ characteristics Kaitlyn Sommer^{*1}, Maci Oelschlager¹, Juliano de Paula Dorigam², Rose Whelan², Julianna Jespersen¹, Laney Froebel¹, Ryan Dilger¹ ¹University of Illinois, Dawson, Illinois, United States; ²Evonik Industries, Essen, Germany

Methionine (Met) is typically the first-limiting amino acid (AA) for broilers and is essential for immune function, growth, and nutrient distribution. Research has indicated that size of immune organs can provide insight into their development and efficacy. This study aimed to determine the Met requirement of broilers (fixed cysteine level) for enhanced immune organ development. A total of 798 male broilers (Ross 308) were housed in cages and allotted to 1 of 7 dietary treatments, with 14 replicate cages each containing 6 birds. Diets were provided *ad libitum* in 2-phases with a common starter diet and experimental grower diets provided d 0-10 and 10-24, respectively. Grower diets included low (i.e., deficient) total sulfur AA (LTSAA; 0.14% and 0.17% total Met and Cys, respectively), a basal diet (0.16% total Met) supplemented with 0.360% L-Cys (0% supplemental Met), and 5 experimental diets with graded levels of supplemental Met (0.072% increments provided as DL-Met, DLM). To ensure the only limiting AA would be Met, other AA were included at 15% above recommendations. On d 24, one bird per cage was

Peanut (PN) skins are a peanut processing by-product, yet they offer nutritional value that can enhance animal diets. This study investigated the effects of incorporating 5% ground PN skin in the diet of broiler chickens on various meat quality parameters. The aim was to assess whether PN Skin supplementation would impact broiler quality metrics, potentially offering an alternative dietary supplement for broiler production. A controlled experiment was conducted with 90 broilers (45/trt, 3 rep/trt, 15/rep). Broilers were fed a 3-phase corn/soy mash diet regimen. Diets were isocaloric and isonitrogenous with the starter providing 3000 kcal/kg and 22% crude protein, the grower providing 3100 kcal/kg and 21% protein, and the finisher providing 3200 kcal/kg and 19% protein. A PN skin-supplemented diet was prepared for half of the broilers by adding 5% ground PN skins to the basal diet at each phase. Five broilers were sampled from each pen (15 birds/trt) for processing after 6 weeks age. Measurements were taken for body weight, hot and chilled carcass weight, and breast muscle weight after first processing. Myopathy, pH at 15 min, 2 h, 24 h and color were measured after slaughter. Data was analyzed by SAS 9.4 with one-way ANOVA and GLM using Duncan's New Multiple Range tests for mean separation with a P -value < 0.05 . Results indicated that chicken from the PN Skin diet exhibited lower body weights (2.21 kg) compared to the control (2.49 kg, $P < 0.05$). Similarly, hot, chilled carcass and breast weights for the PN Skin treatment had lower weights compared to the control ($P < 0.05$). PN skin diet did not impact the myopathy incidence on the chicken meat ($P > 0.05$). There was no difference in pH at 15 min and 24 h for control and PN treatments ($P > 0.05$), but pH at 2 h was lower in PN treatment compared to the control ($P < 0.05$). There was no difference in color between the two treatments ($P > 0.05$). In conclusion, 5% PN Skin impacted live body weight, but it did not adversely impact myopathy, color, or pH, suggesting that PN Skin can be used as a dietary supplement without negatively impacting overall broiler meat quality. While additional feeding trials are needed, these results suggested that PN skins may serve as a suitable poultry feed additive.

Keywords: Broilers, Meat Quality, Peanut Skins, Alternative Poultry Feed Ingredients

ethanized to collect immune organ metrics. Organ weights were analyzed as absolute weights and relative to BW (%). Data were analyzed via a 1-way ANOVA using the Mixed procedure of SAS. Birds receiving 0.216% or 0.288% DLM had heavier ($P < 0.01$) absolute liver weights compared with LTSAA and 0% DLM groups. Furthermore, birds receiving 0.288% DLM had the heaviest ($P < 0.01$) absolute spleen and cecal tonsils compared with LTSAA and 0% DLM. Conversely, relative liver weight increased ($P < 0.05$) in birds receiving 0% DLM compared with 0.360% DLM. Furthermore, relative cecal tonsil weight was higher in birds fed LTSAA or 0% DLM compared with birds receiving 0.216% DLM or higher. In conclusion, broilers fed diets deficient in Met had reduced absolute organ weights, likely linked to decreased BW associated with a Met deficiency. However, Met-deficient diets elicited increased relative liver weights, which may indicate altered nutrient partitioning to maintain a minimum size for adequate liver function, as the liver plays a critical role in both metabolism and immune function.

Keywords: broilers, immune, methionine, organ metrics

P250 Setting the foundation: Preliminary evaluation of digestible lysine requirements in male turkey poults during

early development from 0-4 weeks Darby R. Boontarue*¹, Sophia Bianchi¹, Luis Giron¹, John Boney¹ ¹*Pennsylvania State University, Animal Science, State College, Pennsylvania, United States*

Digestible lysine (dLys) requirements (req) are well-studied in broilers, but research for turkeys, particularly in early development, is outdated. Turkeys have also been slower than broilers in adopting digestible amino acid standards, underscoring a need for updated research. This preliminary study aimed to identify the dLys req of Nicholas Select male turkey poults from 0-28 d for key performance parameters: feed intake (FI), body weights (BW), live weight gain (LWG), and mortality-corrected feed conversion ratio (FCR_m). A broad range of dLys levels ensured the req were within the maximum fed level. Findings will guide the selection of seven dLys levels to further evaluate dLys req. A control diet (CON 1.73% dLys) was formulated to meet breeder standards, while five additional diets adjusted dLys to -10%, +10%, +15%, +20%, and +25% of the control. The six diets were placed in a randomized complete block design across 48 pens with 32 poults/pen. Data were analyzed using one-way ANOVA in JMP Pro 18, with LS means analyzed using quadratic polynomial (QP) regression to estimate dLys req at 95% of the vertex. Results indicated that poults in CON -10% had 13-19g less FI, weighed 14-17g less, and had the lowest LWG from d 0-14 ($P < 0.05$). From d 0-21, CON -10% had the lowest FI, except for CON +25%, which had intermediate FI ($P = 0.012$). By d 0-28, FI was highest in CON 1.73% and CON +10%, consuming 100g more than CON -10%, with intermediate FI in higher dLys treatments (CON +15%, +20%, +25%) ($P = 0.008$). LWG was highest in the CON 1.73% and CON +10%, with reduced LWG in CON -10% and CON +25% ($P = 0.016$). BW were 58-91g higher in CON 1.73% and CON +10% than in CON -10% and CON +25% ($P = 0.016$). FCR_m was most efficient in CON 1.73%, with CON +20% showing a slight decrease in efficiency (+0.021 kg/kg; $P = 0.037$). QP regression estimated dLys req for FI, BW, LWG, and FCR_m at 1.84%, 1.83%, 1.82%, and 1.68%, with R^2 values of 0.914, 0.867, 0.866 ($P < 0.001$), and 0.499 ($P > 0.05$), respectively. These findings suggest an optimal dLys range of 1.77% to 1.99%, with a center around 1.80%. This study provides foundational insight into dLys needs in modern turkey poults, supporting future research to refine the range further between 1.6% and 2.0% for improved growth and feed efficiency.

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

P251 Effect of proline supplementation on gene expression in broiler chickens challenged with *Eimeria* spp. Tatyany S. Mani*¹, Lizia C. Carvalho², Ramon D. Malheiros¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*; ²*Grupo BTZ, Londrina, PR, Brazil*

Proline is an amino acid that plays an important role in gut health and the development of young animals, especially under stress conditions. Previous studies suggest that proline supplementation can strengthen intestinal integrity and modulate the immune response in young animals. This study was conducted to evaluate the effect of proline supplementation on gene expression in broiler chickens challenged with *Eimeria* spp. A total of 24 one-day-old Ross broiler chickens were divided into 4 groups with 6 replicates each. A completely randomized design was used in a 2 x 2 factorial scheme (non-challenged and challenged x 2 diets). The four different groups were as follows: non-challenged and 0% of proline supplementation feed; non-challenged and 1% of proline supplementation feed; challenged and 0% of proline supplementation feed; and challenged and 1% of proline

supplementation feed. The challenged groups were orally inoculated with *Eimeria* spp. on the first day of age. The animals were housed in galvanized wire cages with *ad libitum* access to water and feed throughout the experiment. Birds were euthanized by cervical dislocation at 7, 14, and 28 days of age for sample collection, and gene expression was evaluated for the IL-10, IL-1 β , and MUC2 genes, normalized by the reference gene β -Actin. The data was submitted to analysis of variance using the SAS 9.4, with a 5% level of significance. Proline supplementation showed no significant effect on 1/(IL-10/Bact), 1/(IL-1 β /Bact), 1/(MUC2/Bact), and β -Actin_Ave. However, a significant difference ($P = 0.0177$) was observed in the mean expression of MUC2 between supplemented and non-supplemented groups. Age analysis showed variation in expression over time but no significant differences between the ages tested. The results indicate that proline supplementation may positively influence the expression of the MUC2 gene, related to the intestinal barrier, suggesting a potential benefit in modulating immune response and protecting intestinal integrity in broiler chickens challenged with *Eimeria* spp. In conclusion, these findings suggest that proline may play an important role in modulating immune response and strengthening the intestinal barrier in challenged broiler chickens.

Keywords: Proline, *Eimeria* spp., Broiler chickens, Gene expression, Gut health

P252 Investigation of interactions between phytase and anti-nutritional factors in soybean meal Rebekah L. Drysdale*¹, Benjamin W. Parsons¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*

The objective of this study was to investigate interactions between phytase and trypsin inhibitors in soybean meal. In Experiment 1, growth performance and amino acid (AA) digestibility in broiler chickens was evaluated. Diet 1 was a control corn-soybean meal-based diet. Diet 2 was similar to 1, except that 15% raw soybeans were added to increase trypsin inhibitor activity. Diets 3 and 4 were similar to 2, except that 4,000 U/kg of phytase and 0.75% phytic acid were added, respectively. All diets were formulated to be nutritionally adequate excluding any reductions in nutrient availability caused by anti-nutritional factors. Diets were provided to birds *ad libitum* from 7 to 21 days-of-age. In Experiment 2, a precision-fed rooster assay was conducted using cecectomized Leghorn roosters. Diets 1 through 4 were the same as Experiment 1. Two additional diets were fed that consisted of 100% raw soybeans with and without 4,000 U/kg of phytase. Roosters were fasted for 26 h prior to being precision-fed (crop intubated) 25 g of sample and excreta were quantitatively collected for 48 h. Experiments 1 and 2 contained 6 replicates per treatment (pen of 5 broiler chickens or 1 individually caged rooster). Data were analyzed using a 1-way ANOVA and significance was determined at $P < 0.05$. In Experiment 1 with broiler chickens, feed efficiency and weight gain were reduced ($P < 0.05$) by raw soybeans. The addition of phytase to diet 2 (15% raw soybeans) caused further reductions in weight gain ($P < 0.05$). The standardized ileal digestibility (SID) of amino acids at 21 days-of-age was lower ($P < 0.05$) in diets with 15% raw soybeans compared with diet 1; however, there was no effect ($P > 0.05$) of phytase or phytic acid on SID of AA. In Experiment 2 with precision-fed roosters, standardized AA digestibility was similar ($P > 0.05$) among treatments 1 through 4; however, digestibility of AA was reduced ($P < 0.05$) by 22 to 32 percentage units by feeding 100% raw soybeans. There was no effect ($P > 0.05$) of feeding phytase or phytic acid on AA digestibility. In conclusion, results from this study indicate that phytase may release active anti-nutritional

factors in soybean meal, as shown by the reduction in body weight gain in broiler chickens, which warrants further research.

Keywords: trypsin inhibitors, phytase, soybeans, phytate, amino acid

P253 Impact of almond hulls and a xylanase-cellulase enzyme on cecal microbiota in Lohmann LSL-Lite pullets and laying hens Nelly G. Cribillero Chuquiucacha^{*1}, Craig Wyatt², Kelley Wamsley¹, Timothy Boltz¹, Li Zhang¹, Klinton W. McCafferty³, Pratima Adhikari¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²AB Vista, Feed Ingredients, Plantation, Florida, United States; ³USDA-ARS-Poultry Research Unit, Mississippi State, Mississippi, United States

Three studies evaluated the effects of almond hulls (AH) and cellulase-xylanase enzyme (E) on the cecal microbiota of Lohmann LSL-Lite pullets and laying hens. A randomized complete block design within a 2 E inclusion (yes or no) × 3 AH factorial arrangement + 1 control treatment (No E or AH) was used in each experiment. Low, medium, and high AH levels were: 3%, 6%, and 9% for pullets, and 5%, 10%, and 15% for laying hens. In Experiment 1, 900 pullets (4 weeks) were randomly assigned to 7 trts with 5 rep/trt. In Experiment 2, 504 hens (22 weeks) were assigned to 7 trts, with 18 rep/trt, and in Experiment 3, 315 hens (44 weeks) were assigned to 15 rep/trt. At the end of each phase, a total of 119 cecal contents were collected: 35 samples from pullets (5 birds/trt) and 42 samples from 40 and 70-week-old hens (6

birds/trt each). From these samples, DNA was extracted, and the 16S rRNA gene (V3-V4 region) was sequenced using Illumina Miseq and analyzed with Quantitative Insights into Microbial Ecology 2 (QIIME2). Kruskal-Wallis and PERMANOVA tests were not significant for alpha (Chao1, Evenness, Richness, and Shannon; $P > 0.05$) or beta (Bray Curtis distance; $P > 0.05$) diversity at each phase, except for reduced Evenness and Shannon indices ($P \leq 0.05$) in pullets fed 3% AH+E when compared to control. Age significantly influenced all alpha indices and beta diversity when comparing across phases ($P < 0.0001$). The relative abundance showed that *Firmicutes* and *Bacteroidetes* were the dominant phyla in pullets, while *Actinobacteria* together with *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were dominant in laying phases. Also, in both of the laying phases, *Bacteroidetes* increased while *Firmicutes* decreased. At genus-level, *Bacteroides*, *Lactobacillus*, *Romboutsia* and *Ruminococcus* were enriched almost equally in all three phases. At pullet phase, *Faecalibacterium* and *Erysipelatoclostridium* were abundant, they declined at 40 weeks, and *Blautia* and *Ruminococcus* proliferated. By 70 weeks, *Rikenellaceae* increased. Microbial composition was similar between AH treatments and the control diet at each phase. Microbiota in the pullet phase was less diverse than in the laying phases, despite high-fiber diets. Age was a main factor influencing bacterial population shifts and diversity.

Keywords: almond hull, enzyme, cecal microbiota, pullets, laying hen

Metabolism and Nutrition: Feed Additives

P254 Dietary supplementation of microalgae and xylanase influences nutrient transporter and tight junction gene expression in broiler chickens Pravin Mishra^{*1}, Razib Das¹, Birendra Mishra¹, Rajesh Jha¹ ¹University of Hawaii at Manoa, Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, Honolulu, Hawaii, United States

For sustainable and healthy broiler production, optimizing feed efficiency and improving nutrient utilization by using alternative or novel feed ingredients and additives is essential. However, prior to incorporating these into feeding programs, it is essential to have a comprehensive understanding of their mechanisms. Our previous study found a positive response of microalgae and xylanase on broiler performance, prompting further analysis to understand the expression of genes related to nutrient transporters and tight junction proteins. Therefore, the study aimed to understand the effect of microalgae and xylanase supplementation on biomarkers associated with the gut health of broilers. A total of 162 Cobb 500 one-day-old chicks were allocated to one of three dietary groups: (1) CON: Corn-soybean meal, (2) MAG: CON with 3% microalgae, and (3) MAG+XYN: MAG with xylanase. The trial was conducted over 35 days, comprising two phases based on nutrient requirements: a starter phase (from day 0 to day 21) and a finisher phase (from day 22 to day 35). Total RNA was extracted from ileum tissues, and cDNA was synthesized for qPCR analysis. The expressions of the target genes were normalized using the housekeeping gene β -actin. Statistical analysis was conducted using JMP® Pro 17 with one-way ANOVA and Tukey's HSD test, and significance was reported at $P \leq 0.05$. The expression of *SLC39A13* (zinc transporter) was significantly higher ($P \leq 0.05$) in the MAG group, followed by MAG+XYN and CON groups. The expressions of tight junction protein genes *CLDN2* and *JAM2* were significantly higher ($P \leq 0.05$) in the MAG+XYN group, followed by the MAG and CON groups. This study did not observe any significant differences in the expression of these genes

between the MAG and MAG+XYN diets. In conclusion, incorporating 3% microalgae and xylanase into broiler diets increases the expression of selective nutrient transporter and tight junction protein genes, potentially leading to improved nutrient absorption and gut integrity compared to conventional corn-soybean meal-based diets.

Keywords: Broiler, Gene expression, Gut health, Microalgae, Xylanase

P255 novel precision biotic reduces the negative impact of Eimeria challenge on growth performance and amino acid digestibility in broiler chickens fed wheat-based diets Rama Devi Kolli^{*1}, Bhargavi Kasireddy¹, Cristiano Bortoluzzi², Oluyinka Olukosi¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²dsm-firmenich, Kaiseraugst, Switzerland

Coccidiosis leads to gut damage, impairing nutrient digestibility and modifying the substrates available to the microbiome. This study assessed the supplementation of a precision biotic (PB) on growth performance, ileal digestibility, ceca short-chain fatty acid (SCFA) profile, jejunal histomorphology, and litter ammonia (NH₃) emissions of broilers under *Eimeria* challenge (CH) fed corn-SBM (C) or wheat-corn-SBM (W) diets. One-day-old chicks (1,012) were used (6 treatments with at least 7 replicates) in a randomized complete block design. The treatments were: C diet, no challenge (C-NCH); or with challenge (C-CH); 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. On d12, all birds, except treatment 1, were challenged with mixed *Eimeria* oocysts by feed inoculation to induce enteric stress. Birds and feed were weighed at each feeding phase to assess growth performance. Cecal contents and jejunal tissues were collected on d20 and 42 for SCFA and histomorphology, respectively. On d42, NH₃ emissions were recorded, and ileal

digesta were collected for digestibility. Data were analyzed as one-way ANOVA using JMP and significance was set at $P \leq 0.05$. During the challenge (d12-20) and overall (d0-42) phases, birds in C-NCH were heavier ($P < 0.05$) than the other groups. Birds that received C-NCH or PB inclusion had improved ($P < 0.10$) FCR during the overall phase. On d20, the jejunal VH/CD was higher ($P < 0.05$) in C-NCH than the other groups. On d42, cecal isobutyrate, isovalerate, and total branched-chain fatty acids (BCFA) were lower ($P < 0.05$) in W-CH-600 than in C-CH which suggests lower protein fermentation with possible modulation of ceca microbiota. NH_3 emissions were lower ($P < 0.05$) in W-CH-600 than in (C) diets. The *Eimeria* challenge decreased ($P < 0.01$) N and all AA digestibility, whereas PB supplementation increased ($P < 0.01$) N and all AA digestibility, which may partly account for the improvement in overall growth performance observed with PB supplementation. To conclude, decreased ceca BCFA suggests lower protein fermentation with possible modulation of ceca microbiota, and the increased AA and N digestibility in PB-supplemented diets may partly account for the effect on performance.

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

P256 Lactobacilli modulate the gut microbiome and reduces Campylobacter colonization in chickens Shreeya Sharma*¹, Mostafa Naguib¹, Anna Seekatz², Hosni Hassan³, Khaled Abdelaziz¹ ¹Clemson University, Animal and Veterinary Sciences, Clemson, South Carolina, United States; ²Clemson University, Department of Biological Sciences, Clemson, South Carolina, United States; ³North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States

In the first phase, we investigated how probiotics administered via in ovo injection at embryonic day 18 (ED18), orally, or as a combination of both, influenced the gut microbiome. A total of $n = 200$ Ross 308 eggs were divided into 4 groups: control, in ovo only, in ovo + oral, and oral group. Eggs were inoculated with 10^6 CFU/mL of the lactobacilli cocktail. DNA was extracted from 160 samples (8 samples/group/week) and sent for 16S rRNA gene sequencing. Sequence processing was performed in Mothur v.1.45.2. Alpha diversity and beta diversity were assessed, and significant differences between groups were tested using PERMANOVA (adonis in R). Using LefSe analysis (LDA score > 2), we observed significant enrichment of Romboutsia and Flavonifractor in the dual-treatment group, both genera known for their immunoregulatory properties. Probiotic-treated groups displayed significant increases in beneficial butyrate-producing genera such as Anaerostipes, Anaerotruncus, and Butyrivibrio ($p < 0.05$). Additionally, Lactobacillus abundance was notably increased, and there was a significant reduction in pro-inflammatory cytokines, suggesting a shift toward an anti-inflammatory gut environment. In the second phase, we evaluated the ability of the probiotic cocktail to reduce Campylobacter colonization. Probiotic treatment resulted in a significant reduction of Campylobacter colonization by up to 1.3 log₁₀ ($p < 0.01$). Weekly sampling of bile and sera for antibody levels via ELISA, along with cytokine gene expression analysis in cecal tonsils and spleen at 24- and 48-hours post-challenge, is underway. Statistical significance for gene expression will be determined using GraphPad Prism and the Kruskal-Wallis test. Ongoing 16S rRNA gene sequencing will further explore how these interventions reshape the microbiome in response to the Campylobacter challenge.

Keywords: probiotic, lactobacilli, Campylobacter, broiler, microbiome

P257 Evaluation of precision glycans on growth performance and appetite regulation in broiler chickens Abiola S. Lawal*¹, Olayiwola Adeola¹, Aaron J. Cowieson², Kolapo M. Ajuwon¹ ¹Purdue University, Animal Sciences, Lafayette, Indiana, United States; ²dsm-firmenich, Kaiseraugst, Switzerland

Effective regulation of appetite and energy utilization is important for the growth and overall health of broiler chickens. Therefore, understanding the effects of precision glycans (PG) on these processes is essential for optimizing broiler production. This study aimed to evaluate the effects of PG on growth performance and appetite regulation in broiler chickens. A total of 640 one-day-old chicks (Cobb 500; initial BW = 40.16 ± 0.01 g) were weighed and allocated to 5 dietary treatments in a randomized complete block design to give 16 replicates/treatment, and 8 birds/replicate. The dietary treatments included a Negative Control (NC) without precision glycan (PG) supplementation (Diet 1) and four other PG diets (Diets 2-5) that were supplemented with PG that differed in the composition of monomeric sugars including combinations of glucose, mannose and xylose. The PG were supplemented in the diets at either 0.9 g/kg (Diet 2) or 0.4 g/kg (Diets 3-5). The experiment lasted 21 d with diets provided in 2 phases: d 0 to 10 and d 10 to 21. Growth performance was evaluated at the end of each phase. On d 21, birds were euthanized for blood sampling to measure serum metabolites and hormone concentrations. Results showed a tendency ($0.05 < P \leq 0.1$) for increased overall body weight gain (BWG) and feed intake (FI) with dietary inclusion of PG. Birds fed the Diet 2 showed a tendency ($P = 0.07$) for increased overall BWG compared with Diet 1. However, the BWG of birds on Diets 3, 4 and 5 did not differ from Diets 2 or 1. For the overall FI, birds fed Diet 4 showed a tendency ($P = 0.06$) for increased FI compared to Diet 1. However, the FI of birds on Diets 2, 3, or 5 did not differ from Diets 4 or 1. Dietary PG supplementation decreased ($P < 0.05$) serum concentration of Glucagon-like peptide-1 (GLP-1). Compared with Diet 1, birds fed Diet 3 had decreased ($P < 0.05$) serum GLP-1 concentrations. However, the GLP-1 concentrations of birds on Diets 2, 4 or 5 did not differ from Diet 3 or 1. Serum concentrations of glucose, leptin and peptide tyrosine (PYY) were not affected by the dietary treatments. Taken together, the results suggest that certain PG may influence appetite in broiler chickens; however, additional studies are needed to clarify the underlying mechanisms.

Keywords: broiler chickens, growth performance, precision glycans

P258 Evaluation of two sources of 25-Hydroxyvitamin D3 on early growth performance and bone characteristics on broilers reared on a wire-flooring induction model from D1-56 of age Anh Do*¹², Khawla S. Alharbi¹², Amanda Anthony¹³, Andi Asnayanti¹³, Muhammad Ali Muhammad¹, Abdulaziz Alqahtani¹³, Alexa Thomas¹, Bailey Herberger¹, Hannah McCarver¹, Abigail LeComte¹, Caren Ulcak¹, Kara Hoover¹, Kennedy Hicks¹, Olivia Chapell¹, Wesley Vaught¹, Christiane Matias⁴, Carlos Lozano-Poveda⁵, Estefania Perez-Calvo⁵, Stephanie Kulbacki⁶, Prafulla Regmi⁶, Michael T. Kidd¹³, Adnan Alrubaye¹² ¹University of Arkansas, Fayetteville, Arkansas, United States; ²University of Arkansas, POSC-CEMB, Fayetteville, Arkansas, United States; ³University of Arkansas, POSC, Fayetteville, Arkansas, United States; ⁴dsm-firmenich, São Paulo, Brazil; ⁵dsm-firmenich, Kaiseraugst, Switzerland; ⁶University of Georgia, Poultry Science, Athens, Georgia, United States

Bacterial chondronecrosis with osteomyelitis (BCO) lameness continues to be a major challenge facing the poultry industry, resulting in economic losses and negative consequences on animal

performance and welfare. As a result, preventative measures aimed at reducing the incidences of BCO lameness in broilers have garnered significant attention. This study aims to evaluate 21-day supplementation of two sources of 25-Hydroxyvitamin D₃ on growth performance and the impact on bone characteristics at day 42 using a wire-flooring induction model. A total of 1,260 Cobb 500 chicks were allocated to 21 wire-flooring pens at an initial density of 60 birds/pen and reduced to 50 birds/pen on d21. There were three feeding phases from d1-56: Starter (d1-21), Grower (d22-35), and Finisher (d36-56). Three treatments were involved in the starter phase of the experiment, with additive inclusion levels per treatment as follows: T1 – NC: Basal diet (2,950 IU/kg Vit-D₃); T2 – T1 + 25(OH)D₃ – Source 1: 69 µg/kg of feed; T3 – T1 + 25(OH)D₃ – Source 2: 31.6 µg/kg of feed. On d42 of the experiment, seven birds from each treatment were randomly selected for collection of the tibia for bone characteristic analyses. Bone parameters and performance data were subjected to one-way ANOVA. From d1-21, T3 had significantly lower FI, BWG, and higher FCR compared to T1 and T2 ($P < 0.0001$). In both starter and grower phases, T3 also saw higher cull percentages due to bird health and performance. In terms of tibial parameters, all treatments were mostly similar among experimental treatments at d42, except for T2 with a significantly higher peak breaking force (41% increase vs. T1; $P = 0.026$). These results indicate that supplementation with 25(OH)D₃ from Source 1 at a dose of 69 µg/kg of feed enhanced bird performance up to day 21, which was the supplementation endpoint in the trial, and also increased bone strength at day 42 compared to T1 and T3. This improvement supports better bone quality for birds reaching the end of the cycle. Further research is warranted to determine such effects on broiler performance and bone parameters using other established effective BCO induction models.

Keywords: BCO, lameness, bone quality, broilers, vitamin D

P259 The effect of feeding a new dual-strain probiotic on growth performance of Ross 708 male broilers Kasey E. Young^{*1}, Rosana Hirai¹, Abigail Flores¹, Camryn Wilder¹, Austin O. Silva¹, Addison Elstner¹, Skylar White², Charles Greenwald², Audrey McElroy¹ ¹Texas A&M University, Poultry Science, College Station, Texas, United States; ²NCH Life Sciences, Irving, Texas, United States

The objective of this study was to evaluate the effect of various concentrations of a new dual-strain (DS) dormant *Bacillus* spore additive on Ross 708 by-product male broiler performance. A total of 960 day-of-hatch chicks received 1X dose of Coccivac-B52[®] and were randomly allocated to 6 dietary treatments, with 8 replicates (20 birds/pen, 0.084 m²/bird) in a randomized complete block design. The 6 dietary treatments were: negative control (NC), a corn-soybean meal diet formulated to meet the breeder's recommendations void of probiotic; NC + DS 8x10¹¹cfu/ton; NC + DS 4x10¹¹cfu/ton; NC + DS 2x10¹¹cfu/ton; NC + 400g/ton commercial dual strain probiotic (PRO); and NC + 50g/ton bacitracin methylene disalicylate (BMD). Feeding phases were starter (d0-14), grower (d15-28), finisher I (d29-35), and finisher II (d36-42). Performance data included feed intake (FI), mortality-corrected feed conversion ratio (FCR), mortality %, average body weight (BW), and BW gain (BWG) collected on days 14, 28, 35, and 42. Data were analyzed using SAS 9.4, with means determined by LSMEANS and separated using Tukey's HSD and Fisher's LSD tests, with P-value ≤ 0.05. During d0-14, DS 2x10¹¹ resulted in lower FCR ($P = 0.0048$) than BMD but was similar to the remaining treatments. From d0-28, birds fed DS 8x10¹¹ had lower mortality ($P = 0.0304$) than PRO but was similar to the other treatments. During d29-35, BMD and PRO resulted in higher BWG than DS 8x10¹¹ ($P = 0.0072$), while the remaining treatments

resulted in intermediate results. Birds fed DS 8x10¹¹ had lower FI during d0-35 compared to PRO ($P = 0.0315$), with all other treatments resulting in intermediate results. Additionally, PRO or BMD inclusion resulted in higher d35 BW and d0-35 BWG compared to DS at 2x10¹¹ and 8x10¹¹ ($P = 0.0294$ and 0.0299 , respectively). Birds fed DS 8x10¹¹ had lower d35 BW and d0-35 BWG compared to DS 4x10¹¹, PRO, or BMD, while birds fed NC or DS 2x10¹¹ performed similarly. Overall, PRO inclusion resulted in higher FI ($P = 0.0172$) than DS 8x10¹¹, with all other treatments resulting in similar FI. No significant findings were observed during d15-28 or d36-42 of this study. In conclusion, the results suggest positive effects on early growth performance and overall mortality with inclusion of the new dual-strain probiotic.

Keywords: Bacillus, growth performance, antibiotic alternatives, probiotics, broiler

P260 Effect of synbiotic supplementation on cecal microbiota during exposure to subclinical doses of Fumonisin and Deoxynivalenol in broiler chickens Joseph Rishitha Dasireddy^{*1}, Laharika Kappari¹, Chasity Pender², Doupovec Barabara², Raj Murugesan², Todd J Applegate¹, Revathi Shamugasundaram³ ¹University of Georgia, Poultry science, Athens, Georgia, United States; ²DSM Nutritional Products, Manassas, Virginia, United States; ³USDA, Athens, Georgia, United States

Synbiotics modulate the cecal microbiota in chickens, by promoting the growth of beneficial bacteria, which may have the potential to mitigate the negative effects of mycotoxins such as fumonisins (FUM) and deoxynivalenol (DON). This study aimed to identify the effect of supplementation of synbiotic at 0.05% (PoultryStar[®] BRO, dsm-firmenich) on the cecal microbiota composition, diversity, and short-chain fatty acid (SCFA) profile in broilers exposed to subclinical concentrations of FUM and DON. A total of 360 one-day-old broilers were distributed into 4 treatments: T1 (control), T2 (synbiotic at 0.05%), T3 (8.5 FUM+ 3.8 DON mg/kg diet), and T4 (8.6 FUM+ 3.9 DON mg/kg diet + synbiotic at 0.05% diet). Cecal contents were collected on day 35, and bacterial compositions were identified by analyzing the V3–V4 region of the 16S rRNA gene using Illumina sequencing. Relative abundance of families and alpha diversity indices were analyzed using the Kruskal-Wallis H Test, and SCFAs were analyzed using one-way ANOVA. The results showed that on day 35, the acetate concentrations increased in T2, T3, and T4 by 5.92%, 6.83%, and 5.18%, respectively, compared to that of T1. Total SCFAs increased by 7.07% and 3.56% in T2 and T3 but decreased by 0.78% in T4 compared to the control group ($P > 0.1$). At the family level, the relative abundance fold change for *Bacillaceae* increased by 3.47 and 1.03 in T2 and T4 groups, respectively, whereas it decreased by 0.6 in T3. *Bacteroidaceae* increased by 1.19 in T2, whereas it decreased by 0.86 and 0.67 in T3 and T4 groups compared to the control group ($P < 0.1$). The relative abundance fold change for *Enterobacteriaceae* increased by 1.28 in T2, whereas it decreased by 0.9 and 0.64 in T3 and T4 groups, respectively ($P < 0.1$). *Lactobacillaceae* was increased by 1.45, 2.66, and 2.52 in the T2, T3, and T4 groups, respectively ($P < 0.1$). There were no significant differences in the abundance of *Lachnospiraceae*, *Ruminococcaceae*, and alpha diversity indices between treatment groups ($P > 0.1$). In conclusion, dietary synbiotic supplementation at 0.05% effectively increased the beneficial bacteria, such as *Lactobacillaceae*, and *Bacillaceae* and modulated SCFAs concentrations, thus partially mitigating the adverse impact of mycotoxins within the gut of broilers.

Keywords: Mycotoxins, Synbiotics, Broilers, microbiota

P261 Impact of inclusion of yeast fermentate postbiotic on week-old broiler fecal, cecal, and feed microbiome Ashley A. Tarcin*¹, Elena G. Olson¹, Lindsey A. Wythe², Abe Scheaffer³, Steve Ricke¹ ¹University of Wisconsin- Madison, Animal and Dairy Sciences, Madison, Wisconsin, United States; ²Texas A&M University, Department of Poultry Science, College Station, Texas, United States; ³Harvest Fuel, Inc., Wahalla, North Dakota, United States

The inclusion of feed amendments in poultry production remains an area of study for gastrointestinal tract (GIT) microbiome development, especially in the days closely following hatch. Yeast fermentates (YF), containing various nutrients and metabolites, can be utilized as postbiotic additives to modulate the gut microbiome of the bird. The purpose of this study was to determine the impact of YF on the fecal, cecal, and feed microbiota of broiler chicks at days 6, 8, and 9 of life. Ross 308x308 chicks (N = 400) were divided into 4 treatment groups based on the inclusion rate of YF (0%, 0.2%, 0.5%, 0.75%). On days 6, 8, and 9, fecal and cecal droppings and feed samples from each battery pen (n = 40, n = 10/treatment) were collected for genomic DNA extraction. The V4 region of the 16S rRNA gene was sequenced with Illumina MiSeq, and the data were analyzed using QIIME2-2024.5. Significance was set at $P \leq 0.05$ for main effects and $Q \leq 0.05$ for pairwise differences. The control group did not show significant differences across days for all sample types ($P > 0.05$). While feed microbial composition was not impacted by treatment, it differed by day ($P < 0.05$). Fecal and cecal populations exhibited significant differences for all sampling days in multiple analyses ($P < 0.05$). Cecal microbial richness and evenness increased on days 8 and 9 compared to day 6, resulting in differing phylogenetic diversity and abundance on all days ($P < 0.05$). Cecal richness and evenness were not different to feed on day 9 ($P > 0.05$). Cecal evenness was also increased in 0.2% and 0.5% groups compared to control ($P = 0.02$, $Q = 0.08$). The 0.2% group increased the richness of the GIT on 8 and 9 days and evenness on day 8 compared to day 6 ($P < 0.05$), while the 0.5% group did not affect microbial compositional differences between the days. In the 0.2% group, *Candidatus Arthromitus* and *Clostridia* vadinBB60 which has been associated with improved performance in poultry, was elevated on day 9. These results indicate that including YF at a rate of 0.2% can impact the GIT microbiome in week-old birds. Future research is needed to examine the long-term effects of YF inclusion on the microbial composition of the GIT as broilers reach maturity.

Keywords: Week-Old Broilers, Yeast Fermentate, Feed Additive, Microbiome

P262 Unravelling the Broiler Breeder Paradox: Microbiota-mediated mode of action of probiotics NAPE-EcN Mallory Zumwalt*¹, Mazette Croom¹, Sarah Barnes¹, Rosemary Walzem¹, Samson Oladokun¹ ¹Texas A&M, Bryan, Texas, United States

Feeding restrictions are essential for the health and productivity of breeder hens but can cause chronic hunger and stress-related behaviors, known as the broiler breeder paradox. This study investigates the potential of the probiotic NAPE-EcN to alleviate metabolic hunger in feed-restricted hens via the endocannabinoid signaling system (eCBS) and the gut-brain axis. It evaluates whether NAPE-EcN remains effective three weeks post-administration (week 5 vs. week 8) and whether breed differences influence its mode of action. Fecal samples (n=3/breed/time) were collected from the large intestine of 5-week-old pullets orally dosed with 1×10^8 CFU of NAPE-EcN (*Escherichia coli* Nissle 1917 expressing N-acyl-phosphatidylethanolamine transferase) daily since placement. Probiotic treatment ended at week 5. To assess whether probiotic faunation occurred, fecal samples were again collected at week 8 and analyzed using 16S ribosomal RNA

sequencing and bioinformatics. Results indicated that Firmicutes, Proteobacteria, and Bacteroidota were the most abundant phyla (>95%) across breeds and time points. Dominant genera included *Lactobacillus*, *Escherichia*, *Bacteroides*, and *Clostridium sensu stricto*. While neither breed nor timing of probiotic administration affected microbial alpha diversity ($P > 0.05$), beta diversity analyses showed that Breed 1 and Breed 2 microbiota clustered separately ($P = 0.05$). Breed 1 pullets exhibited a higher abundance of Erysipelotrichales and Erysipelotrichaceae ($P < 0.05$), which are linked to metabolic conditions like obesity. Probiotic administration at week 5 increased the abundance of Erysipelatoclostridiaceae, Oscillospiraceae, and Erysipeloclostridium (short-chain fatty acid-producing taxa) compared to week 8. Summarily, significant breed differences in the microbiota's response to NAPE-EcN were more pronounced than temporal effects, suggesting greater effectiveness in Breed 2 compared to Breed 1. This study offers insights for developing new strategies to enhance gut health and behavior in poultry.

Keywords: Broiler, Breeder, Probiotics, Gut, Microbiota

P263 Evaluating the effect of administering an Enterococcus faecium probiotic spray to newly hatched broiler chicks on the occurrence of bacterial chondronecrosis with osteomyelitis lameness under a Staphylococcus challenge model Abdulaziz Alqahtani*¹, Anh Do², Amanda Anthney¹, Khawla S. Alharbi², Andi Asnayanti¹, Ruvindu Perera², Manel Ben Larbi⁴, Antoine Meuter³, Adnan Alrubaye¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States; ²University of Arkansas, POSC-CEMB, Fayetteville, Arkansas, United States; ³Nononensis, Vertou, France; ⁴Tunisia University, Tunis, Tunisia

Bacterial chondronecrosis with osteomyelitis (BCO) induced lameness presents a significant challenge in poultry production. Rapid growth rates in broilers often lead to microfractures in leg bones or joints, increasing susceptibility to bacterial colonization from environmental sources or compromised gut integrity. Subsequent on farm culls, mortalities, carcass condemnations, and welfare concerns contribute to notable economic losses each year. This study aimed to evaluate the effect of spraying a commercial *Enterococcus faecium* probiotic on day-old broiler chicks at hatch to reduce the incidence of BCO-induced lameness using a *Staphylococcus aureus* challenge model. Four treatments were assessed, each involving 180 Cobb 500 male broiler chicks housed across three isolation chambers, initially at a density of 60 chicks per pen from D0 to D13, then reduced to 50 chicks per pen from D14 onward. Treatment groups were as follows: 1) negative control (no probiotic + no challenge, NC); 2) positive control (no probiotic + challenge, PC); 3) low dosage probiotic + challenge (LOW); and 4) high dosage probiotic + challenge (HIGH). On D5, challenged birds received *Staphylococcus aureus* in drinking water at 1.5×10^6 CFU/ml. Cumulative lameness incidence was monitored daily, with necropsies on lame birds beginning from D22, and was subjected to binomial GLM analysis. Statistical significance was determined at $P < 0.05$. By D57, the cumulative lameness incidence was highest in the PC group at 58.0%, followed by 36.0% in the LOW group, 28.7% in the HIGH group, and 25.3% in the NC group. The PC and HIGH groups differed significantly ($P < 0.05$). These findings suggest that early administration of the *E. faecium* probiotic at hatch can effectively reduce lameness incidence in broilers exposed to *Staphylococcus aureus*. However, further research is recommended to explore the potential of this lactic acid based -probiotic as a promising sustainable solution to address BCO induced lameness in commercial broilers.

Keywords: broiler, probiotic, lameness, bacterial chondronecrosis with osteomyelitis, *Staphylococcus aureus*

P264 Effect of sodium bisulfate inclusion on pellet durability index of broiler diets Jose I. Vargas^{*1}, Joseph P. Gulizia¹, Eva G. Guzman¹, Esteban Rosero¹, Fabio Valenzuela¹, Julianna Jespersen², Craig Coufal², Wilmer J. Pacheco¹ ¹*Auburn University, Poultry Science, Auburn, Alabama, United States*; ²*Jones-Hamilton Co., Rock Island, Illinois, United States*

Sodium bisulfate (SBS) is a mineral acid added to poultry feeds to support growth and intestinal integrity. In addition to lowering feed pH, SBS displays hygroscopic properties that may influence pellet quality as measured by the pellet durability index (PDI). Currently, there is limited research evaluating the effect of SBS inclusion on PDI. Therefore, the objective of this study was to assess the effect of SBS inclusion on the PDI of broiler diets. The experiment used a 3 × 3 factorial design with 3 dietary phases (starter, grower, and finisher) and 3 SBS inclusion levels (0, 0.3, and 0.4%). Nine total diets were mixed using a twin shaft mixer, conditioned at 185°F for 45 seconds, pelleted through a 4.0 x 36 mm (L/D = 9) die at a constant production rate of 816 kg/h, cooled in a counterflow cooler, and bagged. During bagging, representative bags were collected to evaluate PDI using 3 methods: the ASABE method S269.5 and the Holmen NHP 100 (TekPro Ltd, Norfolk, UK) for 30 and 60 seconds. Data were analyzed with a generalized linear model in JMP Pro 16 (SAS, 2021), and means were separated using Tukey's HSD test with statistical significance accepted at $P \leq 0.05$. In the starter diets, no differences ($P > 0.05$) were found among the SBS inclusion levels for any of the PDI methodologies. In the grower diets, inclusion of 0.3% SBS (82.54%) resulted in a higher PDI ($P < 0.05$) for the Holmen NHP tester for 60 seconds, compared to the diet with 0% SBS inclusion (79.01%). Pellet durability index of finisher diets improved ($P < 0.05$) with 0.3 and 0.4% SBS inclusion in comparison to the diet with 0% SBS inclusion for the ASABE method S269.5 (92.04 and 92.03, respectively vs 89.01%), and the Holmen NHP 100 tester for 30 (90.64 and 91.26, respectively vs 87.36%) and 60 (82.16 and 81.54, respectively vs 75.38%) seconds. Overall, it appears a stronger agglomeration of particles due to greater moisture retention during pelleting might explain the improved PDI of the diets containing SBS compared to the 0% SBS diets. Further research is needed to evaluate how these improvements in PDI may influence broiler performance.

Keywords: sodium bisulfate, pellet quality, pellet durability index, broiler, feed

P265 Effect of a direct fed microbial and enzyme blend on the antioxidant status of broiler chickens during

Metabolism and Nutrition: Vitamins and Minerals

P266 Assessing the role of dietary fat on basal endogenous amino acid flow and composition, intestinal morphology and cecal short-chain fatty acid profile in broiler chickens Sujitha Veeraganti^{*1}, Oluyinka Olukosi¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

The quantitative flow of basal endogenous losses of amino acids (AA) is used for the correction of apparent digestibility values of AA to derive their standardized ileal digestibility values. On the other hand, fat digestibility depends on their physical and chemical properties which in turn influence the digestibility of other dietary components, possibly including the AA flow. Therefore, this 21-d experiment investigated the effect of two fat types (tallow or soybean oil) on the amounts of basal endogenous AA losses, gut morphology of the small intestinal sections and cecal short-chain

a *Salmonella* Typhimurium challenge Davis A. Fenster^{*1}, Rasha Qudsieh², Jeng-Jie Wang², Ali Calik³, Rami A. Dalloul¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*; ²*Novus International, Inc., Chesterfield, Missouri, United States*; ³*Ankara University Faculty of Veterinary Medicine, Animal Nutrition and Nutritional Diseases, Ankara, Turkey*

Salmonella Typhimurium is a major issue in the poultry industry, as contaminated products can lead to foodborne human salmonellosis. This study investigated the effects of supplementing 2 different blends of beneficial bacteria and xylanase under low-energy nutritional challenge and nalidixic acid-resistant *S. Typhimurium* (STNR) challenge on the antioxidant status of broilers. Day (d) of hatch Ross 708 male chicks ($n=256$) were randomly divided into 4 dietary treatment groups (8 replicate cages; 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) compared to PC; NC diet supplemented with a commercial multi-strain *Bacillus* spp. probiotic and xylanase (EnzaPro™ Feed Solution; EFS); and NC diet supplemented with non-commercial blend of bacteria and xylanase (BAX). All birds were orally inoculated with 1×10^7 CFU/mL of STNR on d 7. On d 7, 10, 17, and 28, 1 bird per cage (8 birds/treatment) was euthanized to collect the liver. Liver tissues were subjected to 4 assays to measure the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as the malondialdehyde (MDA) content. The data were analyzed using one-way ANOVA and significance ($P \leq 0.05$) between groups was determined by LSD test. BAX showed significantly lower CAT activity compared to NC during peak infection on d 10 ($P = 0.0468$) and d 17 ($P = 0.0374$). BAX also had higher ($P = 0.0298$) activity of SOD compared to EFS prior to the challenge (d 7), whereas EFS had higher ($P = 0.0112$) SOD activity compared to BAX on d 17. At the end of the study (d 28), EFS and BAX had lower ($P < 0.0001$) MDA levels in the liver compared to PC. There were no significant differences ($P > 0.05$) in the GPx activity among the groups at any time point. The lower CAT activity after the challenge in the groups fed the blends of bacteria and xylanase could be caused by lower STNR colonization and thus less need for host antioxidant defenses, which would likely contribute to the reduction in MDA. These results could provide insight into the mechanisms influenced by these blends and help improve products aimed at reducing *Salmonella* loads in broiler chickens and any subsequent foodborne disease outbreaks.

Keywords: *Salmonella* Typhimurium, xylanase, DFMs, broiler, antioxidants

fatty acid (SCFA) profile. A total of 80 broiler chickens were allocated to two treatments consisting of two nitrogen-free diets in which the fat type used is either soybean oil (NFDS) or tallow (NFDT). Each treatment had eight replicates in a randomized complete block design with 5 birds per replicate. All the birds were fed a basal corn-soybean meal diet for 16 d and experimental diets from d 16 to 21. On d 21, the birds were euthanized, and the digesta were collected from the distal ileum. Sections of the duodenum, jejunum and ileum were collected for histomorphology study and the cecal content was collected for analysis of the SCFA profile. Data were analyzed as one-way ANOVA using JMP and significance was set at $P \leq 0.05$. Although there was no significant treatment effect, the analyzed N and all AA content in the ileal digesta (g/kg DM) were greater for the birds fed NFDT. Ileal basal

endogenous flows of N and all AA (g/kg DMI) were not significantly different but were numerically greater for the birds that received NFD. The exceptions were Thr, Cys, Gly, Pro, Ser, and Tyr, which were numerically lower for birds fed NFD. The percentage of individual endogenous AA in recovered endogenous protein was not significantly different except for Trp ($P < 0.01$), which was greater for NFD. There was no significant treatment effect on histomorphology measurements of the small intestine sections or on the cecal SCFA profile. In conclusion, the use of soybean oil or tallow in NFD had only marginal influence on AA flow and composition of individual AA in basal endogenous losses and therefore, may not impact the determination of standardized digestible AA in feedstuffs.

Keywords: soybean oil, tallow, basal endogenous losses, gut morphology, short-chain fatty acids

P267 Practical assessment of model accuracy to predict broiler starter-phase production goals when fed varying feed forms Anna Kathryn Riggs*¹, Nickki Tillman², Kelley Wamsley¹ ¹Mississippi State University, Mississippi State, Mississippi, United States; ²Nutritional Statistics LLC, Buford, Georgia, United States

Feed and feed manufacture represent the greatest cost to rear poultry, with the cost of starter feed being the highest relative to other feeding phases. Thus, optimizing feed intake (FI) can lead to significant cost savings for poultry producers. It is a common practice for commercial poultry producers to assign a target feed amount for birds to consume during this phase (i.e., 645 g/bird). Predicting when the birds will reach this target FI is key to managing feed resources and optimizing growth rates. Models such as linear, quadratic, or cubic regression are commonly used to predict these benchmarks, but they can sometimes be inaccurate when the data distribution is unknown or highly variable. The challenge lies in the fact that the FI data (both daily and cumulative; CFI) can be highly variable due to factors like feed form, feed quality, and individual bird differences. When these factors are not properly accounted for in the modeling process, the resulting predictions can be overly complex or "wiggly," even if the model has a high R^2 value or significant P -value. This means that the model fits the data closely, but the predictions may not be reliable outside the observed range. The smoothing spline model (SSM) is designed to handle data variability by creating a smoother curve that can adapt to the underlying structure of the data, rather than simply fitting a rigid curve (like quadratic or cubic). The current objective was to determine the prediction accuracy of various models, especially where a predictive model is asked to make predictions for inputs (data points) that fall outside the range of values it was trained on, using datasets obtained from previous starter (0-14 d) research varying in feed form (crumble vs pellet) and quality (particle size). Data were fit to quadratic and cubic regression models and predicted the d of target CFI at ~13.5 and 21 d, respectively (both $P < 0.0001$ and $R^2 = 0.99$); however, when using SSM, the predicted d for target CFI was ~14. These results suggest that while traditional regression models (quadratic, cubic) may perform well statistically, they can still misestimate the target day due to overfitting or the inability to account for complex data distributions, especially when predicting beyond the trained range.

Keywords: Feed Intake, Broiler Performance, Starter Phase, Modeling, Feed Quality

P268 Impact of the physical structure of feed on macroscopic anatomical changes in the oropharyngeal cavity of broiler chickens Isabella d. Dias*¹, Suzete d. Melo Neta¹, Ana Carolina B. Doi¹, Maria Letícia B. Mariani¹, Brenda Carolina P. dos Santos¹, Guilherme F. Deda¹, Vivian I. Vieira¹, Everton L.

Krabbe², Alex Maiorka¹ ¹Federal University of Paraná, Animal Science, Curitiba, Paraná, Brazil; ²Embrapa Swine and Poultry, Concórdia, Brazil

The objective of the study was to evaluate possible macroscopic changes in the oropharyngeal cavity of broiler chickens with the different proportions of pelleted feed. A total of 160 male broiler chickens (Ross) from 28 to 42d of age were used. The birds were distributed in a completely randomized design with 4 treatments and 8 replicates of 5 animals each. The treatments varied according to the proportion of pellets in the diets: 100% mash control diet (M100); diet with 34% pellets and 66% fines (P34); diet with 66% pellets and 34% fines (P66); and 100% pelleted diet (P100). The animals were housed in a shed, in 32 pens with wood shavings as litter, tubular feeders and nipple drinkers. From 1 to 26d of age, broilers were fed a mash diet based on corn and soybean meal. After one day of adaptation, the different programs were implemented and followed until 42 days old. To obtain the fines, the pellets were sieved through a sieve with a 1.5 mm. At 42 days of age, one bird per repetition was euthanized by cervical dislocation to collect anatomical material. Thus, the morphological structures evaluated were: salivary ducts, opening of maxillary salivary gland, palatum durum, medial paltine salivary glands, choanal opening, infundibular cleft, sphenopterygoid salivary glands, apex linguae, corpus linguae (tongue), glottis, plica transversa, radix linguae and tomium mandibulare. The data were submitted to the Shapiro-Wilk normality test. Subsequently, the data were analyzed by one-way ANOVA, and when significant, the means were compared using Dunnett's test at a 5% probability, using the mash diet as a control. The different proportions of pellets in the diets evaluated did not macroscopically modify the structures of the broiler oropharyngeal cavity at 42 days of age. In order to detect possible changes in the oropharyngeal cavity of broilers that are not identifiable through macroscopic evaluation, microscopic analysis is recommended. These analyses can contribute to a more detailed understanding of the effects of different types of diets. However, further studies are needed to confirm this hypothesis. In conclusion, the anatomical evaluation of the oropharyngeal cavity was not influenced by the different proportions of pellet.

Keywords: fines, mash, pellet, poultry, salivary ducts

P269 Ahiflower seed and presscake as potential sources of polyunsaturated fatty acids in eggs and nutrients for laying hens during the peak production phase Roseline O. Ogory*¹, Deborah Adewole¹ ¹University of Saskatchewan, Saskatchewan, Canada, Animal and Poultry Science, Saskatoon, Saskatchewan, Canada

The use of ahiflower seed (AS) and ahiflower presscake (APC) in the diet of laying hens during the peak production phase as a source of n-FAs in eggs and nutrients in laying hens has not been evaluated. This study examined the apparent total tract digestibility (ATTD), egg quality (EQ), and yolk fatty acid profile (EYFA) from hens fed diets supplemented with AS and APC. 288 36-week-old white leghorn hens were randomly allocated to 1 of 8 treatments with 6 birds/cage in a completely randomized design for 12 weeks (3 periods; 4 weeks/period). The dietary treatments included the control diet (CD), CD supplemented with flaxseed (10%FS), AS (1, 5, and 10%), and APC (5, 10, and 15%). At the end of the trial, EYFA and ATTD were evaluated, EQ was assessed at the end of every period, and results were analyzed using the SAS Mixed procedure. Dietary treatment did not affect EQ, n-6/n-3, and ATTD of energy. In the egg yolk, steridonic acid was increased ($P < 0.05$) by 10%AS (0.15mg/g) compared to CD (0.01mg/g). The eicosapentaenoic acid was increased by 10%AS (0.28mg/g) and 10%FS (0.29 mg/g) compared to CD (0.05mg/g)

but arachidonic acid was reduced ($P < 0.05$) by 10%FS (2.91mg/g) compared to CD (4.34 mg/g) similar to 10%AS (3.14mg/g). Compared to CD (1.49 mg/g), 10%FS (9.47mg/g) increased α -linolenic acid while docosahexaenoic acid was increased ($P < 0.05$) by 10%AS (5.39mg/g) compared to CD (2.82mg/g) but not different from 5%AS (4.58mg/g) and 5%APC (4.42mg/g). Hens fed 10%FS, 5%, and 15%APC increased ($P < 0.05$) linoleic acid (34mg/g) compared to CD (26.6mg/g), and all AS inclusion levels. Furthermore, the apparent metabolizable energy (AME) and nitrogen-corrected AME were reduced ($P < 0.05$) by 10%AS (3296 and 3309kcal/kg), 5%APC (3297 and 3304kcal/kg), and 15%APC (3268 and 3277kcal/kg) compared to CD (3385 and 3395kcal/kg). Meanwhile, the ATTD of P was reduced by 5%AS (67%) compared to CD (76%), and the ATTD of Ca was significantly reduced by 5%AS (78%) compared to CD (88%), and 10%FS. Conclusively, 10%AS increased n-3 FAs metabolites in egg yolk but reduced AME and AMEn with 5%APC and 15%APC. All inclusion levels of AS and APC did not affect egg quality, n-6/n-3 ratio, and energy digestibility, while 5% AS reduced Ca and P digestibility

Keywords: Ahiflower, apparent metabolizable energy, calcium, and phosphorus digestibility, egg yolk fatty acid profile, laying hens

P270 Performance Evaluation of Ross 708 Broilers Fed Positive or Negative Controls Emmillie Boot*¹, Ramon D. Malheiros¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

The importance of designing suitable diets for broilers is essential to precisely feeding them for optimal growth. Over-supplementation of nutrients that broilers will not utilize is costly for producers, and the environment as these nutrients can leech into waterways when poultry litter is spread on surrounding farmland. Thus, research evaluating various nutrient profiles of diets fed to broilers is essential for nutritionists in the industry to make diet formulation decisions. The objective of this study was to compare litter moisture, foot pad scores, and body weight of broilers fed either a positive control diet (PC), a negative control with reduced methionine and lysine (NC1), or a negative control with further reduction of lysine and threonine with a reduction of 25 kcal of energy (NC2). The three corn and soybean meal diets were designed for all amino acids to maintain a consistent ratio to lysine. A total of 480 male Ross 708 chicks were obtained from a commercial hatchery and transported to North Carolina State University's Chicken Education Unit for placement. Chicks were then allocated to their respective pens and treatments with 20 chicks in each pen and 8 pens per treatment. The broilers were fed starter from 0-14 days, grower from 14-28 days, and finisher 28-42 days of age. At each feed change, remaining feed and pen body weights were measured for calculating feed conversion ratios and body weight change. On days 27 and 42, litter was collected to measure litter moisture, and at days 35 and 42, every bird had their foot pads scored. Data was analyzed in SAS 9.4 as a one-way ANOVA, and Tukey's HSD was used to separate means that were

considered significant at $P \leq 0.05$. On Day 42 the broilers in the PC treatment had significantly higher average body weights than the NC2 treatment ($P = 0.0452$) and lower feed conversion ratios ($P < 0.0001$) than the NC1 and NC2 treatments. However, no significant differences were observed among treatments for litter moisture or footpad scores at either time point. These results support previous research that has been done to create the broiler nutrition guidelines for the breed. This also sets the groundwork for future research to be completed with various feed additives to improve growth parameters.

Keywords: Broiler, Nutrition, Foot Pad, Litter Moisture, Feed Conversion

P271 Phosphorus digestibility in broiler chickens fed autoclaved faba beans and phytase Olumide S. Olowe*¹, Olayiwola Adeola¹ ¹*Purdue University, Animal Sciences, West Lafayette, Indiana, United States*

Faba beans (FB) can partially replace soybean meal in broiler diets, but its use is limited by low phosphorus (P) digestibility due to phytate and other antinutritional factors. Heat treatment, such as autoclaving, can reduce phytate levels, while phytase supplementation has been shown to increase phosphorus digestibility. The aim of this study was to evaluate the effect of autoclaving and supplementation of phytase on the P digestibility for broiler chickens. A 2×2 factorial arrangement was used to formulate diets containing 500 g/kg of faba beans or autoclaved faba beans supplemented with or without 1,000 FTU/kg of phytase. This resulted in four experimental diets: (FB), FB with phytase (FBP), autoclaved FB (AFB), and AFB with phytase (AFBP). Birds were fed a starter diet until day 19 post hatching when 256 Cobb 500 male broiler chickens were individually weighed and allotted to the four dietary treatments. The experiment consisted of 8 replicate cages with 8 birds per cage in a randomized complete block design, with weight as a blocking factor. Chromic oxide was included as an indigestible index marker to determine apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of P and calcium (Ca). Data were analyzed using the mixed procedure of SAS. Inclusion of phytase resulted in increased body weight gain ($P = 0.041$) with a tendency to improve gain-to-feed ratio ($P = 0.060$). Birds fed AFB had higher feed intake ($P = 0.017$). Phytase supplementation improved the digestibility of P and Ca, with an increase shown for AID of P ($P < 0.001$), and ATTD of Ca ($P < 0.001$). Additionally, ileal digestible P was improved by phytase supplementation ($P < 0.0001$), and retainable phosphorus intake was also increased ($P = 0.032$), with the highest values observed in the AFBP diet. Autoclaving improved the ileal digestible P intake ($P = 0.025$) with a tendency to improve the utilizable P ($P = 0.09$), however, there was no interaction between autoclaving and phytase. In conclusion, autoclaving did not affect P digestibility, but phytase supplementation increased the AID of P, regardless of whether the faba beans were autoclaved.

Keywords: Autoclaving, Broiler, Digestibility, Phosphorus, Phytase

Physiology

P272 A potential role of the yolk sac tissue as an endocrine organ during embryonic development Grant G. Bennett*¹, Jordan Castle¹, Kloie Varnadoe¹, Laura E. Ellestad¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Advancements in the broiler industry have reduced rearing time while increasing carcass yields, placing emphasis on

understanding the factors regulating embryonic growth and development. The yolk sac tissue (YST) transports nutrients to the embryo during incubation, and prior work in our lab suggests it also serves as a potential endocrine mediator of growth and development. This study aimed to determine how the YST might affect hormonal action throughout embryogenesis. Broiler eggs were incubated at standard conditions, and the entire YST was

collected on embryonic development days (E) 3, 6, 9, 12, 15, 18, and day of hatch (DOH). Male embryos were used for mRNA analysis by RT-qPCR ($n=8/\text{age}$) to determine relative expression of genes responsible for production, transport, and metabolism of hormones within the somatotrophic and thyrotrophic axes. Data were analyzed using a one-way ANOVA and Fisher's LSD test. Deiodinase (DIO) 2 and 3 are responsible for the activation and inactivation of thyroid hormones (THs), respectively. *DIO2* exhibited lowest expression on E15 and E18 before increasing at DOH ($P \leq 0.05$). *DIO3* showed greater expression from E3 to E9 before decreasing after E12 through DOH ($P \leq 0.05$). Thyroid hormone receptor (THR) alpha increased gradually throughout development, while THR beta was consistent from E3 to E12 and increased at E15, E18, and DOH ($P \leq 0.05$). Insulin-like growth factor (IGF) 2 increased expression towards late development from E15 onwards ($P \leq 0.05$). IGF binding proteins (IGFBPs) 2 and 7 increased relative expression between E18 and DOH ($P \leq 0.05$), while *IGFBP1* increased expression until E15 before levels decreased at E18 ($P \leq 0.05$). Higher expression of *DIO2* and *DIO3* during early development suggests the YST metabolizes maternal THs in the yolk prior to thyroid gland development and regulation of available THs in target tissues. Dynamic expression of IGFBPs shows the YST's capacity for regulating its own growth as well as the embryo's through modulating IGF availability and action. These data provide insight into the broader role of the YST during embryonic growth and development and highlight it as a target for novel strategies aimed at increasing broiler hatchability and post-hatch performance.

Keywords: embryogenesis, somatotrophic axis, thyrotrophic axis, IGFBPs, Deiodinases

P273 Early thermal programming and post-hatch baicalin supplementation enhanced immune response in broiler chickens Sadid Al Amaz^{*1}, Md Ahsanul Shahid¹, Rajesh Jha¹, Birendra Mishra¹ ¹*University of Hawaii at Manoa, Human Nutrition Food and Animal Sciences, Honolulu, Hawaii, United States*

Developing novel heat stress (HS) mitigation strategies for sustaining broiler production is critically needed. Previously, we reported that pre-hatch embryonic thermal manipulation (TM) and post-hatch baicalin supplementation mitigated the deleterious effect of HS in broiler chickens. This study aimed to evaluate the effects of these interventions on the immune status in the blood, spleen, bursa, and thymus of broiler chickens exposed to HS. A total of 600 fertile Cobb 500 eggs were incubated for 21 d. At candling, 238 eggs were selected for TM and incubated at 38.5°C with 55% relative humidity (RH) from embryonic day (ED) 12 to 18. From ED 19 to 21, the eggs were transferred to a hatcher at 37.5°C. In parallel, 236 eggs were incubated at the standard temperature of 37.5°C until hatched. After hatch, 180-day-old chicks from both groups were raised in thirty-six pens (10 birds per pen, six replicates per treatment). The treatments consisted of 1) Control, 2) Thermal manipulation (TM), 3) Control heat stress (CHS), 4) TM heat stress (TMHS), 5) Control heat stress supplement (CHSS), and 6) TM heat stress supplement (TMHSS). The chicks were raised under normal environmental conditions for the first 21 d. From d 22 to d 35, CHS, TMHS, CHSS, and TMHSS groups were subjected to chronic HS (32-33°C for 8 h/d). The Control and TM groups were raised at 22-24°C. TM significantly increased ($P < 0.05$) *AvBD11*, *IL4*, and *TLR21* expression in the spleen. TM and baicalin supplementation significantly decreased ($P < 0.05$) *TLR15* expression in the spleen. In the bursa, TM significantly increased ($P < 0.05$) *IL4* expression. The combination of TM with baicalin supplementation significantly increased ($P < 0.05$) *CD3* and significantly decreased (P

< 0.05) *TLR1* expression in the immune organs. Interestingly, TM alone significantly decreased ($P < 0.05$) *IFN γ* expression under HS. In the thymus, TM significantly decreased ($P < 0.05$) *IL10* and *TLR15* expression, while incorporating baicalin with TM decreased ($P < 0.05$) *AvBD6* expression. TM alone improved the immune status of broiler chickens in normal conditions, and dietary baicalin supplementation with TM helped reduce the adverse effects of HS by boosting the expression of critical immune-related genes.

Keywords: Broilers, Heat stress, Thermal manipulation, gene expression, Antibody

P274 Effects of chronic heat stress and antibiotic-growth promoter supplementation on ileal morphology and expression of nutrient transporters in modern and legacy broilers Colin A. Barcelo^{*1}, Iyanni Stevens¹, Audrey Knoper¹, Laura E. Ellestad¹ ¹*University of Georgia, Poultry Science, Pooler, Georgia, United States*

Proper intestinal function is necessary to maintain optimal broiler production. Selective breeding has led to more efficient intestinal nutrient uptake and utilization but decreased capability to withstand heat stress (HS). Antibiotic-growth promoters (AGPs) improve performance in modern broilers during HS, however, the mechanisms by which this occurs are unclear. This study evaluated the effects of bacitracin methylene disalicylate (BMD) at AGP levels on growth performance, expression of ileal carbohydrate & amino acid transporters, and ileal morphology in modern and legacy broilers subjected to chronic HS. Day (D) of hatch male chicks were randomly assigned into 8 groups ($n=6$ pens/group) with two lines [Ross 708 (ROSS) or Athens-Canadian Random Bred (ACRB)], two diets [antibiotic-free (ABF) or BMD-supplemented], and two temperatures [thermoneutral (TN, 75°F) or HS (95°F for 8h/D)]. Treatment diets were fed for the entire experiment, and birds were subjected to temperature treatments from D32-D38. Birds and feed were weighed on D0, D14, D32, and D38. On D38, ileal sections and mucosa were collected from one bird per pen. Ileal sections were analyzed to measure villus height (VH), crypt depth (CD), and VH:CD. Ileal mucosa was analyzed for mRNA levels of glucose transporter (*GLUT*) 1, 5, 8, and 12, sodium-linked glucose transporter 1 (*SGLT1*), and L-type amino acid transporter (*LAT*) 1 & 3 via RT-qPCR. Data were analyzed by ANOVA and Fisher's LSD test when ANOVA indicated significance. Average daily gain (ADG) from D32-38 was decreased during HS in ROSS, with BMD-supplementation during HS improving ADG compared to ABF-ROSS ($P \leq 0.05$). In ROSS, VH:CD was decreased in HS-ABF birds but not HS-BMD birds ($P \leq 0.05$). Line-by-temperature interactions were present for *GLUT1*, 5, and 8, *SGLT1*, and *LAT3*, where ROSS had lower expression during HS while expression in ACRBs was unaffected by temperature treatment ($P \leq 0.05$). Expression of *GLUT12* and *LAT1* was decreased in ABF-ROSS during HS but not affected in BMD-ROSS ($P \leq 0.05$). Decreased ileal nutrient uptake during HS may contribute to decreased growth performance in modern broilers. Taken together, BMD-supplementation during HS likely improves broiler performance through improved ileal morphology and nutrient transport.

Keywords: Intestinal health, Amino acid transport, Carbohydrate transport, Villus height, Crypt depth

P275 Embryonic thermal manipulation improved gut-brain axis coordination (appetite and taste) with or without heat stress in broiler chickens Sadid Al Amaz²¹, Suman Poudel¹, Rajesh Jha¹, Birendra Mishra^{*1} ¹*University of Hawaii at Manoa, Human Nutrition Food and Animal Sciences, Honolulu, Hawaii,*

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Exposure to high ambient temperatures leads to heat stress (HS) in broiler chickens, which adversely impacts their health and production efficiency. Ensuring sustainable broiler production requires developing innovative solutions to counter the harmful effects of HS. This study aims to investigate the impact of embryonic thermal manipulation (TM) on gut-brain axis neuropeptides of birds under HS. A total of 600 fertile Cobb 500 eggs were incubated for 21 d. After candling, 238 eggs underwent thermal manipulation (TM) at 38.5°C with 55% relative humidity (RH) from embryonic day (ED) 12 to 18, followed by transfer to a hatcher at 37.5°C from ED 19 to 21. Meanwhile, 236 eggs were incubated continuously at 37.5°C until hatching. After hatching, 180-day-old chicks from both groups were housed in 24 pens (10 birds per pen, with 6 replicates per treatment). The treatments included 1) Control, 2) TM, 3) Control heat stress (CHS), and 4) Thermal manipulation heat stress (TMHS). All birds were raised under standard conditions for the first 21 d. then, the birds were exposed to chronic HS (32–33°C for 8 h/d) from day 22 to 35, and the thermoneutral environment (22–24°C) remained after the HS period. Throughout the experiment, RH was kept constant at 50 ± 5%. In the hypothalamus, *Ghrelin*, *AgRP*, *CART*, *TIR1*, and *TIR3* expression significantly increased ($P < 0.05$) in the TMHS group compared to the Control group. *POMC* expression significantly increased ($P < 0.05$) in the TM group than in the Control group. In the duodenum, *NPY2R* and *POMC* expression was significantly lower in the TMHS group compared to the Control group. *TIR1* and *TIR3* expressions were significantly higher ($P < 0.05$) in the TMHS group than in the Control and CHS groups. In the jejunum, *NPY*, *NPY2R*, *PYY*, *POMC*, *TIR1*, *MC4R*, and *Pepro-orexin* expression was significantly higher ($P < 0.05$) in the Control group than in the TM and TMHS groups. *AgRP* expression was significantly lower ($P < 0.05$) in the TM group, and *TIR1* expression was significantly lower ($P < 0.05$) in both the TM and TMHS groups compared to the Control group. In summary, embryonic TM enhanced the coordination of the gut-brain axis regarding appetite and taste receptors by increasing their markers, with or without HS.

Keywords: Broilers, Heat stress, Thermal manipulation, Hypothalamus, Neuropeptide

P276 Continuous monitoring of light intensity in commercial broiler houses providing natural light Justus Ilemobayo*¹, John Linhoss¹, Jeremiah D. Davis^{1,2}, Brendan Higgins¹ ¹Auburn University, Biosystems Engineering, Auburn, Alabama, United States; ²Auburn University, National Poultry Technology Center, Auburn, Alabama, United States

Light intensity significantly affects broiler welfare, behavior, and growth, yet continuous monitoring over a flock remains challenging due to dust accumulation on the sensors. The goal of this study was to continuously monitor light intensity over two 42 day flocks in two commercial broiler houses (18.2 x 182.9 m) providing natural light with different window configurations. The one-sided window (1SW) house had 23 translucent windows (1.42 x 1.09 m) on the north sidewall, while the two-sided window (2SW) house had 58 translucent windows (0.95 x 0.60 m) on both sidewalls and two on the west end wall. Data acquisition units (DAQs) were constructed to measure light intensity at two locations in each house during two seasons (spring and summer) and production stages (brooding and growout). Each DAQ was equipped with five photometric light sensors facing the following directions: evaporative pad end wall, fan end wall, north and south-facing sidewalls, ceiling. Data was collected on a 1 min interval

and each sensor was cleaned daily by the grower. Light intensity in each house was compared as a two-way comparison (season and production stage) using PROC GLIMMIX in SAS. Means were separated using Tukey's adjustment and significance was considered at $p \leq 0.05$. During brooding and growout, mean light intensity was significantly higher during summer than spring in both the 1SW and 2SW houses. Mean light intensities in the 1SW and 2SW houses were also significantly higher during brooding than growout in both seasons. Seasonal changes in sun angle and azimuth influenced light distribution. In the 2SW house, the south-facing sensor recorded a higher average growout light intensity than the north-facing sensor (north = 25.73 lx; south = 32.07 lx) during spring, while the opposite was true during summer (north = 48.53 lx; south = 46.14 lx). These findings show that window configuration and seasonal variations affect light intensity in commercial broiler houses using natural light and that the overall lighting environment experienced by broilers in these houses will vary temporally.

Keywords: light intensity, broiler, brooding, growout

P277 Transcriptional regulation of albumen biosynthesis in the magnum of broiler breeder hens Prem Lal L. Mahato*¹, Vedbar Khadka³, Kenneth Macklin², Rajesh Jha¹, Birendra Mishra¹ ¹University of Hawaii at Manoa, Human Nutrition Food and Animal Sciences, Honolulu, Hawaii, United States; ²Mississippi State University, Department of Poultry Science, Starkville, Mississippi, United States; ³University of Hawaii at Manoa, Quantitative Health Sciences Tropical Medicine and Medical Microbiology, Honolulu, Hawaii, United States

The production of a large number of fertile eggs is the desired trait for the broiler breeder industry. Broiler breeder hens exhibit hyperphagia, leading to overweight, which negatively affects egg production, fertility, and hatchability. Egg albumen provides essential nutrients for growing chicks, and its quality is crucial for post-hatch performance. The magnum, part of the oviduct, produces and releases egg albumen, and its quality declines with age. This study aimed to determine the differentially expressed genes (DEGs), and pathways involved in albumen biosynthesis in the magnum (young vs. aged). Broiler breeder hens were sourced from commercial breeder farms at peak-lay (35 weeks of age; termed as "young") and late-lay phases (50 weeks of age; termed as "aged") (n=30/group). The hens were euthanized, and magnum tissues were collected. To identify the genes and pathways related to albumen biosynthesis, total RNAs were extracted from the magnum of young and aged hens at 2 to 3 hours post-ovulation (yolk present in the magnum) and analyzed using RNA sequencing (n=6/group), followed by validation with real-time qPCR. DESeq2 package in RStudio was used to analyze the data, and genes with $|\log_2\text{foldchange}| > 2$ and $P < 0.05$ were considered DEGs. The expressions of *AMDHD1* (essential for stabilizing lysozyme in albumin), *ZMYM3* (regulates cell morphology and cytoskeletal), and *UHRF2* (DNA methylation, histone modification), *STAM2* (aids in the development and survival of T cells) and *OvoDA3* (regulates defense mechanism) were significantly upregulated ($P < 0.05$) in the young hens, whereas *FER* (cell adhesion and migration) and *CTNNA3* (cell to cell adhesion) expressions were significantly downregulated ($P < 0.05$) in young hens compared to aged hens. The upregulation of *AMDHD1*, *ZMYM3*, and *UHRF2* suggests superior albumen quality, while *STAM2* and *OvoDA3* indicate enhanced defense capabilities in young hens. The reduction in *FER* and *CTNNA3* expression impairs cell-to-cell adhesion in the magnum, essential for the secretion of the largest protein, mucin. These results suggest that genes and biological pathways associated with albumin

biosynthesis and defense mechanisms were enriched in young breeder hens, suggesting that aging alters transcriptional regulation and albumen quality.

Keywords: Breeder hen, magnum, albumen, defense, gene

P278 Methodology of egg white preparation for analysis on benchtop blood analyzer Catherine Fudge^{*1}, Romel Ortiz¹, Nicolas Mejia Abaunza², Chongxiao Chen¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*; ²*University of Georgia, Poultry Science, Lawrenceville, Georgia, United States*

Egg whites can reflect the health status of laying hens, but analysis of egg whites is a slow and costly process. The ability to run egg whites on a benchtop blood analyzer would allow for new ways to monitor flock health and nutritional status. This study aimed to explore a method for egg white analysis on a benchtop blood analyzer (Randox Daytona+). In study 1, the egg whites (EW) of 10 eggs were pooled as one sample and homogenized in a bead beater to destroy the ovomucin and reduce viscosity. 6 tubes of EW were placed on a bead mill homogenizer and shaken for 10 s, 15 s, 30 s, 1 min, or 15 min. EWs were spun on a mini centrifuge for 30 s to reduce foam. Samples (6 replicates) were loaded on the Randox Daytona+ and analyzed for phosphorus (mmol/L),

potassium (mmol/L), sodium (mmol/L), calcium (mmol/L), total protein (g/L), and albumin (g/L). In study 2, pooled EW samples were homogenized (30s from study 1), diluted (100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125%), and measured to determine dilution effects on analyzer readings. Homogenate results were analyzed using one-way ANOVA in JMP Pro 16 with Tukey's HSD for mean separation. A linear model was used for study 2. Sodium, total protein, and albumin were not altered by homogenization for up to 1 minute but were significantly reduced when homogenized for 15 minutes ($P < 0.0001$). Calcium remained stable at all time points ($P = 0.0430$). Phosphorous levels were most consistent between 15s and 1 minute ($P < 0.0001$). The readings from the dilution trial show all parameters herein fit in a linear model ($R^2 > 0.99$), making the diluted egg white consistent for future analysis. Without dilution, potassium falls above the measurement range of the Daytona+ meanwhile diluting below 25% reduces sodium below the machine's range. In summary, homogenizing times of 15 s to 1 min reduced the viscosity of EW without impacting the components. A 25% dilution brought all parameters of interest into detectable ranges. In future, dilution should consider the machines' detection limits and the expected levels of the parameter within the EW.

Keywords: Egg white, Randox, Egg

Welfare and Behavior

P279 Comparative Microstructure of Clinically Lamé and Normal Bones of Broilers Venkata Sesha Reddy Choppa^{*1}, Hamid Reza Rafieian Naeini¹, Hemanth Reddy Katha¹, Woo Kyun Kim¹ ¹*University Of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Lameness in modern day broilers is one of the huge problems in terms of welfare and economic losses. This study focused on microstructural differences between clinically normal and lame broilers of 30, 35, and 40 days of age addressing lacunae in understanding lameness in broilers. Clinically normal (Score 0) and lame broilers (Score 2) were selected by gait score for this comparative study. For Dual Energy X-ray Absorptiometry (DEXA) and micro-computed tomography (micro-CT) analyses, 14 birds (7 normal vs. 7 lame) on day 30, 10 birds (5 normal vs. 5 lame) on day 35, and 12 birds (6 normal vs. 6 lame) on day 40 were selected. Data were analyzed using one-way ANOVA with P value fixed at < 0.05 . Data from DEXA revealed insignificant differences for all the broilers which could be due to localization of changes to femur between normal and lame birds. Interestingly, total bone 3-dimensional (3D) data by micro-CT revealed a significant decrease in trabecular BMD along with increased trabecular separation in bones from clinically lame broilers compared to ones from normal broilers. Furthermore, trabecular 3D parameters such as bone volume to tissue volume ratio and number of trabeculae in lame birds decreased significantly, whereas increase in total porosity % was observed in lame broilers. Surprisingly, total bone 3D analyses from micro-CT on day 40 exhibited an increase in cortical BMD in lame broilers but bone volume and closed pores number has decreased significantly compared to normal birds. Additionally, cortical 3D analyses again revealed a decrease in bone volume along with decrease in closed pores in clinically lame broilers compared to normal birds. Furthermore, Trabecular 3D analyses revealed lower trabecular thickness and tissue and bone volume in lame birds compared to normal birds. Finally, this study found a decrease in bone 3D parameters clinically lame broilers on day 30 and 40, indicating a weaker bone microarchitecture compared to normal birds. Current study revealed key differences among the clinically lame and normal broilers in terms of cortical and trabecular

parameters, providing insights into use of therapeutic products to improve bone health in modern day broilers

Keywords: Clinically lame, Broilers, Micro-CT, Dual Energy X-ray Absorptiometry

P280 Deep Learning Model for Automated Key Point Estimation of Cage-Free Chickens Anjan Dhungana^{*1}, Xiao Yang¹, Bidur Paneru¹, Samin Dahal¹, Lilong Chai¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Cage-free systems offer a more natural and welfare-friendly environment for the chickens and introduce unique challenges for animal wellbeing monitoring due to increased movements and social interactions. Therefore, with the transition of caged production systems to cage-free, it is important to develop suitable precision systems to monitor health, behavior, and welfare status. Detecting key points (e.g., comb, beak, body center, tail, etc.) on a chicken's body is an essential first step for automated systems such as pose estimation and helps develop accurate monitoring systems to predict health through behavior analysis. This also eliminates the need of traditional invasive body markers which requires human-bird interaction and can be stressful to chickens. The purpose of this study was to develop an accurate and markerless chicken key point detection system for cage-free settings using YOLO (You Only Look Once) model. "YOLO_{V11}-pose" family of models were custom trained on chicken images annotated with ten major body key points for each chicken. The images were obtained from four cage-free research facilities with 200 chickens each, resembling commercial settings. Each image included a variety of chicken postures, providing diversity to the dataset. Performance evaluation of five YOLO_{V11} model variants (YOLO_{V11N}-pose, YOLO_{V11S}-pose, YOLO_{V11M}-pose, YOLO_{V11L}-pose, and YOLO_{V11X}-pose) on the same dataset showed that YOLO_{V11S}-pose had the highest mean average precision (mAP) and recall of 92% and 92.6% respectively, with a precision of 89.5%. One-way ANOVA revealed a statistically significant difference ($P < 0.0001$) in these performance indicators among the five models. These findings suggest that YOLO_{V11} family of pose estimation

models, particularly YOLOv11s-pose can be used as a reliable framework for key point estimation due to the highest mAP and further developed into pose monitoring systems for commercial poultry production, hence supporting welfare-focused management practices.

Keywords: key point detection, laying hen welfare, machine vision, pose estimation, precision poultry farming

P281 Monitoring feather loss using thermoimaging technology in cage-free laying hens Samin Dahal^{*1}, Xiao Yang¹, Bidur Paneru², Anjan Dhungana¹, Lilong Chai¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*; ²*University of Georgia, Poultry Science, Athens, Georgia, United States*

U.S. egg production is gradually shifting towards a cage-free housing system due to concerns about animal welfare. However, severe feather pecking has become a persistent problem causing feather damage and losses. Feather loss impacts welfare and results in decreased productivity, because of higher metabolic loss in thermoregulation. Monitoring this damage using conventional manual assessment tools is laborious and subjective. This study explores the use of thermal imaging technology for the detection of feather loss. Thermal cameras detect the temperature of a target by evaluating its radiative heat loss from the surface. Hens at the age of 50 weeks were randomly selected from four identical cage-free rooms (25 hens from each room). A dorsal and a ventral view picture of each hen was captured using the 'FLIR T530 Thermal Imaging Camera' at an emissivity of 0.95 and room temperature of 68 °F. FLIR tools software was used to compare the average temperature of the randomly selected featherless areas (except the feet and head) to that of the feathered areas. The average temperature of the low-feather or bald area (88.09 ± 0.41 °F) was significantly higher than that of the feathered area (82.55 ± 0.24 °F) ($p < 0.001$). In addition, the distinct pattern of feathered and non-feathered areas of the hen could be observed from the thermal images. This study showed that thermal imaging is a reliable tool for the detection of feather loss in hens, paving the way for the development of machine learning models to detect feather damage in real-time automatically.

Keywords: Infrared thermography, cage-free housing, animal welfare, poultry feather, pecking behavior

P282 A short longitudinal evaluation of stress states in two broiler breeds in a pasture poultry system Aliyah N. Bonner^{*1}, Ayodeji Aderibigbe¹ ¹*Florida A&M University, College Agriculture and Food Sciences, Tallahassee, Florida, United States*

Broiler chickens encounter various stressors throughout their lifespan, with pasture-raised birds especially vulnerable due to additional exposure to predators and environmental fluctuations. These stressors can impair growth, increase disease susceptibility, and reduce feed efficiency. Understanding breed-specific stress responses is essential for optimizing breed selection and promoting sustainable practices in the evolving poultry systems. This study assessed the stress profiles of slow-growing Freedom Ranger (SGB) and fast-growing Cornish Cross (FGB) broilers raised on two local pasture-based farms in Tallahassee, Florida, with consistent management practices across both farms in terms of feed, housing, stocking density, and environmental exposure. In a randomized complete block design, feather samples were collected weekly from the wings of 20 birds to measure feather corticosterone (fCORT) concentrations, a biomarker for chronic stress. Sampling was conducted from weeks 3 to 8 and 6 to 11 for FGB and SGB, respectively, ending just before processing. Using

PROC MIXED in SAS, the effects of breed, sampling week, and their interaction on fCORT levels were analyzed, with initial bird weight included as a covariate. Pairwise comparisons between groups were evaluated with Tukey's HSD test, setting statistical significance at $p < 0.05$. The main effect of breed was not significant, indicating no overall difference in stress levels between SGB and FGB (6.17 vs. 6.21 pg/mg fCORT) over the sampling period. However, stress levels increased significantly over the five-week period ($P = 0.026$) regardless of breed. Within breeds, fCORT levels increased over time in SGB ($P < 0.05$), whereas FGB levels remained stable, possibly reflecting cumulative stressors related to breed-specific activity level, handling, or physiological development. The lack of a breed-by-week interaction suggests that both breeds experienced similar patterns of time-related stress increases. This study highlights that temporal factors are key drivers of stress in pasture-raised broilers, while breed does not significantly influence overall stress levels. Future research should explore interventions to mitigate stress, particularly in later growth stages, to enhance welfare and production outcomes.

Keywords: Broiler, Corticosterone, Pasture Poultry, Stress

P283 Effect of Dietary Tryptophan Supplementation on Tonic Immobility Behavior of Pasture-Raised Broilers Before and After Heat Stress Jaelen Cherry^{*1}, Jennifer Timmons², Shawna Weimer¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*; ²*University of Maryland - Eastern Shore, Department of Agriculture, Food, and Resource Sciences, Princess Anne, Maryland, United States*

Dietary tryptophan (TRP) supplementation has been shown to mitigate the negative effects of heat stress (HS), but the effects of tryptophan and HS on the behavioral responses of broilers during the tonic immobility test remain underexplored. This study assessed the influence of TRP supplementation on broiler chicken behavior during the tonic immobility (TI) test before and after HS. Fourteen-day-old male Ross 708 broiler chicks ($N=336$ birds) were randomly assigned to a 2x2 factorial split-plot design consisting of diet as the main plot: TRP-supplemented (T, 0.4% TRP) or control (C, 0% TRP) and heat stress as the subplot when birds were exposed to either thermoneutral (TN, 25.5°C) or heat stress (HS, 41°C) temperatures for 6h on day (D) 20. Birds were raised in pasture pens ($N=28$ pens, 12 birds/pen) for 7 pen replicates/TRP*HS treatment combination (TTN, CTN, THS, and CHS). On D15 and D22, one bird per pen ($N=28$) was subjected to the TI test. The latency to right, first head movement, leg movement, and vocalization, and the frequency of each behavior were measured from video recordings. Data were analyzed using R version 4.4.1 using a linear mixed effects model, with random effects of shelter and plot. A one-way ANOVA was used for the fixed effect of diet on D15, and a two-way ANOVA for the fixed effects of diet and environment and their interaction on D22. On D15, T-diet broilers displayed lower respiration rates (62.8 vs. 68.2 breaths/min, $P<0.02$) compared to C-diet birds. In addition, D15 T-diet birds tended to have shorter latencies to first leg movement (107 sec) and more leg movements (1.34) than C-diet birds (206 sec and 0.18, respectively, $P=0.07$). On D22, THS birds had a greater ($P<0.03$) frequency of leg movements (2.5) compared to CHS and CTN birds (0.22 and 0.89, respectively), with TTN birds showing intermediate frequency. These results suggest that tryptophan supplementation may reduce fear-related behaviors, such as respiration rate, and increase vigilance behaviors (i.e., leg movement latency and frequency) during the TI Test. Further research is needed to explore the long-term effects of tryptophan supplementation during heat stress on broiler behavior.

Keywords: heat stress, behavior, tonic immobility, fear, vigilance

P284 The relationship between body weight and skeletal traits in a strain of commercial turkey toms Stephanie Kulbacki*¹, Nathan Griffith², Gokul Selvaraj¹, Marisa Erasmus², Luiz Brito², Prafulla Regmi¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Purdue University, Animal Sciences, West Lafayette, Indiana, United States

Genetic selection has resulted in turkeys having heavier body weights and larger breast muscles. To support their larger body mass, turkey toms require a corresponding increase in the size and strength of their skeletal structure. However, contemporary turkey strains are at risk for gait abnormalities and lameness, indicating that the skeletal structure may not have been proportionally favored by genetic selection that was focused solely on performance traits. This study aimed to quantify the relationship between body weight (BW) and skeletal parameters at different ages in male turkeys. At day of hatch, 400 male Nicholas Select turkeys were randomly divided among 24 pens. A total of 48 and 155 toms without overt gait abnormalities were weighed and euthanized to collect the right tibia and femur at 8 and 18 wk, respectively. Morphological (bone weight, length, and mid-diameter) and biomechanical (peak breaking force; PF) traits were measured for each bone sample. Peak force was quantified using a 3-point bending test (TA.HDPlus, Stable Micro Systems) with a 250 Kg and 750 Kg load cell at 8 and 18 wk, respectively, applied at the rate of 1 mm/sec. The correlations between phenotypic parameters at each age were calculated using the JMP package. Toms weighed 2.9 ± 0.38 kg on average at 8 wk and their BW increased to 17.3 ± 1.9 kg at 18 wk. At 8 wk, BW was strongly correlated with femur weight ($r^2 = 0.75$; $p < 0.01$), while BW was only moderately correlated with the PF ($r^2 = 0.46$; $p < 0.01$). Similar results were observed for the tibia with BW accounting for 62% and 63% of the variability in tibia weight and PF, respectively ($p < 0.01$). At 18 wk, the correlation was quite weak between BW and bone weight (9% for femur and 5% for tibia; $p < 0.05$). Further, BW could only explain 29% of the variability in the PF of the femur ($p < 0.01$) at 18 wk. Interestingly, there was no correlation between BW and tibia PF ($r^2 = -0.03$; $p = 0.75$) in 18 wk old toms. These findings show that market BW is weakly correlated or not related to skeletal traits of turkey toms indicating that heavier toms may not always have the strongest bones. Further, there is a possibility that genetic selection for optimum bone traits may be feasible while having little impact on performance traits in turkeys.

Keywords: Turkey, Tom, Fracture, Leg bones, Peak force

P285 Validating a methodology for broiler bird activity Brenda Hernandez Tapia*¹, Katy J. Tarrant¹ ¹California State University, Animal Science and Agriculture Education, Fresno, California, United States

The usage of novel technology within the industry sets the stage to allow managerial practices to advance, enhancing animal management and welfare. In this study, we aim to understand the ability and limitations of a method to measure bird activity during personnel walkthroughs utilizing EthoVision software within a commercial broiler facility. The purpose of this pilot trail was to observe how total activity could be measured within a commercial size facility that aligns with normal operational procedures - namely walking with and without a bucket used to pick up mortality. Thirty sec walkthrough videos were recorded from a CCTV system installed in the ceiling of a 1486 m² commercial broiler facility housing 20,400 fast-growing broilers, facing in direction the litter; videos were viewed with the Blue Iris v4 software. For each video the same individual proceeded to walk through the video frame in a consistent time manner. Six videos taken at 1 wk of age in which the employee was carrying a bucket in 3 videos and had hands free in 3 videos. This was replicated at 5 wk of age. The videos were recorded at 1630 for 30 sec. Within each video, total activity was quantified using Noldus Ethovision software. Means were separated using a student's t-test. Data show that overall activity level was significantly higher at 5 wk at a quantified mean activity of 4.253 vs 0.655 at 1 wk ($P < 0.0001$). Within the 1wk age, activity level when holding a bucket was higher at an activity mean rate of 0.835 vs 0.475 with no bucket ($P = 0.0042$). Mean activity level did not vary between management practices at wk 5 ($P = 0.4035$). As expected, results indicate that mean activity level increases when a bucket is carried during a walk through. Additionally, activity level does vary between broilers studied at 1 and 5 wk of age. Overall, using EthoVision can analyze activity within a facility, though additional study is needed to better understand the accuracy of these measurements. Size of person walking, bucket being carried, and speed of walking can contribute to a difference in activity levels. Future work will consider how we can analyze broiler activity and managerial practices by only relying on this software.

Keywords: Artificial intelligence, behavior

Pathology

P286 Coinfection effects of *Histomonas meleagridis* and *Salmonella typhimurium* on *Salmonella* colonization, liver apoptotic genes in Turkey Poults Hamid Reza Rafieian-Naeini*¹, Venkata Sessa Reddy Choppa¹, Hemanth Reddy Katha¹, Seshidhar Reddy Gudidoddi¹, Woo Kyun Kim¹ ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States

Histomonas meleagridis (HM) causes necrosis in the ceca and liver of turkeys. However, the mutual interaction of HM and *Salmonella* has not been investigated much. This study aimed to evaluate the effects of HM coinfection challenge with *Salmonella Typhimurium* on the mortality, ceca and liver lesion score, *Salmonella* colonization in ceca and liver, and mRNA expression of apoptotic, cytokine, tight junction, and nutritional absorption genes in the jejunum and liver of turkey poults. A total of 336 one-day-old male turkey poults were randomly allocated into 4 treatments with six replicates. Treatments consisted of NC, negative control; HM, challenged with 10^5 HM/bird at d 18; ST, challenged with 10^8 *Salmonella Typhimurium*/bird at d 8; and HM+ST, challenged with HM and

ST. Bird mortality was recorded daily, liver and jejunum samples were collected on day 28, and ceca and liver lesion score was measured for all of the birds. The data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The results showed that the mortality significantly increased in HM and HM+ST, compared to NC and ST. Additionally, HM and HM+ST had about 10% lower BW compared to NC ($P < 0.05$). All ST challenge groups regardless of coinfection with *Histomonas* had significantly higher *Salmonella* colonization in the ceca compared to NC and HM. Ceca and liver lesion scores in HM challenge groups (HM and HM+ST) were significantly higher than ones in NC and ST. All challenge groups had significantly lower mRNA expression of *CASPASE3*, *BAX*, and *CASPASE8* in the liver compared to NC. However, *JAM-A*, *GLUT2*, and *IL1RN* expression was significantly upregulated in the jejunum of HM+ST compared to NC. In conclusion, *Histomonas meleagridis*, regardless of co-infection with *Salmonella Typhimurium*, has a severe impact on turkey poults' body weight, mortality rate, and lesion scores in the ceca and liver. However, *Histomonas* does not exacerbate *Salmonella Typhimurium* colonization in the ceca and liver.

Keywords: Histomonas, Salmonella, Mortality, Lesion score

P287 Effect of an herbal product (Vermi) on the embryonation and eggshell integrity of *Heterakis gallinarum* Lillie Romines*¹, Catherine Fudge¹, Dan Gray², Thilo Borchart², Chongxiao Chen¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²DOSTOFARM GmbH, Westerstede, Germany

Heterakis gallinarum is a common cecal worm of Galliformes and is the paratenic host for *Histomonas meleagridis*, the protozoan parasite known to cause histomoniasis (blackhead disease). Control of *H. gallinarum* is one of the keys to controlling histomoniasis. *H. gallinarum* eggs can survive in the environment for up to 5 years and remain infective. Control methods currently focus on the adult nematode, but no treatments are available to reduce egg viability. In this study, the effects of an herbal product (Vermi) against *H. gallinarum* eggs were examined. Gravid female *H. gallinarum* was collected from the ceca of spent broiler breeder hens. Approximately 50 eggs from the dissected oviducts were pipetted into each well of 24-well plates. A total of 8 levels of the product were tested: 0 (control), 125, 250, 500, 1,000, 2,000, 4,000, 8,000, and 16,000 ppm Vermi at 6 incubation time points: 12 hours, 24 hours, 2 days, 7 days, 14 days and 21 days. Each treatment and time point consisted of 6 replicates. At 21 days, eggs were examined under a microscope for embryonation. To determine eggshell integrity, eggs were exposed to 200 μ L of 0.4% trypan blue for 2 minutes and subsequently washed. Clear eggs (intact) and eggs stained blue (compromised) were counted under a dissection microscope. Results were analyzed using one-way ANOVA, JMP pro16, and Dunnett's test to compare each mean to the negative control with a significance of $P \leq 0.05$. *Heterakis* egg embryonation was reduced by 12h treatments of 125 ppm Vermi, 24h treatments of 125, 250, 500, and 1,000 ppm Vermi, 2d treatments of 125 ppm Vermi, 14d treatments of 250 ppm Vermi, and 21d treatments of 125 ppm and 250 ppm Vermi ($P < 0.0001$). Eggshell integrity was compromised at 7d treatment of 2,000, 4,000, 8,000, and 16,000 ppm Vermi, 14d treatments of 500, 1,000, 4,000, 8,000, 16,000 ppm Vermi, and 21d treatments at levels above 125 ppm ($P < 0.0001$). These data suggest that Vermi affects the embryonation of *H. gallinarum* eggs as early as 12 hours and is more effective at lower dosages. Meanwhile, most dosages above 500 ppm impact *H. gallinarum* eggshell integrity with treatment durations exceeding 7 days. Further studies should focus on the interaction between embryonation and eggshell integrity and its effects in vivo.

Keywords: *Heterakis gallinarum*, histomoniasis, embryonation, eggshell integrity

P288 Effect of an herbal product (Vermi) against *Histomonas meleagridis* in vitro Romel Ortiz*¹, Catherine Fudge¹, Dan Gray², Thilo Borchart², Chongxiao Chen¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²DOSTOFARM GmbH, Westerstede, Germany

Histomonas meleagridis infects Galliformes and causes histomoniasis (blackhead disease) with high mortality rates in turkeys and, to a lesser extent, chickens. The removal of effective drugs against this disease has left the industry with no control methods and renewed the search for alternative treatments. The current study aims to study an herbal product (Vermi) against *H. meleagridis* in vitro. A total of 8 levels of the product were tested: positive control (PC, 12.5 ppm dimetridazole), 0 (NC, negative control), 125, 250, 500, 1,000, 2,000, 4,000, 8,000, and 16,000 ppm Vermi. The treatments were added into flasks containing 100,000 histomonads in 10 mL of culture media and incubated at 42 °C for 48 hours. *H. meleagridis* was counted 3 times per flask

using a hemocytometer to evaluate the inhibitory effects of treatments. 5 mL of culture from each flask was taken and spun down at 500 g for 1 minute to remove *H. meleagridis*, and the remaining supernatant was spun at 4,000 g for 10 minutes to form a bacterial pellet. The pellets were then homogenized in 5 mL of distilled water, and bacterial suspensions were measured at OD600 on a spectrophotometer to determine the bacterial cell density in the culture. Results were analyzed using one-way ANOVA, JMP pro16, and Dunnett's test to compare each mean to the NC with a significance of $P \leq 0.05$. Vermi at 8,000 ppm and 16,000 ppm for 48 hours led to no live *H. meleagridis* in the culture, similar to the PC group. 1,000, 2,000, and 4,000 ppm levels reduced *H. meleagridis* by approximately 95% ($P < 0.0001$) compared to the NC. However, 125, 250, and 500 ppm of Vermi did not significantly reduce *H. meleagridis* counts. Bacterial density was around two times higher at 2,000, 4,000, and 8,000 ppm compared to the NC, while 125, 250, 500, 1,000, and 16,000 ppm did not alter bacterial density ($P < 0.0001$). In summary, Vermi at more than 1,000 ppm directly inhibited *H. meleagridis* without affecting the bacteria level in the culture (indirect effects). Further studies are needed to investigate the median lethal dose, as well as the effects of Vermi in vivo, to characterize its anti-histomonal activity.

Keywords: blackhead disease, In vitro, *Histomonas meleagridis*, phytochemicals

P289 Genotyping *Salmonella* spp. strains of avian origin through Oxford Nanopore Technologies Tirado Agreda Daniel Wagner*¹, Maria Belen Cevallos Almeida¹, Maria Ines Baquero Cardenas¹, Freddy Proaño Pérez¹, Carlos Gomez¹, Francis Xavier Acosta Villafuerte¹, Vallejo Xavier Gerardo Apolo¹, Natalia Naidelyn Muentes Buenaventura¹, Marco Cisneros Tamayo¹ ¹Universidad Central del Ecuador, Facultad de Medicina Veterinaria y Zootecnia, Quito, Ecuador

Third-generation sequencing (TGS) by Oxford Nanopore Technologies (ONT) stands out for its real-time long-read genotyping capability, providing rapid and accurate results. This greatly enhances the diagnosis of *Salmonella* spp. and aids in decision-making within the poultry sector, establishing itself as a valuable support tool for veterinarians. The main objective of this descriptive qualitative study is the genotyping of avian-origin *Salmonella* spp. strains using ONT sequencing and subsequent bioinformatic analysis. Four *Salmonella* spp. strains from the Bacteriology and Mycology Laboratory collection at the Faculty of Veterinary Medicine and Zootechnics, Central University of Ecuador (UCE), were used. DNA was extracted using the commercial "Wizard® SV Genomic DNA Purification System" kit, following the manufacturer's instructions. Quality control of the DNA was then conducted by assessing its concentration and purity. Sequencing was performed with the MinION Mk1C device and the FLO-MIN106 R9.4.1 flow cell. At the end of the process, output files in "Fastq.gz" format were generated and used for bioinformatic analysis on the Galaxy Europe platform, applying specific workflows and tools. Using MLST v2.22.0, SeqSero2 v1.1.0, and SISTR v1.1.1 tools, the serotypes *S. Typhimurium*, *S. Infantis*, and *S. Corvallis* were identified. Additionally, the ResFinder v4.5.0 tool detected resistance genes for sulfonamides, aminoglycosides, quinolones, beta-lactams, tetracyclines, phenicols, nitrofurans, macrolides, and fosfomicin. In conclusion, precise identification of serotypes and antimicrobial resistance genes in each *Salmonella* spp. strain was achieved, showing consistency with previous regional studies. These findings highlight the applicability of Oxford Nanopore technology as a versatile and efficient tool in the veterinary field, enabling detailed pathogen analysis and supporting informed

decision-making in the diagnosis, treatment, and control of infectious diseases of public health importance.

Keywords: ONT, Salmonella spp., Sequence of nanopores MinION, Bio-informatic tools, Genotyping

P290 Novel sequencing-based method for *E. coli* O-Serogroup typing enhances poultry disease surveillance Sunita Shrestha*¹, Linan Jia¹, Chuan-Yu Hsu³, Mark A Arick II³, Kelsy Robinson², Li Zhang¹ ¹Mississippi State University, Department of Poultry Science, Starkville, Mississippi, United States; ²USDA, Agriculture Research Service, Poultry Research Unit, Starkville, Mississippi, United States; ³Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, Mississippi, United States

Avian pathogenic *E. coli*, especially serotypes O1, O2, and O78, cause significant economic losses in the poultry industry due to decreased productivity and increased mortality. Early and accurate detection of these infectious serotypes is critical for controlling their transmission. This research aims to develop an accurate sequencing-based O-serogroup typing method for *E. coli* using the Oxford Nanopore Technologies (ONT) platform. We targeted the O-antigen biosynthesis gene clusters (O-AGCs), which exhibit a wide size range from 10 kbp to 30 kbp in *E. coli*. Twelve *E. coli* isolates with O-types previously determined from whole-genome sequencing were selected, comprising nine reference strains and three field strains with O-AGCs ranging from 10 to 21 kbp. Genomic DNA was extracted, and O-AGCs were amplified by PCR using the specific primers. Native barcoded amplicon libraries were prepared and sequenced with Flongle flowcell on a GridION sequencer. The sequences were compared to reference O-AGC sequences using minimap2. Z-tests were performed using the mean depth and coverage of the O-AGC sequences for each barcode to find any sequence significantly (BH adj. P-value ≤ 0.05) above the baseline similarity. Each barcode had a single serotype found to be significant in the alignments, with all samples except one being correctly typed. The novel ONT-based O-typing method demonstrated high reliability for serotype identification in *E. coli*. Further optimization and validation with a larger sample size are required to enhance the method's robustness and applicability. Successful validation of this method could enable early detection of pathogenic strains in poultry, supporting rapid intervention and risk reduction for poultry stakeholders.

Keywords: Serotype identification, Oxford Nanopore Technologies, Avian pathogenic *E. coli*, O-antigen biosynthesis gene cluster, epidemiological surveillance

P291 Phylogenetic characterization and genotyping of non-S1 Avian Reovirus Genomic Segments Vicente A. Avila-Reyes*¹, Kelsey T. Young³, Holly Sellers², Morgan L. Cunningham¹, JoAnna Bowers¹, Crosby Casto¹, James Stanton¹ ¹University of Georgia, Pathology, Athens, Georgia, United States; ²University of Georgia, Population Health, Athens, Georgia, United States; ³Virginia-Maryland College of Veterinary Medicine, Biomedical Sciences and Pathobiology, Blacksburg, Virginia, United States

Avian orthoreoviruses (ARV) are ubiquitous among multiple avian species worldwide, and show a wide range of strain pathogenicity, with some resulting in significant health issues. Although reoviral disease can be of concern for different bird populations the most affected and economically important are poultry. Diagnosis of ARV is difficult due to the genomic reassortment permitted by its segmented genome. The lack of full-genome sequencing and characterization has also hindered understanding the genomic determinants of pathogenesis. While

genotypic classification is currently based on the sC gene (on S1 segment) and this gene's product is an important determinant of pathogenicity, data suggest other segments (e.g., M2) may contribute to pathogenicity. Thus, expanding the phylogenetic knowledge will improve the understanding avian reovirus disease, viral diversity, ancestral relationships, potential new variants, as well as identification of genetic clusters. The objective of this study was to create a genotyping scheme for all non-S1 ARV segments using available ARV genome data. ARV genome segments were downloaded from GenBank, and aligned (ClustalO) by segment. Maximum likelihood phylogenetic analyses were performed using the IQTREE software with an automated fast best model selection (ModelFinder) for accurate phylogenetic estimates, and an ultrafast bootstrap approximation for assessing branch support (1000 ultrafast bootstraps). Previously published genotyping criteria were applied to the M and L segments. In all segments, occasional individual sequences failed to fall within genotypic clusters, representing potential additional genotypes. In all segments, turkey and duck isolates frequently clustered within the same genotype. The results demonstrate the ability to genotype all segments of the ARV genome, which can then be used in conjunction with full genome sequencing to better understand reassortment and genetic determinants of pathogenicity within ARV.

Keywords: Avian orthoreovirus, ARV phylogenetics, genotyping, IQ-TREE

P292 Detection of pathogenicity factors in field-isolated *Enterococcus cecorum* strains from chickens across different ages Thaciane Amaral*³, Renata Gomes¹, Latasha Gray², Philip Yeboah³, Demilade I. Ibiwoye³, James Higuaita², Aaron Forga², Danielle Graham², Lisa Bielke³ ¹Universidade Federal de Goiás, Escola de Veterinária e Zootecnia, Goiânia, GO, Brazil; ²University of Arkansas, Department of Poultry Science, Fayetteville, Arkansas, United States; ³North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States

Enterococcus cecorum (EC), once a commensal species in the GI tract of chickens, has emerged as a significant pathogen of the poultry industry. Strains isolated from extraintestinal organs have been linked to outbreaks of enterococcal spondylitis, septicemia, and early mortality. Comparative genomic analyses suggest that commensal EC strains may originate from distinct phylogenomic lineages than pathogenic strains, contributing to those clinical outcomes. This study investigated the prevalence of six virulence factors in 26 field-isolated pathogenic EC strains from flocks aged 1 to 85 days, divided into two groups: ≤ 21 days (n=12) and >21 days (n=14). The following key genes were evaluated: ECS3_0196 (CapI gene), ECS3_0200 (NAD-dependent epimerase), ECS3_0662 (Lipoyl synthase), ECS3_2296 (epa M-like gene), ECS3_2299 (epa P-like gene), and ECS3_2316 (LPXTG cell wall anchor domain-containing protein). Isolates were cultured on Tryptic Soy Agar, and DNA was extracted using an Invitrogen PureLink™ Genomic DNA Kit. Gene-specific primers were designed from EC genome sequences (NCBI), PCR amplification run, and products analyzed using 1.5% agarose gel electrophoresis with EtBr staining. Chi-square analysis (JMP Pro 17) was performed to assess the relationship between age and gene presence at $P \leq 0.05$. Prevalence of factors across age groups are as follows: ECS3_0196: 75% in ≤ 21 d and 64.3% in >21 d; ECS3_0200: 83.3% in ≤ 21 d and 78.6% in >21 d; ECS3_0662: 75% in ≤ 21 d and 78.6% in >21 d; ECS3_2296: 100% in both ≤ 21 d and >21 d; ECS3_2299: 66.7% in ≤ 21 d and 57.1% in >21 d; ECS3_2316: 91.7% in ≤ 21 d and 92.9% in >21 d. No significant age-related variations were observed in the expression of

pathogenicity factors among samples, suggesting that early- and late-infection strains may be genotypically related and that infection with pathogenic EC strains can occur as young as one day of age. While EC pathogenesis remains unclear, the consistency of certain virulence markers across groups confirms their role in bird morbidity and mortality. Further research is

needed to clarify clinical outcomes from those strains and develop strategies to mitigate EC infections in poultry.

Keywords: Enterococcus cecorum, lameness, PCR, septicemia, pathogenicity factors

SCAD

P293 Early postbiotic administration regulates the expression of intestinal genes in broilers during subclinical necrotic enteritis Bingqi Dong*¹, Ali Calik², Candice E. Blue¹, Rami A. Dalloul¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Ankara University, Animal Nutrition & Nutritional Diseases, Ankara, Ankara, Turkey

Necrotic enteritis (NE) is a major intestinal disease of poultry caused by the bacteria *Clostridium perfringens* that damages the epithelial lining and compromises its integrity. Intestinal stem cell proliferation is crucial for maintaining gut integrity, which supports its defenses by providing a robust barrier against pathogens. Postbiotics can improve bird performance, intestinal barrier function, and reduce NE-associated pathology. This study evaluated the effects of early postbiotic supplementation in ovo and in drinking water on intestinal stem cells and immune-related 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) were randomly assigned to (n=72; 6 cages/group): 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 d14, 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 d19 and d20. Intestinal tissues were collected to assess mRNA abundance of stem cell markers and immune response genes from 6 birds/group on day of hatch (DOH), d7, d14, and d21. Data were analyzed by student's *t*-test and followed by two-way ANOVA with significance set at $P \leq 0.05$. On DOH, mRNA abundance of LGR5 was significantly greater in the jejunum of postbiotic-supplemented birds compared to NC birds. OLFM4 mRNA level was greater in PIW birds in the jejunum on d7 and ileum on d14. On d14, mRNA abundance of TNF α , iNOS, IFN γ , and IL-10 in the ileum were significantly lower in the PIW groups. No significant interactions in mRNA abundance of stem cell markers were observed in either the jejunum or ileum on d21. However, a significant interaction was observed in mRNA level of TNF α in the jejunum, and iNOS in cecal tonsils between the postbiotic treatment and NE challenge with greater mRNA in PIW+ compared to NC+. In conclusion, early postbiotic supplementation positively influences stem cell activity prior to NE challenge, and regulates immune responses in broilers during a subclinical NE challenge.

Keywords: necrotic enteritis, postbiotic, in ovo, stem cell, immune response

P294 Immune Gene Expression in Cecal Tonsils, Spleen, and Jejunum Tissue During Coccidiosis Infection in Poults Demilade I. Ibiwoye*¹, Philip Yeboah¹, Carissa Gaghan², Thaciane Amaral¹, Shelly Nolin¹, Raveendra Kulkarni², Pradyut Paul³, Kimberly Livingston³, Lisa Bielke¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²North Carolina State University, Population Health and Pathobiology, Raleigh, North Carolina, United States; ³Optum Immunity, Waterloo, Wisconsin, United States

The U.S. turkey industry faces significant challenges from coccidiosis, leading to substantial economic losses due to reduced growth rates, increased mortality, and elevated costs of prevention and treatment. Investigating the immune response of turkey poults to coccidiosis is essential for assessing its impact and devising effective interventions. A promising approach involves using a dried-egg product to modulate the gut immune response, offering potential for improved management and prevention strategies. This study examined the immune response of turkey poults challenged with mixed-species *Eimeria* on day 14, focusing on gene expression in the cecal tonsils, spleen, and jejunum. Three groups were compared: negative control (NC), challenge control (CC), and 300 CBU dried-egg treatment (300 CBU Ct). Genes analyzed were IFN- γ , IL-10, IL-18, and IL1RN expression five days post-infection. Results showed that IL-10 expression in the cecal tonsils exhibited a significant increase in the CC compared to the 300 CBU Ct groups ($p < 0.05$), indicating a heightened anti-inflammatory response in the cecal region to *Eimeria* infection. The increase in IL-10 suggests an effort to regulate local immune responses and potentially mitigate tissue damage caused by the infection. Conversely, IL-18 expression was significantly elevated in the jejunum of the 300 CBU Ct group compared to the CC group ($p < 0.05$), indicating enhanced activation of the innate immune response and potentially improved immune protection. However, IFN- γ and IL1RN expression showed no significant variation across groups, suggesting these cytokines may not play pivotal roles in the localized immune response to *Eimeria* in turkeys. In conclusion, the study demonstrated tissue-specific immune responses in *Eimeria*-infected turkey poults, with notable differences in IL-10 expression in the cecal tonsil and IL-18 expression in the jejunum. These findings suggest that the immune modulation in these tissues plays a critical role in the overall response to *Eimeria* infection and may be influenced by adding dried egg product. Further research is warranted to explore the mechanistic pathways regulating these cytokine responses and their implications for better coccidiosis control in turkey poults.

Keywords: Coccidiosis, immune response, turkeys, *Eimeria*, cytokines

P295 *Eimeria*-induced predisposition of chickens to necrotic enteritis is characterized by increased expression of pro-inflammatory and immunoregulatory genes Matthew Browning*¹, Pok Man Chan², Carissa Gaghan² ¹North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina, United States; ²North Carolina State University, Department of Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, North Carolina, United States

Clostridium perfringens, a Gram-positive, anaerobic and toxin-producing bacterium, causes Necrotic enteritis (NE), an enteric disease of chickens that negatively impacts the economy of the broiler industry worldwide. Although NE has been such an economic burden, no effective vaccines are currently available. This is perhaps largely because of a poor understanding of the host responses during NE. In the present study we used two NE predisposing models, namely the 'dietary' and 'dietary + Coccidia (a mixture of *E. maxima*, *E. tenella* and *E. acervulina*)', to evaluate the mucosal (duodenum and jejunum) and lymphoid (cecal tonsil

'CT' and bursa of Fabricius ('Bursa') immune gene expression in broiler chickens during NE. We also used two virulent strains of *C. perfringens*, Str. CP44 and Str. CP64, to reproduce NE. Results showed that *Coccidia*-predisposition followed by CP44 or CP64 infection induced increased ($P < 0.05$) expression of IL-1 β , 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 ($P < 0.05$) IFN γ 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-1 β /IL-6/IFN γ) in both mucosal and lymphoid tissues, as well as increased immunoregulatory transcription factors such as FoxP3. Further work to devise novel vaccines against NE in chickens is currently underway.

Keywords: Necrotic Enteritis, Chickens, Immune Response, *Clostridium perfringens*, *Eimeria*

P296 Selective pre-enrichment reduces time to confirmed *Salmonella*-positive isolate in poultry preharvest samples Amy T. Siceloff^{*1}, Nikki Shariat¹ ¹*University of Georgia, Population Health, Athens, Georgia, United States*

The current, culture-based method for detecting *Salmonella* is time and resource intensive, as it involves a 24-hour pre-enrichment step in buffered peptone water (BPW), followed by a 24-hour selective enrichment in either Rappaport Vassiliadis (RV) or tetrathionate (TT) broths before streaking onto selective

Salmonella isolation by 24 hours.

Keywords: *Salmonella*, preharvest, selective pre-enrichment

P297 Nanopore amplicon sequencing as an alternative for characterizing *Salmonella* complex serovar populations David Ayala-Velastegui^{*1}, Amy T. Siceloff², Nikki Shariat¹ ¹*University of Georgia, Population Health, Athens, Georgia, United States*; ²*University of Georgia, Population Health, Athens, Georgia, United States*

Salmonella is a foodborne pathogen that causes an estimated 1.35 million illnesses annually in the United States. *Salmonella enterica* is comprised of ~2,600 serovars, but only 20 serovars are associated with 70% of human illnesses. In food animal production systems, including poultry, *Salmonella* can be found in mixed populations of multiple serovars. Therefore, culture-based approaches that rely on selecting a few colonies can underestimate serovar complexity in a sample, which can limit food safety testing. Molecular deep serotyping methods like CRISPR-SeroSeq provide a high resolution of *Salmonella* populations by detecting multiple serovars in a single sample. The objective of this project was to determine if a more rapid deep serotyping approach could be developed using the Oxford Nanopore Technology (ONT) platform. To accomplish this, a database was built containing the sequences of two conserved *Salmonella* virulence genes from 1,121 genomes, representing 36 serovars (including polyphyletic lineages for 10 of these serovars). Total genomic DNA was isolated from three serovars of concern (Typhimurium, Enteritidis, Infantis) and mixed in ratios of 1:10, 1:100, 1:1000 with serovar Kentucky, with the latter as the majority to recreate the most common dynamic in poultry products and environments. The virulence genes were amplified and barcoded with the ONT native barcoding kit. Pooled samples were sequenced with the ONT miniION sequencer. Following basecalling and demultiplexing, the

indicator agar. Previous work has shortened this process by adding novobiocin (Nv) and some selective ingredients from RV (malachite green (MG); 0.1 g/L) and TT (bile salts (BS); 1 g/L) to BPW in parallel, creating an all-encompassing selective pre-enrichment step. This approach was successfully used to recover *Salmonella* from commercial poultry processing samples but had not been applied to pre-harvest samples where the background microflora is significantly higher and can negatively affect *Salmonella* recovery. The objective here was to validate the use of selective pre-enrichment on poultry live production samples. Two pairs of boot sock samples were collected from 23 houses, representing 11 different broiler or breeder farms, for a total of 46 samples. The samples were cultured under two different selective pre-enrichment conditions (Nv + MG, Nv + BS) in parallel with two controls, pre-enrichment in BPW alone and BPW followed by RV and TT selective enrichment. Molecular enumeration was done to quantify the amount of *Salmonella* present. Overall, *Salmonella* was found in 67.4% (31/46) of samples. The selective pre-enrichment and selective enrichment conditions each recovered *Salmonella* in 9/11 farms, and on the house level, *Salmonella* was detected in 65.3% (15/23) and 69.6% (16/23) of houses, respectively; the difference in *Salmonella* recovery between the two methods was not statistically significant ($p = 0.30$, Fisher's exact test). CRISPR-SeroSeq was used to quantify the relative frequency of *Salmonella* serovars in each sample and culture condition, and overall, the identities were conserved between selective pre-enrichment and traditional enrichment methods. These findings suggest that increasing the selectivity of the *Salmonella* pre-enrichment step may eliminate the need for a separate selective enrichment step without compromising the recovered serovar diversity, thus reducing the time required for

amplicons were compared to the database to identify the serovars. Due to the high error rate in ONT reads, 15mer sequences were generated ($n=392$) to reflect serovar-specific SNPs in the virulence genes and sequence reads were screened against these kmers. Serovar proportions were calculated, and no differences were observed between the observed (proportions from long-read sequences) and expected serovar ratios of the different mixes (Wilcoxon Signed-Rank Test, $p=0.8976$). Overall, this study demonstrates that long-read amplicon sequencing can be used to characterize the relative abundance of multiple serovars in a sample.

Keywords: *Salmonella*, poultry, next-generation sequencing

P298 In-vitro screening of turkey host-specific *Lactobacillus* strains possessing immunomodulatory and anti-*Clostridium septicum* properties Carissa Gaghan^{*1}, Sarah O'Flaherty², Ravi Kulkarni¹ ¹*North Carolina State University, Population Health and Pathobiology, Raleigh, North Carolina, United States*; ²*North Carolina State University, Raleigh, North Carolina, United States*

Clostridial Dermatitis (CD) in turkeys is an economically important disease caused by *Clostridium septicum* bacteria. In the current era of 'no-antibiotics-ever' farming, CD incidences are increasing, and unfortunately, there are no effective non-antibiotic means, such as probiotic administration, currently available to prevent CD in turkeys. In the present study, we isolated 16 strains from the family *Lactobacillaceae* belonging to the species of *L. saerimneri*, *L. ingluvies*, *L. animalis*, *L. salivarius*, *L. reuteri*, and *L. vaginalis* from healthy turkey intestines and sought to screen and select those possessing immunomodulatory properties. An in-vitro avian macrophage cell-based assay was used to treat the cells with the 16 strains followed by measuring expression of immune genes (IL-1 β , IL-6, IL-10, TGF β) by real-time PCR, MHC-II (antigen-presenting molecule) expression by

Flow Cytometry, and Nitric Oxide (NO) production by Griess assay techniques. The results showed that the strains NCKL-11 (*L. salivarius*), 22 (*L. reuteri*), and 32 (*L. reuteri*) showed superior immunomodulatory properties based on an upregulation ($P<0.05$) in the expression of anti-inflammatory cytokines (IL-10 and/or TGF β) or decreased ($P<0.05$) expression of IL-1 β and/or IL-6 when compared to the untreated control. Additionally, while all 16 strains induced increased ($P<0.05$) MHC-II surface expression by macrophages compared to the untreated control, NCKL-22 induced higher expression of MHC-II than the other treatment groups ($P<0.05$). Furthermore, the macrophage NO production was higher ($P<0.05$) in cells receiving Str. NCKL-1 (*L. saerimneri*), 2 (*L. reuteri*), 6 (*L. reuteri*), 22, and 41 (*L. reuteri*) compared to the control. Str. NCKL-22 in determining its anti-*C. septicum* property, showed a reduction ($P<0.05$) in bacterial growth inhibition in a blood agar spot test. Collectively, our results to date indicate that it is possible to screen potential probiotic strains possessing immunomodulatory and anti-*C. septicum* properties in-vitro using a macrophage cell-based assay such that efficacious probiotic candidates for in-vivo work can be selected to test their ability to prevent CD in turkeys.

Keywords: Clostridial dermatitis, Turkey, Probiotics, Immunomodulation, Clostridium septicum

P299 Evaluating the impact of a tannin-based feed additive on bird performance and commercial *Salmonella* vaccine recovery in four-day-old broiler chicks William C. Grisham^{*1}, Emily Magee², Luis Muñoz¹, Timothy Cummings³, Kalen Cookson⁵, Phil Stayer⁴, Timothy Boltz¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ³Zoetis, Parsippany, New Jersey, United States; ⁴Dr Phil Stayer Poultry Consulting, LLC, Hattiesburg, Mississippi, United States; ⁵Zoetis, Lawrenceville, Georgia, United States

Salmonella contamination can occur at multiple stages of poultry production. Its prevalence and control are significant concerns within the poultry industry due to bird health and food safety concerns. Various factors, including vaccination status and feed additives, can help manage *Salmonella* prevalence. The current study aimed to determine broiler performance parameters (FI, LWG, FCR) and the relationship between *Salmonella* vaccine recovery in 4-day-old chicks depending on the inclusion of a tannin-based feed additive. Two hundred birds allocated in 20 pens were used between two separate rooms (10 pens per room) based on vaccination status (vaccinated or unvaccinated). Birds were fed one of two mash diets (tannin or no tannin) to create a 2x2 factorial design. Vaccination was performed via ocular and nasal routes following the manufacturer's dosing suggestions. On day 4, intact ceca and liver samples and lung swabs were taken from 5 birds per pen. Intact spleen samples were taken from all 10 birds per pen. All samples were used to determine vaccine recovery and *Salmonella* prevalence. All data was analyzed using SAS with a two-way ANOVA for broiler performance and organ weights, and Chi-square for *Salmonella* prevalence. Significance was set at $\alpha \leq 0.05$. No significant differences were observed between treatments for broiler performance ($P>0.05$). No significant differences were observed for the ceca and spleen weights ($P>0.05$), but a trend was observed for the heavier liver weights ($P=0.1057$) in unvaccinated birds fed no tannins. No differences in *Salmonella* prevalence between all treatments were observed

for spleen samples and lung swabs ($P>0.05$). The *Salmonella* prevalence trend observed in the liver samples ($P=0.0734$) were between the unvaccinated birds fed tannins and unvaccinated fed no tannins. The second trend observed was in the ceca samples ($P=0.0893$) for vaccinated birds compared to unvaccinated birds. The vaccine was recovered via PCR from the organs collected from the vaccinated birds. From these, 100% of ceca, 96-100% of livers, and 72-76% of spleens were positive for the vaccine. Data from this study suggest that inclusion of a tannin-based product does not negatively impact the effectiveness of the *Salmonella* vaccine in broiler chicks.

Keywords: Salmonella, Live Vaccine, Tannin, Prevalence, Broiler

P300 Differences in cellular and humoral immune responses to a primary and secondary immunization with herpesvirus of turkey (HVT) vaccine in layer pullets Allie K. Bowerman^{*1}, Chrysta N. Beck¹, Jossie M. Santamaria¹, Gisela F. Erf¹ ¹University of Arkansas System Division of Agriculture, Center of Excellence for Poultry Science, Fayetteville, Arkansas, United States

In poultry production, chickens are routinely vaccinated with live herpesvirus vaccines for protection from lymphoma causing Marek's disease virus. However, information on the immune responses to these vaccines is limited. This study examined cellular and antibody responses to primary and secondary vaccinations with herpesvirus of turkey (HVT). Six unvaccinated 9-wk-old, and six HVT-vaccinated (s.c. at hatch) 19-wk-old, layer pullets received a first (V1) or a second (V2) HVT vaccination, respectively, by intradermal injection of HVT-vaccine into the pulp of growing feathers (GFs; 10 μ L/GF; 16 GFs/bird). GFs were collected before (0h), and at 0.25, 1, 2, 3, 5, 7, and 10d post-pulp-injection (p.i.) and, blood, at 0, 3, 5, 7, 10, 14, 21, and 28d p.i. To assess leukocyte profiles in GF-pulps, cell suspensions were prepared for immunofluorescent staining and leukocyte population analysis by FACS. ELISA was used to measure plasma levels of HVT-specific IgM and IgG. GF and blood data were analyzed by 2-way ANOVA and 2-way repeated measures ANOVA, respectively, to test effects of vaccine, time, and their interactions, followed by Tukey's HSD tests when appropriate ($P\leq 0.05$ for all tests). Both V1 and V2 recruited leukocytes, primarily lymphocytes, by 1d p.i. ($P\leq 0.05$) with peak levels (% pulp cells) observed by 3d that were higher ($P\leq 0.001$) with V1 (54%) than with V2 (24%) and declined gradually to near 0h levels by 10d. Heterophils and macrophages peaked (4% each; $P\leq 0.05$) at 0.25d with both V1 and V2. However, V1 and V2 differed greatly in recruitment of T- and B-cells, with V1 recruiting much higher levels at 3, 5, and 7d ($P\leq 0.05$). While CD4⁺ T cells dominated the T cell response to V1, with elevated levels from 3 to 10d, CD8⁺ T cells were the most abundant T cells responding to V2, with peak levels on 1 to 3d p.i. ($P\leq 0.05$). Plasma HVT-IgM levels were elevated ($P\leq 0.05$) on 10 and 28d post V1 but did not change post V2. Similarly, HVT-IgG levels increased steadily post V1 ($P\leq 0.05$), reaching near maximal levels by 14d, while V2 did not stimulate a further increase. The temporal, qualitative, and quantitative differences in leukocyte presence at the site of HVT injection post V1 versus V2, suggest development of cell mediated immunity in response to HVT vaccination.

Keywords: herpesvirus of turkey vaccine, immune response, cell-mediated immunity, antibodies, chickens

POSTER ABSTRACTS – NON-COMPETITION

Environment and Management

P301 Orange corn diet decreased serum corticosterone and improved gait, egg production, egg quality and fertility of breeder Pekin ducks exposed to heat stress Esther M. Oluwabenga¹, Evan Rocheford², Torbert Rocheford¹, Darrin Karcher¹, Jenna M. Schober¹, Melanie Bergman¹, Serena Wesley¹, Gregory S. Fraley*¹ ¹*Purdue University, West Lafayette, Indiana, United States*; ²*Nutramaze, West Lafayette, Indiana, United States*

Heat stress (HS) negatively affects poultry making it essential to find mitigation strategies. We hypothesized that orange corn (OC) diet would reduce 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 4 rooms, 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, body weight (BW), feed intake (FI), egg production, egg quality, and welfare assessment were collected weekly before and during HS. Fertility and hatchability were assessed at the end of HS, relative organ weights were collected before and at the end of HS. Data were analyzed with ANOVA, t-tests, and chi-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 drakes' glucocorticoids were observed. Diet had no effect on the heterophil to lymphocyte ratio (HLR), but HS increased HLR at 48 hours ($p < 0.001$). Diet did not affect BW, although HS decreased BW in hens ($p < 0.001$), with no effect on drakes. FI was similar between diets, but HS led to a decrease ($p < 0.001$). A higher percentage of ducks exhibited poor gait in the YC treatment at weeks -1 ($p < 0.05$), 2 ($p = 0.07$), and 3 ($p = 0.07$), while poor feather quality was more common in the OC treatment at week -2 ($p = 0.08$). Egg production was higher in the OC treatment during the first week of HS ($p < 0.05$). 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. Shell strength was higher in the YC treatment at week 1 ($p < 0.05$). Fertility was higher in the OC group ($p = 0.07$), although hatchability was not different. No diet effects were observed on relative organ weights, however, HS reduced relative liver weight in hens ($p < 0.001$), with no effect on drakes. OC diet may help alleviate the effect of HS in breeder ducks.

Keywords: orange corn, carotenoid, climate change, poultry

P302 Investigation of poultry manure removal efficiency and volume estimation on grooved-floor panels Siya Chen*², Yu Wang¹, Gregory Archer¹, Ziteng Xu¹ ¹*Texas A&M University, Poultry Science, Bryan, Texas, United States*; ²*Texas A&M University, College Station, Texas, United States*

Litter flooring is primarily used in cage-free broiler production to absorb moisture and excreta. Management of litter materials in poultry production is labor-intensive. Poorly managed litter material leads to welfare issues such as respiratory disorders and contact dermatitis. To address these issues, we aim to investigate the feasibility of collecting poultry manure and assessing its volume from non-porous grooved floor panels that are designed to reduce the direct contact between the birds and the manure. The experiment was conducted for 2 days. Each day, 14 grooved-floor panels were placed underneath layer cages for 18 and 24 hours to

collect manure samples. A mobile platform equipped with an electric cylindrical brush was developed and tested for manure removal efficiency at 6 cm/sec. An algorithm was developed to compute manure volume using point cloud data collected before and after the manure removal operations. Results indicated that $89.95 \pm 2.16\%$ of the collected manure was removed by the platform. According to ANOVA tests, no significant difference in cleaning efficiency was found between two or three birds ($p > 0.05$). Also, no significant difference was observed in manure residuals (9.6% for Day 1, 10.53% for Day 2) between two consecutive days. This suggests that despite the system not being able to collect 100% of the manure, the system still provides a highly thorough cleaning, the small amount of residual left is minimal and should not affect the overall efficiency, or exceed the floor's manure-holding capacity. In addition, linear regression analysis showed a strong correlation between manure weight and volume ($R^2 = 0.94$). In the analysis of variance, significant differences ($p < 0.05$) in manure weight and volume were found between 2 and 3 birds/cage. These findings suggest that the developed method can accurately estimate manure weight, and the distribution of manure weight may provide spatial assessments of birds' stock density and potentially detect dead birds in caged systems. Overall, this study demonstrated a method to remove manure from grooved floor surfaces, which is a critical component for moving away from the litter floor system. The developed algorithm for manure volume assessment can provide accurate estimations of manure weight.

Keywords: Manure Management, Machine Vision

P303 Analysis of Nine Laying Hen Production in Cage and Cage-Free Environments: 41st North Carolina Layer Performance and Management Test Ishab Poudel¹, Taylor O'Leary Reid¹, Becca Wysocky¹, Kenneth Anderson*¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States*

The 41st North Carolina Layer Performance and Management Test was initiated to provide an unbiased assessment of current white and brown egg strains available to egg producers. This current study has 9 strains in conventional cages and enriched colony systems, collecting data on hen-day egg production (HDEP), hen-housed egg production (HHEP), feed intake (FI), feed conversion ratio (FCR), egg quality parameters, and mortality from 17 to 69 weeks of age. The egg quality parameters measured were egg weight (EW), shell color, albumen height (AH), haugh unit (HU), yolk color (YC), eggshell breaking strength, and vitelline membrane strength. 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 $P < 0.01$, with means separated using Tukey's HSD test. Results showed significant differences among white-egg strains in both cage and cage-free systems for HDEP and HHEP ($P < 0.01$). In cages, the Shaver strain had the highest HDEP (93.0%) and HHEP (91.5%), while the W-36 had the lowest (82.5% and 80.6%, respectively). Feed intake and FCR varied significantly among strains. Egg quality parameters like EW, AH, and HU also differed significantly ($P < 0.01$) among strains in both housing systems. In cages, the Babcock strain exhibited the highest egg weight (63.07 g), AH (8.95 mm), and HU (93.05), significantly surpassing other strains. The Tetra-White and W-36 strains had lower EW and AH. In cage-free systems, the Shaver strain showed the whitest shell with a reflectance value of

79.51 and AH (8.59 mm). For brown-egg strains, significant differences were observed in feed intake and FCR in cages ($P < 0.01$). In cage-free systems, HDEP and HHEP differed significantly among strains ($P < 0.01$ and $P < 0.01$, respectively), with Bovans-Brown showing higher production rates. Significant differences in production and egg quality traits among strains underscore the importance of selecting appropriate strains to optimize productivity, aiding North Carolina egg producers in making informed decisions on strain selection and management practices to enhance efficiency.

Keywords: Housing Condition, Strain Selection, Egg Production, Egg Quality

P304 Accessing utilization of platform enrichments in broilers by introducing the platforms at different ages Jose Millan*¹, Katy J. Tarrant¹ ¹*California State University, Fresno, Fresno, California, United States*

Introducing environmental enrichments that increase environmental complexity can benefit poultry welfare. Past research has shown that broilers in commercial houses utilize platform enrichments of different designs at different rates yet, little is known about the utilization rate of the platform enrichments if introduced at different ages. This study aimed to evaluate the interaction levels between broilers and platforms by introducing the platform at different ages in a commercial broiler facility. Platforms were introduced equal distances from waters and feeders before placement, day 7, 21 and 35. These 4 platforms were all placed next to each other and under one camera. A picture was taken automatically every day at times 6:30, 9:30, 13:30, 17:30 and 21:30. This process was repeated in two more cameras, giving us a total of 3 replicates for each control. The number of birds using the platforms, active and inactive on the platform and around the platform were counted and used for a statistical analysis. An ANOVA was performed and means were separated using Tukey HSD in Rstudio. Platforms placed at day 7 had the highest bird usage, activity in the platform and inactivity in the platform with a mean of (6.54 ± 4.88 , 1.43 ± 1.67 and 5.10 ± 3.99). While platforms placed at day 35 had the lowest bird usage, activity in the platform and inactivity in the platform with a mean of (2.46 ± 1.11 , 0.574 ± 0.709 and 1.90 ± 1.08 ; $P < 0.0001$). However, platforms placed at day 35 had the highest birds around the platforms with a mean of (11.6 ± 2.89), while platforms placed at day 7 had the lowest with a mean of (8.58 ± 3.41 ; $P < 0.0001$). All means between the 4 platforms were significantly different in number of birds using the platforms and birds inactive on the platform ($P < 0.04$). The difference between means in the birds around the platform and active in the platform were also significantly different ($P < 0.03$), except for the means between platforms placed before placement and day 7 ($P > 0.05$). While birds around the platform for platforms placed at day 35 showed the opposite results from the rest of our study, it is important to note that this could be due to the birds getting bigger and filling more space of the house.

Keywords: environmental complexity, broiler, environmental enrichment, production system

P305 Standardizing microbiome practices of poultry for practical application Jean E. de Oliveira*¹⁰, Diana Ayala¹, Sami Dridi², Daniel Grum¹, Tim Johnson³, Jannigje G. Kers⁴, Michael Kogut⁵, Joshua M. Lyte⁶, Joshua Rehberger⁷, Mitiku Seyoum², Alexandra H. Smith⁷, Guolong Zhang⁸, Monika Proszkowiec-Weglarz⁹ ¹*Land O'Lakes, Purina Animal Nutrition Center, Gray Summit, Missouri, United States*; ²*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States*; ³*University of Minnesota, Veterinary and Biomedical Sciences, Saint Paul,*

Minnesota, United States; ⁴*Utrecht University, Faculty of Veterinary Medicine, Utrecht, Netherlands*; ⁵*Agricultural Research Center USDA-ARS, Southern Plains, College Station, Texas, United States*; ⁶*Agricultural Research Service, USDA, Poultry Production and Product Safety Research, Fayetteville, Arkansas, United States*; ⁷*Arm and Hammer, Animal Nutrition, Waukesha, Wisconsin, United States*; ⁸*Oklahoma State University, Department of Animal and Food Sciences, Stillwater, Oklahoma, United States*; ⁹*USDA-ARS, Animal Biosciences Biotechnology Laboratory, Beltsville, Maryland, United States*; ¹⁰*Cargill R&D Centre Europe, Core R&D Nutrition, Vilvoorde, Belgium*

Poultry intestinal microbiome information can provide valuable insights to understanding performance, digestion, health, and management of broilers, layers, and breeders. Microbiome is frequently included in academic and commercial research, but its interpretation is seen as complex, and practical application is often unclear. It is difficult to compare results from different publications, limiting building of knowledge on the field. Considering that 16S rRNA gene amplicon sequencing is the most frequently employed method to characterize poultry microbiota taxonomic composition and diversity, there is a need for standardization of this experimental workflow. In 2023, a panel of microbiome specialists from academia, industry and government was formed with the goal to propose guidelines for poultry microbiome research, similar to STORMS guidelines for human studies. One key aspect addressed is study design, which should match its purpose such as observational cohort study or cross-sectional or case control experiment. Each option will change the required sample size. To demonstrate the effect of sample size, a broiler ceca microbiome dataset from birds fed two diets and 35 samples per group was used. Power was calculated as the fraction of time the null hypothesis was rejected over 100 repetitions of the test and $p > 0.01$. Results for diversity (power=0.80) showed that Shannon index and Bray-Curtis metrics are the most sensitive indices to find differences, requiring between 12 and 15 samples. Another approach used a multi-kernel machine learning classification model. It started with 100 samples in each class and reduced the number of samples by randomly removing one at a time until model accuracy, after 100 permutations, dropped below 85%. Results showed that a minimal of 14 individual samples are needed to maintain model accuracy. Ileum and cloaca swabs or fecal samples required higher number of samples compared to ceca content or droppings. Based on this effort, guidelines for the appropriate design, corresponding sample size, and a standardized protocol for 16S sequencing, library construction and bioinformatics will be published soon. These guidelines will be instrumental for poultry research and subsequent practical application of microbiome-based insights

Keywords: microbiome, guidelines, method, sampling, analysis

P306 The impact of group size and spiking on fertility and hatchability in broiler breeder flocks Patricia Quino*¹, Marcela Quino¹, Madison Berger¹, Kristian Almendares¹, Olivia McGuire¹, Nicole Dundur¹, Emsho Alvarado¹, Bethany Baker-Cook¹, Charlene Hanlon¹ ¹*Auburn University, Department of Poultry Science, Auburn, Alabama, United States*

Reproductive efficiency in broiler breeders is a critical factor for maximizing broiler production. Fertility and hatchability rates are influenced by numerous variables, including male-to-female (MF) ratio, group size, spiking, and flock age. Therefore, this study aims to determine the influence of group size and spiking on fertility and hatchability rates. A total of 396 Ross 308AP female and 64 Ross 344 male broiler breeders were distributed among large pens (L; 15'x6') and small pens (S; 4'x6') at 20 weeks of age (woa). Birds were randomly allocated into 4 treatments (n=4

pens/treatment): L with spiking (LY), L without spiking (LN), S with spiking (SY), and S without spiking (SN). Birds were photostimulated at 21 woa. All pens maintained a 1:9 MF ratio with identical stocking densities, resulting in 4 males in L and 1 male in S. L was reduced to 3 males at 32 woa to maintain industry MF ratios. At 45 woa, the single male in SY was replaced with a spiker male, while in LY, one of the three males was exchanged. Eggs were collected per pen over 3 days at 30, 40, and 50 woa and stored in an egg cooler (20°C and 65% relative humidity; RH). Eggs were set in a single-stage NatureForm 1080 incubator (37.5°C and 65% RH). Eggs were candled at transfer (embryonic day 18) to assess fertility and embryonic mortality (EM). A three-way ANOVA using a linear mixed model in SAS v9.4 included age, group size, and spiking as fixed effects. Fertility rates declined with age ($P<0.01$) and smaller group size ($P<0.01$). There was also an interaction ($P<0.01$), with L exhibiting higher fertility rates than S at 40 and 50 woa. While there was no effect on early-EM, mid-EM was affected by group size ($P<0.05$), as L had higher mid-EM rates than S. Late-EM increased with age ($P<0.01$), with rates at 50 woa (20.8%) higher than 30 (9.6%) or 40 (8.3%) woa. Group size also affected late-EM ($P<0.01$), as S displayed higher EM at this stage than L. An interaction between age and group size ($P<0.01$) demonstrated that while L demonstrated increased late-EM at only 50 woa, S had a higher late-EM rate than L at all ages. As birds age, fertility declines and late-EM increases, particularly in S. These findings underscore the critical role of age and group size in influencing reproductive efficiency.

Keywords: Fertility, Broiler Breeders, Spiking, Hatchability, Embryonic Mortality

P307 Impact of Restricted Feeding Regimens on the Fertility and Hatchability of Broiler Breeder Flocks Kristian Almendares^{*1}, Kristin Diehl², Brenda Flack³, Madison Berger¹, Patricia Quino¹, Olivia McGuire¹, Charlene Hanlon¹ ¹Auburn University, Department of Poultry Science, Auburn, Alabama, United States; ²USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Maryland, United States; ³Cobb-Vantress, Siloam Springs, Arkansas, United States

With rising demand for chicks from the broiler industry, broiler breeder management focuses on reproductive performance and chick quality through strategies like optimized feeding regimens (FR). This study aims to determine the impact of pullet and cockerel FR on fertility, hatchability, and chick quality. At 3 weeks of age (woa), 90 Cobb 500 FF pullets ($n=30/FR$) and 9 Cobb male ($n=3/FR$) broiler breeder cockerels were randomly placed in individual cages, and at 4 woa, birds were assigned to one of the three treatments: (1) daily control diet (CON), (2) skip-a-day control diet (SKIP) or (3) daily high-fiber diet (ALT). At 19 woa, SKIP birds moved to a daily allocation. Birds were reared under an 8L:16D photoperiod and photostimulated at 21 woa. Artificial insemination (AI) was conducted weekly, with all hens receiving 1×10^9 sperm/dose. Eggs were collected 1, 2, 5, 6, and 7 days post-AI (dp-AI) to assess the duration of fertility (DF) at 30, 40, and 50 woa. Eggs were stored in a fertile egg cooler (20°C and 65% relative humidity; RH). All eggs were set in a single-stage incubator (NatureForm 1080; 37.5°C and 65% RH). On embryonic day 18, all eggs were candled, assessed for fertility and mortality, and transferred. On hatch day, quantitative (chick weight) and qualitative methods (Pasgar; PS and Tona; TS) were used to determine chick quality. A subset of chicks was selected for yolk-free body mass (YFBM). Statistical analyses were performed using the MIXED procedure in SAS version 9.4. FR ($P<0.001$) had an effect on fertility, as ALT fertility rates were higher than CON. There were also effects of age ($P<0.001$), dp-AI ($P<0.001$), and an interaction ($P<0.05$) on fertility rates. Eggs collected at 40 woa had

the best fertility rates and DF. However, 30 woa eggs had lower fertility at 1 dp-AI than 2 dp-AI, while 50 woa eggs displayed reduced DF with an earlier decline between 2 dp-AI and 6 dp-AI. Age, FR, and dp-AI did not affect hatch of fertile. There was a trend indicating an effect of FR on TS ($P=0.055$), with ALT chicks scoring higher than SKIP, while PS was affected by age ($p<0.001$), as 40 woa chicks scored lower than those at 30 and 50 woa. This study has demonstrated that different FR can alter fertility and chick quality while hatchability remains unaffected.

Keywords: Fertility, Hatchability, Embryonic Mortality, Feeding Regimens, Chick Quality

P308 The effects of acidifying agents on the growth, antimicrobial resistance, and plasmid genome of *Salmonella* Infantis Sohyun Cho^{*1}, Taejung Chung¹, Gregory Zock¹, Adelumola Oladeinde¹, Michael Rothrock¹, Xiang Li¹, Jodie Lawrence¹, Walid G. Al Hakeem^{1,2} ¹USDA-ARS, Athens, Georgia, United States; ²ORISE, Oak Ridge, Tennessee, United States

Salmonella Infantis has become one of the main serotypes found in chicken products in the US and across the globe. *S. Infantis* strains that harbor the pESI plasmid are prevalent in litter samples from broiler farms, indicating that pre-harvest management practices cannot efficiently eradicate *S. Infantis*. The objective of this study was to evaluate if acidifying agents that are extensively used to acidify litter and water for poultry house ammonia control and pathogen reduction have a deleterious effect on the survival and growth of *S. Infantis*. We also investigated if there are fitness effects of pESI on *S. Infantis* when grown in the presence of acidifiers by comparing the plasmid-carrying strains with the plasmid-free strains. When *S. Infantis* was exposed to sodium bisulfate (SBS) and acidified copper sulfate (ACS) at levels recommended by the manufacturers, there was a bacteriostatic effect, but when exposed to ACS at double the recommended concentration, *S. Infantis* growth was totally suppressed. This suggests that SBS and ACS prepared at the recommended concentrations to be used in the poultry industry do not prevent the growth of *Salmonella*. When the pESI-positive strains and pESI-negative strains were cultured under various conditions of acidifiers and pH, there were no statistical differences in the maximum growth and lag phase time ($P > 0.05$), using the Kruskal-Wallis test, followed by Dunn's multiple comparison test, indicating that there was no fitness cost or advantage to a pESI carriage. However, when exposed to SBS at pH of 4, both the strains exhibited reduced growth with a significantly long lag phase time ($P < 0.05$). In addition, changes in antimicrobial resistance phenotypes and pESI plasmid genome compositions were detected in pESI-carrying isolates; they lost either sulfamethoxazole (SOX) or trimethoprim-sulfamethoxazole (SXT) resistance phenotypes as well as the genes on the plasmid that were associated with SOX and SXT resistance. The media containing SBS posed a selective pressure on *S. Infantis* to drop SOX and SXT resistance, and further studies are needed to determine why SOX or SXT resistance and the *sulI* or *dfrA14* genes were lost in the presence of SBS.

Keywords: Salmonella, acidifying agents, antimicrobial resistance, plasmid, fitness

P309 Could a specific blend of essential oils and oleoresins of spices be part of a nutritional strategy to lower broiler feed environmental footprint? Guillaume Desrousseaux¹, Ivan Girard², Alain Autret³, Jean François Gabarrou^{*1} ¹Phodé, Animal Care, Terssac, France; ²Probiotech, QUEBEC, Quebec, Canada; ³DuraConsult, Toulouse, France

Considering that the environmental footprint of broiler production might be reduced, research for alternative diets combined with feed additives is incentivised. This study aimed at evaluating the efficiency of a blend of essential oils and oleoresins of spices on performance and environmental footprint of broilers fed a low specification diet. 288 one day chickens (Ross-308) were randomly distributed into two groups with 6 replicates (6x24 birds) each. They received Coxivac-B at 5 times the normal dose of vaccine on arrival. Control group was fed a standard feed program with normal nutrient specifications: 3003, 3150 and 3024 kcal AME ; 21.5, 19.4 and 18.7 % CP ; 0.55, 0.53 and 0.5% of phosphorus, respectively for starter (day 1 to 21), grower (day 22 to 28) and finisher feed (day 29 to 36)). Oleo group was fed a diet containing 2.5% less energy, amino acids and phosphorus. A plant extract (Oleobiotec Poultry, Laboratoires PHODE, France) was incorporated into feeds at a rate of at 100 g/Mt. Live weight and feed intake at 36 days were considered to estimate the effects of this nutritional strategy on the environment thanks to life cycle assessments according to PEF CR Feed method. Statistical analysis was carried out using the Mixed procedure of SAS® software. No significant difference in mortality was observed during the test (3.52% in average). Live weight at 36 days was increased by 53 g in Oleo group (2622 vs. 2568 g) while feed conversion ratio was decreased (1.672 vs. 1.740). Life cycle assessments of feed formulas in Oleo group compared to those in control to get 1 kg LW showed a lower carbon footprint by 2.24% (1.31 vs. 1.34 kg of CO₂-eq). That represents 9.4 Mt of CO₂-eq saved per year for a 25,000-broiler barn. Water use, fossil resource and eutrophication of fresh water were also reduced by 2.42% (1.21 vs. 1.24 m³), by 4.22% (6.35 vs. 6.63 MJ) and by 6.86% (326 vs. 350 mg P equivalent) respectively. The plant extract feed additive Oleobiotec Poultry allowed a better performance despite a 2.5% reduction of diet specifications. This nutritional strategy appears interesting to cope with the environmental footprint of broiler production. Further analysis needs to be conducted to complete the life cycle assessment of 1 kg broiler LW at farm gate.

Keywords: broiler, life circle assessment, essential oil

P310 Effect of biochar litter treatment on cecal microbiome composition of broiler chickens Kelsy Robinson^{*1}, Stephanie Whitham¹, Joseph Purswell¹, Jeffrey Evans¹, Maryam Mohammadi-Aragh² ¹USDA-ARS, Poultry Research, Starkville, Mississippi, United States; ²Mississippi State University, Mississippi State, Mississippi, United States

Chicks often consume litter either directly from the floor or through contamination of feed which can lead to the spread of gastrointestinal diseases such as necrotic enteritis. Biochar is a carbon rich litter amendment that has been proposed as an effective means to reduce pathogen spread and improve bird health through its ability to decrease microbial loads in litter. In this study, we sought to understand the effect of biochar litter treatment on the total microbial profile of both the litter and broiler cecal content. Broiler chicks were divided into three treatments and reared for 42 days in identical individually enclosed rooms with 44 birds per room and five rooms per treatment. Treatments included reused litter that was either untreated (control) or top dressed with biochar or commercial poultry litter treatment (PLT). At 42 days of age, two litter samples were collected from each room. Litter was obtained from multiple sites throughout the front and back of the room. Additionally, cecal samples were collected from two birds per room. Samples were thoroughly mixed and subjected to DNA isolation. High quality DNA was subjected to V4 16S rRNA sequencing on an Illumina NovaSeq via the bTEFAP® DNA analysis service and analyzed using QIIME2. No difference in

alpha diversity among treatment was observed in the litter samples. However, a significant decrease in species richness was observed in the cecum of broilers reared on biochar treated litter versus control ($P = 0.041$). Similarly, beta diversity analysis found no difference in litter samples but did determine a significant difference in cecum composition between birds reared on biochar treated litter versus controls ($P = 0.028$, $R = 0.165$). Investigation into abundance of specific genera revealed a decrease in *Acetatifactor*, *Enterococcus*, and *Bacteroides* members in birds reared on biochar and PLT treated litter versus control. These results indicate the ability of biochar litter treatment to significantly affect cecal microbiome composition of broiler chickens. While the reduction in *Enterococcus* members is most likely beneficial to poultry health, concomitant decreases in the beneficial *Bacteroides* members is concerning for overall gut health indicating a need for further research.

Keywords: Broiler, Biochar, Microbiome, Enterococcus, Litter

P311 Evolution of Brown breeder pullet hatchability from 2016 to 2023 and influencing factors Caleb M. Marshall^{*1}, Daniel Valbuena², Edgar O. Oviedo-Rondón¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²Hy-Line International, Des Moines, Iowa, United States

Quantitative assessment of reproductive parameters is critical for improvement. This study analyzed pullet hatchability (PH) in commercial brown breeder flocks over 8 years (2016-2023). The dataset included 16,650 observations from 92 flocks across three hatcheries. This data represents 219.4 million eggs set and 88.3 million pullets hatched. The PH was evaluated in relation to flock age (FA), egg storage duration (ES), and short periods of incubation during storage (SPIDES). Eggs stored for less than 7 d were classified as non-SPIDES, while eggs stored for greater than 7 d received SPIDES. The mean ES across all years was 6.81 d ($SD = 3.07$), with non-SPIDES eggs averaging 5.01 d ($SD = 1.32$) and SPIDES eggs averaging 9.89 d ($SD = 2.75$). ES practices were relatively consistent until 2022-2023, when ES increased, reaching 9.68 d ($SD = 4.52$) in 2022 and 9.42 d ($SD = 4.15$) in 2023. A mixed model analysis was conducted in JMP 17, with FA and ES as response surface predictors. Year and SPIDES were fixed effects, while hatchery, flock, and FA nested within the flock were included as random effects to account for variability in management practices. Over the lifecycle of flocks, there was no difference ($P = 0.083$) in average PH between SPIDES (41.14%) and non-SPIDES (41.27%) eggs ($R^2 = 0.61$). For non-SPIDES eggs ($R^2 = 0.58$), year was a significant factor ($P < 0.001$) in PH, with 2016 showing the highest PH (42.54%), whereas PH was lowest in 2022 (40.74%) and 2023 (40.38%). The final non-SPIDES model removed the linear, quadratic, and FA*ES terms, resulting in the equation: $PH (\%) = 31.362 + 0.584*FA - 0.008*FA^2$, with a maximum PH of 43.61% predicted at 37.5 wk FA. In SPIDES-treated eggs ($R^2 = 0.60$), the year effect remained significant ($P < 0.001$), with the highest PH in 2016 (42.01%) and lower values in 2017 (40.63%), 2021 (39.90%), and 2023 (39.94%). The response surface for SPIDES-treated eggs was $PH (\%) = 36.561 + 0.572*FA - 0.913*ES - 0.008*FA^2 + 0.004*FA*ES + 0.024*ES^2$, achieving a maximum predicted PH of 43.80% at an FA of 37.7 wk and ES of 7 d ($P < 0.001$). In conclusion, the PH decreased independently of SPIDES. Quadratic effects of FA described the PH of non-SPIDES eggs, while the PH of SPIDES eggs had quadratic effects of FA, ES, and their interaction.

Keywords: pullet hatchability, data analysis, brown breeders, flock age, SPIDES

Food Safety

P312 Synergistic effects of peracetic acid combined with other oxidizing sanitizers approved for use in poultry meat processing on five serotypes of *Salmonella enterica* in pure culture Elizabeth McMillan*¹, Jaci Hawkins², Eric S. Adams¹, Quentin Read³, Trevor Mitchell², Anthony Pokoo-Aikins², Caitlin Harris¹, Michael D. Hughes¹, Anthony Glenn², Richard Meinersmann¹, Jonathan Frye¹ ¹USDA-ARS, Poultry Microbiological Safety and Processing Research Unit, Athens, Georgia, United States; ²USDA-ARS, Toxicology and Mycotoxin Research Unit, Athens, Georgia, United States; ³USDA-ARS, Southeast Area, Raleigh, North Carolina, United States

Current estimates attribute ~23% of U.S. human salmonellosis cases to consumption of undercooked contaminated poultry products. Effective interventions are needed to reduce *Salmonella* contamination in poultry processing plants. While many investigations have evaluated the efficacy of biocide sanitizers for *Salmonella* reduction, few have investigated these interventions in combination, despite the use of multiple sanitizers in processing. We evaluated the efficacy of peracetic acid (PAA) in combination with three other oxidizing sanitizers: cetylpyridinium chloride (CPC), calcium hypochlorite, and sodium hypochlorite. Serotype specific mixtures (n=5) of *Salmonella* strains were exposed to 2 oxidizing sanitizers in serial, for 15 seconds each. Experiments were performed in 96-well plates which allowed for a gradient of up to 64 different combinations of biocide exposures. Briefly, plates were filled with up to 8 different concentrations of the first sanitizer and then bacterial cultures were exposed for 15 seconds to simulate spray or dip applications. Bacterial cultures were then exposed to the second sanitizer for an additional 15 seconds after which, all chemical activity was neutralized. Plates were incubated overnight, and recovery was determined by a color change due to a pH indicator in the neutralizing media. Results were analyzed using a cumulative logistic mixed model conducted in R. Combined biocide tolerance was variable for all sanitizers tested, regardless of serotype. Tolerance was more uniform, across serotype, to the sanitizer applied first, with the exception of CPC, where serotype I,4,5,12,i had a statistically higher tolerance to PAA when applied after CPC (p<0.01). Responses to PAA in combination with either calcium or sodium hypochlorite were statistically similar across serotype. In general, PAA tolerance was significantly lower when applied after a hypochlorite compound rather than before (p<0.01). Given the variability of results, we concluded that tolerance variability was likely due to a dilution effect of combining sanitizers. We also concluded that, in pure culture, PAA was most effective when added after a hypochlorite compound. Results will be used to design experiments for applications on meat.

Keywords: Salmonella, processing, biocides, efficacy

P313 A computer vision based automatic system for egg grading and defect detection in cage-free facilities Xiao Yang¹, Ramesh Bist¹, Sachin Subedi*¹, Lilong Chai¹ ¹University of Georgia, Athens, Georgia, United States

Egg defects can decrease food quality and market value of laying hen production, especially in cage-free houses where have more floor eggs. Automatic grading and defect detection have been developed using machine vision and image processing technology. Egg weight is also an important aspect of egg quality and market value, with automated egg measurement systems being developed to improve efficiency and accuracy. Previous studies primarily focused on using computer vision techniques for egg classification, but few have combined deep learning and machine vision techniques for joint egg classification and weighing. To

address this gap, a two-stage model was developed based on real-time multitask detection (RTMDet) and random forest networks for predicting egg category and weight. The model uses deep learning and machine learning techniques to perform joint egg classification and weighing. RTMDet was used to extract egg features for classification, and a Random Forest algorithm is used to regress egg weight data based on the extracted features. The results of the study showed that the best accuracy achieved was 94.8% and best R² is 96.0%. Statistical analysis, including model evaluation and validation, was conducted using JMP Pro 16 to ensure the reliability of these results. In addition, the model can be used to automatically exclude non-standard size eggs and eggs with exterior issues (e.g., calcium deposit, stains, and cracks). This detector is among the first models that perform the joint function of egg sorting and weighing. By implementing the findings of this study, the poultry industry can reduce costs and increase productivity, ultimately leading to better quality products for consumers.

Keywords: Cage-free system, egg classification, egg weight, deep learning

P314 In vitro Testing for Effectiveness Against *Salmonella* of Two Natural Products for Litter Application Virginia Baxter*¹, Charles Hofacre¹, Matthew K. Jones¹, Lanny Weaver², Earnie Porta², Neil Teevan² ¹Southern Poultry Research Group, Watkinsville, Georgia, United States; ²Arkion Life Sciences, New Castle, Delaware, United States

The recent U.S.D.A.-FSIS food safety proposed framework document has increased focus on control of *Salmonella* on the farm. It has also classified specific *Salmonella* strains in raw poultry as adulterants. This regulatory shift emphasizes the need for effective on farm treatments for reducing *Salmonella*. Chitosan and essential oil based products have shown promise in reducing *Salmonella* loads, enhancing intestinal health, and optimizing production performance in poultry. The present study evaluated two products, a darkling beetle spray which is a combination of essential oil with a spreader, and a chitosan-essential oil spray for their potential to reduce *Salmonella* growth. *In vitro* testing is an efficient way to evaluate a product's antimicrobial effectiveness prior to application in the poultry house. In this *in vitro* assay, a darkling beetle spray and a chitosan-essential oil spray were tested against *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella infantis*. The bacterial isolates were grown and added into tubes with the test products. Each tube was incubated and plated to XLT4 agar at different time periods over 24 hours while maintaining temperature at 37° C. Negative and positive controls were included in the assay to determine if the product slows or inhibits the growth of the test bacteria. The results were analyzed to evaluate whether the darkling beetle spray and the chitosan-essential oil spray were effective at inhibiting the growth of the test bacteria. In this study, both products inhibited the growth of *S. enteritidis*, *S. typhimurium*, and *S. infantis* as early as 2 hours post inoculation and continued to inhibit *Salmonella* growth to 24 hours. The data was analyzed by ANOVA and the Bonferroni procedure was used to separate groups. These results demonstrated that chitosan and essential oil-based sprays as well as the darkling beetle (essential oil + spreader) significantly reduced *Salmonella* growth. These products may be good candidates for lowering the *Salmonella* loads on broiler litter thus reducing the load of *Salmonella* brought to the processing plant from broiler farms.

Keywords: In vitro, Salmonella, Essential Oil, Chitosan

P315 Local calibrations for the monitoring of nutritional quality of plant protein meals by using Near Infrared Reflectance Spectroscopy A.B.M. Khaleduzzaman^{*2}, Shariful Haque¹, Zobaidul Kabir¹, H.M. Salim² ¹Ministry of Fisheries and Livestock (MoFL), Livestock Unit, Dhaka, Dhaka, Bangladesh; ²Director, Production, Department of Livestock Services, Dhaka, Dhaka, Bangladesh

Due to the strong link between feed and food, the quality of plant protein materials is of public health and food safety concern. The study was to develop local calibrations by using Near Infrared Reflectance Spectroscopy (NIRS) and validation of calibrations for the prediction of nutrient contents in imported plant protein meals in Bangladesh. Soybean meal (SBM), full fat soybean (FFSB), rape seed meal (RSM) and DDGS samples were collected from different locations in Bangladesh during 2022 to 2024. NIRS (MPA II, Fourier transform, Bruker, Germany) spectrum were obtained in duplicate (scanning number 32, resolution 8) with systems monochromator (700-2400 nm) using a Quartz cup sampling device. Multivariate analyses were performed for the development of calibration equations of SBM, FFSB, RSM and DDGS. Data were centered using PLS algorithm and spectral outliers were identified from each calibration. Calibration models were validated by the root mean square error cross validation (RMSECV), ratio of performance to deviation (RPD) and correlation coefficient (R^2) between the measured values determined by wet chemistry vs predicted values of NIRS. The RMSECV for the prediction of moisture (0.253%), protein (0.369%), fat (0.221%), fiber (0.313%) and ash (0.267%) contents in SBM indicating the potentiality of the models for nutritional evaluation. Besides, the RPD values (> 2.5) and R^2 (> 0.82) for the evaluation of SBM were proved the accuracy of the model. Similarly, RMSECV ranged between 0.170%-0.456%, RPD (> 3.00) and R^2 (> 0.91) indicating the efficiency of the model for FFSB. For the nutritional evaluation of RSM, RMSECV ranged between 0.218%-0.543%, RPD (> 2.5) and R^2 (> 0.82) values proved the potentiality of the NIRS model. In evaluating DDGS, the RMSECV ranged between 0.333% to 0.678% and the RPD (> 2.50) and R^2 (> 0.85) proved the efficiency of the model. However, the correlation coefficient ($R^2 = 0.69$) and the RPD = 1.80 might not be suitable for the evaluation of ash contents in DDGS. NIRS could be potential for the monitoring and nutritional evaluation of nutrient contents of plant protein meals except the ash contents of DDGS in which the calibration equation could be improved by including large number of samples in the calibration set for the evaluation.

Keywords: NIRS, Nutritional quality, Feed safety, Plant protein meals

P316 Salmonella Reduction Efficacy and Nutritional Influence of In-Feed Antimicrobial Agents with or without Sodium Bisulfate Matthew K. Jones^{*2}, Charles Hofacre², Virginia Baxter², Roy Berghaus¹, Adam J. Davis³, Juan Suarez⁴, Craig Coufal⁴, Julianna Jespersen⁴ ¹University of Georgia, Department of Population Health, College of Veterinary Medicine, Athens, Georgia, United States; ²Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States; ³University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ⁴Jones-Hamilton Co., Walbridge, Ohio, United States

FSIS has proposed a framework document which will add greater pressure to decrease the load of *Salmonella* on the broiler farm. Based on decades of *Salmonella* experimentation, there is not a “silver bullet” solution to eliminate *Salmonella*. Some feed mill interventions decrease dietary bacteria, but there are questions regarding the degree of reduction, the impact on digestibility, and

the interaction between other ingredients. In this trial, two products proposed to decrease bacterial load were evaluated with and without sodium bisulfate (SBS) in broiler breeder diets. Treatments included a control diet formulated with sodium bicarbonate (SBC), the same diet plus an organic acid product (3 kg/MT) or a formaldehyde product (3 kg/MT), and each of these diets formulated with SBS at 4 kg/MT in place of SBC, for a total of 6 dietary treatments. Each treatment was fed to 5 replicates each containing 4 intact or cecectomized roosters to determine TMEn and amino acid digestibility, respectively. After samples for nutritional analysis were submitted, each treatment was contaminated with *Salmonella* Enteritidis (SE) by meat and bone meal method to achieve a concentration of 10^5 CFU/g of finished feed. Five replicate bags of contaminated feed represented each treatment. On day 0, 1, 2, and 7, SE was enumerated in these samples in duplicate. Log-transformed *Salmonella* CFU were analyzed with a linear mixed model and Bonferroni procedure separated means at a P -value of < 0.05 . Nutrition data was subjected to ANOVA and Tukey's HSD procedure was used to separate means, $P < 0.05$. The SBS diet with formaldehyde had significantly lower SE CFU on day 0 compared to the SBS control. This pattern continued for the remaining timepoints with significant reduction in SE in groups treated with formaldehyde relative to other treatments. By day 7, there was no *Salmonella* cultured from formaldehyde treatments. TMEn was not different between treatments. Arginine and proline had lower digestibility coefficients in the SBS group with the organic acid relative to the SBS control treatment. The choice of sodium-based ingredient had no negative impact on efficacy of *Salmonella* reduction in feed, however, there may be an interaction between some organic acids and amino acid digestibility.

Keywords: Feed Sanitation, *Salmonella*, Digestibility, Sodium Bisulfate, Arginine

P317 Microbial Volatile Organic Compounds (mVOCs) as Early Indicators of Contamination in Incubating Poultry Eggs Stephanie Richter^{*1}, Christopher Heist¹, Olga Kemenova¹, Daniel Sabo¹, Caitlin Harris² ¹Georgia Tech Research Institute, Atlanta, Georgia, United States; ²USDA-ARS, Athens, Georgia, United States

This study aims to identify and characterize microbial volatile organic compounds (mVOCs) produced by *Salmonella* enteritidis (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 embryonic 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. By developing a low-concentration detection technique, we aim to identify contaminated eggs early, enabling 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): 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 PBS or PBS containing SE at 10^3 or 10^5 CFU/mL, and VOCs were collected on days 0, 7, and 14 of incubation using a sorption extraction device in sterile glass jars over 60 minutes. On day 16, embryo viability was assessed via breakout analysis. No SE inoculated yolk eggs were viable while most of 10^2 SE albumen 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. Results reveal that amine- and sulfur-containing compounds are significantly associated with contamination. Principal Component Analysis (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 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: Salmonella, Volatile Organic Compounds, Incubation, Early Detection

P318 Use of a yeast blend product to mitigate *Salmonella* Enteritidis in laying hens Carlos A. Guardado*¹, Pratima Adhikari¹, Fozol Ovi², Theresia Lavergne³, Charlie Elrod³ ¹Mississippi State, Poultry Science, Starkville, Mississippi, United States; ²PantheraVax, Ames, Iowa, United States; ³Natural Biologics, Inc., Newfield, New York, United States

The objective of this study was to evaluate the effect of Cascade (Natural Biologics, Newfield, NY), an all-natural blend of yeast complex carbohydrates and concentrated yeast culture, derived from *Saccharomyces cerevisiae*, on fecal shedding, organ translocation, and egg production in hens challenged with *Salmonella* Enteritidis (SE). A total of 54 hens were completely randomized into three groups, 1) Negative Control (NC; no SE challenge), 2) Positive Control (PC; SE challenge), and 3) Cascade (SE challenge with Cascade at 100g/ton of complete feed). All groups were orally challenged for two consecutive days with approximately 10⁹ colony-forming units (CFU) of nalidixic acid-resistant SE. Fecal samples were collected at 0-, 3-, and 7-days post-inoculation (dpi) and organs were collected at 7-dpi. Samples were pre-enriched in buffer peptone water (BPW) at 37°C for 24 hours. Following pre-enrichment, 1 mL of the sample was transferred to 10 mL of Tetrathionate (TT) broth. Both enriched samples were incubated at 42°C for 24 hours. After enrichment, samples were serially diluted in phosphate-buffered saline (PBS), and 100 µL of each dilution was plated onto XLT4 agar containing 200 ppm nalidixic acid to select for nalidixic acid-resistant SE. Plates were incubated at 37°C for an additional 24 hours, and black-centered colonies were counted to quantify SE concentrations. Data were log-transformed and analyzed using the PROC GLM procedure in SAS 9.4. By 7 dpi, fecal SE shedding was lower (P=0.0269) in the Cascade (0.0939 log CFU/g) and NC (0.0209 log CFU/g) groups compared to the PC (0.1657 log CFU/g) group. At 7 dpi, SE colonization in the Cascade group was numerically lower (P>0.10) in the liver, ovary, and spleen than in the PC group. Although not significant (P>0.10) cecal SE was reduced by 1.019 log CFU/g in the Cascade group (1.594 log CFU/g) compared to the PC group (2.613 log CFU/g). During the post-challenge, the NC group had an egg production rate of 89.2%, while the PC group had 92.4% production. Egg production in the Cascade group was 90.4%. These findings highlight the potential of this dietary intervention as a practical strategy for poultry producers to manage SE risks more effectively, helping to maintain both bird health and production performance.

Keywords: Laying hens, *Salmonella* Enteritidis, *Saccharomyces cerevisiae*, prebiotics, complex carbohydrates

P319 A study comparing vaccination strategies to protect long-lived birds against *Salmonella* Enteritidis and *S. Infantis* using commercially licensed products Kalen Cookson*¹, John Dickson¹, Jon Schaeffer¹ ¹Zoetis, Lawrenceville, Georgia, United States

Inactivated *Salmonella* vaccines are critical to providing long-lasting protection to hens during lay as well as limiting shedding in eggs and passing maternal antibodies to progeny. The goal of this study was to compare how the two inactivated SE vaccine types protect against SE and cross protect against *S. Infantis* (SI) as well as explore the additive effect of including a live ST. Study Design: SPF Female 6-week-old leghorns were raised on litter to 10 weeks of age and then vaccinated by intramuscular injection (IM) according to the following treatments: T01) No vaccine (Controls), T02) SE/E. coli subunit vaccine, T03) SE-ND-IB whole cell vaccine and T04) SE-ND-IB + Live ST. Birds were bled at 16 weeks to measure Biochek *Salmonella* Group B/D serology. At 17 weeks of age, 19 birds per treatment from T01-T04 were comingled and challenged with SE or SI at 10⁹ CFU/bird orally. At 18 weeks, ceca and liver/spleen were collected for Most Probable Number (MPN) enumeration and enrichment for prevalence if negative on MPN testing. All hypotheses were conducted at the p≤0.05 level of significance with the Shaffer simulated method used to adjust for multiple comparisons. Against the SE challenge, all three vaccines gave non-significant reductions in liver/spleen loads and % super shedders. The subunit vaccine did not reduce cecal loads or % super shedders but the two whole cell treatments did with the addition of Live ST having the lowest numbers. Against SI challenge, the subunit vaccines showed no reductions in any tissues. The whole cell bacterin significantly reduced ceca loads but the addition of Live ST consistently resulted in the greatest reductions that were significant in liver/spleen loads (3.4log10), % positive and super shedder liver/spleens, as well as cecal loads and % super shedders. This study showed both commercial inactivated SE vaccines reduced SE loads in liver/spleens but only the whole cell bacterin reduced ceca loads. The whole cell bacterin was also able to reduce SI loads in ceca and the addition of Live ST further reduced ceca loads while also reducing % positive liver/spleens and loads. The combination of whole cell bacterin and live ST providing a level of cross protection to a Group C of human concern is noteworthy.

Keywords: SE, vaccine, colonization, loads, *Infantis*

P320 Evaluation of sensory qualities, consumer acceptance, and microbiological aspects of cold-smoked eggs with oak and cherry wood chips Ahmet Y. Pekel*¹, Alp E. Yildiz², Abdurrahman Kizil¹, Ali Aydin² ¹Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Istanbul, Avclar, Turkey; ²Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Istanbul, Avclar, Turkey

A study was conducted to evaluate the organoleptic characteristics, color, and microbiological quality of eggs cold-smoked with oak and cherry wood chips for 15 min, prior to hard boiling. Fresh medium-sized eggs were randomly collected from those laid by 60-week-old Lohmann Brown hens (n=10). The eggs were positioned inside an airtight chamber to maintain the smoke concentration for 15 min. The wood chips were burnt with the help of a lighter on an external smoke generator and the smoke was collected into a chamber containing eggs. Then, they were hard boiled. A group of panelists (n=10) evaluated the attributes using a 5-point or 9-point scale. Each participant received a set of four coded samples in a balanced, randomized order and was asked to rate each sample. Color was measured using a colorimeter. The surface of each egg was swabbed with a sterile wet swab, and the total populations of total mesophilic aerobic bacteria (TMAB), coliforms, and yeasts and molds were determined. A one-way ANOVA was conducted for normally distributed data, while the Kruskal-Wallis test was used for non-normally distributed data in

SPSS. Appearance liking was lower ($P < 0.05$) for cherry-smoked egg whites and yolks compared to both control and oak-smoked egg whites and yolks. The yolk of the cherry smoked eggs received lower score ($P < 0.01$) for taste than control and oak smoked yolks. Purchase intent was higher for oak-smoked eggs than for cherry-smoked eggs. In cherry smoked egg whites, redness and yellowness increased significantly compared to non-smoked eggs. On the other hand, lightness of the egg whites of cherry smoked eggs were lower than the non smoked egg whites. Egg yolk lightness and yellowness were both significantly lower in smoked eggs compared to control egg yolks. The TMAB level on the eggshell was significantly reduced with the use of cherry smoke compared to control eggs without smoke application, after 8 days of storage at room temperature. In conclusion, eggs smoked with oak chips had higher overall liking characteristics (appearance, texture, and flavor) which were very close to non-smoked eggs when compared to cherry smoked eggs. These data also showed that cherry chips can be used to decrease the overall bacteria count on the eggshell.

Keywords: Smoked egg, Sensory evaluation, Eggshell microbial activity, Cherry wood chips, Oak wood chips

P321 Effect of different supplementation methods of a triple strain Bacillus-based probiotic on Salmonella colonization and carcass contamination in broilers Hanseo Ko^{*1}, Indira Neupane¹, Venkata Prathap Reddy Keshavareddy¹, Antoine Meuter², Meghan Schwartz², Woo Kyun Kim¹ ¹University of Georgia, Poultry Science, Athens, Georgia, United States; ²Novonosis, Milwaukee, Wisconsin, United States

The aim of this study was to evaluate the effect of different probiotic supplementation methods on growth performance, water consumption, and concentrations of *Salmonella* in the ceca and carcass of broilers challenged with *Salmonella* Typhimurium (ST). A total of 408 0-d old male Cobb 500 broilers were randomly allocated into 2×2 factorial arrangement with 6 replicates (17 birds per pen) in a floor pen house and raised for 35 d. The main factors were probiotic supplementation in diet (yes vs. no) and in water (yes vs. no) with a commercial triple strain Bacillus-based probiotic. Dietary probiotic supplementation groups received the diet containing probiotics (2 lbs per ton from d 0 to 7; 1 lbs per ton from d 8 to 35), and probiotic water supplementation groups received water containing probiotics (30 g/1,000 birds/day from d 1 to 7 and d 28 to 35). All birds were orally challenged with ST (1×10^9 CFU/mL) on d 30. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and water consumption (WC) were examined weekly. Cecal ST loads were measured on d 34 and 35. ST loads in carcass rinsate and carcass traits were measured on d 35. All data were analyzed using a two-way ANOVA. Probiotic water supplementation improved BWG by 5.0% from d 1 to 35 compared to the unsupplemented groups ($P < 0.05$). In addition, probiotic supplementation in feed or water improved FCR by 0.07 points from d 1 to 35 ($P < 0.05$). Water supplementation with probiotics significantly reduced ST loads in the ceca and carcass rinsate compared to the unsupplemented groups ($P < 0.05$). The present study revealed that probiotic supplementation in feed or water improved growth performance and carcass characteristics (increase in carcass weight and reduction of abdominal fat) without any adverse effects on FI and WC despite the late-phase ST challenge ($P < 0.05$). Finally, after the ST challenge, reduced ST loads in the ceca at 4 and 5 days post-infection and carcass were found in probiotic water supplement groups ($P < 0.05$). Therefore, the results of this study indicate that probiotic supplementation in water, alone or as a complement to the supplementation in the diet, would be a potential strategy to minimize *Salmonella* contamination in broiler production.

Keywords: Probiotics, Water supplement, Foodborne pathogens, Salmonella Typhimurium, Broiler

P322 Random Forest Modeling to Identify Key Farm to Fork Factors Influencing Campylobacter Level in Pastured Poultry Systems Minh Kim^{*1}, Walid G. Al Hakeem², Michael Rothrock¹ ¹USDA-ARS, Athens, Georgia, United States; ²ORISE-USDA, Athens, Georgia, United States

Campylobacter in poultry flocks poses significant food safety challenges, yet risk factors across different production systems remain unclear. Specifically, the impact of farming and environmental factors on *Campylobacter* dynamics in poultry production is not well established. This study aims to address these knowledge gaps by using random forest models to rank the importance of different factors influencing *Campylobacter* levels in pastured poultry systems. Data were collected from 11 pastured poultry farms in southeastern United States from 2014 to 2017. Two separate random forest models were developed using different sets of predictor variables: (1) 32 farming practices and 26 soil physicochemical constituents and (2) 80 meteorological variables were used. Five sample types were used for modeling: soil, feces, ceca, whole carcass rinse after processing (WCR-P), and whole carcass rinse after chilling and storage (WCR-F) samples. The variable importance was measured based on the mean increase in Mean Squared Error (%incMSE). Soil, feces, and ceca samples showed that farm was the most influential factor (%incMSE=29.2, 58.4, 34.6% respectively, $p < 0.01$ for all). These samples consistently predicted Farms A and B as having higher levels of *Campylobacter*. WCR-F samples also indicated farm was the most influential factor (%incMSE=25.1%, $p < 0.01$). However, WCR-F samples predicted higher levels in Farms E and J, indicating that preharvest conditions do not necessarily correlate with postharvest samples due to processing interventions. WCR-P samples indicated rinse water was an important factor (%incMSE=25.9%, $p < 0.01$), with organic acids-treated rinse water having the lowest level. The second model, focusing on meteorological factors, revealed that maximum humidity two days prior to sampling (%incMSE=20.1%, $p < 0.01$) was the most important predictors for soil samples. For feces samples, the daily temperature range three days prior to sampling (%incMSE=27.4%, $p < 0.01$) was the most influential factor. This study provides valuable insights into the complex dynamics of *Campylobacter* in pastured poultry systems, identifying key predictors. Future research should focus on developing targeted control strategies that account for identified key factors.

Keywords: Microbial Ecology, Predictive Modeling, Poultry Management

P323 Effect of chicken breed on Campylobacter and Salmonella load and prevalence in pasture-raised farms Walid G. Al Hakeem^{*1}, Minh Kim², Sohyun Cho³, Xiang Li⁴, Adelumola Oladeinde², Michael Rothrock² ¹ORISE-USDA, Athens, Georgia, United States; ²USDA-ARS, Athens, Georgia, United States; ³USDA, Egg and Poultry Production Safety Research Unit, Athens, Georgia, United States; ⁴USDA-ARS, Athens, Georgia, United States

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 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 and *Salmonella* prevalence were determined by cultural methods. 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 higher in the Cornish Cross breed compared to Freedom Ranger during the midgrowth period (6.3 vs. 4.7 log₁₀CFU/ml) and end of the 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) ($p < 0.05$). *Salmonella* was isolated from 53 of a total of 412 samples, representing an overall prevalence of 12.86%. *Salmonella* prevalence was higher in Cornish Cross (18.51%) compared to the Freedom Ranger (8.1%) ($p < 0.05$). *Salmonella* prevalence was the highest in the flies (45%) and lowest in feed samples (0%) ($p < 0.05$). These results indicate that the Cornish Cross breed can potentially harbor a higher load and prevalence of *Campylobacter* and *Salmonella*, respectively, and that flies can serve as a source for transmission of *Campylobacter* and *Salmonella* within a pasture-raised flock.

Keywords: Pasture-raised, *Campylobacter*, *Salmonella*, Cornish Cross, Freedom Ranger

P324 Foodborne pathogens prevalence, load and ecology on pasture-raised broilers processed on-farm or at a small USDA-inspected facility Walid G. Al Hakeem^{*1}, Taejung Chung², Minh Kim², Adelumola Oladeinde², Michael Rothrock² ¹ORISE-USDA, Athens, Georgia, United States; ²USDA-ARS, Athens, Georgia, United States

Gaining access to the US retail market poses a significant challenge for small pasture-raised farms as a USDA-inspected status is required, for which a lot of these producers are ineligible. Therefore, many farmers process their birds on-site (on the farm), or transport them to small-scale USDA-inspected slaughter facilities. There is a scare of information regarding the food safety of these methods. Therefore, the aim of this experiment is to investigate the association between microbial communities and foodborne pathogen in pasture raised birds that are processed with two different methods. Preharvest (feces, pasture soil), processing (ceca, whole carcass rinses) and final product (whole carcass rinses on farm or USDA-inspected facility) samples were collected from 4 flocks from the same pastured poultry farms. 16S rDNA microbiome sequencing was conducted. *E. coli*, *Campylobacter*, *Salmonella*, and *Listeria* were identified using cultural methods. One-way ANOVA with farm location or sample type as the main effect, followed by Tukey's multiple comparison method for pairwise comparison. Bacterial differential abundance was analyzed using ANCOM-BC2. The feces showed the highest ($p < 0.05$) *E. coli* and *Campylobacter* loads (6.54 and 3.90 log₁₀ CFU/mL, respectively) and prevalence (100% and 93.3%, respectively), while the final product whole carcass rinses had the lowest ($p < 0.05$) *E. coli* and *Campylobacter* loads (1.63 and 0 log₁₀ CFU/mL, respectively) and prevalence (85.21% and 0%, respectively), showing a downward trend observed from preharvest to postprocessing samples. *Salmonella* prevalence was low (3%) across all samples. Among the 4 tested pathogens, *Listeria* was significantly more prevalent in final product

processed at sampling plant. Overall, microbiomes in soil and ceca samples harbored a higher taxonomic richness. Beta-diversity analysis demonstrated significant differences between the microbiomes of feces and ceca, but not for onfarm and USDA-facility final product. The final product on farm and in a USDA-inspected facility showed no significant differences in their bacterial communities; however, the high *Listeria* prevalence on the USDA-inspected facility indicates that chicken slaughterhouses can be a source of foodborne pathogens detected in the final product.

Keywords: Pasture-raised, Microbiome

P325 E. coli diversity along the Farm-to-Fork continuum of pastured poultry flocks in the Southeastern United States Walid G. Al Hakeem^{*1}, Minh Kim², Michael Rothrock² ¹ORISE-USDA, Athens, Georgia, United States; ²USDA-ARS, Athens, Georgia, United States

Greater consumer demand for antibiotic-free, locally produced poultry products has increased pastured poultry operations in the United States. Given the increased level of environmental interaction and the potential increase in exposure to foodborne pathogens, a greater understanding of the prevalence and diversity of *E. coli* populations inherent within pastured poultry flocks is required. Therefore, this study aimed to characterize *E. coli* throughout the farm-to-fork continuum to see how different management or environmental variables affect the ecology of this bacteria. Forty-two pastured poultry flocks from 11 farms were sampled using a farm-to-fork strategy, and *E. coli* was isolated and characterized through pre-harvest (feces, soil) to post-harvest (ceca, whole carcass rinse) to the final product (whole carcass rinse) the consumer would purchase. One-way ANOVA with farm location or sample type as the main effect, followed by Tukey's multiple comparison method for pairwise comparison, was performed using R software. *E. coli* was isolated from 1897 of 1935 samples, representing an overall prevalence of 98%. The feces showed the highest ($p < 0.05$) *E. coli* load (6.54 log₁₀ CFU/mL) and prevalence (100%), while the final product whole carcass rinses had the lowest ($p < 0.05$) *E. coli* load (1.63 log₁₀ CFU/mL) and prevalence (85.21%), showing a downward trend observed from preharvest to postprocessing samples. Of the 1897 positive samples, 860 *E. coli* isolates were selected for further characterization. Even though these flocks were raised antibiotic-free, *E. coli* isolates exhibited resistance to a variety of antibiotics, with the two most common resistances being toward tetracycline and streptomycin (43.83 and 13.13%, respectively). Multidrug resistance phenotypes (=3 antibiotic classes) were relatively low for *E. coli* isolates (9.53%). *E. coli* load and prevalence were more affected by farm location than by the type of sample from which the *E. coli* was isolated. Overall, these results indicated a need for farm-specific *E. coli* mitigation strategies to ensure the safety of these increasingly in-demand poultry products.

Keywords: Pasture raised, *E. coli*

P326 Key Farm to Fork Factors Influencing E. coli Level in Pastured Poultry Production Minh Kim^{*1}, Walid G. Al Hakeem², Michael Rothrock¹ ¹USDA-ARS, Athens, Georgia, United States; ²ORISE-USDA, Athens, Georgia, United States

Pasture poultry farms offer a unique model for investigating microbial ecology in less controlled environments, presenting both challenges and opportunities for food safety management. *E. coli* can serve as an indicator organism for environmental contamination and exploring the efficacy of interventions. This study aims to identify and understand the factors that influence *E. coli* level with two modeling approaches: random forest (RF)

model and a linear mixed-effect model (LMM). Data were collected from 11 pastured poultry farms in southeastern United States from 2014 to 2017. Five sample types were analyzed: soil, feces, ceca, whole carcass rinse after processing (WCR-P), and whole carcass rinse after chilling and storage (WCR-F). Two different sets of predictor variables were used separately: (1) 32 Farming practices and 26 soil physiochemical constituents, and (2) 80 meteorological data. For farming practices and soil constituents, both RF model and LMM identified pH in soil and feces and Na in soil as key factors influencing *E. coli* levels. The LMM also showed significant positive correlations for pH in soil ($t=3.9$) and feces ($t=9.2$), and for Na in soil ($t=3.5$). Bird age was associated with lower *E. coli* levels in feces and ceca samples according to the RF model, while the LMM partially supported this, showing significantly lower levels at mid-pasture time compared to end ($t=-2.6$). During processing, RF model with WCR-P samples showed that carcasses washed with organic acid had lower *E. coli* level while similar result was predicted from WCR-F samples with longer storage time. For meteorological factors, daily temperature range was important in both soil and feces showing higher daily temperature range reducing *E. coli* level. LMM was not used for meteorological factors due to the multicollinearity. This study identifies key predictors influencing *E. coli* levels in farm-to-fork continuum. The study can help stakeholders developing data driven management strategies to reduce food safety risk and explain correlations between specific weather events and level of the pathogen in preharvest environment.

Keywords: Predictive Modeling, Poultry Management, Microbial Ecology

P327 Impact of sodium bisulfate and water activity on *Salmonella* survival in poultry litter Yabaiz Tahir*¹, Justin Lowery¹, Mary Mendoza¹, Christina S. Sigmon¹, Jeyam Subbiah², Nanje Gowda³, Craig Coufal⁴, Lin L. Walker¹ ¹North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ²University of Arkansas System Division of Agriculture, Dale Bumpers College of Agricultural, Food and Life Sciences Food Science, Fayetteville, Arkansas, United States; ³University of Arkansas Division of Agriculture, Department of Food Science, Fayetteville, Arkansas, United States; ⁴Jones-Hamilton Co., Walbridge, Ohio, United States

Salmonella, a harmful bacterial species commonly found in broiler litter, has been shown to decrease in populations in litter treated with sodium bisulfate (SBS). This study aimed to evaluate the impact of SBS and water activity (a_w) on *Salmonella* survival in poultry litter. Windrowed used litter from commercial broiler houses was dried and adjusted into 3 a_w levels (0.85, 0.9, and 0.95) by adding water. Litter was placed in 100.33 x 51.44 cm 66 qt plastic bins with lids at a depth of 16.5 cm (6.5 in.). The litter at each a_w level was further divided into 3 groups for different SBS surface application rates: 0, 252, and 378 g/tub (0, 100, and 150 lb/1000 sq ft). Prior to SBS application on d 0, a *Salmonella* cocktail at 8 log₁₀ CFU/mL, comprised of nalidixic acid-resistant *S. Enteritidis*, Typhimurium, and Heidelberg was used as an inoculum. On d 0, 1, 2, 3, 4, 6, and 8, 2 random samples (25 g) at a time were collected from a section of the litter surface for *Salmonella* enumeration, and original samples in buffered peptone water were further enriched for the detection of *Salmonella*. The pH, moisture, and a_w were also measured at each sampling day. The entire experiment was repeated in 2 independent trials with fresh windrowed litter acquired for each trial. Data was analyzed in JMP software using 2-way ANOVA to assess the effects of a_w and SBS on *Salmonella* counts and Tukey's

HSD for mean separation. *Salmonella* reduction, with and without SBS application, was more rapid in the 0.85 and 0.90 a_w treatments compared to the 0.95 a_w treatments. The application of SBS significantly reduced the litter pH from 8 to 2-5, with a lower litter pH obtained with SBS application in the drier litter, although the pH gradually increased over time. Both a_w and SBS played significant roles in *Salmonella* reduction ($p<0.05$). This study demonstrated the importance of keeping poultry litter dry to control *Salmonella* and maximize the litter pH reduction potential of acidifying agents such as SBS. Long-term bacterial control in poultry litter should focus on controlling the litter moisture.

Keywords: Litter, Salmonella, Sodium bisulfate, Water activity, Poultry

P328 16s amplicon sequence analysis finds comparable microbial community dynamics in *Salmonella* Heidelberg-challenged broilers with or without antibiotic alternatives Dhananjai Muringattu Prabhakaran*¹, Shijina Manjankattil¹, Claire Peichel¹, Anup K. Johny¹ ¹University of Minnesota, Animal Science, Saint Paul, Minnesota, United States

Previously, we determined the efficacy of *trans*-cinnamaldehyde (TC) and caprylic acid (CA), two antibiotic alternatives (AA), in reducing *Salmonella* Heidelberg (SH) colonization in 5-week-old broiler chickens. We found that TC and CA reduced ~2.0 log₁₀ – and 3.2 log₁₀ – CFU/g of SH, respectively, in broiler ceca. The current study investigated the effect of TC and CA on the cecal microbial composition and microbial network association and predicted metagenomic function in SH-challenged broilers exposed to both AAs. Birds were randomly distributed in BSL2 isolation units (3 chickens/unit per space requirements, two experiments; n=6/treatment): Negative control (NC; standard diet [SD], no SH challenge), Positive Control (PC; SD, +SH challenge), antibiotic group (AB; SD with 50g/ton bacitracin +SH challenge), CA (SD with CA 1% v/v, +SH challenge), and TC (SD with 0.5% TC +SH challenge). All groups except NC were challenged with SH (3.8 log₁₀ CFU/bird) on the 4th week, and samples were collected on the 5th week for sequencing the V4 region of 16s rRNA gene using the Miseq platform. Amplicon sequence variants were assigned to sample sequences using DADA2. ANCOM-BC2 was used to find differentially abundant genera. Association network analysis and functional prediction were done using NetCoMi and PICRUSt2, respectively. The AA groups yielded comparable microbial composition, association network, and predicted metagenomic functions with PC, but not AB. The α and β diversity between AB and PC differed significantly (Wilcoxon rank-sum and PERMANOVA, respectively, $P<0.05$). Bacilli RF39 was the differentially abundant genera in AB-PC, AB-NC, and AB-TC comparisons, and the Clostridia vadinBB60 group was differentially abundant in AB-TC comparison ($P<0.05$). Furthermore, a significant difference in key taxa in the microbial association network and differential abundance of pathways (nucleic acid and lipid biosynthesis pathways) in predicted metagenomic function was observed in the AB-PC comparison. Dietary AAs did not adversely affect the broiler cecal microbiome but also suggested that microbiome-associated immune modulation could not be an antimicrobial mechanism by which TC and CA reduced SH colonization (MIN-16-071; USDA #2020-69012-31823).

Keywords: Microbiome, trans-cinnamaldehyde, Salmonella, caprylic acid, Food safety

P329 *Propionibacterium freudenreichii* and *S. Typhimurium* vaccine elicit comparable colonization resistance to multidrug-resistant *Salmonella* Infantis in broiler chickens Dhananjai Muringattu Prabhakaran*¹, Amritha Ajayan¹, Hamza Javaid¹, Tim

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Salmonella Infantis (SI) is one of the top serovars of foodborne *Salmonella* isolated from chickens. Reducing SI colonization in chickens could reduce the incidence of SI outbreaks. This study evaluated *Propionibacterium freudenreichii* subsp. *freudenreichii* (PF), a dairy-origin probiotic, against multidrug-resistant (MDR) strains of SI *in vitro* and commercial broiler chickens. The effect of PF on growth (co-culture media [CCM] and cecal contents [CC]), motility, cell adhesion, and invasion of MDR SI strains (CDC AR0919, AR0923) alone or in combination was evaluated. The treatments were: Negative Control (NC: -PF, -SI), Positive Control (PC: +SI), and treatment group (SPF: +PF, +SI) (Duplicate samples/group, experiment repeated three times [n=6/group]). For growth assay in CCM, the effect of three doses of PF (10⁵, 10⁷, 10⁹ CFU/mL) on SI growth at different incubation times (0, 4, 12, 24 hours) was evaluated. In motility and cell culture studies, PF at 10⁹ CFU/mL was assessed on ~10⁵ CFU/mL of SI. A multiplicity of infection of 10 was used in cell culture studies. In the *in vivo* study, day-old

broiler chicks were randomly distributed into 5 treatments (6 birds/group, 2 experiments, n=12): NC (-SI, -PF, -vaccine), PC (+SI, -PF, -vaccine), PF (+SI, +PF, -vaccine), VC (+SI, -PF, +vaccine), PV (+SI, +PF, +vaccine). PF and PV groups received 10⁷ CFU/mL PF via drinking water. VC and PV were vaccinated with the *S. Typhimurium* vaccine. Treatments except NC were challenged with SI (AR0923) in the 4th week, and ceca, liver, and spleen samples were collected in the 5th week. ANOVA, *t*-test, and Chi-squared test were appropriately used to analyze data with significance tested at *P*<0.05. PF reduced the growth in CCM and CC (two-way ANOVA), motility (one-way ANOVA), and cell adhesion and invasion (*t*-test) of SI strains alone or in combination (*P*<0.05). A significant reduction in cecal colonization of SI (AR0923) in PF, VC, and PV groups compared to PC was observed in chickens (one-way ANOVA; *P*<0.05). The dissemination of SI to the liver and spleen was also significantly reduced by PF, VC, and PV (Chi-squared test; *P*<0.05). The study suggests that PF could be a preharvest anti-*Salmonella* strategy in broiler farms to reduce SI in broiler chickens (USDA #2020-67017-30787).

Keywords: Broiler chicken, *Salmonella*, Cell culture, probiotics, Food safety

Processing and Products

P330 The effect of rearing systems and heat treatments on liquid egg white functionality Leah Dexter-Boone^{*1}, Skye Freeland¹, Masahiko Taniguchi³, Haotian Zheng², Kenneth Anderson¹, Taylor O'Lear Reid¹, Aaron Kiess¹, Yan L. Campbell¹ ¹North Carolina State University, Prestage Family Department of Poultry Science, Raleigh, North Carolina, United States; ²North Carolina State University, Department of Food, Bioprocessing and Nutrition Sciences, Raleigh, North Carolina, United States; ³North Carolina State University, Department of Chemistry, Raleigh, North Carolina, United States

More consumers are choosing cage-free (aviary) eggs over conventional (cage) eggs due to perceptions of enhanced animal welfare. This research aimed to investigate the effect of the rearing system and batch pasteurization on functionalities of liquid egg whites. Thirty eggs were collected from two different rearing systems (cage and aviary) at 32, 52, and 82 weeks of age. The liquid egg whites were pasteurized (heat) or non-heat treated. Liquid egg whites (LEWs) were separated from the yolk and were characterized for protein content (bovine serum albumin protein assay), dry matter content (drying oven), particle size, zeta potential, surface hydrophobicity, free and total sulfhydryl (SH) groups, and thermal properties of conalbumin and ovalbumin. Free and total SH were quantified using a UV-Vis spectrometer and protein thermal properties with a differential scanning calorimeter. Rearing system and heat treatment were evaluated in a 2x2 factorial and blocked by age. Analyses were conducted in triplicate and data was analyzed in SAS 9.4 by a two-way ANOVA and reported as different if *P*<0.05, with mean separation using Tukey's test. The protein content, particle size, surface hydrophobicity, and total SH in liquid egg whites were unaffected by the rearing system and temperature (*P*>0.05). The dry matter was greater in heated aviary egg whites (12.76%) compared to non-heated cage egg whites (11.90%) (*P*<0.05). Only non-heated cage egg whites had lower zeta potential (-14.38 mV) compared to the heated aviary egg whites (-18.45 mV) (*P*<0.05). The free SH concentration in heated/non-heated aviary and heat egg whites (0.83-0.96 mM) was greater compared to non-heated cage (0.47 mM) egg whites (*P*<0.05). It took more energy to denature ovalbumin in heated egg whites (0.59 J/g) with respect to non-heated egg whites (0.65 J/g) (*P*<0.05), but no other factors affected temperatures and enthalpy (*P*>0.05). Rearing systems and heat

treatments affected several egg functionality properties including dry matter content, zeta potential, free SH concentrations, and protein enthalpy of ovalbumin, which may affect sensory and ingredient functionality of egg whites.

Keywords: liquid egg whites, cage rearing systems, aviary rearing systems, LEW, protein functionality

P331 Meat quality of hens fed an unblanched high-oleic peanut diet Afsana Rahaman Munmun^{*12}, Jean C. Caceres³, Christina S. Sigmon², Ishab Poudel⁴, Thien Vu⁵, Becca Wysocky³, Ramon Malheiros², Kenneth Anderson², Ondulla T. Toomer⁵, Yan L. Campbell² ¹North Carolina State University, Food, Bioprocessing & Nutrition Sciences Department, Raleigh, North Carolina, United States; ²North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States; ³North Carolina State University, Prestage Poultry Science Department, Raleigh, North Carolina, United States; ⁴Mississippi State University, Poultry Science, Raleigh, North Carolina, United States; ⁵United States Department of Agriculture, Raleigh, North Carolina, United States

High-oleic peanuts offer an energy dense, high protein alternative to conventional poultry feed ingredients and can potentially improve meat quality in spent layers. Previous studies showed that a 15% inclusion of high-oleic peanuts in layer diets optimizes egg production and feed efficiency without impacting egg quality. Spent layers are most often used for rendering, composting or waste and do not generally enter the human food chain. This study investigated the effects of unblanched high-oleic peanuts in 5 varying levels (0%, 5%, 10%, 15%, and 20%) into layer diets for 8 weeks on meat quality parameters in laying hens. We conjecture that including unblanched high-oleic peanuts in diets can enhance layer meat quality and potential for human consumption. Diets were formulated to be isonitrogenous and isocaloric, providing 18% crude protein and 3080 kcal/kg metabolizable energy. A total of 200 laying hens were acclimated for 7 days before random assignment to dietary treatments, each comprising 5 reps of 8 hens. Hens (10/trt – 2/rep, randomly selected) were processed at the end of the trial. Body weight, carcass weight, breast muscle weight, pH, and color metrics were recorded to assess the influence of peanut inclusion on meat quality. The data were analyzed using

SAS 9.4 software, employing one-way ANOVA and the General Linear Model (GLM), with mean separation conducted through Duncan's New Multiple Range Test at a significance level of $P < 0.05$. Results indicated no significant difference in body weight, hot carcass weight, chilled carcass weight, or breast weight across the treatments ($P > 0.05$). Carcass pH measurements at 2 h and 24 h and drip loss percentage also showed no notable differences ($P > 0.05$) among the dietary groups. Additionally, breast color parameters remained consistent across treatments, indicating that high-oleic peanut inclusion did not adversely affect meat color stability ($P > 0.05$). These findings suggest that high-oleic peanut crumbles can replace soybean meals up to 20% in layer diets without adversely impacting meat quality, carcass yield, or color attributes. Although additional trials are necessary, this study supports the potential for peanut-based feed ingredients as sustainable alternatives in poultry nutrition.

Keywords: Layers, Meat Quality, High-Oleic Peanuts, Poultry Feeding Trial

P332 Effect of alternative additives to formaldehyde on reducing *Salmonella* in animal feed Adrieli Biava^{*1}, Silvana de Castro Caldas², Liris Kindlein¹ ¹UFRGS, Encantado, RS, Brazil; ²UFRGS, Encantado, RS, Brazil

Salmonella in animal feed not only poses a significant threat to animal health and welfare, but also can compromise sanitary conditions along the production chain. Therefore, it is essential to assess the mitigating efficacy of additives used in animal feed processing to reduce contamination by this pathogen. This study aimed to evaluate the efficacy of used of alternatives additives to formaldehyde for reducing *Salmonella* in poultry feed. In vitro tests were conducted under three treatments: T1 (control) –

formaldehyde-based; T2 - formic acid, propionic acid, and lactic acid; and T3 - other organic acids (excluding formic acid, propionic acid, lactic acid, or formaldehyde). A total of 40 chemicals were tested. Also, were considered two presentation forms (powder and liquid), different concentrations of the chemicals (1, 1.5, 2.0, 2.5g per kg of feed) that were tried on different *Salmonella* serotypes (*S. Anatum*, *S. Ouakam*, and *S. Senftenberg*). Data were analyzed via a 3 x 2 x 4 x 3 factorial experiment using the Pearson's Chi-squared test, Chi-square Test of Independence and Fisher's Exact Test (R Software, version 4.2). Pearson's Chi-squared test showed significant difference between chemical groups. The absence of *Salmonella* was 86.65% in formaldehyde group (T1), compared to T2 (1.48%) and T3 (0.61%) (p-value < 2.2e-1). Regarding product concentrations, no significant differences were observed (p-value = 0.5886) for the different proportions of kg/ton of feed, indicating that *Salmonella* presence does not depend on the chemicals' concentration. Analyzing different strain types, no significant differences (p-value = 0.5257) were found among pathogen isolates in Fisher's Exact Test. For product presentation, a statistically significant difference was noted between liquid and powdered products, with liquid products showing higher *Salmonella* inhibition (p < 0.001). Formaldehyde (T1) was more effective in mitigating the serotypes tested compared to the other acids (T2 and T3), confirming that the combinations of tested compounds cannot be considered alternatives to replace formaldehyde. However, considering its toxicity and environmental impact, along with its ban in the European Union, exploring safer alternatives is essential and must be studied.

Keywords: feed sanitation, feed manufacturing, *Salmonella* Anatum, *Salmonella* control

Metabolism and Nutrition: Amino Acids and Enzymes

P333 Productive energy (Arkansas net energy) as a key indicator: evaluating full-fat soybean meal for enhanced broiler protein gain and feed conversion Abdullah Scott²¹, Cole Umberson², Callie Selby², Jordan Weli², Nawin Suesuttajet², Ehsan Sheikhsamani², Diego A. Martinez^{*2}, Mian Riaz³, Craig Coon² ¹Salahaddin University, Animal Resources, Erbil, Erbil, Iraq; ²University of Arkansas, Fayetteville, Arkansas, United States; ³Texas A&M University, College Station, Texas, United States

This study evaluated the characteristics, nutrient digestibility, and performance effects of six Full-Fat Soybean Meal (FFSBM) sources from six different U.S. states. A 42-day feeding trial was conducted with 1,800 male Cobb-500 broilers assigned to 90-floor pens. Birds were fed a corn-soybean meal basal diet incorporating 4, 8, or 12, percent of each FFSBM source across different feeding phases. The Apparent Metabolizable Energy corrected for nitrogen (AMEn), Classic Net Energy (CNE), and Productive Energy (PE) values were determined, along with the Standardized Ileal Digestibility (SID) of protein, amino acids, and lipids. Data were analyzed using one-way ANOVA and Tukey's HSD test (p < 0.05). Quality assessments showed Potassium hydroxide solubility (KOH) between 65–75% across FFSBM sources. Trypsin inhibitor activity ranged from 2.33 to 3.56 TIU/g without significant variation. Lipid quality analysis indicated Peroxide Values of 4.93–5.57 meq/kg, Thiobarbituric Acid values of 1.22–1.69 mg/kg, and Anisidine Values consistently below 1%. Energy values averaged 3,527 kcal/kg for AMEn, 2,759 kcal/kg for CNE, and 3,272 kcal/kg for PE. Among the sources, FFSBM-D, with 0.85% digestible total sulfur amino acids (TSAA), 5.23% digestible branched-chain amino acids (BCAA), 2.06% digestible lysine, 30.31% digestible crude protein (CP), and 3,342 kcal/kg PE, yielded the best broiler

performance. This source achieved the lowest feed conversion ratio (FCR) of 1.34 over 42 days. The coefficient of determination (R^2) was calculated to assess energy systems' predictive accuracy for broiler performance and nutrient contributions. The PE system showed superior accuracy, with R^2 values of 0.68 for body weight, 0.76 for body weight gain, 0.90 for feed intake, and 0.92 for FCR, outperforming AME and CNE systems. Digestible CP contributions to AME, CNE, and PE systems were 0.14, 0.24, and 0.70, respectively. The PE system also exhibited the strongest correlations with BCAA ($R^2 = 0.76$) and TSAA ($R^2 = 0.86$). Overall, the PE system emerged as the most accurate predictor of nutrient utilization and performance metrics. These findings underscore the importance of selecting high-quality FFSBM to optimize nutrient digestibility, enhance FCR, and improve broiler performance.

Keywords: Productive Energy, Full-Fat soybean meal, Amino Acid, Digestibility, Broiler

P334 Understanding the branched-chain amino acid interactions on bone morphology of pullets at 18 weeks of age Charis A. Waters^{*1}, Roshan Adhikari², Jason T. Lee², Deependra Paneru³, Woo Kyun Kim³, Ruben Kriseldi⁴, Michael Elliot⁵, Li Zhang¹, Kelley Wamsley¹, Pratima Adhikari¹ ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²CJ Bio America, Downers Grove, Illinois, United States; ³University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ⁴Aviagen, Huntsville, Alabama, United States; ⁵A&E Nutrition Services, LLC, Lititz, Pennsylvania, United States

Branched-chain amino acids (BCAAs) are critical nutrients that support bone health in layer pullets during key developmental stages. The objective of this study was to investigate the impact of BCAA ratios on femur morphology in Hy-Line W-36 pullets from 12 to 18 weeks of age. A total of 1,440 pullets were assigned to 120 pens in a circumscribed central composite design (CCD) with 20 treatments. Each treatment consisted of varying digestible Val:Lys (65 to 95), Ile:Lys (58 to 88), and Leu:Lys (120 to 220) ratios; with digestible Lys set at 0.80% per the Hy-Line management guide (2020). At 18 weeks of age, one bird per pen was randomly selected, and euthanized, and the right femur was collected for analysis. Data were analyzed as a CCD using the surface response option of JMP v. 15. Due to repeated observance of effects in CCD, means were considered significant at $P < 0.10$. Cortical BMD showed a trend toward significance (1.1303 g/cm^3 ; $P = 0.1057$; $R^2 = 0.67$) and tended to increase at the highest Val:Lys (95) and Leu:Lys (220) ratios combined with the lowest Ile:Lys (58) ratio. There was a significant interaction ($P = 0.0316$) between Val:Lys and Ile:Lys ratios on cortical BMD. Keeping the Leu:Lys (220) and Ile:Lys (58) ratios constant, and decreasing the Val:Lys ratio from 95 to 80 and 65 reduced BMD by 14.6 and 16.8% respectively, stating importance of Val on BCAA balance. Medullary BMD also showed a trend toward significance (0.1548 g/cm^3 ; $P = 0.1073$; $R^2 = 0.67$) at the lowest Val:Lys (65) and Leu:Lys (120) and intermediate Ile:Lys (70) ratios. The model showed a significant interaction ($P = 0.0215$) between Val:Lys and Leu:Lys ratios on medullary BMD. Increasing Val:Lys from 65 to 80 and 95 keeping Ile:Lys (73) and Leu:Lys (120) ratios constant decreased BMD by 14.79 and 17.59%. Increasing Leu:Lys from 120 to 220 keeping Val:Lys (65) and Ile:Lys (73) ratios constant decreased BMD by 17.83%. However, increasing the Val:Lys from 65 to 80 and 95, at elevated Leu:Lys (220) and Ile:Lys at (73) ratios improved BMD by 5.49 and 20.78% respectively. These findings suggest that adjusting Val, Ile, and Leu ratios according to the dietary BCAA levels on 12 to 18 weeks of age pullets may affect bone structure, potentially improving skeletal health in laying hen pullets.

Keywords: branched-chain amino acids, bone quality, microstructure, pullets, surface response

P335 The effect of a sfericase protease on protein solubility of soybean meal and trypsin inhibitor degradation Camila D. Cardoso*¹, Diogo Rosso², Tine V. Jensen², Jacqueline C. de Souza¹, Vitor B. Fascina³ ¹Novonosis LA, Araucária, PR, Brazil; ²Novonosis A/S, Lyngby, Denmark; ³dsm-firmenich, São Paulo, Brazil

Trypsin inhibitor (TI) content is a critical quality factor for soybean meal (SBM), which can negatively impact nutrient absorption. Two SBMs, with low (2mg/g) and high TI levels (14.1 mg/g), were submitted to exogenous sfericase protease (500 ppm) *in vitro* hydrolysis, with and without the presence of trypsin (500 ppm), as well as a negative control. Assays were carried out in triplicates, in 50 mL tubes, 15% total solids, pH 6.5 at 40°C for 4 hours under 25 rpm agitation. Soluble protein (SB) was obtained from processing of dried pellets in nitrogen analyzer by Dumas methodology. Supernatant collected was analyzed using OPA (o-phthalaldehyde) colorimetric assay to obtain a relative degree of hydrolysis (DH). Data was analyzed as one-way ANOVA and means were separated by treatment using All Pairs, Tukey-Kramer HSD test on JMP 16 software with significance set at $P < 0.05$. For the SBM with low TI level, trypsin treatment delivered $14.68\% \pm 0.41\%$ of SB, while protease presented $16.70\% \pm 1.59\%$, with no statistical difference ($P = 0.1210$). Combination of trypsin and protease resulted in $26.68\% \pm 0.82\%$, highlighting the additive effect with statistical significance compared to other treatments

($P < 0.001$). This pattern was also observed with the analysis of relative DH. For substrate with high TI level, results showed that the combination of enzymes increased 35% the relative DH over control ($P < 0.001$), while trypsin alone showed an increase of 3% ($P = 0.9410$). Moreover, we evaluated the efficacy of the sfericase protease on degrading TI. Four doses of protease (1, 2, 4, 8 mg/mL) and one level of TI (3 mg/mL) were incubated at 40°C for 2 and 4 hours, on buffered media (pH 7.8) in the presence of 1mM calcium. Hydrolytic profile was evaluated by SDS-Page gel. Protease was able to degrade 93% and 97% of trypsin inhibitors, dosed at 1 and 2 mg/L, respectively, after 2 hours of incubation. Within 4 hours, protease was able to degrade 98% of TI in both dosages (calculations based upon band intensity on gel). In conclusion, the exogenous sfericase protease acts in combination with trypsin to improve protein solubility, even in the presence of high contents of TI. Beyond that, results of dose response suggest that this protease is also effective on degrading TI.

Keywords: protease, protein solubility, trypsin inhibitor, soybean meal

P336 Xylanase supplementation: Influence on growth performance and carcass characteristics of broiler chickens Kamel Mahmoud*¹, Basheer Nusairat², Omar Al-Qudah¹ ¹Animal Production, Jordan University of Science and Technology, Irbid, Jordan, ²Department of Animal Production, Jordan University of Science and Technology, Irbid, Jordan

Two studies were conducted sequentially to evaluate the effect of adding xylanase to the diets of broiler chickens on growth performance, carcass characteristics, and meat quality. Corn-soy based diets were used in both trials, but the second trial was formulated to contain 15% wheat. Seven hundred and fifty of one-day-old unsexed Ross 308 broiler chicks were randomly allocated into 6 dietary treatments with 5 replicates pen of 25 chicks each. Data was analyzed using Completely Random Blocked Design (CRBD), and means were separated by LSM. Control diets were formulated according to Ross 308 manual recommendation designated as positive control (PC and PW for corn-based diets and corn/wheat-based diets, respectively); control diet with 90 kcal/kg less energy designated as negative controls (NC and NW for corn-based diets and corn/wheat-based diets, respectively). Xylanase at 0.01% inclusion rate with 15 XU/g of feed was added to negative control diets (XC and XW for corn-based diets and corn/wheat-based diets, respectively). All diets were fed as mash, starter phase was from 1-14 days of age and the grower phase was from 15-28 days of age in experiment one and 15-35 in experiment two. Results showed that only body weight gain for the period of 15-28 d and overall period were higher ($P < 0.03$) in wheat-NC diet with enzyme addition. Meat lightness (L^*) was lower ($P < 0.02$) in meat from birds fed wheat PC, while water holding capacity was improved ($P < 0.02$) by adding xylanase to control diets. In conclusion, under the current experimental conditions, it can be concluded that xylanase supplementation in broiler diets can be used as a mean for reducing energy content of the diets. Results were better in wheat-based diets, possibly due to a higher substrate concentration.

Keywords: Broiler, Wheat, xylanase, carcass

P337 Blood biochemistry influenced by dietary available phosphorus and phytase supplementation Xaviere Rousseau*¹, Gilson Gomes¹, Michelle Stewart¹, Mohammad Pilevar², Bhargavi Kasireddy², Oluyinka Olukosi² ¹AB Vista, Marlborough, United Kingdom; ²University of Georgia, Poultry Science, Athens, Georgia, United States

Phytase represents one of the major tool for nutritionist to reduce inorganic phosphates and has also been reported to reduce the severity of wooden breast myopathy via the modulation of oxygen transport. This study explored the effect of graded levels of phytase supplemented on a phosphate-free diets on growth performance, bone mineralization and blood biochemistry. A total of 360 Cobb 500 broilers were raised up to 21 days of age and randomly distributed to 9 dietary treatments. Five treatments with gradual levels of available phosphorus (avP) and calcium (Ca) (avP at 4.2, 3.5, 2.7, 2.0, and 1.2 g/kg and Ca at 8.4, 7.6, 6.8, 5.9, and 5.1g/kg, respectively). Four levels of phytase (250, 500, 1000, and 2000 FTU/kg, Quantum Blue, AB Vista, UK) were added on top of the 1.2g/kg avP diet. In addition to growth performance and bone mineralization, 2 birds per pen were euthanized at 20 days of age for blood biochemistry analysis. A blood analyzer (iSTAT, Zoetis) was used to determine Haematocrit (Hct) as a component for oxygen transport, hemoglobin (Hb) as a protein ensuring oxygen transport, oxygen saturation (sO₂) as the fraction of Hb able to bind oxygen, carbon dioxide (sCO₂) and bicarbonate (HCO₃) as the indicator of the buffering capacity. A one-way ANOVA was

performed (JMP Pro 16.2)). Polynomial orthogonal contrasts (linear, logarithmical, quadratic) were assessed. Results concluded that higher doses of phytase can be used on low avP diet for broilers from 6-21 days of age without impairing performance and bone mineralization. Hb and Hct are both influenced in a logarithmical way by avP ($p < 0.001$); phytase ($p < 0.001$) with the highest levels observed at the lowest dietary avP. sO₂ increases as the dietary avP increases (linear, $p < 0.001$) while the supplementation of phytase increased the sO₂ (logarithmical, $p < 0.001$). Increasing dietary avP (linear, $p < 0.001$) and phytase (linear, $p < 0.001$) linearly reduced TCO₂ in blood ($p < 0.001$). HCO₃ decreased as the dietary avP increased (linear, $p < 0.001$) while phytase reduced HCO₃ in blood (linear, $p < 0.001$). Blood analytes in this study were all influenced by the dietary avP, Ca and phytase supplementation. Blood analytes for phytase supplemented birds has been shown to reach same levels than birds fed the highest avP diet.

Keywords: phosphorus, phytase, blood oxygen

Metabolism and Nutrition: Feed Additives

P338 Fructooligosaccharides dose optimization for improving growth performance and intestinal development in broiler chickens Viviana Parada Roa¹, Alvaro Uribe-Serrano¹, Blanca C. Martinez¹, Loufrantz Parra-Mendez¹, Jaime A. Angel-Isaza*¹ ¹Promitec Santander SAS, Unidad de Innovacion y Desarrollo Biotecnologico, Bucaramanga, Colombia

In cutting-edge nutrition, the use of non-digestible carbohydrates, such as certain types of oligosaccharides like short-chain fructooligosaccharides (scFOS) or resistant starches, is increasingly being adopted. This trend is driven by advancements in the understanding of intestinal microbial communities and their relationship with productivity and gut health. There has been an intensified search for compounds that can modulate microbial balance to positively impact the profitability of broiler chickens. The present study aimed to evaluate the effect of increasing doses of scFOS on productive parameters and intestinal histological development in broiler chickens. The study was conducted at the San Nicolas Experimental Farm of the Innovation Unit of Promitec, located in Bucaramanga, Colombia. A total of 200, one-day-old ROSS AP broiler chickens were used in a completely randomized design. Five treatments were tested, consisting of different inclusion levels of scFOS. A basal diet containing Enramycin (10 ppm) was supplemented with increasing doses (0, 700, 1050, 1400, and 1950 ppm) of scFOS. On days 21 and 42, productive variables were measured, and duodenum and jejunum samples were collected for the evaluation of intestinal morphometric variables. Statistical analyses were performed using a generalized linear model to compare means between groups for the productive and histological variables. The results showed significant differences ($p < 0.05$) between treatments. On day 21, the doses of 1050, 1400, and 1950 ppm showed a significant increase ($p < 0.05$) in live weight compared to the lower doses, without showing differences in feed conversion ($p > 0.05$). By day 42, all scFOS inclusion doses resulted in significantly higher ($p < 0.05$) live weight compared to the control group, with the 1400 ppm dose being significantly superior ($p < 0.05$) to the other doses. In the morphometric analysis, a significant linear effect ($p < 0.05$) of scFOS doses was observed. Furthermore, the villus-to-crypt ratio was significantly higher at the 1400 ppm dose compared to the other inclusion levels. In conclusion, the use of scFOS improves live weight and intestinal development in broiler chickens, with inclusion levels between 1050 and 1400 ppm being optimal at 21 and 42 days of age.

Keywords: poultry, prebiotic, eubiosis, productivity, fiber

P339 Enhancing broiler chicken growth, immunological response, and intestinal morphology through organic copper supplementation combined with synbiotics Mohsen Afsharmanesh*¹ ¹Shahid Bahonar University of Kerman, Department of Animal Science, Kerman, Kerman, Iran (the Islamic Republic of)

A study was conducted to investigate the effects of different levels of organic copper (copper-methionine chelate) in combination with synbiotics (SYN) on the growth performance, morphology, intestinal microbial population, immune response, and meat quality of broiler chickens. The study involved a total of 360 one-day-old broiler chickens randomly assigned to three levels of organic Cu (8, 16, and 32 mg/kg) and two levels of SYN (0 and 200 mg/kg) in a 3×2 factorial arrangement of treatments, with five replications and 12 birds per replication. Over the entire breeding period, incorporating higher Cu levels with SYN led to improved body weight gain and feed conversion ratio ($P < 0.05$). Diets supplemented with SYN resulted in lower relative weights of the gizzard and abdominal fat but higher relative weights of the spleen ($P < 0.05$). Furthermore, broiler chickens fed diets with higher Cu levels or SYN had lower counts of coliforms (COL) and pH levels, as well as higher lactic acid bacteria/COL ratios in the ileum ($P < 0.05$). Interaction effects indicated that chickens fed a diet containing 8 mg/kg of Cu without SYN exhibited the highest COL population and pH compared to other treatments ($P < 0.05$). Additionally, elevated Cu levels or SYN supplementation improved intestinal morphology, particularly increasing villus surface area and the ratio of villus height to crypt depth ($P < 0.05$). The total antibody titer and IgM in the serum of chickens fed a diet containing 16 mg/kg of Cu along with SYN were significantly higher compared to those fed diets containing 8 and 16 mg/kg of Cu without SYN ($P < 0.05$). Meat analysis showed that the water holding capacity, yellowness, and chroma values were higher in chickens fed 32 mg/kg of Cu compared to those fed 8 mg/kg of Cu ($P < 0.05$). Moreover, the percentage of cooking loss in the meat of chickens fed diets containing 32 mg/kg of Cu with SYN was lower compared to chickens fed diets containing 8 mg/kg of Cu without SYN ($P < 0.05$). In conclusion, the inclusion of Cu-methionine chelate alongside SYN significantly improved the performance, morphology, intestinal microbial population, immune response, and meat quality of broiler chickens.

Keywords: Broiler Chickens, Copper, Gut Morphology, Lactic Acid Bacteria, Synbiotic

P340 Evaluation of a sodium butyrate stabilized by calcium salts against a fat coated calcium butyrate on broiler performance and economic profitability Octavi C. Balaña*¹, Housseem Kadardar¹, Ekaitz Maguregui¹ *Igusol, Technical, Barcelona, Catalonia, Spain*

Butyric acid is important for gut health and animal growth. However, due to its volatility, fat encapsulation is often used to ensure stability and ease of handling whilst potentially improving performance. BUTYRON is a sodium butyrate stabilized with a novel technique using calcium salts without fats. This improves butyric acid bioavailability without needing lipase, making it beneficial for young animals and enhancing performance. This study aimed to evaluate the growth performance and the economic profitability of broilers from 0 to 42 d of age when using BUTYRON compared to vegetable fat-coated calcium butyrate (FCB). A total of 240 Ross 308 broilers were assigned to 4 dietary treatments. Diet A used as control, diet B was supplemented with BUTYRON, diet C was supplemented with a FCB, and diet D received double the quantity of BUTYRON used in diet B (90% of the cost of the FCB). Both BUTYRON in diet B and the FCB in diet C were included at isobutyric dosages, ensuring comparable butyric acid levels. The feeding program consisted of 3 phases: starter (0 to 10 d), grower (11 to 24 d), and finisher (25 to 42 d). Growth performance [body weight (BW; g), average daily gain (ADG; g), feed intake (FI; g/d), and feed conversion ratio (FCR; g/g)] were measured by feeding phase and cumulatively. Each treatment was replicated 6 times, and the experimental unit was a pen with 10 chicks. Data was analyzed using a completely randomized design using the MIXED procedure of SAS. Cumulatively, BW and ADG tended to be higher ($P = 0.100$) in birds fed diets D and C compared to birds fed diets B and A but no significant effect on FI was detected ($P > 0.05$). Broilers fed diet D had a numerically better FCR compared to bird fed diets C, B, and A, respectively (1.408 vs. 1.424 vs. 1.425 vs. 1.432 g/g) but these differences were not statistically significant ($P > 0.05$). Economically, birds fed diet D demonstrated the highest income over feed cost compared to bird fed diets C, B, and A, respectively (3.99 vs. 3.96 vs. 3.87 vs. 3.85 \$/bird). In conclusion, while statistical significance was not achieved, the numerical trends suggest that BUTYRON has the potential to improve growth performance and profitability in broiler production, making it an economically viable option.

Keywords: Broiler performance, Butyric acid, Fat-coated calcium butyrate, Saturated sodium butyrate, Income over feed cost

P341 Efficacy of a liquid solution containing grape and olive extracts on broiler chickens challenged by aflatoxin B1, ochratoxin A and fumonisins: Effects on animal performance, biochemical and liver gene analysis Insaf Riahi*¹, Antonella Della Badia¹, Catalina Nicholls¹, Raquel Codina¹, Eva León¹, Amrita K. Dhara², Anirvid Sarkar², Sayantani Arora², Sudipto Halder² *BIONTE Nutrition S.L., Technical Department, Reu, Tarragona, Spain; ²Agrivet Research and Advisory P Ltd., Lake Town, South Dum Dum, West Bengal, India, Kolkata, India*

Grape and olive extracts have shown to mitigate the detrimental effects induced by mycotoxin exposure, as oxidative stress and liver damage (Antonissen et al., 2015). This study evaluates the efficacy of a liquid solution (LS) with grape and olive extracts in mitigating the negative effects of mycotoxin exposure on performance, biochemical parameters, and liver gene expression in broiler chickens fed a multi-mycotoxin-contaminated diet. A total of 72 d broiler chickens Ross 308 were randomly distributed

in 2 groups (G1 and G2), with 3 replicates/12 broilers, during 42d. The experimental diet (ED) was contaminated with different levels of aflatoxin B1 (AFB1), fumonisins (FBs), and ochratoxin A (OTA) during all the nutritional phases (on average: 4.8, 855.6, 9.5 ppb for AFB1, FBS and OTA respectively). Then, G1 received ED without any supplementation (n=36), and G2 received ED plus 2L/1000L of the LS through drinking water, during all the experiment (n= 36). Feed intake (FI), body weight (BW), average daily gain (ADG) and feed conversion ratio (FCR) were recorded on 10, 24 and 42d. On 10 and 24d, blood was collected to analyze stress biomarkers (SOD, GSH-Px) and liver tissue samples for hepatic gene analysis (Nrf-2, EPHX1). On 42 d mortality was recorded and carcass traits were analyzed (liver, gizzard, giblet, drumstick and thigh). Statistical analyses were performed using the PROC GML of SAS. Mycotoxin exposure decreased FI, BW and ADG in G1 on 42d (-2.5% on average; $P < 0.05$). This effect was counteracted in G2 animals, where these parameters significantly increased due to the supplementation with LS on 42 d ($P < 0.001$). The same trend was observed for carcass traits, which were significantly improved in G2 compared to G1 ($P < 0.003$). SOD levels significantly increased in G2 at 24 d (by 62%; $P < 0.001$), thus demonstrating the antioxidant efficacy of the the LS. The hepatoprotective effect of LS was confirmed by the increase in hepatic gene expression analysis observed in G2 on 42 d (Nrf-2: $P < 0.022$). The results suggest that the LS beneficially impacts on the performance, biochemical parameters, and hepatic gene expression in broiler chickens challenged by a multi-mycotoxin contaminated diet.

Keywords: multi-mycotoxin contamination, broiler chickens, gut health, anti-mycotoxins agent, grape and olive extracts

P342 A combination of essential oils from oregano, cinnamon, and clove improves growth performance, gut morphology, and intestinal barrier function in broilers In H. Kim*², Md Raihanul Hoque¹, Chae Bin Lim¹, Lane Pineda³, Marlien de Kock³, Ellen Hambrecht³ *¹Dankook University, Animal Biotechnology, Cheonan, South Korea, Korea (the Republic of); ²Dankook University, Animal Biotechnology, Cheonan, Choongnam, Korea (the Republic of); ³Trouw Nutrition, Amsterdam, Netherlands*

Plant-derived products, including essential oils (EO), have long been recognized for their beneficial effects on animal health and physiology. However, effects vary depending on the source, structure, and form of use of the EO, but also on animal characteristics and other factors. This study was designed to compare the effects of different blends of EO on broiler performance, intestinal morphology, and gene expression related to immunity and gut barrier function. Broiler chicks (n=1680) were randomly assigned to four dietary treatments with 21 replicates of 20 chicks each. The treatments included a basal diet (NC), a basal diet + a blend of oregano, clove, and cinnamon essential oils (FP), a basal diet + a thyme and star anise extracted essential oil blend and quillaja bark powder (BS), and a basal diet + blend of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (DS). Between days 22 and 28, 2.5% dextran sulphate sodium was orally administered via drinking water to induce enteric inflammation. The performance parameters were recorded from day 1 to 42, and for markers related to intestinal morphology and gene expression, samples were taken on day 28 (n=2 birds/replicate). Supplementation of FP increased average daily gains (+4.6%, $p=0.003$) and final body weight (+112 g, $p=0.003$) of broilers compared to NC. Additionally, FP supplementation reduced gene expression of the pro-inflammatory cytokines interleukin 1 and interleukin 8 in the ileum tissue ($p < 0.05$) and increased ileal villous height ($p=0.01$), villous:crypt ratio ($p < 0.10$)

and expression of genes coding for mucin and zona occludens in the cecum ($p < 0.05$) and ileum ($p < 0.10$). On the other hand, performance, intestinal morphology and gene expression were statistically not significantly different between NC, BS, and DS ($p > 0.10$). These results suggest that the specific blend of oregano, clove, and cinnamon essential oils used in the present study can enhance growth performance, improve gut morphology, strengthen intestinal barrier function, and mitigate intestinal inflammation in broilers.

Keywords: essential oils, MUC2, ZO1, Clove, intestinal infection

P343 Influence of bacillus-based probiotic and yeast cell wall-derived prebiotic supplementation across various laying stages on performance, egg quality, and immune response Ala E. Abudabos*¹ *Alcorn State University, Agriculture, Lorman, Mississippi, United States*

This study explored the impacts of probiotics (*Bacillus* spp. Strains) and prebiotic (yeast cell wall) supplements on the performance, egg quality, and serum inflammatory markers of laying hens across various stages. Over 16 weeks, 500 Hisex white layers at 37 weeks were allocated in a randomized complete block design into 5 dietary regimens (5 replications of 20 birds each): T1, control; T2, control + 0.5 g/kg *Bacillus subtilis*; T3, control + 0.5 g/kg *Bacillus subtilis* and *Bacillus licheniformis*; T4, control + 0.4 g/kg *Bacillus coagulans*; T5, control + 0.25 g/kg yeast cell wall. For the cumulative performance ($P < 0.001$), T1 exhibited heightened feed consumption compared to all other treatments. Conversely, T4 and T5 demonstrated greater egg production than T1 and T3, with T1 exhibiting the lowest production rate. Markedly, T3 recorded the highest egg weight while T2 had the lowest. Additionally, T3, T4, and T5 demonstrated elevated egg mass compared to T1 and T2, with T1 showing the lowest mass. Furthermore, the feed conversion ratio was enhanced across all supplemented groups relative to the control group. The wet yolk percentage in T5 was elevated compared to T1, T2, and T3 ($P < 0.001$), whereas dry albumin percentages in T2 and T5 surpassed that of the other treatments ($P < 0.05$). Circulating pro-inflammatory cytokines (IL-1 β ($P < 0.05$), IL-6 ($P < 0.01$), TNF- α ($P < 0.01$)) were diminished in hens receiving supplemented diets compared to those on an un-supplemented diet. Moreover, the feed supplements resulted in an elevation of the levels of anti-inflammatory cytokines (IL-10 ($P < 0.01$) and IFN- γ ($P < 0.05$)) in contrast to the un-supplemented control. In conclusion, the dietary inclusion of probiotic *Bacillus* spp. strains and yeast-derived prebiotic hold great promise for boosting productivity and supporting the health status of layers raised on antibiotic-free diets.

Keywords: Probiotic, Prebiotic, Performance, Inflammatory response, Egg quality

P344 Bacterial competitive exclusion product compatibility and synergy with 3 *Salmonella* live vaccines Alison Johnson², Sally Gibson², Florence Barbe¹, Audrey Sacy¹, Anaïs Nozeran¹, Pierre Lebreton*¹, Eric Chevaux¹, Victor Pain³ *Lallemand SAS, Blagnac Cedex, France*; ²*Lallemand SAS UK, Malvern, United Kingdom*; ³*SOCSA Elevage, L'Union, France*

In modern poultry production, vaccination is an efficient strategy to control *Salmonella* prevalence. The objectives of this study were to demonstrate the compatibility of a Bacterial Competitive Exclusion (CE) product (AviGuard, Lallemand) with 3 *Salmonella* live vaccines (LV) and to evaluate the synergistic effects of both solutions in *Salmonella* challenge model. The recovery in the liver of chicks of the *Salmonella* strain used in LV was used to demonstrate vaccine uptake and counts reduction of *S. kedougou* in ceca as a proof of efficacy/synergy. Firstly, a model

was developed to test the compatibility of the co-administration of CE and LV on the same day. 60 chicks were conjointly dosed by oral gavage at d1 with 3 LV (A: *S. enteritidis*; B & C: *S. typhimurium*) and with either water or CE (6 groups, $n = 10$ birds/group). The number of birds positive for LV strains in liver was determined for each group. Secondly, the synergistic effect of each LV with CE was evaluated with a challenge model. 144 chicks were allocated in 16 boxes of 9 chicks: control- (PBS, $n=1$), control+ (*Salmonella kedougou*, $n=1$), CE ($n=2$), gavage at d1 of the 3 LV (A, B, C) with or without CE ($n=2$ /condition). Each LV (+/- CE) was administered by oral gavage at d1, *S. kedougou* was administered orally at d2 (6.95e3 cfu/chick) for all groups except control- and *S. kedougou* was numerated in ceca at d8. Infection factor (IF) was calculated for each box as: $\sum(\text{nb birds} \times \log \text{number}) / \text{nb birds}$ and 3 categories of protection were defined: not protected: % of birds colonized at $> 10^5$ cfu/g organ; partially protected: % of birds colonized between 10^1 and 10^5 cfu/g organ; fully protected: % of negative birds. Results were analyzed by Chi2 tests (IBM SPSS Statistics 26.0). Aviguard (CE) and the 3 *Salmonella* LV compatibility is confirmed with similar *Salmonella* LV strain recovery in the liver in the 2 groups (vaccine and vaccine + CE). IF is reduced and more birds are fully protected when each *Salmonella* LV is combined with CE ($P < 0.05$). Improved and synergistic protective effects are then demonstrated in *S. kedougou*-challenged birds, when CE and *Salmonella* LV are used together, which may be an interesting approach under commercial conditions to better protect chicks from early multi-pathogenic contamination.

Keywords: *Salmonella*, live vaccine, competitive exclusion, compatibility, synergy

P345 Compatibility of a competitive exclusion solution with a *Salmonella* live vaccine administered at different ages Victor Pain², Jonny Harrison¹, Anaïs Nozeran¹, Sophie Huppert¹, Pierre Lebreton*¹, Florence Barbe¹, Audrey Sacy¹ *Lallemand SAS, Blagnac cedex, France*; ²*SOCSA Elevage, L'Union, France*

Salmonella live vaccines (LV) and Competitive Exclusion Products (CE) are 2 strategies used in poultry production systems to control *Salmonella* prevalence. The compatibility between these 2 solutions was confirmed when co-administered on the same day or when CE is administered 24 hours after the vaccine. The objective of this study was then to demonstrate the compatibility of a CE product (AviGuard, Lallemand) with a *Salmonella* LV administered at different ages. 54 chicks were randomly allocated to 6 groups of 7 birds each: control (*Salmonella* LV alone administered at D1: Cevac Salmovac: *S. enteritidis*), and 5 groups receiving CE at D1 and *Salmonella* LV at D1, D3, D5, D7 and D9, respectively. In each group, 2 sentinel birds were additionally raised along with the treated chicks (CE sentinel receiving CE at D1 but no LV and blank sentinel, receiving neither CE nor LV). All birds were necropsied at D10. The efficacy of vaccine uptake was measured by quantifying the recovery of LV strain in chick tissues (liver & ceca). One-way ANOVA and Tukey post-hoc tests were used to compare the different groups for *Salmonella* count in each organ (liver, ceca) (IBM SPSS Statistics 26.0). The compatibility of AviGuard and *Salmonella* LV was confirmed based on the successful recovery of LV strain from the liver of all tested groups regardless of the age of LV administration. The numeration of *Salmonella* vaccine strain in the ceca showed decreasing *Salmonella* count, control and D1 groups presenting the highest counts and D5, D7, D9 groups presenting the lowest counts ($P < 0.001$). It could be due to: 1) less time for *Salmonella* to invade the ceca as *Salmonella* LV was administered at later stages and all birds were sacrificed at D10; 2) a competitive exclusion effect in the gut between CE and LV as microbiota provided by CE

had more time to settle when LV is administered later. Interestingly, *Salmonella* LV strain was not detected in the sentinel birds demonstrating that there was no cross-contamination between vaccinated and unvaccinated contact birds. This suggests that the *Salmonella* LV strain has to be orally administered to infect the birds. This study adds valuable information and confirms the compatibility of this CE product with *Salmonella* LV administered at different ages.

Keywords: compatibility, competitive exclusion, *Salmonella*, live vaccine, age

P346 Effects of live yeast *Saccharomyces cerevisiae boulardii* on broilers growth performances and food safety Dariusz Mikulski², Jan Jankowski², Pierre Lebreton*¹, Florence Barbe¹, Vanessa Demey¹ ¹Lallemand SAS, Blagnac Cedex, France; ²Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Department of Poultry Science, Olsztyn, Poland

This study aimed to evaluate the impact of live yeast supplementation on the growth performance of broilers and the carriage of *Salmonella* and *Campylobacter*, focusing on pathogen control and food safety. A total of 2,000 Ross 308 broilers were divided into four groups with 10 replicates of 50 birds each: T1 (Unchallenged Control), T2 (Challenged Control), T3 (Unchallenged Live Yeast) and T4 (Challenged Live Yeast). The live yeast (*Saccharomyces cerevisiae boulardii* CNCM I-1079) was incorporated at a rate of 1×10^9 CFU/kg of complete feed. The birds were housed in separate buildings for unchallenged and challenged groups. Challenges included a co-inoculation with *S. enteritidis* (10^5 CFU/bird) via drinking water and *C. jejuni* (3×10^5 CFU/bird) via oral gavage at 7 and 28 days of rearing. Feed and water were provided *ad libitum*, and the diet composition was adjusted in two phases (0-14 days and 15-38 days). Zootechnical parameters (body weight and feed intake) were recorded on days 0, 14, and 38. Microbiological analysis of caeca content was performed on days 14 and 38 to determine the presence of *Salmonella* and *Campylobacter*. Data were analyzed appropriately either by GLM-ANOVA (performance) or with non-parametric procedures (microbiology). Live yeast supplementation improved zootechnical performance, with 1.6% increase in body weight gain and average daily gain ($P < 0.05$) and 1.2% improvement in feed conversion ratio ($P < 0.05$). The challenge significantly reduced performance in the control group (T2 vs. T1) and the challenged live yeast group (T4) showed similar performance to the unchallenged control (T1). Microbiological analysis revealed numerically lower number of *Salmonella*-positive samples in the live yeast groups. No *Campylobacter* was detected in the unchallenged groups and a lower number of *Campylobacter*-positive samples were observed in the live yeast group at day 38. It can be concluded that supplementation with *Saccharomyces cerevisiae boulardii* enhances broiler growth performance and mitigates the negative effects of *Salmonella* and *Campylobacter* challenges. It also shows potential in reducing the carriage of these pathogens, contributing to improved food safety.

Keywords: live yeast, growth performance, *Salmonella*, *Campylobacter*, food safety

P347 Comparative Effects of Ionophore Anticoccidials with or without the addition of a QY phytogenic on Performance and Coccidia Lesion Scores in Broilers Challenged with Contemporary Coccidia Strains Luis B. Gomez*¹, Chad Malinak¹, Blair Telg¹, Sandra Bonaspetti¹, Vasil Stanev¹, Ana Villegas¹, Ashley Halowell¹, Brett Lumpkins² ¹Phibro Animal Health, Technical Services, Watkinsville, Georgia, United

States; ²Southern Poultry Research and Feed, Athens, Georgia, United States

The U.S. poultry industry's recent shift from marketing poultry products under "No Antibiotics Ever" (NAE) claims to "No Antibiotics Important to Human Medicine" (NAIHM) claims has driven a return to ionophores as the backbone of coccidia control. This resurgence coincides with both increasing acceptance and continued field performance of Magni-Phi® nutritional specialty product, a proprietary blend of *Quillaja* and *Yucca* saponins (QY). This study compared the impact on broiler performance and acquired coccidia immunity when broilers were fed ionophores with or without QY in a strict challenge of coccidia strains contemporary to the southeastern United States. The study employed completely randomized floor pens, 8 treatments with 8 replicates each, and dividers between pens to limit cross-contamination of pathogens. T1 and T2 represented non-medicated/non-challenged (NMNC) and non-medicated/challenged (NMC) controls. Three ionophores (salinomycin (SAL) at 50 g/ton; lasalocid (LAS) at 68 g/ton; and narasin (NAR) at 72 g/ton) were included alone in T3, T5 and T7 respectively, and combined with 250 g/ton of QY in T4, T6 and T8 respectively. Birds and feed were weighed and oocyst per gram of feces was measured weekly. Coccidia lesion scoring was performed on day 27. Statistical analysis employed the General Linear Models procedure with $P < 0.05$ to determine least significant differences. Compared to SAL and NAR, LAS had the lowest corrected FCR used alone or with QY, a significant difference alone but not with QY. The addition of QY to LAS resulted in a six-point lower FCR, though the difference was not significant. When used alone, all ionophores statistically reduced lesion score (LS) compared to the NMC (19.3% to 48.7% reduction). Their results were further improved by addition of QY (10.0% to 48.7% reduction), statistically significant differences in the SAL and NAR treatments. LAS was the only ionophore to statistically lower OPG compared to NMC at d28 when used alone. All three showed statistically lower OPG when combined with QY. Although ionophores still serve as the main tool for coccidia control, the benefit from feeding a phyto-genic with an ionophore was obvious in this trial where contemporary strains of coccidia were used.

Keywords: Ionophores, Phyto-genics, Coccidia, Broilers, Lesion Score

P348 Impact of sodium bisulfate supplementation, alone or in combination with in-feed anticoccidials, on broiler performance during a coccidiosis challenge Bradley Turner², Craig Coufal¹, Julianna Jespersen*¹ ¹Jones-Hamilton Co., Rock Island, Illinois, United States; ²Poultry Research Partners, LLC, Hoschton, Georgia, United States

Research has demonstrated that animal feed grade (AFG®) sodium bisulfate (SBS) can ameliorate performance losses in poultry during coccidiosis challenges. However, the impact of SBS inclusion in feeds in combination with anticoccidials has not been fully investigated. Therefore, this experiment assessed the effect of SBS supplementation, alone or in combination with other anticoccidial feed additives, on performance and intestinal health in broilers during a coccidiosis challenge. Day-old, male Cobb 500 by-product broiler chicks were randomly assigned to 6 dietary treatments with 10 replicate pens per treatment. Forty chicks were placed in each floor pen containing used litter (2,400 total). *Ad libitum* corn-soybean meal-based diets for each treatment were provided in 3 phases: starter (d 0-14), grower (d 14-28), and finisher (d 28-42). Treatments were formulated to be nutritionally equivalent and varied by feed additive as follows: control (no additive), SBS (0.3% SBS, all phases), Shuttle (0.04% nicarbazin

25% in starter and grower, 0.05% zoalene in finisher), SAL (0.05% salinomycin sodium, all phases), SBS-Shuttle (SBS+Shuttle), and SBS-SAL (SBS+SAL). Diets with SBS were balanced for sodium at the expense of salt and sodium sesquicarbonate. On d 0, a coccidiosis challenge was administered by spraying sporulated oocysts onto the feed in each pen (50,000 *Eimeria maxima* oocysts/pen). Growth performance was measured throughout the trial and intestinal lesion scoring (2 birds/pen) was conducted on d 28. Data were analyzed using a 1-way ANOVA with means separated by a Duncan's Multiple Range Test when appropriate. Means were considered significantly different at $P \leq 0.05$. Feed conversion ratio was highest (i.e., worst; $P < 0.05$) in the control treatment through the grower phase. Day 42 BW was greater ($P < 0.05$) for all additive treatments compared to the control. Birds fed diets with SAL had numerically higher BW on d 42 but were not different from treatments containing SBS. Day 42 feed conversion ratio was improved ($P < 0.05$) in all diets containing SBS compared to the control. These results indicate that incorporating SBS at 0.3% is advantageous to bird performance during coccidiosis and does not have negative interactions with in-feed anticoccidials.

Keywords: sodium bisulfate, gut health, coccidiosis, feed additive, anticoccidial

P349 Efficacy of gut microbiota modulators from yeasts on performance of broiler chickens under a necrotic enteritis challenge Elise Nacer-Khodja¹, Raquel Pereira¹, Amrita K. Dhara², Maria Angeles Rodriguez¹, Mariel Neves Tavares¹ ¹*Olmix SA, Brehan, France;* ²*Agrivet Research & Advisory, Kolkata, India*

Necrotic enteritis (NE) is a severe inflammation of the small intestine that can be artificially induced by inoculation of coccidia and *Clostridium perfringens* (CP). Gut microbiota modulators derived from yeast cell wall are among the additives that improve broilers performance and confer protection against NE by regulating the intestinal microbiota and modulating immunity. The aim of this study was to evaluate the efficacy of gut microflora modulators (MOS and β -glucans) combined with prebiotics (FOS and GOS) in reducing the effects of NE caused by coccidia and CP infections in broilers. A total of 770 male DOC (Cobb 500) were divided into 7 treatments with 10 replicates: A negative control (NC) group without challenge; a positive control (PC) group with NE challenge without any additive in the diet; an antibiotic group (PCAGP) with 150 g/T of enramycin; a gut microflora modulator group (PCG) with MOS and β -glucans; a gut microflora modulator + prebiotics group (PCGG) with FOS, GOS, MOS and β -glucans; and 2 competitor groups (PCC1 and PCC2) with similar products containing β -glucans and/or MOS. All the birds, except those in the NC group, were challenged by oral gavage with 5,000 oocysts of *Eimeria maxima* at d15 and with CP at d18, d19 and d20. On d42, all the remaining birds were euthanized and productive performance, histomorphometry, and TMBES (Total Mean Bacterial 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 d42, the weight of the birds in the PCGG group was significantly higher than all the others, and not significantly different from the NC group. Although there weren't significant differences, the PC group had a lower viability than any of the other groups, showing the effect of the challenge. The PCAGP, PCG and PCGG groups had the highest viability. On d42, VH:CD was significantly higher in the PCGG group. In this study, the use of gut microflora modulators combined with prebiotics (PCGG group) offered the highest protection, allowing animals to significantly improve their

performance against the PC, and even (numerically) compared to the NC and the PCAGP.

Keywords: yeasts, necrotic enteritis, broilers

P350 Bioactive compounds from Passion fruit seed oil act as a natural antioxidant in laying hens Lais Cordeiro¹, Elis O. Castillo¹, Gabrieli A. Lima¹, Paola A. Rodrigues¹, Joyce A. Silva¹, Júlia d. Lopes¹, Luane B. Andrade¹, William d. Xavier¹, Matheus G. Guimarães¹, José R. Sartori¹ ¹*UNESP, FMVZ, Animal Breeding and Nutrition, Botucatu, SP, Brazil*

Feed additives have been extensively studied in poultry nutrition aiming to improve animal health and production. A great source of bioactive compounds is the agro-industrial waste, such as the passion fruit seed, which after cold pressing, results in a sustainable and safe oil rich in carotenoids (β -carotene, lycopene, pro-lycopene, β -cryptoxanthin), phytosterols (campesterol, stigmasterol, β -sitosterol and stigmasterol) and phenolic compounds (caffeic, salicylic and p-coumaric acids), which give it a high antioxidant capacity. Therefore, the current study was designed to evaluate the potential antioxidant capacity of passion fruit seed oil (PFSO) on laying hens. A completely randomized design was adopted with 192 Lohmann White laying hens aged 25 weeks, divided into three treatments with eight replicates of eight hens each. The groups were fed a corn-soybean-basal diet containing 0.00%, 0.45% and 0.90% PFSO for 16 weeks. At 41 weeks old, blood and liver samples were collected from eight hens per treatment and malonaldehyde and antioxidant enzyme activity of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione peroxidase (GPx; EC 1.11.1.9) were analyzed. Data were submitted to UNIVARIATE procedure and, whether the residual distribution was normal, the results were analyzed with simple regression by REG procedure, using SODA (SAS), to verify which regression model (linear or quadratic) best describes the results, considering a significance level of 0.05. PFSO inclusion decreased the plasma malonaldehyde in a linear regression ($P = 0.012$; $y = -0.0379x + 0.0962$, $R^2 = 0.29$) and there was no linear or quadratic effect of PFSO in the activity of SOD ($P = 0.302$; $P = 0.920$), GPx ($P = 0.384$; $P = 0.975$), and CAT ($P = 0.078$; $P = 0.270$). These results indicate that the bioactive compounds, especially carotenoids and phenolic compounds, of the PFSO acted as antioxidants, reducing the lipoperoxidation, without significantly demanding the enzymatic antioxidant system of the laying hens. In conclusion, PFSO can be included in layers diet at a level up to 0.90% to provide beneficial health responses by its antioxidant activity.

Keywords: animal health, antioxidant activity, functional additive, *Passiflora edulis*

P351 Effects of Pearlzyme® (Protease) supplementation on growth performance, nitrogen-corrected apparent metabolizable energy, and amino acid digestibility in broiler chickens fed reduced crude protein and amino acid diets Deependra Paneru¹, Oscar Tejada¹, Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Proteolytic enzymes are added to broiler diets to help break down proteins into smaller amino acids and peptides, thereby improving the digestion and absorption of nutrients. A study was conducted to evaluate the effects of Pearlzyme® (Pearlzyme Inc., Incheon, South Korea), a natural proteolytic enzyme derived from *Bacillus spp.*, on growth performance, nitrogen-corrected apparent metabolizable energy (AMEn), and amino acid (AA) digestibility in broiler chickens fed low crude protein (CP) and reduced AA diets. A total of 400 one-day old male Cobb500 broilers were

randomly allocated to 5 dietary treatments with 8 replicates and 10 birds per replicate for 21 days. The treatments included: (1) positive control with standard CP and AA (PC), (2) first negative control with 1.5% point lower CP and 10% reduced AA (NC1), (3) NC1 supplemented with 0.1% Pearlzyme® (NC1+PAR), (4) second negative control with 3% point lower CP and 20% reduced AA (NC2), and (5) NC2 supplemented with 0.1% Pearlzyme® (NC2+PAR). Growth performance, AMEn, and AA digestibility were analyzed on day 21. Statistical analyses were conducted using one-way ANOVA followed by Tukey's HSD post-hoc test, with significance set at $P < 0.05$, and trends were considered at $0.10 < P < 0.05$. The results showed that Pearlzyme® supplementation in 3% point lower CP and 20% reduced AA diet (NC2+PAR) significantly improved the body weight gain and feed conversion ratio compared to NC2 diet ($P < 0.001$). Pearlzyme® supplementation also improved AMEn in both low CP and reduced AA diets (NC1+PAR and NC2+PAR) compared to NC1 and NC2 diets ($P = 0.068$). Additionally, digestibility of energy, CP, and dry matter improved significantly in broilers fed Pearlzyme® supplemented diets (NC1+PAR and NC2+PAR) compared to both non-supplemented diets (NC1 and NC2) and PC diet ($P < 0.001$). Furthermore, the digestibility of AA, particularly, tryptophan, phenylalanine, leucine, and glutamate were significantly higher in broilers fed Pearlzyme® supplemented diets (NC1+PAR and NC2+PAR) compared to the PC ($P < 0.05$). In conclusion, our results indicate that Pearlzyme® supplementation at 0.1% can improve growth performance, AMEn, and AA digestibility in broiler chickens fed diets with low crude protein and reduced amino acids.

Keywords: Pearlzyme®, broiler chickens, AMEn, crude protein, amino acids

P352 Effects of Pearlzyme® (Protease) supplementation on growth performance, nutrient utilization, and immune response in broiler chickens Deependra Paneru*¹, Venkata Sesha Reddy Choppa¹, Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States*

Functional feed additives are becoming increasingly popular in the poultry industry due to their potential beneficial effects on bird health and performance. A study was conducted to evaluate the effects of two Pearlzyme® (Pearlzyme Inc., Incheon, South Korea) products, a feed additive containing a protease enzyme derived from a unique strain of *Bacillus* bacteria with/without probiotics, on broiler performance, nutrient utilization, and immune response. A total of 240 one-day-old male Cobb500 broiler chicks were randomly assigned to three dietary treatments: a control diet (CON) and two experimental diets supplemented with 0.1% Pearlzyme 1 (PAR1: protease alone) and 0.1% Pearlzyme 2 (PAR2: combination of protease and probiotics), provided for 21 days with 8 replicates of 10 birds each. Growth performance, nitrogen-corrected apparent metabolizable energy (AMEn), and amino acid (AA) digestibility were assessed in non-challenged birds on day 21. Additionally, a subset of birds was challenged with lipopolysaccharide (LPS) on day 19 to evaluate the effects of Pearlzyme® on gut health and immune response. Statistical analyses were performed using one-way ANOVA followed by Tukey's HSD post-hoc test, with significance set at $P < 0.05$ and trends noted at $0.05 < P < 0.10$. Results indicated that both PAR1 and PAR2 significantly increased AMEn compared to CON ($P = 0.0027$). PAR2 supplementation led to a 3.93% increase in feed intake ($P = 0.086$) and a 5.86% increase in body weight gain ($P = 0.090$) compared to CON. Furthermore, PAR2 supplementation improved the digestibility of phenylalanine, and tryptophan compared to CON ($P < 0.05$). In LPS-challenged birds, PAR2 supplementation significantly downregulated the expression of

interleukin 1 β (IL-1 β) and interleukin 10 (IL-10) in the ileum compared to the CON ($P < 0.001$). Both PAR1 and PAR2 tended to downregulate claudin-1 (CLDN1) expression ($P = 0.095$) compared to CON, although intestinal morphology remained unaffected. In conclusion, Pearlzyme® supplementation at 0.1% can improve energy utilization and AA digestibility, thereby improving the growth performance in broilers. Additionally, Pearlzyme® mitigates the inflammatory response under LPS challenge, demonstrating its potential as a functional feed additive in poultry.

Keywords: Pearlzyme®, AMEn, nutrient digestibility, immune response, broiler chickens

P353 Replacement of choline chloride with natural choline in broiler chicken diets Bernardo F. Iglesias*^{1,2}, Maria Viviana Charriere¹, Virginia Fain Binda¹, Silvina Pinto³, Gonzalo Vicente⁴, Christian Vagedes⁴ ¹*Instituto Nacional de Tecnología Agropecuaria - INTA, Sección Avicultura, Pergamino, Buenos Aires, Argentina;* ²*UNNOBA, Área de Producción Animal, Pergamino, Buenos Aires, Argentina;* ³*Universidad de Buenos Aires, Fac. de Cs. Veterinarias, Buenos Aires, Argentina;* ⁴*Adinova, Coronda, Santa Fe, Argentina*

Choline is a water-soluble compound with vitamin-like properties, found in all plant and animal cells. This dietary compound performs essential metabolic functions, such as serving as a precursor for acetylcholine synthesis and as a component of phospholipids. Among its non-essential metabolic functions, choline acts as a methyl donor and helps prevent fatty liver and perosis. The aim of this trial was to evaluate the effect of two sources (synthetic and natural) on broiler performance and intestinal histopathology. A total of 672 one-day-old male broiler chicks (Cobb-500) were allocated in floor pens in a completely randomized block design (14 replicates/treatment). Starter (1-14 d), grower (15-28 d), finisher (29-35 d) and last week (36-42 d) diets were used. Three treatments were evaluated, 1. Basal (BSL) formulated according to Cobb-500 requirements without choline addition; 2. Synthetic (choline chloride, ChCl at 800 ppm 1-14 d; 500 ppm 15-35 d and 300 ppm 36-42 d) and 3. Natural (Colmax®, ChNat, at 250 ppm 1-28 d and 200 ppm 29-42 d). Birds and feed were weighed weekly individually and by pen, respectively, and the mortality recorded daily. At the end of the trial one bird per pen was selected by average weight and euthanized. The duodenum and jejunum were fixed in formalin 10% for histopathological examination: integrity lost (IL) and inflammation (Infl). Parametric data were analyzed using ANOVA and non-parametric data through Kolmogorov-Smirnov test. Compared to the BSL, at 42 d, the addition of ChCl improved the body weight (+3.4%, $P < 0.05$) and FCR (-2.6%, $P < 0.10$), but not affect the feed intake ($P > 0.10$), and ChNat increased the body weight (+3.3%, $P < 0.05$) and feed intake (+1.5%, $P < 0.10$). Compared to ChCl, ChNat only differed by showing higher feed intake (+1.3%, $P < 0.10$). Histopathological traits were not different between treatments ($P > 0.10$), however with ChNat, no chickens were found to have IL in either the duodenum or jejunum. Additionally, broilers in this treatment group showed the lowest frequencies of Infl (duodenum and jejunum). In conclusion, natural choline effectively replaced choline chloride without compromising broiler performance, potentially offering benefits in intestinal histopathology.

Keywords: Poultry, Broiler performance, Choline chloride alternative, Feed additive, Intestinal histopathology

P354 Understanding the effectiveness of short and medium chain fatty acid glycerides in Cocci-Challenged broilers: a 57-day commercial field evaluation Ariel Bergeron*¹, Rob

Shirley¹, Nicholas P. Evans¹ ¹*Adisseo USA, Alpharetta, Georgia, United States*

Using 1,800 Ross 708/YPM chicks that were sourced from a single breeder flock, a 57-day commercial field was conducted to determine the effect of feeding blends of short- and medium-chain fatty acid (SMCFA) products on the performance of broilers that were challenged with 20x cocci vaccine and raised on used litter. Across 12 replicate pens per treatment and 50 chicks/pen, broilers were allocated to 3 treatments (TRT 1: Negative Control (no SMCFA), TRT 2: SMCFA Blend 1 (FRA Gut Balance; glycerides of Butyric, Citric, Caprylic, and Capric acids), and TRT 3 SMCFA Blend 2 (FRA Gut Protect; glycerides of Butyric, Citric, Caprylic, Capric, and Lauric acids). The feed additives in TRT 2 and TRT 3 were provided in a four-phase step-down feeding program (Starter (0-14 days): 0.1%; Grower (15-30 days): 0.075%; Finisher (31-44 days): 0.05%; Withdrawal (45-57 days): 0.025%). All trial birds were challenged [RS1] on day 15 with a 20x vaccine dose of Cocci-Vac B52. Performance measurements included 35- and 57-day-old body weight gain (BWG), feed intake (FI) and feed conversion (FCR). These data were evaluated by ANOVA using JMP18; and, using a post-hoc Student's t-test, comparisons were made between the control and two SMCFA treatments. On d-35, significant improvements in BWG ($p=0.0005$) and FCR ($p=0.001$) were observed for TRT 3 compared to TRT 1 and TRT 2 (BWG - TRT 1: 1.88 kg, TRT 2: 1.83 kg, and TRT 3: 2.06 kg; FCR - TRT1: 1.57, TRT 2: 1.56, and TRT 3: 1.50). The same response in BWG ($p=0.0067$) and FCR ($p=0.0387$) between TRT 3 and TRT 1 and 2 was observed on d-57 (BWG - TRT 1: 4.15 kg, TRT 2: 4.02 kg, and TRT 3: 4.36 kg; FCR - TRT 1: 1.71, TRT 2: 1.70, and TRT 3: 1.67). Defining the mode of action, it is theorized that the improvements in post-challenge performance of TRT 3 was due to the presence of Lauric acid (C12). Lauric acid is observed to aid immune modulation under a bacterial and/ or viral challenge. Given the typical dysbiosis and necrotic enteritis that follows a heavy cocci challenge, it is possible that C12 may have mediated a positive shift in the gut's microflora, resulting in less of a performance drag. Further research is required to better understand the mode of action for short and medium chain fatty acids during a vaccine challenge.

Keywords: short- and medium-chain fatty acid (SMCFA), broilers, performance, cocci vaccine, cocci challenge

P355 Efficacy of a toxin binder in mitigating aflatoxin-induced damage in broiler chickens Mariana Ornaghi¹, Caio Tellini¹, Rodrigo Bressan¹, Bruna Troian¹, Jéssica Zandonai¹, Jociane Boeck¹ ¹*Safeeds, Research and Development, Cascavel, Paraná, Brazil*

Aflatoxins are toxic metabolites produced by fungi such as *Aspergillus parasiticus* and pose serious risks to poultry health by causing hepatotoxic, immunosuppressive, and carcinogenic effects. These toxins impair animal performance and threaten both economic sustainability and food safety. Toxin binders can mitigate these effects by binding toxins, reducing absorption, and promoting excretion through faeces. This study evaluated the efficacy of a toxin binder (Safetox[®]) in improving the performance of broiler chickens fed diets contaminated with 2.8 ppm aflatoxins. The experiment was conducted at the SAMITEC Institute with 300 male COBB broilers distributed into five treatments (6 replicates of 10 birds per treatment) over 21 days. Treatments included a control diet without aflatoxins (T1), a contaminated diet with aflatoxins (T2; 2.8 ppm), and contaminated (2.8 ppm) diets supplemented with Safetox[®] at two dosages, 0.15% (T3) and 0.30% (T4). Birds had *ad libitum* access to feed and water in an environmentally controlled condition. Performance metrics (body weight, feed intake, and feed conversion ratio) were assessed on

days 7, 14, and 21. Relative liver weight and plasma protein levels (PPT) were evaluated to determine hepatic health. Statistical analyses were performed using ANOVA and Bonferroni's test ($P \leq 0.05$). The results showed that aflatoxins significantly reduced broiler performance on days 14 and 21, from 415.92 g to 317.92 g, T1 and T2 respectively. However, the two treatments with toxin binder inclusion (T3 and T4) improved growth parameters, liver function, and PPT levels compared to birds fed the contaminated diet ($P < 0.01$) the level of 0.30% were numerically higher compared to the low dosage, although the FCR was not significantly different between the treatments. Including the toxin binder at both levels ($P < 0.01$) reduced liver weight and increased PPT, indicating positive effects on liver health. These improvements are likely associated with the enhanced feed intake observed in the treatments with toxin binder usage ($P < 0.01$). These findings demonstrate that using a toxin binder is an effective strategy to mitigate the negative effects of aflatoxins in broiler's diet, promoting animal health and production efficiency.

Keywords: Health, Liver, Mycotoxins, Poultry

P356 Enhancing broiler health and performance: toxin binders as a solution against fumonisin-induced damage Mariana Ornaghi¹, Maigel Dreyer¹, Andressa Flores¹, Mayara Gall¹, Osmair Stuani^{2,1}, Ricardo Castilho¹ ¹*Safeeds, Research and Development, Cascavel, Paraná, Brazil*; ²*Safeeds, Cascavel, Paraná, Brazil*

Fumonisin is a mycotoxin produced primarily by *Fusarium* species, commonly found in corn and other cereals, and pose significant health risks to animals and humans. Toxin binders are widely used in animal nutrition to adsorb toxic molecules, potentially reducing adverse effects on the gastrointestinal tract by preventing mycotoxin absorption and mitigating health damage. Additionally, substances with hepatoprotective properties support metabolism in eliminating toxic compounds. This study aimed to evaluate the efficacy of the toxin binder Safetox[®] (containing montmorillonite, mineral coal, and hepatoprotective agents) in broiler chicken diets challenged with fumonisins (FUM), focusing on animal performance. The treatments included a control diet without FUM (T1), a FUM-contaminated diet (T2; 100 ppm), and FUM-contaminated diets supplemented with two dosages of the toxin binder: 0.20% (T3) and 0.50% (T4). Performance metrics (body weight, feed intake, and feed conversion ratio) were evaluated on days 7, 14, and 21. Additionally, relative liver weight, plasma protein levels (PPT), and the sphinganine/sphingosine (SPA/SPO) ratio were assessed to determine liver health. Statistical analyses were conducted using ANOVA and Bonferroni's test ($P \leq 0.05$). Results showed that FUM significantly reduced broiler performance, with body weight declining from 443g to 364g on day 14 and 855g to 685g on day 21 in T1 and T2, respectively ($P < 0.01$). However, toxin binder treatments at both concentrations (T3 and T4) improved body weight compared to the FUM-only group (T2; $P < 0.01$). Feed efficiency was higher in T1, T3, and T4 treatments, with values of 1.31, 1.34, and 1.36, respectively, versus 1.44 for T2 ($P < 0.01$). Liver health metrics also improved with toxin binder inclusion ($P < 0.01$), as shown by reduced liver weight, higher PPT levels, and a lower SPA/SPO ratio. These findings suggest that toxin binder supplementation can enhance liver health and broiler performance under the FUM challenge, offering effective animal protection against fumonisin toxicity. Overall, this study highlights the importance of mycotoxin management in animal nutrition, emphasizing that effective detoxification approaches can secure productivity and animal health.

Keywords: Mycotoxins, hepatic health, poultry

P357 Evaluating the impact of probiotic and multivariant vaccination strategies in mitigating BCO lameness using a hybrid challenge model Khawla S. Alharbi*¹, Amanda Anthney¹, Ruvindu Perera¹, Reginald Onyema², Sara Reichelt², Antoine Meuter³, Palmy Jesudhasan⁴, Adnan Alrubaye¹
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Bacterial chondronecrosis with osteomyelitis (BCO) lameness is the most critical animal health and welfare issue facing the broiler industry worldwide. It is estimated that 1 to 2% of bird condemnation at marketing age is caused by BCO lameness, resulting in tens of millions of dollars in annual losses. Therefore, this study aims to evaluate the efficacy of a probiotic program associating an *Enterococcus faecium*-based probiotic (*E. faecium* 669 sprayed on day old chicks at 2×10^9 CFU/bird) and a triple-strain *Bacillus*-based product (probiotic combining *B. subtilis* 597, *B. subtilis* 600 and *B. amyloliquefaciens* 516 included in the drinking water at 1.0×10^9 CFU/bird/day from day 1 to day 56), both in isolation and in conjunction with a multivalent electron beam (eBeam)-inactivated vaccine, on the reduction of BCO lameness in broiler chickens. The experimental design involved using two wire-flooring pens (T1) that served as the source of infection, simulating the aerosol transmission of bacteria typically in commercial poultry houses. A negative control group (T2) was exclusively maintained on standard feed, while the remaining birds were allocated to three treatment groups: T3 (six pens receiving the probiotic program), T4 (six pens receiving the multivariant vaccine), and T5 (six pens receiving both the probiotic program and the multivariant vaccine). A total of 1,300 fertile eggs were randomized across treatments. Cumulative lameness incidence was monitored from day 21 to 56 through daily evaluations and necropsies. Data were analyzed using ANOVA with Tukey's HSD ($p < 0.05$). By day 56, T3, T4, and T5 exhibited significantly lower lameness incidence (43.7%, 40.3%, and 40.7%, respectively) compared to T2 (71.0%) and T1 (83.0%) ($p < 0.05$). The combination of probiotics and vaccine (T5) demonstrated a synergistic reduction in lameness, significantly improving outcomes compared to individual treatments ($p < 0.05$). These results indicate that using probiotics alone or combined with a multivalent vaccine offers a promising strategy to reduce BCO lameness prevalence in broilers. Further studies are needed to clarify the mechanisms and assess long-term benefits in commercial production, enhancing animal welfare and mitigating economic losses.

Keywords: Broiler, Lameness, BCO, Probiotic, eBeam-inactivated Vaccine

P358 Effect of early-life electrolyte supplementation on broilers under commercial housing conditions Caio Tellini*², Mariana Ornaghi², Francisco Bertoli¹, Juliano Sganzerla³, Carla Rodrigues³
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Dehydration in chicks, particularly during the first-week post-hatch, can significantly impact growth and performance. Excessive fluid loss relative to intake reduces circulatory volume and tissue hydration, leading to an imbalance often associated with acid-base and electrolyte disturbances that impair physiological functions. Severe dehydration can also suppress immune function and increase susceptibility to infections. This study evaluated the efficacy of a rehydrating electrolyte supplement administered during the first week of rearing. The experiment was conducted across twenty commercial farms in northern Paraná, Brazil, using

2,000 Ross male broilers. Birds were randomly assigned to two treatments: a control group without supplementation and a group receiving an electrolyte supplement (Hydrat[®] Lyt) in drinking water. Each treatment included 10 replicates with 100 birds per replicate. Birds were weighed upon arrival and randomly placed in one of three aviary locations: near the evaporative cooling pads, in the middle, or near the exhaust fans. Birds were reweighed 7 days after treatment, and mortality was recorded. Additionally, batch uniformity was observed. Data were analyzed using one-way ANOVA in SAS 9.4, with means compared using Tukey's test. Results were considered significant at $P \leq 0.05$. Animals receiving electrolyte supplementation achieved a significantly higher average weight (201.28 g) compared to the control group (196.13 g; $P < 0.001$), with similar mortality rates across treatments (1.5%; $P = 0.927$). Batch uniformity also tended to improve with electrolyte supplementation in drinking water ($P = 0.063$). These findings suggest that dehydration during the first week of housing can substantially impact growth and can potentially impact health responses in broilers. Administering electrolytes via drinking water in the early stages can be an effective strategy to improve weight gain, uniformity, and resilience under commercial housing conditions, particularly in hot climates or environments with a high risk of dehydration. Implementing this approach may enhance overall productivity and reduce health complications associated with early dehydration, supporting the welfare and performance of the birds.

Keywords: Dehydration, growth uniformity, poultry

P359 Supplementation of a synbiotic improves the growth performance of broiler chickens submitted to coccidiosis vaccination Cristiano Bortoluzzi*¹, Chasity Pender²
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Coccidiosis vaccination may be included in coccidia control programs to induce immune response, thereby generating protection against subsequent *Eimeria* challenges and reducing the severity of coccidiosis. However, coccidiosis vaccination may induce intestinal damage that could impair the growth performance of chickens. The objective of the present study was to evaluate the impact of the supplementation of a Synbiotic (SYN) on the growth performance of fast-growing broiler chickens vaccinated against coccidiosis. A total of 720 one-day-old Ross 308 broiler chicks were placed in 24 floor pens (3 treatments, 8 replicates, 30 birds/replicate). The three treatment groups were: unvaccinated control, vaccinated control, and vaccinated birds fed diets supplemented with SYN at 500 g/MT from 1-21d (PoultryStar[®] me^{EU}). The coccidiosis vaccination (Paracox[®]) was applied, to the respective groups, by spray at the hatchery. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were calculated for each feeding phase (starter, 1-14d; grower, 15-27d; finisher, 28-42d). Cumulative performance was calculated from 1-28d and for the entire growth cycle. Growth performance data were subjected to a one-way ANOVA with blocks and, when appropriate, means were separated by Tukey's test. It was observed that, as expected, the coccidiosis vaccination impaired the FCR ($P = 0.03$) of the birds at d 28 by 3.5 points, but the supplementation of SYN promoted an improvement of 5 points in FCR compared to the coccidiosis vaccinated group. Considering the entire experimental period (1-42d), the coccidiosis vaccination impaired ($P = 0.05$) the FCR by 3 points compared with the non-vaccinated group (1.593 vs 1.623), but the supplementation of SYN improved the FCR by 4 points (1.583 vs 1.623). Although not significant ($P = 0.25$), the supplementation of SYN reduced the overall mortality by 57% vs the vaccinated group. In conclusion, the supplementation of dietary SYN improved the growth

performance and should be considered as an effective solution when adopting vaccination as the program of choice for the control of coccidiosis in broiler chickens.

Keywords: broilers, coccidiosis vaccination, symbiotic

P360 The effect of NCH Direct-Fed Microbes incorporated into poultry feed with a coccidia vaccine program Addison Elstner^{*1}, Rosana Hirai¹, Austin O. Silva², Skylar White³, Charles Greenwald³, Audrey McElroy¹ ¹Texas A&M University, Poultry Science, College Station, Texas, United States; ²Texas A&M University, Poultry Science, Bryan, Texas, United States; ³NCH Technology, College Station, Texas, United States

Probiotics, such as *Bacillus*-based direct-fed microbes (DFM), have been shown to improve gut health and growth performance of poultry by modulating gut microbiota. This study evaluated the efficacy of NCH *Bacillus* probiotics for enhanced performance in Ross male-broilers under a challenge. Birds were fed a corn-soy diet during a 42d grow-out with 90-floor pens (20 birds/pen, 0.084 m²) using a random-block design. All birds received a 1X-dose of Coccivac-B52[®] before being assigned to 1 of 9 treatments: negative control (NC) void of DFM, positive control (PC) with 50g/ton of bacitracin methylene disalicylate (BMD), a competitor's dual-strain product (1.3x10⁶ CFU/g), and 3 NCH probiotics: dormant *B. subtilis* (DS), dormant-dual *B. subtilis* + *B. licheniformis* (DD), or active *B. subtilis* (AS) at 1.3x10⁶ or 5x10⁶ CFU/g. Performance metrics, including average body weight (BW), BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured on days 14, 28, and 42. Data was analyzed using PROC GLM SAS 9.4 with 2-way ANOVA and Tukey's test (P<0.05) for means separation. From d0-14, the dormant dual (DD) strain at 1.3x10⁶ or competitor product resulted in the lowest FCR (P<0.05) when compared to other treatments. In the grower phase, the DD-strain at 1.3x10⁶ continued to improve (P<0.05) BW, BWG, FI, and FCR. The PC had the best d29-42 FCR, while the NC, competitor product, dormant (DS) strain (5x10⁶), and active (AS) strain (1.3x10⁶) had comparable FCR. DD-strain included at 1.3x10⁶ resulted in a higher FI from d29-42 and d0-42 than the other treatments, with the competitor product performing similarly to the DD-strain. Birds fed the DD-strain at 1.3x10⁶ had a higher d42 BW (P<0.05) than those fed the single DS or AS (regardless of inclusion) and DD-strain at 5x10⁶, with the NC, PC, and competitor product performing similarly. Overall, PC-fed birds had reduced FCR (P<0.05) compared to birds fed the DD (5x10⁶) or AS (1.3x10⁶). DD-strain inclusion at 1.3x10⁶ had a higher d0-42 BWG than the DS or AS (regardless of inclusion) and DD at 5x10⁶. Results suggest early-stage inclusion of NCH *Bacillus* products benefit chicks by promoting gut-microflora diversity. Future studies should test higher vaccine doses and/or additional challenge to explore product limits.

Keywords: growth performance, antibiotic alternatives, *Bacillus*, direct-fed microbial

P361 A proprietary blend of Phenylpropanoid and vanilloid oleoresins ameliorate growth performance of broilers raised with non-medically important antibiotics (NMIA) Bertrand Medina^{*1}, Stijn Bruwier², Ivan Girard¹ ¹Probiotech International Inc., St-Hyacinthe, Quebec, Canada; ²PONCO Poultry Research Station, Vliedden, Netherlands

While recently some US broiler integrations reintroduced some NMIA (chemicals and ionophores coccidiostats) into their feeding programs, botanicals like spice extracts with reviewed positive effects on gastric function, gut integrity and activity, inflammation and immunity might have beneficial effect on birds' performance.

A total of 176 Ross 308 male broiler chickens were assigned to two dietary treatments: basal diet (CTL) and basal diet supplemented with 35 g/TM of botanicals based on phenylpropanoid and vanilloid rich oleoresins (phenylpropanoids:vanilloids ratio=3) granulated in a mineral matrix (C2PG, Probiotech International Inc). Grains-based (no animal by-products) diets were offered *ad libitum* to 6 replicates of 22 birds from 0 to 35 days (d) of age. 500 g/MT of robenidine HCL premix (0-28 d) were used as non-medically important chemical drugs. A microbial challenge from dirty litter suspension was completed at d7 on the litter (200 ml/pen). Live-weights (LW) and feed intakes were recorded on d10, 21, 28 and 35 (slaughter), and mortality rates were reported per period. LSMeans with repeated measures were performed to compare the variable (SAS, 2024). Final LW of broilers fed with the blend oleoresins (2411 g) was heavier compared to CTL (2358 g, i.e. +53 g, P<0,1). The highest effect on growth was measured at phase (d21-29) with +752 vs +719 (P<0.05) for C2PG and CTL groups, respectively. Similarly, feed conversion ratio (FCR) measured at d28 was optimised in favor of supplemented birds (1.436 vs 1.481, P<0.05), whereas only a numeric difference was observed at d35 (1.50 vs 1.52 for C2PG and CTL, respectively). Despite an higher cumulative mortality in C2PG (6,9%) compared to CTL (2,3%), calculated net profit was in favor the oleoresins supplemented group (0.899 vs 0.837 \$/bird). These results are consistent with previous ones observed on standard (avilamycin + coccidiostats) fed broilers (Avila et al., 2021) and validate this proprietary blend of oleoresins as a complementary aid to bolster the growth performance of birds raised with non-medically important antibiotics. Further investigations on biomarkers of gut integrity and activity could point out any complementary mechanisms between spice oleoresins and non-medically important antibiotics.

Keywords: botanicals, spices, broiler, non-medically important antibiotics, robenidine

P362 Potentiation of antioxidant defenses in broilers with a standardized dry grape extract supplementation Paul Engler^{*12}, Damian Bien³, Sekhoo Cissé¹², Sławomir Jaworski⁴, Agata Lange⁴, Patrycja Ciborowska⁵, Anna Zalewska⁵, Jakub Urban⁵, Monika Michalczuk⁵, Mohammed el Amine BENARBIA¹², Arkadiusz Matuszewski³ ¹Nor-Feed, Beaucauzé, France; ²FeedInTech, Angers, France; ³Warsaw University of Life Sciences, Institute of Animal Sciences, Warsaw, Poland; ⁴Instytut Nauk o Zwierzętach SGGW w Warszawie, Department of Animal Breeding, Warsaw, Poland; ⁵Warsaw University of Life Sciences, Institute of Biology, Warsaw, Poland

Antioxidant defenses are crucial for livestock resilience against many stressors. Over the last few decades, a strong emphasis has been put on potential antioxidants from natural sources. Whilst they are often studied *in vitro*, this only offers a partial vision of the activity of such solutions. Thus, the aim of the present study was to evaluate the effect of a dietary supplementation of broiler chickens with a commercial standardized dry grape extract (SDGE) on zootechnical performances and physiological antioxidant parameters. 756 day-old all-male Ross 308 broiler chickens were randomly divided into two groups and raised for 35 days. Control birds (CTL, n=392) received a classic feeding program. The rest of the chickens (NG, n=364) were fed the same dietary program with the addition of 30ppm of a commercial SDGE (Nor-Grape®, Nor-Feed, France) from D1 to D35. Body weight was measured weekly for each replicate and feed intake and FCR per period. Blood samples were collected on D11 and D35 to measure global antioxidant protection (TEAC) and oxidative damage levels (MDA). Endogenous antioxidant levels were also monitored to assess the potential modulatory effect of the

supplementation on CAT, SOD and GSH-Px. On D35, mean liveweight of NG chickens was significantly greater than the CTL (+5.8%, $p < 0.001$) and feed intake ($p < 0.01$). No differences ($p > 0.05$) were found in terms of FCR or mortality between groups. The European Efficiency Index was also significantly higher in the NG group compared to the CTL ($p < 0.05$). CAT activity was higher on D11 ($p < 0.001$) and D35 ($p < 0.001$) in the NG broilers. On D35, NG birds had higher SOD activity ($p < 0.05$), GSH-Px activity ($p < 0.05$), and antioxidant potential (TEAC, $p < 0.05$). Overall, these results confirm previous findings showing that a dietary addition of 30ppm of a commercial SDGE potentiates the antioxidant defense system, improving the chickens' resilience. This translates into improved performance as illustrated in this work.

Keywords: Broiler, grape extract, antioxidant, polyphenol

P363 Effects of different dose of chestnut extract on performance and intestinal health in broilers exposed to *Clostridium perfringens* and coccidia challenge Clarisse Techer*¹, William Lambert¹, André N. Mayer³, Eveline Berwanger³, Jovanir Fernandes² ¹*miXscience, Bruz, France;* ²*UFPR Federal University of Parana, Parana, Brazil;* ³*Salus Animal Nutrition, São Paulo, Brazil*

To face to restrictions on the use of in-feed antibiotic growth promoters (AGP), the research for alternatives remains a key topic in poultry production. Phytobiotics such as chestnut extracts, have shown to enhance productivity and animal health thanks to their antioxidant, anti-inflammatory and anti-microbial properties. However, contrasting results on the effective supplementation of chestnut tannins have been observed. This study analyses the effects of chestnut tannins on the growth performance and intestinal health in order to clarify the appropriate dosage and responses in broiler production. A total of 2,580 male Cobb 500 broilers were subjected to different treatments from 0 to 42 days: Uninfected Untreated Control (UUC, no challenge); Infected Untreated Control (IUC, challenge-no supplementation); Infected Treated Control (ITC, with virginamycin); 3 treatments infected and supplemented with KingBrown (KB), a purified source of chestnut tannins : KB0.5 (500g/T); KB1 (1kg/T) or a program (KB2) with 2kg/T, 1 kg/T and 0.5kg/T in starter, grower and finisher. Infected broilers challenge consisted with a 10x coccidiosis vaccine and a *Clostridium perfringens* inoculation. Zootechnical and intestinal health parameters, carcass and meat yields were evaluated. Statistical analysis was performed using R Software. At 42d, live weight of broilers of UUC was significantly higher than IUC, ITC and KB2, while KB0.5 and KB1 were intermediate. For FCR, ITC was significantly lower than IUC and UUC while KB0.5, KB1 and KB2 were intermediate. The ADG of broilers was quadratically affected by KB supplementation, driven by a feed intake response. KB1 was significantly higher in carcass yield compared to IUC. The ISI scoring, combining 8 gut health indicators, confirmed expected effects of the challenge on gut health with IUC having the worst score (7.98), then ITC (7.27) and UUC (5.07). Regarding the KB treatments, the lowest score was obtained with KB1 (7.08) while the other KB treatments were higher (8.17 and 8.22 for KB0.5 and KB2, respectively). In conclusion, this study highlighted the beneficial effects of chestnut extract at 1 kg/T on broiler intestinal health and performance under challenged conditions and comfort its interest as an alternative to AGPs.

Keywords: Antibiotic growth promoter alternatives, Broiler intestinal health, Growth performance, Hydrolysable tannins, ISI score

P364 Evaluation of a quillaja saponin-based product, Enviro QSTM_{DF}, against a quillaja and yucca saponin-based product or

a yucca saponin-based product in broilers Maci Oelschlager*¹, Jason McCamy¹, Megan Koppen¹, Manuel Da Costa¹ ¹*Cargill Animal Nutrition, Wayzata, Minnesota, United States*

The use of phytogetic products, especially saponin-based ones from the *Yucca schidigera* plant and *Quillaja saponaria* tree, is becoming more prevalent among broiler producers due to their plant-derived nature and ability to support bird performance and health. Saponins offer anti-microbial, anti-inflammatory, and antioxidant properties and can help promote intestinal health, nutrient digestibility, and the immune system. The objectives of the present four trials were to evaluate the effects of a quillaja saponin-based product, Enviro QSTM_{DF} (EQS). This was achieved by evaluating EQS vs a quillaja and yucca saponin-based product (QYS) or a yucca saponin-based product (YS), either with or without a probiotic, on growth performance. Trial 1 (T1) tested EQS (0.60 lb/ton; d 0 – 33) vs QYS (0.50 lb/ton; d 0 – 28) and a probiotic (1.0 lb/ton; d 0 – 33). Trial 2 (T2) tested EQS (0.60 lb/ton; d 0 – 28) vs QYS (0.50 lb/ton; d 0 – 28). Trial 3 (T3) tested EQS (0.60 lb/ton; d 0 – 33) vs YS (0.50 lb/ton; d 0 – 33) and a probiotic (1.0 lb/ton; d 0 – 33). Trial 4 (T4) tested EQS (0.75 lb/ton; d 0 – 33) vs QYS (0.50 lb/ton; d 0 – 33). All trials used day-old male broiler chicks and ran for 33 days in floor pens (4'x4') at a broiler integrator facility. Each trial had 18 birds/pen and 12 replicate pens/treatment, except for T2, which had 22 birds/pen. Birds were fed a standard three-phase program that met nutrient requirements and diets contained a coccidiostat. Data from the four trials were analyzed independently as RCBD using treatment as the main factor. Final bodyweight at d 33 for T4 was increased ($P = 0.0095$) by 0.194 lb for the EQS treatment compared to the QYS treatment. Feed conversion ratio (FCR) d 0 – 33 for T3 was improved ($P = 0.0396$) by +3 points for the EQS treatment compared to the YS+probiotic treatment. Bodyweight (4.75 lb) adjusted FCR d 0 – 33 for T4 was improved ($P = 0.0056$) by +3 points for the EQS treatment compared to the QYS treatment. Mortality d 0 – 33 was not impacted ($P > 0.05$) by treatment for any trial. Our data demonstrate that EQS performs similarly to marginally better than a quillaja and yucca saponin-based product and better than a yucca based-saponin product, which can potentially result in improved financial gains for broiler producers.

Keywords: broiler, saponin, feed additive, phytogetic, feed efficiency

P365 Effect of dietary supplementation of a postbiotic and phytogetic blend on laying performance and colibacillosis in laying hens challenged with avian pathogenic *Escherichia coli* Serotype O78 Hanseo Ko*¹, Deependra Paneru¹, Manuel Da Costa², Vivek Kuttappan², Woo Kyun Kim¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States;* ²*Cargill Animal Nutrition, Minneapolis, Minnesota, United States*

The present study aimed to evaluate the effect of a dietary postbiotic (*Saccharomyces cerevisiae* fermentation product; SCFP) and phytogetic blend on laying performance, body weight (BW), liver lesion score, and *Escherichia coli* colonization in laying hens challenged with Avian Pathogenic *E. coli* Serotype O78 (APEC). A total of 84 Hy-Line W-36 laying hens (79 weeks old) were allocated into 42 cages (2 birds per cage) for 56 d. From d 1 to 28, the control group ($n = 21$) received the basal diet, while the treatment group ($n = 21$) received a diet containing postbiotic and phytogetic blend (SCFP+; BiostrongTM, Cedar Rapids, IA) on top of the basal diet (1.15 lb of SCFP+ per US ton of feed). From d 28 to 56, the control group was divided into non-challenge control (NC, $n = 7$) and APEC challenge control (CC, $n = 14$), while the SCFP+ group was divided into non-challenge with dietary SCFP+ supplementation (NC+P, $n = 7$) and APEC

challenge with dietary SCFP+ supplementation (CC+P, n = 14). On d 28 (0-day post-inoculation, dpi), NC and CC groups were challenged with PBS and APEC (0.5 mL of 10^{10} CFU/mL) via intratracheal route, respectively. Laying performance and BW were measured weekly. Liver lesions and *E. coli* loads in the liver and lung were measured on 7 dpi. The data from d 1 to 28 and 1 to 28 dpi were analyzed using t-test and One-way ANOVA with Scheffé's post hoc test, respectively. Before the challenge, dietary SCFP+ supplementation minimized BW change from d 0 to 14 and d 0 to 21 ($P < 0.05$) and tended to improve feed conversion ratio from d 8 to 14 and d 21 to 28 ($P = 0.070$ and $P = 0.097$, respectively). After the APEC challenge, dietary SCFP+ supplementation tended to alleviate the BW loss from 0 to 7 dpi ($P = 0.076$). Hen day egg production and egg mass in the NC+P and CC+P groups were similar between two, and both were higher ($P < 0.01$) than in the CC group from 1 to 28 dpi. The APEC challenge increased the liver lesion scores and the incidence of fibrinous exudate in the liver ($P < 0.001$), which was higher in the CC than in the CC+P (100% vs. 83.3%). In conclusion, the results implied that dietary supplementation of the unique postbiotic and phyto-genic blend could be effective to support alleviating the adverse effects of colibacillosis on laying performance.

Keywords: Postbiotic, APEC, Laying hens, Colibacillosis, Feed supplement

P366 The effects of an unblanched high-oleic peanut diet on layer performance, and the fatty acid profile and quality of the eggs produced Ondulla T. Toomer*¹, Ishab Poudel², Thien Vu¹, Ramon Malheiros², Rebecca Wysocky², Kenneth Anderson² ¹Agricultural Research Service/ USDA, Food Science and Market Quality & Handling Research Unit, Raleigh, North Carolina, United States; ²North Carolina State University, Prestage Dept. of Poultry Science, Raleigh, North Carolina, United States

High-oleic peanuts are energetically dense and high in monounsaturated fats which may serve as a promising alternative to conventional poultry feed ingredients. In this study we aimed to determine the effects of incorporating unblanched high-oleic peanuts in a layer diet on performance and egg quality parameters in an 8-week feeding trial. Two hundred hens were fed a conventional diet with 14:10 (L:D) and allowed to acclimate for 7 days. Subsequently hens were randomly assigned to one of five treatments with various levels of unblanched high-oleic peanuts (0%, 5%, 10%, 15% and 20%), with 40 hens per treatment (5 replicates/treatment). Hens were provided free access to feed and water. Body weights were recorded at the onset and termination of the study. Feed weights were measured weekly to calculate the total feed intake (FI) and feed conversion ratio (FCR). Eggs were collected daily and weighed weekly. Bi-weekly egg quality parameters were measured. All data were statistically analyzed for variation using a combination of generalized linear mixed models (GLIMMIX) in SAS 9.4 to evaluate the effects of treatments and time (fixed effects) and block as random effect on various response variables in a randomized complete block-design experiment at $P < 0.05$ significance level. There were no significant treatment differences in body weights at either the onset or termination of the study ($P > 0.05$). Moreover, there were no significant treatment differences in the egg quality parameters (egg weight, haugh unit, shell strength, vitelline membrane strength) or hen day egg production ($P > 0.05$). Feed intake was significantly reduced in hens fed the 5%, 10%, 15% and 20% treatment groups as compared to the controls ($P < 0.001$), with hens fed the 15% and 20% having the most improved FCR relative to the other treatment groups ($P < 0.001$). There was a dose-dependent increase in egg yolk oleic and omega 9 fatty acid content and a significant

reduction of palmitic and stearic saturated fats at week 6 and week 8 with increasing dietary inclusion of unblanched high-oleic peanuts ($P < 0.05$). These results support the potential use of unblanched high-oleic peanuts as a sustainable alternative poultry feed ingredient.

Keywords: layers, alternative poultry feed ingredients, peanuts, egg fatty acid profile

P367 Enhancing Heat Stress Tolerance in Laying Hens through dietary supplementation with Vitamin C, Choline, and Betaine Waqas Ahmed*¹, Sara Mubeen¹, Usma Sher¹, Iram Sadiq¹, Muhammad Shoab¹, Zeshan Zulfiqar² ¹University of Agriculture Faisalabad, Institute of Animal and Dairy Sciences, Punjab, Pakistan; ²Henan Agricultural University, Department of Animal Nutrition and Feed Science, Zhengzhou, China

Heat stress is a major challenge in poultry farming, particularly for laying hens, as it negatively impacts their productivity, egg quality, and overall health. The study aimed to investigate the role of dietary supplementation with Vitamin C, Choline, and Betaine in mitigating heat stress and their effects on egg production and liver health in laying hens. The study was carried out with 500 laying hens, aged 33 weeks, over 42 days. The experimental period was divided into four treatment groups, with birds randomly allocated to four dietary treatments, each containing five replicates. Each replicate consisted of 25 birds. The treatments included: T1 (control) – a diet without any additives; T2 – a control diet supplemented with Vitamin C at a rate of 300 mg/kg of feed; T3 – a control diet supplemented with Choline at a rate of 1000 mg/kg of feed; and T4 – a control diet supplemented with Betaine at a rate of 1000 mg/kg of feed. All the findings were analyzed statistically using analysis of variance (ANOVA) under a completely randomized design and means were compared using Tukey's test. Egg production, feed intake, liver health indicators, and plasma levels of stress-related biomarkers (such as corticosterone and cortisol) were assessed. The results indicated that supplementing with Vitamin C, Choline, and Betaine significantly enhanced ($P < 0.05$) egg production and feed conversion efficiency under heat stress conditions. Birds receiving these supplements exhibited a substantial decrease in body temperature and lower ($P < 0.05$) stress hormone levels compared to the control group. Additionally, liver function was notably improved ($P < 0.05$), with reduced serum liver enzymes and less histopathological damage observed in the supplemented groups. These findings suggest that the combination of Vitamin C, Choline, and Betaine can help alleviate the adverse effects of heat stress by boosting egg production, supporting liver health, and reducing oxidative stress. Overall, this study showed the potential of these nutrients as effective strategies for increasing the resilience of laying hens to heat stress, thereby improving both welfare and productivity in poultry farming.

Keywords: Heat Stress, Vitamin C, Choline, Betaine, Liver Health

P368 Effects of Bacillus based probiotics on improving feed conversion and lowering mortality of broilers during a Coccidiosis challenge Sharon H. Miller*¹, Kristy Dorton¹, John Schleifer¹, Shameer Rasheed¹, Mark LaVorgna¹, Mahmoud Masadeh¹, Gary Reznik¹ ¹Devenish Nutrition, Fairmont, Minnesota, United States

Two studies were conducted to determine the effects of *Bacillus* based probiotics on growth performance and mortality of Cobb 500 (Study 1) or Ross 708 (Study 2) broilers challenged with a 20x dose of Coccivac B-52 (Merck Animal Health) on d 4 or 3, respectively. Day-old broiler chicks (as hatched; n = 1200 in Study 1 or 900 in Study 2) were randomly allocated to floor pens

(12 or 9 reps/treatment; 50 birds/pen; 0.88 to 1.08 ft²/bird). Treatments in Study 1 and 2 were: Control (CON; no probiotics) and ValuPro-L, a single-strain *Bacillus licheniformis*-based probiotic (VPL; 1.5 x 10⁶ CFU/g of feed; Devenish Nutrition, Fairmont, MN). Broilers were fed corn/soy based pelleted diets. Body weight (BW), feed intake, and mortality were measured. Feed conversion (FCR_M) and BW adjusted feed conversion (FCR_{M+BW}) were calculated. Data were analyzed as a one-way ANOVA using the Mixed procedure of SAS with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$. Supplementation of VPL improved FCR_M by 2.3 points in Study 1 ($P = 0.04$) and 1.5 points in Study 2 ($P = 0.05$) and FCR_{M+BW} by 4.9 points in Study 2 ($P = 0.04$) compared to control birds. Although only a numerical difference ($P = 0.17$), mortality was 43% lower in broilers supplemented with VPL compared to broilers in the control treatment in Study 1. As a result of better feed conversion, estimated feed cost savings ($\$/lb$ live weight) were increased with supplementation of VPL. This could result in substantial feed cost savings over the course of the year. Overall, the results showed that supplementing VPL to Ross 708 (big bird program) or Cobb 500 (small bird program) broilers improved feed conversion and feed cost savings, and minimized mortality.

Keywords: Probiotics, broiler, feed conversion, mortality

P369 Silymarin Improves Performance, Liver Health, and Diet Productive Energy Value in Commercial Broilers: A Mechanistic Model Diego A. Martinez¹, Carol Ponce-de-Leon², Patricia Acuna^{*3} ¹University of Arkansas, Fayetteville, Arkansas, United States; ²Independent Researcher, Fayetteville, Arkansas, United States; ³CKM SAC, Lima, Peru

A two-phase study assessed a silymarin formulation in commercial broilers under a subclinical hepatic challenge and developed a mechanistic model. Twelve houses (180,000 broilers) were assigned half to each treatment: T1, control diet; T2, +35 ppm of phytoextract (SIL; from Liverin-M, 7% silymarin; reformulated to 28%, Liverin-NFC; CKM SAC, Peru) 21-35 days. Feed intake, BW gain (BWG), feed conversion ratio (FCR), protein accretion (PAC, g/bird/d) and efficiency (PACe, %), net energy (NE) for gain (NEg, kcal/kg), fasting heat production (FHP, kcal/kg^{0.75}/d), NE for maintenance (NEm, kcal/kg), and productive energy (PE, =NEg+NEm) were assessed (22-28, 29-35, 22-35 d). Mortality (%), liver lesion prevalence (LLP, %) and intensity (LLI, score 0-3), and European Broiler Index (EBI) were assessed (29-35 d). Gompertz curve was modeled per house with a set asymptote. Growth rate (GR), inflection point (IP), and age at 2.7 kg (CA) were calculated. Processing weights (PWs, breast fillet, tenderloins, leg quarters, wings) and market value (MKV) were estimated (42 d). A mechanistic model was developed and complemented with literature data. A flexible P-value criterion was used. ANOVA and modeling were run in JMP Pro 16. SIL improved BW, BWG ($P=0.053$), FCR ($P=0.024$), EBI ($P=0.037$), PAC ($P=0.058$), PACe ($P=0.028$), NEg (29-35 d: $P=0.033$, +65.3 kcal/kg; 22-35 d: $P=0.068$, +47.7), and reduced FHP ($P=0.052$), transferring the extra NEg kcals into PE (29-35: +52.9 kcal/kg; 22-35: +51.9). SIL increased PWs ($P \leq 0.034$), MKV ($P=0.033$; +0.129 \$/bird), GR ($P=0.081$), and CA ($P=0.043$; -0.68 d), and reduced LLP, LLI ($P < 0.001$) and mortality ($P=0.007$). The mechanistic model indicates SIL reduces liver lesions, protein degradation rate, maintenance expenditure, FCR, FHP, and mortality while increasing protein synthesis, PAC, BWG, leanness, NEg, and PE, reducing the age at commercial BW, lowering feeding cost, and increasing MKV and feed ROI. It suggests that these responses do not necessarily depend on toxic/pathological challenges but that

they support the high metabolic activity of modern lines, and that the bioavailability and combination of flavolignans are crucial. In conclusion, SIL reduced subclinical liver damage and mortality while improving performance and diet energy value.

Keywords: silymarin, hepatotoxicity, antioxidant, efficiency, productive energy

P370 Effects of dietary inclusion of bentonite clay in reducing aflatoxin B1 toxicity on production performance, immune response, and intestinal health of broilers Waqas Ahmed^{*1}, Sara Mubeen¹, Usma Sher¹, Iram Sadiq¹, Sajid Mehmood¹, Muhammad Shoaib¹ ¹University of Agriculture Faisalabad Punjab Pakistan, Institute of Animal and Dairy Sciences, Rawalpindi, Pakistan

This study was conducted to check the effects of dietary inclusion of commercial bentonite clay (BC) in reducing aflatoxin B1 toxicity on production performance, immune response, intestinal and hepatic health of broilers. For this purpose, 360-day-old broiler birds were randomly classified into three dietary treatments having eight replicates and each replicate contained 15 birds per replicate. The whole trial was conducted for 35 days and treatments were; (C) control (basal diet containing aflatoxin B1 @ 1 ppm/kg); (T1) = C + 200 mg/kg bentonite; (T2) = C + 600 mg/kg bentonite. All the findings were analyzed statistically using the analysis of variance (ANOVA) technique under a completely randomized design and means were compared using Tukey's test at a 5% significance level. The data on feed intake, body weight gain, and feed conversion ratio were recorded weekly. Based on the findings, birds fed a control diet showed poor weight gain and lower feed conversion ratio (FCR) compared to other treatments. Birds fed T2 diets showed significantly higher ($P < 0.05$) weight gain and better ($P < 0.05$) FCR when compared to other treatments. Birds fed the diet supplemented with BC showed increased ($P < 0.05$) levels of IgG and IgM as compared to the control diet. The T2 group showed significantly improved ($P < 0.05$) villus length, villus width, and crypt depth as compared to the other treatments. Therefore, it can be concluded that supplementing broilers' diets with bentonite clay as toxin binders to prevent them from aflatoxin B1 toxicity can boost bird's growth performance, cellular and humoral immunity levels, and intestinal health.

Keywords: Bentonite, Aflatoxins, Growth performance, Immune response, Intestinal health

P371 A blend of probiotic and xylanase can ameliorate the impact of coccidiosis on broilers Basheer Nusairat^{*1}, Rahaf Asaad¹, Mohammad Gharaibeh³, Jeng-Jie Wang² ¹Department of Animal Production, Jordan University of Science and Technology, Irbid, Jordan, ²Novus International, Inc., Chesterfield, Missouri, United States, ³Basic Veterinary Medical Science, Jordan University of Science and Technology, Irbid, Jordan

This study was designed to investigate the effect of a zootechnical feed additive comprised of multi-strain *Bacillus* spp. probiotic and xylanase (EnzaProTM Feed Solution; EFS) on growth performance, *Eimeria* spp. oocyst shedding, and intestinal lesion score of broilers subjected to a coccidia vaccine challenge at 25X the recommended dose and raised to 32 d. Three experimental treatments with 10 pens/treatment and 25 birds/pen were tested. The treatments were: non-challenged control (NC), challenged control (NC), and NC+EFS. Both NC and NC received same diet while NC+EFS received same diet supplemented with 100g/MT EnzaProTM Feed Solution fed as crumble and pellet for starter and grower, respectively. The challenge was delivered via oral gavage on d18. Performance parameters were measured before (d0-14), during (d14-28), and after (d28-32) challenge. Oocyst shedding in

feces was measured on 10th and 11th days post infection (dpi) and expressed as oocyst per gram (opg). Intestinal lesions were scored on 12 and 18 dpi. Data were analyzed as one-way ANOVA, and means were separated by Tukey's. Data showed that the challenge model was successful and there was no cross-contamination from challenged to non-challenged treatments. During the challenge period, FCR was worse ($P<0.0001$) in challenged treatments compared to non-challenged control; values were 1.84, 2.22, and 2.23 for PC, NC, and NC+EFS, respectively. At 32 d, FCR was 1.85, 2.04, and 1.86 for PC, NC, and NC+EFS, respectively. Furthermore, during the challenge period, mortality was approximately 1.7% lower in birds fed NC+EFS compared to challenged control. Oocyst shedding in fecal material was higher ($P<0.0001$) in challenged control compared to NC+EFS (48625 vs 23625 opg) when evaluated on 10dpi. Feeding NC+EFS decreased ($P<0.0001$) intestinal lesion scores by 59% and 55% compared to NC on 12 and 18 dpi, respectively. Based on findings of this research, it can be concluded that birds were able to recover faster and more efficiently when fed diets supplemented with EnzaPro™ Feed Solution. The coccidiosis impact was ameliorated as evidenced by the improvement in FCR during the recovery period, reduction of oocyst shedding, and intestinal lesions, as well as improving livability, compared to challenged control.

Keywords: Probiotic, xylanase, coccidia vaccine, broiler, lesion score

P372 Evaluation of the impact of feed additives on pathogens associated with necrotic enteritis in broilers raised on used litter seeded with cocci Firmin Delago^{*1}, Elizabeth Galbraith¹, Mike E. Persia², Adrienne Woodward³, Michael King¹, Sona Son¹, Brian Dirks³ ¹Microbial Discovery Group, Oak Creek, Wisconsin, United States; ²Virginia Tech, Blacksburg, Virginia, United States; ³United Animal Health, Audubon, Pennsylvania, United States

Necrotic enteritis (NE), primarily caused by *Clostridium perfringens* following *Eimeria* spp. intestinal damage, severely impacts broiler health and productivity. This study examined the effects of an antibiotic (BMD, 55 ppm) or a direct-fed microbial (DFM, Novela®ECL at 3.68×10^5 CFU/g of feed) on virulence genes associated with NE, coccidiosis, and colibacillosis in Ross 708 broilers (264 birds/treatment in 12 replicate pens). The design included a fresh litter control (FL) and 3 treatments with used litter generated before the trial by raising broilers on shavings for 21d and administering CoccoVac® B52 for the first 3d: a used litter control (UL), antibiotic (BMD), and Novela®ECL (DFM). At 21d, ileum swabs were obtained from 12 birds and 3 pooled litter homogenates per treatment. DNA was extracted from swabs and litter and genes from avian pathogenic *E. coli* (APEC; *iss*, *iutA*, *hlyF*, *ompT*, *iroN*), *C. perfringens* (*cpa*, *netB*) and *Eimeria* spp. (*E. tenella*, *E. maxima*, *E. acervulina*) were quantified by qPCR. Groups were compared via ANOVA with Tukey's post-hoc test in JASP 0.18.3 (significant at $P<0.05$). *E. acervulina* gene quantities were elevated in UL, BMD, and DFM litter vs. FL, indicating that seeded litter harbored higher cocci as expected. The BMD group exhibited higher levels of APEC genes ($P<0.05$) than UL, while DFM did not differ from controls. APEC genes *hlyF*, *iss*, *ompT*, *iroN* were reduced in DFM and UL litter samples vs. FL ($P<0.05$), while BMD was intermediate. Clostridial genes did not differ in the ileum, but BMD reduced clostridial genes (*cpa*, *netB*) in litter compared to FL ($P<0.05$), and DFM and UL were intermediate. Overall, DFM did not stimulate *E. coli* overgrowth in the gut and litter of broiler chickens exposed to cocci, and coccidial inoculation did not appear to impact *C. perfringens* populations in the ileum or litter. At 21d, mortality-adjusted FCR improved ($P<0.05$, 1-way ANOVA and Fisher's

LSD in SAS 9.4) for DFM compared to FL, UL, and BMD (1.297 vs. 1.334, 1.338 and 1.322 respectively). Feed intake decreased in UL and DFM groups vs. FL ($P<0.05$) and weight gain was reduced across all three used litter groups ($P<0.05$). DFM treatment shows promise in improving feed efficiency while maintaining lower APEC quantities in birds exposed to cocci.

Keywords: Broiler, Necrotic enteritis, Cocci, Antibiotic, Probiotic

P373 Polyphenols decrease oxidative stress and occurrence of breast myopathy Vivian A. Castilho Heiss^{*1}, Leticia C. Garcia¹, Maria Fernanda d. Burbarelli¹, Elizabeth Santin², João B. Lancini³, Rodrigo G. Garcia¹, Claudia M. Komiyama¹ ¹Universidade Federal da Grande Dourados, Dourados, Ms, Brazil; ²Kupono Assessoria e Consultoria, Curitiba, Brazil; ³Silvafeed, Porto Alegre, Brazil

The development of pectoral myopathies in broiler chickens is primarily linked to increased oxidative stress. It is hypothesized that a polyphenol blend with strong antioxidant properties could reduce white striping and wooden breast. This study assessed the impact of different levels of the polyphenol blend (Silvafeed ATX® - Silvateam, Itália) on pectoral myopathies, lipid peroxidation, and antioxidant capacity in the breast muscle. A total of 1,280 male Ross AP91 broilers were divided into 4 groups (Control, 250, 500, and 1,000 g/t of Silvafeed ATX®) with 8 replicates of 40 birds each. At 21 and 42 days, two birds per replicate were selected for slaughter to evaluate the incidence of white striping (WS) and wooden breast (WB). Breast meat samples were also collected for malondialdehyde (MDA) analysis and antioxidant capacity measurement via DPPH inhibition. Myopathy assessment was based on scores and analyzed using generalized linear models and the SAS GLIMMIX procedure (SAS, 2003). Data were analyzed using ANOVA and the Dunnett test (5%). At 21 days, 500 g/t and 1,000 g/t reduced severe wooden breast scores by 41.6% and 27.4%, respectively, compared to the control ($p=0.0127$). At 42 days, severe WB scores decreased by 45.5% and 58.4% with 250 g/t and 500 g/t, respectively, compared to the control ($p=0.0211$). Polyphenol supplementation reduced lipid peroxidation in breast meat, indicated by lower MDA levels (nmol/ml). At 21 days, MDA values were 1430 (control), 659 (250 g/t), 571 (500 g/t), and 516 (1000 g/t) ($p=0.0290$). At 42 days, MDA values were 3232 (control), 1254 (250 g/t), 665 (500 g/t), and 447 (1000 g/t) ($p=0.0106$). Silvafeed ATX improved the antioxidant capacity of breast meat at both 21 and 42 days. At 21 days, DPPH inhibition was 15.12%, 18.94%, and 23.29% for 250, 500, and 1000 g/t, respectively, compared to the control (11.07%) ($p<0.0001$). At 42 days, DPPH inhibition was 16.01%, 19.40%, and 25.09% for 250, 500, and 1000 g/t, respectively, compared to the control (11.52%) ($p<0.0001$). Polyphenol supplementation reduced pectoral myopathies, lipid peroxidation, and improved antioxidant capacity in breast meat, suggesting its potential to mitigate oxidative stress effects in broiler chickens.

Keywords: lipid peroxidation, wooden breast, white striping, antioxidant capacity, meat quality

P374 The effects of feeding supplemental tryptophan or probiotic on stress, blood serotonin and brain neurotransmitter response in broilers and Pekin ducks Emily Jiral¹, Melanie Bergman², Gregory S. Fraley², Gregory Archer^{*1} ¹Texas A&M University, College Station, Texas, United States; ²Purdue University, West Lafayette, Indiana, United States

This study evaluated the effects of adding L-tryptophan or a probiotic to the diet on welfare and fear response in male broilers and straight-run Pekin ducks. Three treatments were tested: 1) standard broiler or duck diet (CON), 2) CON with L-Tryptophan

at 1lb/ton (Trp), 3) CON with probiotic at 5lb/ton (PB). Each treatment had 30 broilers or 24 Pekin ducks. On d35, heterophil/lymphocyte ratio (HL) and plasma corticosterone concentrations (Cort), and plasma serotonin (PSer) determined from a subset of birds. Also on d35, brain tissues from the caudal and rostral mesencephalon (CM, RM) were analyzed for static serotonin and dopamine as well as turnover for both. All data was analyzed using a one-way ANOVA test. Fisher's LSD was used for mean separation. In broilers, CON had the greatest HL and Cort compared to both Trp and PB ($P < 0.05$). In ducks, CON had higher HL and Cort than the PB ($P < 0.05$) but the Trp was intermediate. In broilers, CON had the lowest plasma serotonin compared to both Trp and PB ($P < 0.05$). In ducks, CON had lower plasma serotonin than the PB ($P < 0.05$) but the Trp was intermediate. In broilers, higher static serotonin was observed in the CM in the Trp compared to the CON with PB intermediate. Trends for static dopamine in the CM and serotonin turnover in the RM were also observed. In ducks, only trends for static dopamine in the CM and turnover for both serotonin and dopamine in the RM were observed. The results indicate that both Trp and PB supplementation may reduce stress susceptibility and increase plasma serotonin concentrations. However, neither of the two supplementations appeared to alter the serotonin in the brain. Therefore, it appears that while systemic serotonin may be altered by feeding it does not translate to altered brain physiology.

Keywords: Tryptophan, Probiotic, Serotonin, Corticosterone, Brain

P375 Efficacy of a three-strain *Bacillus* DFM in a group-housed performance study in laying hens Sasha van der Klein^{*1}, Elizabeth Kim¹, Pam Utterback², Jennifer Blair², Minoy Cristobal², Bekah Drysdale², Kirsty Gibbs¹, Carl M. Parsons², Janet Remus¹ ¹Danisco Animal Nutrition & Health, Oegstgeest, Netherlands; ²University of Illinois, Department of Animal Sciences, Urbana, Illinois, United States

Direct-fed microbials (DFM) are known for their beneficial effects, but long-term performance studies in laying hens are scarce. Therefore, the objective of this study was to investigate differences in productive performance of group-housed laying hens fed a *Bacillus*-based DFM over a 40 wk production period. A commercially relevant corn-soy based control diet containing DDGS was formulated to be nutritionally adequate for each phase (Control) and tested against the same diet including three strain *Bacillus* DFM (Control+PRO; Enviva[®] PRO) at a dose level of 150,000 CFU per gram of feed (60 g/ton). Lohmann LSL Lite birds were randomly assigned to cages at 22 wk (7 birds/cage, 2 cages per lot) and lots randomized to treatment (10 lots/treatment) based on approximate equal BW and egg production after a 1-wk assessment period. Diets were fed *ad libitum*. BW, hen-day egg production, egg weight, egg mass, feed intake, feed efficiency and specific gravity were monitored over a period of 40 weeks (wk) and data analyzed on a 4-week basis. Statistical analysis was performed on single 4-week time periods using ANOVA including treatment as a fixed effect in the lm function in R and on repeated measures over time including treatment as a fixed effect and period as a random effect within the lmer function in R. In the repeated measures analysis (23 to 62 wk), egg mass, egg weight, feed efficiency and egg specific gravity were all increased in Control+PRO- vs. Control-fed birds ($P < 0.05$; by 1.3 g/bird or 2.2%, 1.33 g or 2.2%, 3.5% and 1.1%, respectively). Mortality was 7.9% in control-fed birds and 5.7% in Control+PRO fed birds but did not significantly differ by treatment ($P > 0.05$). Single time periods analysis indicated that improvement in performance was most apparent during challenging stages, such as peak production (31-34 wk) and production drop (51-54 wk). The Control+PRO

treatment had numerically higher hen-day egg production from wk 39 onward. These results demonstrate the efficacy of the three-strain *Bacillus* DFM for improving the productive performance of laying hens, when added to a corn-soybean meal-based diet. The combined effect of this DFM on egg weight, feed efficiency, production level, and liveability can support more economic and sustainable egg production.

Keywords: probiotic, bacillus, egg production, feed efficiency, laying hens

P376 Protected calcium butyrate improves broiler performance under commercial conditions versus tributyrin for the equivalent supplementation of butyric acid David Harrington^{*1}, Yann Lever², Nemanja Todorovic¹, Jean Francois Le Roux² ¹nuance Biotechnology, Lausanne, Switzerland; ²Global Nutrition International, Fougères, Veuillez Sélectionner Une Région, Une état ou Une Province, France

Butyrate salts and tributyrin have shown beneficial effects on bird performance when used as a dietary supplement. However, the form of butyric acid source impacts its efficacy. The objective of the study was to evaluate the effect of protected calcium butyrate and tributyrin on broiler performance under commercial conditions. A total of 1280 as-hatched Ross 308 broilers were allocated to 4 treatments across 32 pens (40 birds/pen; 8 pens/treatment) in a commercial poultry house: CON: control (basal diet, no additives); CAB: basal diet + coated calcium butyrate (250 g/t); TRI1: basal diet + tributyrin (480g /t); TRI2: basal diet + tributyrin (750g /t). CAB and TRI1 delivered equivalent 250g/t butyric acid, TRI2 delivered 390 g/t. Diets contained a natural anticoccidial. Body weight gain (BWG) was measured on days 11, 21, and 32 and FCR determined for days 0-11, 0-21 and 0-32. Litter quality (score 0-5; best to worst) was assessed on days 16 and 27. Data were analyzed via ANOVA and statistical significance declared at $P < 0.05$. Litter quality was better ($P < 0.05$) in CAB than CON at 16 days (1.79 and 1.12, respectively), while TRI1 and TRI2 (1.46 and 1.33, respectively) did not differ ($P > 0.05$) from CAB and CON. At 27 days, litter quality did not differ ($P > 0.05$) between treatments, although TRI1 was numerically lower (2.67 versus 3.08, 3.37 and 3.33 for TRI1, CON, CAB and TRI2, respectively). BWG in both CAB and TRI2 was greater ($P < 0.05$) than CON for all test periods and numerically higher than TRI1. BWG on day 32 was 1902, 1873, 2071 and 2025g for CON, TRI1, CAB and TRI2, respectively; TRI1 and CON did not differ significantly. FCR was better ($P < 0.05$) in CAB and TRI2 than CON for all test periods. Final FCR was 1.62, 1.66, 1.52 and 1.54 for CON, TRI1, CAB and TRI2, respectively; FCR in TRI1 was numerically worse than CON on day 32 and significantly lower than CAB and TRI2. In conclusion, supplementation of protected calcium butyrate led to superior broiler performance versus tributyrin for the equivalent level of butyric acid. Furthermore, calcium butyrate performance was similar to a 56% higher butyric acid equivalence from tributyrin, suggesting protected calcium salt is more effective than a butyric acid ester to improve broiler productivity.

Keywords: calcium butyrate, tributyrin, broilers, litter quality, performance

P377 Efficacy of a blend of organic acids in water in reducing *Salmonella Heidelberg* contamination in broilers Karolina V. Z. Augusto², Giseli Heim^{*1}, Antonio J. Piantino Ferreira³ ¹Selko, Amersfoort, Netherlands; ²Trouw Nutrition, R&D, Campinas, Brazil; ³University of Sao Paulo, Poultry Pathology, Sao Paulo, Brazil

Salmonella Heidelberg (SH) is a significant public health concern due to its antibiotic resistance and potential to infect humans. Any broiler carcass testing positive for *Salmonella* can be condemned. Pre-slaughter fasting is commonly used to reduce gastrointestinal contents and lower *Salmonella* contamination risks. However, it may also lead to recontamination as birds may ingest contaminated bedding material. This 42 days-study evaluated the effects of a blend of free and buffered organic acids (OA [Selko Alpha, a Selko water acidifier – SWA]) in water on reducing SH at slaughter. The study was conducted at the University of São Paulo, Brazil. A total of 288 Ross male chicks were assigned to 18 independent pens, with 16 birds per pen, and randomly divided into three groups: negative control – basal diet (NC), positive control – basal diet + oral challenge with 2.0×10^6 CFU of *S. Heidelberg* on day 10 (PC), and PC + 1 L/1000 L water of OA (SWA). Samples from the liver, spleen, caecum and crop were collected on days 14, 28 and 42 for *Salmonella* isolation. A Kruskal-Wallis' test was performed to compare treatments with a

significance level of $\alpha = 0.05$. All birds in the NC group remained free of SH during all the collection points. The percentage of birds contaminated with SH, either in the liver, spleen, or caecum, on day 14 was 83% for PC and 89% for SWA; on day 28 was 61% for PC and 22% for SWA; and on day 42 was 44% for PC and 6% for SWA. Significant differences between groups were observed in the prevalence of SH in infected organs: cecum (6, 11 and 39% vs 33, 22 and 6%) and liver/spleen (83, 56 and 44% vs 89, 6 and 0%) for PC vs SWA on days 14, 28 and 42. The SWA effectively reduced SH contamination, particularly in the crop (39 vs 0% for PC and SWA on day 42). Lowering bacterial prevalence, especially in the crop, is essential to prevent carcass condemnation during slaughter, thereby reducing economic losses. These findings suggest that SWA could be a viable strategy to enhance food safety and quality in poultry production.

Keywords: *Salmonella Heidelberg*, organic acids, water acidifier

Metabolism and Nutrition: Vitamins and Minerals

P378 Effect of different metabolizable energy on Ross 308 broiler growth performance, nutrient digestibility, organ weight, fecal score and lesion score Kye j. Lee^{1,2}, Usman Kolawole^{1,2}, In H. Kim^{*1,2} ¹Dankook University, *Animal Biotechnology, Cheonan, Choongnam, Korea (the Republic of); ²Smart Animal Bio Institute, *Cheonan, Choongnam, Korea (the Republic of)**

Advances in genetics research have enabled an improvement in feed conversion rates and carcass yield of broilers, which are determining factors from a nutritional point of view, considering that the phenotypic expression of genetic potential is influenced by the environment and nutrition. Thus, knowledge of the nutritional requirements for growth and development is important to obtain the maximum performance from the poultry. Metabolizable energy (ME) is the term used to express the energy available and is commonly used in calculations of the nutritional value of poultry feed (Nunes et al., 2008). The requirements for crude protein, amino acids and other nutrients are usually expressed in terms of levels of ME in feed (Silva et al., 2003). Therefore, the focal aim of this study is to establish the best level of ME for broiler performance. A total of 1134 one-day-old Ross 308 broiler chicks (44.33 ± 1.70 g) were randomly assigned to 3 treatment groups: control (CON), basal diet (3200 kcal/kg), TRT1, basal diet - 50 kcal/kg ME (3150 kcal/kg), and TRT2, basal diet + 50 kcal/kg ME (3250 kcal/kg). Each treatment has 21 replicates of 18 male birds/pen. Birds had *ad libitum* access to water and a three-phase diet: starter (d 1–7), grower (d 8–20), and finisher (d 21–35). Growth performance [body weight (BW), average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR)] were recorded from d1-35. While, nutrient digestibility, fecal score, and foot pad lesion score were assessed at the end of d 35. Data were analyzed using the MIXED procedure in SAS, and Tukey's range test was used to determine the significance between treatment means ($P < 0.05$). During the growing phase, the TRT2 group showed a reduced feed conversion ratio (FCR) compared to the TRT1 group. In addition, during the overall experimental period, TRT2 group broilers showed an increased average daily gain (ADG) and a reduced FCR than in the TRT1 group. However, there were no differences observed on feed intake (FI) and body weight gain (BWG), nutrient digestibility, fecal score, organ weight, and lesion score. In conclusion, feeding a diet at 3250 kcal/kg ME from days 1–34 could be suitable for increasing both average daily gain and feed efficiency in broiler chickens.

Keywords: broilers, growth performance, nutrient retention., energy variation, organ weight

P379 The detection and co-occurrence of mycotoxins in poultry feed using HPLC-MS/MS analysis Anthony Pokoo-Aikins^{*1}, Jaci Hawkins¹, Trevor Mitchell¹, Lincoln Adams¹, Revathi Shamugasundaram¹, Quentin Read², Scott P. Gold¹, Anthony Glenn¹ ¹USDA, ARS, Athens, Georgia, United States; ²Southeast Area, ARS, USDA, Raleigh, North Carolina, United States

Mycotoxins are secondary metabolites that pose problems when ingested by poultry including reduced appetite, suppressed growth, poor feed efficiency, compromised immune systems, predisposition to secondary infections, and ultimately poor production performance. Co-contamination of mycotoxins has been widely reported to exert synergistic enhancing these problems. The objective of this surveillance study was to detect mycotoxin co-contamination in poultry feed and develop effective procedures to consistently quantify the mixed mycotoxin content of feed samples. We quantified the levels and combinations of Deoxynivalenol (DON), Zearalenone (ZEA), Aflatoxins (AFLA), and Fumonisin (FB's) in 137 breeder (n=6), broiler (n=63), and layer (n=68) feed samples. Samples were analyzed via HPLC-MS/MS. Of those tested, 100% contained FB's, 74% contained DON, 73% contained ZEA, and 13% contained AFLA. In terms of co-contamination per sample, 14% contained only FB's, 16% contained 2 mycotoxins (DON+FB's, ZEA+FB's, and AFLA+FB's), 66.4% contained 3 mycotoxins (DON+ZEA+FB's and DON+AFLA+FB's), and 3.6% contained all 4 mycotoxins. When samples were analyzed by feed type, the co-contamination levels in broiler vs layer feed were similar with the exception of AFLA, with 5% and 22% respectively. Additionally, the mean level of FB's in the broiler feed was 2.5 $\mu\text{g/g}$, while the layer diets had a mean level of 0.4 $\mu\text{g/g}$. Approximately 45% of both broiler and layer feed samples had DON+ZEA+FB's. Marginal means of mycotoxin concentration were estimated from a Gamma generalized linear mixed model. Comparisons were done using z-tests. The p-values were adjusted using the Tukey adjustment for multiple comparisons for all 4 mycotoxins, but only AFLA was significant between feed types. AFLA differed significantly between feed types ($\chi^2_5 = 32.6$, $p < 0.0001$). We conclude that multiple mycotoxin contamination, particularly DON+ZEA+FB's, is the rule in feed. All levels observed were below FDA guidance for poultry. Because combinations of mycotoxins may be synergistic well below their individual guidance, as seen in

previous studies with these combinations, knowledge of their co-occurrence and combined action is important for maximal poultry productivity and mycotoxin mitigation.

Keywords: Poultry, Nutrition, Mycotoxin, Feed safety, LCMS

P380 The effect of *Lagenaria Siceraria* (bottle gourd) based seed meal on growth and carcass characteristics of Boschveld chickens Siphonkosi¹, Margaret Taona Moyo¹ ¹*Lupane State University, Agriculture, Bulawayo, Zimbabwe*

A study was carried out to test a ration which included locally produced but underutilised bottle gourds (*Lagenaria Siceraria*) seeds as a supplement to indigenous free ranging chickens. Research has shown the need to formulate rations using locally available ingredients to supplement chickens in order to improve productivity and hence the nutrition and livelihood of the rural populace. Feed supplementation especially with protein has been identified as key to the improvement of indigenous chicken production. The objectives of the study were to determine the effect of bottle gourd seed based ration on growth performance of Boschveld chickens and to determine the effects of bottle gourds seed based ration on carcass characteristics of Boschveld chickens. The experiment was a two by two factorial experiment in a completely randomized design with three replicates. The two factors were sex and diets. Factor 1 was sex with two levels, male and female. Factor two was feeding regimes with two levels that is; birds on range supplemented using commercial soya bean meal/maize ration, (CM) and birds on range supplemented with gourd seed based ration (GBM). All data was analysed by using a two way analysis of variance (ANOVA) using MINITAB version 14. Results from this research showed overall weight gain, dressing percentage and carcass weight did not show significant difference between the control and gourd based feed meal. Feeding regime had no significant effect on thigh weight, however cockrels had significantly heavier thighs than pullets ($p < 0.05$). There was no interaction of sex and feeding regime for growth and carcass characteristics. These results suggest that gourd seed meal can be used as replacement to commercial meal to supplement free ranging indigenous chickens. It therefore can be concluded that it is feasible to use of gourd based seed meal as supplement to free ranging indigenous chickens.

Keywords: supplementation, curcubitacea, Indigenous chickens, scavenging

P381 Impact of bulk density in broiler diets on performance, digestive organs, and litter composition Muhammad Naem^{*1}, Dianna Bourassa¹, supplementation, curcubitacea, Indigenous chickens, scavenging Samuel J. Rochell¹, Shijinaraj Manjankattil Rajan¹, Emily Burton², Ashraf Alkhtib² ¹*Auburn University, Auburn, Alabama, United States*; ²*Nottingham Trent University, Nottingham, United Kingdom*

There is increasing concern about the ability of modern broilers to adapt to higher feed intake and energy levels in their diets. The digestive system of broilers has been shown to adjust and expand when fed diets with bulky ingredients. Oat hulls, being inert and bulky fibres, have been found to promote the development of broiler digestive systems when included in their diets at moderate levels. The present study investigated the impact of modifying diet bulk density (BD) by incorporating oat hulls on broiler production performance, digestive tract development, and litter quality. A total of 168 male Ross 308 broiler chicks were randomly assigned to 24-floor pens (12 pens per treatment, with 7 chicks per pen) in an environmentally controlled room. The chicks were fed wheat-soy-based mash diets that met the nutritional needs of Ross 308 broilers up to day 35. The diets were isonitrogenous and isocaloric,

but their BD was adjusted by incorporating oat hulls in either whole or ground form. This resulted in two different BDs: 800 and 650 kg per cubic metre. On day 35, production performance data were collected, and the weights of the empty digestive organs (proventriculus, gizzard, and small intestine) were recorded from two cervical dislocated birds per pen. Litter nitrogen and moisture content were analyzed on day 34 using AOAC (1990) methods. The independent samples t-test was employed to evaluate the impact of dietary treatment on response variables at $P < 0.05$ using SPSSv26. The study found that altering the diet's bulk density did not significantly affect feed intake, growth performance, digestive tract development, and litter nitrogen and moisture. However, the diet with a bulk density of 650 kg per cubic metre significantly increased gizzard weight (33.53 vs 30.12, $P = 0.029$) compared to the diet with a bulk density of 800 kg per cubic metre. Reducing the bulk density from 800 to 650 kg per cubic meter with whole oat hulls appears to be beneficial for gizzard development. Nevertheless, a key challenge remains in identifying a specific characteristic of "bulkiness" that can accurately predict feed intake in broilers fed diets with significant amounts of bulky materials, given the variability in the type and particle size of these materials.

Keywords: bulk density, gastrointestinal tract, gizzard, litter characteristics, broiler

P382 Apparent ileal amino acid digestibility and relative mRNA expression of tight junction proteins and cytokines in broiler chickens fed diets with inclusion of raw potato starch or high-amylose corn starch in a mixed *Eimeria*-challenge model Iyabo W. Oluseyifunmi^{*1}, Oluyinka Olukosi¹ ¹*University of Georgia, Poultry Science, Athens, Georgia, United States*

A total of 1,056 male broiler chicks (by-products) were used to evaluate the effects of dietary inclusion of raw potato starch (RPS) or high-amylose corn starch (HCS) on the apparent ileal digestibility (AID) of amino acid, and relative mRNA expression of tight junction proteins and cytokines in chickens challenged with mixed *Eimeria* oocysts. Birds were randomly allocated to eight treatments in a 4×2 factorial arrangement with a corn-soybean meal-based diet containing 0, 25, or 50 g/kg RPS, or 50 g/kg HCS, with or without *Eimeria* infection. All diets were isonitrogenous, isocaloric, and fed for 35 days. On day 13, the challenged groups were orally gavaged with *Eimeria* oocysts (*E. maxima*, *E. acervulina*, *E. tenella*). On day 20, ileal digesta, jejunal tissues, and ceca tonsils were collected to determine the AID of amino acids and mRNA expression of target proteins. Results showed no significant ($P > 0.05$) diet \times infection interaction or main effects of diet for dry matter digestibility (DMD) or AID of indispensable amino acids, except for Trp. Birds fed 50 g/kg HCS had higher ($P = 0.018$) AID of Trp than those on the control diet. The AID of Ile, Lys, Met, Phe, and Val were reduced ($P < 0.05$) and that of Trp was greater ($P < 0.001$) in challenged birds. Birds fed 25 g/kg RPS and 50 g/kg HCS had greater ($P = 0.005$) AID of Cys than the control, whereas AID of Ala decreased ($P = 0.002$) in challenged birds. The mRNA expression of tight junction proteins showed no diet \times infection interaction, but *Eimeria* challenge reduced ($P = 0.001$) JAM-2 expression and showed a tendency ($P = 0.069$) to reduce occludin. The main effects of diets were significant ($P < 0.05$) for TNF α and IL-10, and the main effects of infection for IL β . Birds fed 50 g/kg RPS and HCS showed increased TNF α expression ($P = 0.014$) compared to the control, and IL-10 was higher ($P = 0.035$) in birds fed control or 25 g/kg RPS diets. *Eimeria* challenge reduced IL- β expression ($P = 0.003$), whereas annexin A1 expression was higher in uninfected birds fed 50 g/kg RPS or HCS diets ($P = 0.001$). Dietary inclusion of resistant starch in corn-soybean meal-based diets may, therefore,

offer a nutritional strategy to support digestive efficiency and resilience in broiler chickens facing enteric stress.

Keywords: Raw potato starch, High-amylose corn starch, *Eimeria*, Amino acid digestibility, mRNA expression

P383 Determination of the effects of quantitative feed restriction along with oats fodder on meat quality parameters of native geese in Bangladesh Umme S. Ety*², Md. Sazedul Karim Sarker², Farhana Sharmin¹, Nushrat Nourin², Aminul Islam², Sadman S. Zihan² ¹Bangladesh Jute Research Institute, Savar, Dhaka, Bangladesh; ²Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

The present study was designed to assess the efficacy of Oats (*Avena sativa*) fodder as a supplementation along with concentrate feed for determining meat color and texture, pH, drip loss, cooking loss, water holding capacity (WHC) and Thiobarbituric acid reactive substances (TBARS) value of Bangladeshi White and Grey geese varieties. A total of 60 adult geese from White and Grey varieties were reared in a semi-intensive production system in Bangladesh Livestock Research Institute, Savar. After 3 months of rearing (8 weeks to 20 weeks) on a restricted concentrate diet supplemented with oats fodder, they were slaughtered at 20 weeks of age. Birds were randomly divided into 4 groups with 3 replicates having 5 geese in each replication. Maize soya-based diet was considered as Basal diet and marked as T₀- 200gm concentrated feed, T₁= 180 gm Basal diet+ 138 gm oats fodder, T₂= 160 gm Basal diet+ 276 gm oats fodder, T₃= 140 gm Basal diet + 415 gm oats fodder daily. A calibrated waterproof pH meter equipped with a spear tip probe was 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 were determined using a texture analyzer (Tx-700, France). TBARS value was determined using UV-VIS spectrophotometer. These value were determined for both breast and thigh meat. Data were statistically analysed using SPSS software (23 version). Treatment remarkably affected breast meat cohesiveness value ($p < .05$). Breast meat cohesiveness tended to be lower in the T₂ group. However T₂ group showed lower drip loss and cooking loss, higher WHC and also higher a* (redness) value but these parameters were not significantly affected by treatments. Moreover, TBARS value on different weeks among treatment groups were not significantly varied but T₂ group showed lower value. Supplementation of native Bangladeshi adult geese with 160 gm concentrate feed+ 276 gm oats fodder daily was found to be beneficial for improving meat quality.

Keywords: Geese, Oats fodder, Meat quality, Restricted feeding

P384 Evaluation of the amount of pre-starter feed on broiler growth uniformity and gastrointestinal tract development Mirian Leticia Ramos Provin¹, Max W. Calasans¹, Silvana de Castro Caldas*¹, Liris Kindlein¹ ¹UFRGS, Encantado, RS, Brazil

The poultry commercial companies are continuously striving to improve broiler performance and reduce production cost. It's well known that the nutrition provided to chicks during the pre-starter phase affects the performance and immune system. Also, the feed given during this period should be highly nutritious and digestible to meet the nutrient demands of the animals and to promote the development of the gastrointestinal tract, influencing animal growth and weight gain. The objective of this study was to evaluate the impact of reducing pre-starter feed intake on the gastrointestinal tract (GIT) development and growth uniformity of broiler chickens. A total of 640 one-day-old male broilers (Ross AP95) were randomly distributed into two treatment groups with

five replicates. The experimental groups were established with different daily feed amounts: Treatment 1 (T1) provided each bird with 8g, 6g, and 6g on days 1, 2, and 3, respectively, while Treatment 2 (T2) provided 6g, 4g, and 4g feed per day. Data collection involved measuring body weight and calculating the coefficient of variation (CV) at days 0, 7, 14, and 35. The CV was associated with the batch uniformity condition considering its importance in the final result of the process and the products produced. Relative intestinal weight and length measurements were also taken on day 7 using a sample of 20 birds per replicate, totaling 100 birds per treatment. Data were analyzed via a 2 × 4 factorial ANOVA and Tukey's test with α set at 0.05. Results indicated no significant differences in growth uniformity between T1 and T2 ($p > 0.05$). Both treatments exhibited similar CV trends, with an increase at day 14 (T1: 14.19%; T2: 14.55%) followed by a decrease at day 35 (T1: 12.44%, T2: 12.10%). Significant differences in the relative intestinal weight and length measurements were not found. The findings suggest that reducing pre-starter feed does not adversely impact growth uniformity, offering a viable approach for reducing feed costs without compromising broiler performance. Regarding GIT development, more studies related to the morphological structure and cellular renewal of the organ are necessary for a better conclusion of the findings.

Keywords: broiler chicks, feed restriction, growth performance, poultry nutrition, weight uniformity

P385 Trends in mycotoxin contamination in 2024 United States corn Chasity Pender*¹, Paige N. Gott¹, Lan Zheng¹, Shelby Corray¹ ¹dsm-firmenich, Animal Nutrition & Health, Plainsboro, New Jersey, United States

Historically, feed ingredients have been shown to be contaminated by mycotoxins, secondary fungal metabolites that may be detrimental to animal health. Classic signs such as reduced feed intake and oral and intestinal lesions often underestimate other costs of mycotoxicosis, including increased frequency and severity of disease, inflammation, and modulation of the gastrointestinal environment. The objective of this study was to evaluate mycotoxin prevalence and contamination levels in corn from US 2024 harvest and compare with prior years. Samples were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A (OTA). Statistical analysis was performed using GLIMMIX procedure of SAS with harvest year as a fixed effect and sample as the experimental unit. Means were separated using Tukey-Kramer with significance reported at $P \leq 0.05$. A limited number of samples are available thus far ($n = 48$) as harvest is still progressing, therefore the risk profile of this crop year is likely to change as the sample pool expands. To date, 94% of samples evaluated contained at least one mycotoxin, which is similar to the prevalence observed in 2023. Co-occurrence in corn thus far is comparable with 2023 (77 vs. 78%), though co-contamination has been increasing since the 2020 harvest (2020: 49%, 2021: 57%, 2022: 69%). Mean B-Trich, ZEN, FUM, OTA, and Afla contamination levels have remained consistent between the 2023 and 2024 harvest years with no statistical differences observed. Currently, ZEN is the most prevalent group (81 vs. 73% in 2023), followed by B-Trich (69 vs. 64% in 2023), and FUM (67 vs. 82% in 2023). The occurrence of A-Trich and Afla was numerically decreased in 2024 vs. 2023 (8 vs. 18% and 4 vs. 10%, respectively). As the mycotoxin risk of this harvest season is still coming into focus, preliminary results of the 2024 survey indicate a continued risk of multi-mycotoxin contamination. Continued

surveillance is advised to assess risk as new crop corn is fed out over the coming months.

Keywords: feed ingredients, mycotoxins, temporal, corn, United States

P386 Impact of increased particle size of wheat on broiler performance, gastrointestinal development, nutrient utilization, and welfare Muhammad Naeem*¹, Dianna Bourassa¹, Samuel J. Rochell¹, Shijinaraj Manjankattil Rajan¹, Emily Burton², Ashraf Alkhtib² ¹*Auburn University, Auburn, Alabama, United States;* ²*Nottingham Trent University, Nottingham, United Kingdom*

This study aimed to assess the impact of increasing the particle size of a single main cereal ingredient (wheat) in a wheat-based mash diet on the growth performance, gastrointestinal tract, nitrogen utilization, and welfare parameters of broiler chickens. Two dietary treatments were formulated using wheat ground through screens of 2.00mm and 5.00mm using a hammer mill. A total of 240 broiler chickens were assigned to 48 pens, with 5 birds per pen. The pens were randomly allocated to receive either the 2mm or 5mm wheat treatment. Birds had free access to feed and water for 35 days. At the end of the trial (Day 35), one bird from each pen was euthanized by cervical dislocation to collect data on digestive organs. Growth performance, nitrogen utilization, and carbon footprint data were recorded throughout the trial (Days 0–35). The independent samples t-test was applied to evaluate the impact of dietary treatment on response variables at $P < 0.05$ using IBM SPSS27. The results showed that the diet containing 5mm ground wheat significantly reduced feed intake ($P < 0.05$) and feed conversion ratio (FCR), but did not affect body weight gain ($P > 0.05$). The 5mm treatment significantly increased ($P < 0.05$) the absolute weight of the proventriculus and the absolute length of the small intestine and decreased ($P < 0.05$) the relative gastric isthmus diameter (mm/100g proventriculus). The 5mm treatment also led to a significant increase ($P < 0.05$) in both the absolute and relative weight (g/kg BW) of the gizzard digesta. Additionally, the 5mm treatment reduced carbon footprint (kg CO₂/kg BWG), nitrogen intake (g/bird), and nitrogen excretion (g/bird and g/kg BWG), while improving nitrogen efficiency (%). Litter moisture, excreta moisture, and nitrogen content were all significantly reduced in the 5mm treatment group in addition to a significant reduction ($P = 0.004$) in pododermatitis scores. Overall, grinding wheat through a 5mm screen in a wheat-based mash diet was found to be more beneficial than using a 2mm screen which can reduce processing cost and improve digestive tract development.

Keywords: wheat, particle size, gastrointestinal tract, gizzard, broiler

P387 Dietary Productive Energy Recommendations for Broilers up to 56 Days of Age Diego A. Martinez*¹, Nawin Suesuttajit¹, Cole Umberson¹, Craig Coon¹ ¹*University of Arkansas, Fayetteville, Arkansas, United States*

This study determined dietary productive energy (PE) recommendations for broilers. Four experiments (E1 to E4) fed broilers a total of 60 pelleted diets within 1-56 d (4 experiments, 5 phases each, 3 treatments per phase) following Cobb 500 guidelines. They included replacements of soybean meal with cellulose (E1) or corn (E2) and inclusion levels of oil (E3) or total digestible amino acids (E4). One treatment consisted of a fully balanced diet in each experiment and feeding phase. A total of 9,600 birds were used (20 birds/pen, 8 pens –the experimental unit– per treatment, 3 treatments per feeding phase, 5 phases assessed, 4 experiments). In all feeding experiments and feeding phases, BW, BW gain (BWG), feed intake (FI), and feed

conversion ratio (FCR) were determined. Birds were scanned with Dual-Energy X-Ray Absorptiometry (DEXA) at the beginning and end of each feeding phase to determine the change in body composition and energy retention. Net energy (NE) for gain (NE_g, kcal/kg) was calculated. Close to the end of each phase, birds from each treatment were placed in indirect calorimetry chambers to determine fasting heat production (FHP), and the NE for maintenance (NE_m; kcal/kg) was calculated. PE (kcal/kg) was determined as NE_g+NE_m. A standard digestibility study was conducted to determine the N-corrected apparent metabolizable energy (AMEn). A database with 60 data rows was constructed, including all performance, body composition, and AMEn and PE measured values. The PE recommendation was defined as the PE value of the diet that maximized the response in each experiment and feeding phase. Data was analyzed using ANOVA and Tukey test. AMEn varied 2,468 – 3,061 kcal/kg, and PE 1,758 – 3,193 kcal/kg. Best response was observed in diets that contained mean 2,802, 2,829, 2,856, 2,887, and 2,826 AMEn kcal/kg in Starter, Grower, and Finisher 1, 2, and 3, respectively, while containing 2,884, 3,031, 2,914, 2,763, and 2,315 PE kcal/kg in the same phases. All values were adjusted to Cobb 500 AMEn recommendations, obtaining PE recommendations of 2,991, 3,163, 3,111, 2,967, and 2,579 PE kcal/kg for Starter, Grower, and Finisher 1, 2, and 3, respectively, following a quadratic trend ($P < 0.05$). PE values showed a higher ($P < 0.05$) correlation with FI and FCR than AMEn.

Keywords: Productive energy, Net energy, Nutritional recommendations, Broilers, Performance

P388 The Productive Energy Value of Corn as Determined by its Content of Digestible Nutrients and Broiler Age Diego A. Martinez*¹, Nawin Suesuttajit¹, Cole Umberson¹, Craig Coon¹ ¹*University of Arkansas, Fayetteville, Arkansas, United States*

Energy represents a major cost in feed formulation. This study intended to develop a model to predict the productive energy (PE) of corn based on its content of digestible nutrients, including digestible crude protein (dCP, %), digestible fat (dFA, %), digestible starch (dST, %), and broiler age, compared to apparent metabolizable (AMEn) and classic net (CNE) energy. Four experiments (E1 to E4) were conducted 1-56 d in floor pens with diets formulated following Cobb 500 guidelines. Pelleted treatment diets (5-phase feeding program) included SBM dilutions with cellulose (E1) or corn (E2) and graded levels of oil (E3) or total digestible amino acids (E4). Body composition (Dual Energy X-Ray Absorptiometry, DEXA), heat production (calorimetry chambers), performance, and digestible nutrients of 60 test diets were assessed. The PE value of corn was determined by regression (E1, E2), and the digestible nutrient profiles of 4 corn batches were characterized (E1 to E4). Nutritional values of corn batches varied in dCP (7.54–9.26%), dFA (2.41–3.42%), and dST (52.5–56.8%). A database was built up (n=646), and data were analyzed in JMP using mixed models. A multiple linear model was obtained (E1 to E4) applying a stratified random data-splitting with a refitting approach to predict corn PE considering dCP, dFA, dST, and age (d) as fixed predictors: PE (kcal/kg) = 136.5 + 80.55 dCP + 65.29 dFA + 35.49 dST – 13.66 age – 0.454 (age – 31.1548)² (every predictor $P < 0.0001$; adj. $R^2 = 0.62$). The model was validated. dST, dCP, and dFA in corn explained 72.8, 20.8, and 6.4% of its PE value. Mean predicted corn PE values were 2,755, 2,792, 2,656, 2,435, and 2,183 kcal/kg in Starter, Grower, Finisher 1, Finisher 2, and Finisher 3, respectively. The mean PE values of 4 corn batches used in the experiments were 2,379, 2,491, 2,379, and 2,497 kcal/kg. FCR and feed intake were correlated more with PE than AME or CNE ($> \text{adj. } R^2$; $< P$ value). In conclusion, the PE value of

corn can be predicted for diverse feeding programs based on its digestible nutrient content and broiler age, and it is a better predictor of performance.

Keywords: Productive energy, net energy, Prediction, Digestible nutrients, Broilers

P389 Distinct Responses of Two Broiler Genetic Lines to Different Feed Restriction Programs at High Altitude up to 39 Days of Age Rodolfo Riboty^{*1}, Diego A. Martinez², Carlos Vilchez¹ ¹*Universidad Nacional Agraria La Molina, Pichincha, Ecuador;* ²*University of Arkansas, Fayetteville, Arkansas, United States*

This study assessed mortality and ascites biomarkers, performance, organ development, and carcass yield of 2 broiler lines at 2500 m above sea level and fed 5 feeding levels. 810 day-old male chicks from each line were placed in 45 floor pens (18 chicks each). Pens were allocated to 10 treatments under a 2x5 factorial arrangement (2 lines, 5 feed restriction programs). Restrictions were: 70% of the corresponding line standard (ST), 80% ST, 90% ST, 100% ST, and 110% ST. Each treatment had 9 pen replications. A 3-phase feeding program with corn-soybean meal mash diets was used, following each genetic line recommendations. Performance, including feed conversion ratio (FCR) and ascites or pericardial effusion mortalities, (%), was assessed weekly, blood biochemistry (urea nitrogen, BUN; uric acid, UA; hematocrit, HCT) and organs weights (proventriculus, gizzard, liver, spleen, bursa) at 21 and 39 d, and processing weights (breast fillet, BRE; tenderloins, TEN; drumsticks, DRU; thighs, THI) at 39 d. ANOVA and Tukey's test were ran. Line B showed higher BWG ($P<0.001$) than line A. FCR of line B was better than line A across all weeks ($P<0.001$) and cumulatively ($P<0.001$). Higher feeding levels (100% ST, 110% ST) produced the highest BW. Up to 14 d, 70% ST produced the best FCR, whereas between 15–35 d, 80% ST showed better FCR ($P=0.044$). No mortality difference was detected between lines ($P>0.05$). From 22 d, onward, ascites mortality increased with higher feeding levels ($P=0.034$). Pericardial effusion cumulative mortality was higher with 100% ST only ($P=0.016$). Heart/BW ratio, BUN, UA, and HCT values were lower in line B at both 21 and 39 d ($P<0.05$). Gizzard, liver, and bursa weights were influenced by line at 21 d ($P<0.016$), and gizzards were heavier at 39 d with 70% ST and 80% ST ($P<0.001$). BRE (16.59 vs. 15.51%; $P=0.004$) and thighs (8.92 vs. 8.38%; $P=0.001$) were larger in line B compared to line A. 70% ST resulted in the lowest BRE% ($P=0.013$) and highest DRU% ($P<0.001$). As feeding levels increased, these trends reversed. In conclusion, results suggest that although birds from both modern genetic lines exhibit similar ascites-related mortality when raised at high altitudes, differences in performance and carcass yield are evident between the two genetic lines.

Keywords: Ascites, Feed restriction, Processing yield, High altitude, Biomarkers

P390 Compensatory Growth, Mortality and Carcass Yield of Broilers from 39 to 46 Days of Age at High Altitude Rodolfo Riboty^{*1}, Diego A. Martinez², Carlos Vilchez¹ ¹*Universidad Nacional Agraria La Molina, Lima, Peru;* ²*University of Arkansas, Fayetteville, Arkansas, United States*

Feed restriction effectively reduces the incidence of ascites but prevents maximum performance. This study evaluated compensatory growth, mortality, and processing yield during the last week of life in broilers previously fed different levels of feed restriction at 2500 meters above sea level. 810 day-old male chicks A diet with 25% SBM will possess 1 mg/g TI activity if the SBM TI activity is 4.0 mg/g. Among the 526 solvent extract SBM

from each of 2 genetic lines were distributed in 45 floor pens (18 chicks each). During the previous 39 days, birds received one of five feed restriction programs according to each genetic line recommendation standard feeding recommendation (ST): 70% ST, 80% ST, 90% ST, 100% ST, and 110% ST for each genetic line. From 39 to 46 days of age, the same birds were re-assigned to ad libitum feeding. In both genetic lines, birds previously fed at 70%, 80%, and 90% ST were divided into two subgroups and received two high feed allocation levels: 100% ST (4 pens each) and 150% ST (5 pens each), based on each line standard. Birds previously receiving 100% ST and 110% ST were maintained at 100% ST. At 46 days of age, performance, ascites or pericardial effusion mortality (%), and carcass yield were recorded. Statistical significance was evaluated using ANOVA with Tukey's test for multiple comparisons. Results indicated similar compensatory BW gain for both lines but lower feed intake ($P<0.001$) and better feed conversion ratio (FCR; $P<0.001$) for line B. Birds in the 150% ST group showed higher feed intake than those in 100% ST ($P<0.001$), allowing them to gain more weight compared to birds fed 100% ST ($P<0.001$), whether previously restricted or not. FCR and mortality did not differ between genetic lines or feeding programs ($P>0.05$). For carcass variables, only thigh yield was higher in line B (11.59% vs. 10.79%; $P<0.001$). The 150% ST feeding program for birds that were been more restricted (70% ST and 80% ST) resulted in greater *Pectoralis major* ($P<0.001$), thigh ($P=0.002$), and drumstick ($P=0.001$) yields compared to birds consuming 100% ST. In conclusion, compensatory growth may be a useful tool to enhance productive response and meat production, which can be maximized by using specific feeding levels and genetic lines.

Keywords: Ascites, Feed restriction, Processing yield, Compensatory growth, High altitude

P391 Evaluation of trypsin inhibitor activity in 2021-2024 United States soybean meal Mike Schulz¹, Emily Jackson¹, Frances Yan^{*1} ¹*Novus International Inc., Chesterfield, Missouri, United States*

Residual trypsin inhibitors (TI) in soybean meal (SBM) inhibit endogenous trypsin activity, reduce overall dietary protein digestibility and compromise growth performance and health of poultry. Previous in vivo studies have indicated that dietary TI activity greater than 1 mg/g of feed may cause potential issues in reducing AA digestibility and performance of broilers. The objective of this study was to evaluate TI activity of the United States SBM samples from 2021-2024 to understand their risk in poultry production. A total of 748 SBM samples from 17 companies were analyzed for TI by NIR technology. Wet chemistry TI activity values analyzed by the AOCS Official Method Ba 12a-2020 were used to establish NIR prediction equations. Among the 748 samples, 526 were solvent extract SBM, 165 were expeller SBM, and 57 were full-fat SBM. Due to unequal sample size and unequal variance, data were subject to Welch ANOVA to evaluate TI activity as affected by SBM type, year and company; means were separated by Tukey Cramer test with a significant level of 0.05. Full-fat SBM had significantly higher TI than expeller SBM, and expeller SBM had significantly higher TI than solvent extract SBM (16.7, 9.4, and 5.2 mg/g trypsin inhibited for full-fat, expeller, and solvent extract SBM respectively; $P<0.05$). Expeller SBM TI activity decreased from 2022 to 2024 ($P<0.05$), whereas no change was observed for full fat or solvent extract SBM with year ($P>0.05$). Trypsin inhibitor activity of solvent extract SBM varied among companies ($P<0.05$) with the highest averaging 7.4 mg/mg and the lowest averaging 3.0 mg/g.

samples, 79% had TI activity higher than 4.0 mg/g. In summary, the study revealed a large variation in TI activity of the United

States SBM samples, many with TI activity high enough to potentially compromise bird performance and health. Therefore, to achieve precision feeding and improve production efficiency, it is necessary to monitor SBM TI activity with NIR technology to better manage variation in SBM quality through supplier selection, formulation adjustment, and/or supplementation of exogenous proteases effective in hydrolyzing TI in SBM.

Keywords: trypsin inhibitor, soybean meal quality, NIR technology

P392 Evaluation of Microencapsulated Vitamin and Trace Minerals on Growth Performance, Blood Biochemistry, and Welfare Indicators in Broilers Dimitri Malheiros^{*1}, Ludovic Lahaye¹, Miguel Barrios¹, Peter Feket³, Dan Moore², Monique Archibeque², Jean Fontaine¹, Derek Detzler¹ ¹*Jefo, Saint-Hyacinthe, Quebec, Canada;* ²*Colorado Quality Research, Wellington, Colorado, United States;* ³*North Carolina State University, Raleigh, North Carolina, United States*

Lipid-based microencapsulation of vitamins and trace minerals (PVTM) using Jefo Matrix Technology (JMT) can significantly enhance broiler growth performance, even at reduced composition levels compared to conventional free vitamin and trace mineral (FVTM) premixes. The objective of this study was to further assess the effects of PVTM, at lower composition levels than industry-standard FVTM, on broiler performance, welfare, and blood biochemistry. It is hypothesized that the PVTM will allow for slower release in the gut, allowing for better VTM absorption leading to better performance. A total of 1008-day-old male Cobb 500 broiler chicks were housed in an environmentally controlled facility across 48 floor pens divided into 4 treatments (12 pens per treatment). The treatments were as follows: FVTM100 (100% commercial FVTM), PVTM75, PVTM50 and PVTM25 (75, 50 and 25% of FVTM100, respectively, but protected with JMT). Feed and water were provided ad libitum. Body weight and feed intake were measured on days 0, 14, 28, and 42, while water intake was recorded weekly. Body weight gain, feed intake, and Feed conversion ratio were calculated across 0–14, 0–28, 0–42, and 28–42 days. On day 28, blood biochemistry samples were collected via an iStat device from one bird per pen. On day 42, welfare indicators such as litter condition, footpad lesions, shank pigmentation, feather coverage, and mobility were assessed. Statistical analysis was conducted using ANCOVA in XLSTAT with block as a covariate, and with significance set at $P \leq 0.05$. Tukey's HSD was used for mean comparisons. No significant differences in water intake, body weight gain, or feed conversion ratio were observed among treatments during days 0–14 and 0–42. However, on days 0–28, birds in the PVTM50 group exhibited lower body weight gain compared to other groups. Blood biochemistry on day 28 did not differ significantly across treatments. Welfare indicators on day 42 also showed no statistical differences, except for footpad scores, where PVTM50 and PVTM25 had better scores than PVTM75 ($P=0.013$). In conclusion, JMT microencapsulation is a viable alternative to conventional FVTM, demonstrating comparable performance, blood biochemistry, and welfare outcomes with reduced vitamin and trace mineral levels.

Keywords: Microencapsulation, Nutrition, Mineral, Vitamin, Broiler

P393 Niacin and nicotinamide riboside supplementation effects on growth performance in turkey hens Victoria Wilson^{*1}, John Gonzalez², Scott Beyer³, Chad Paulk¹, Haley Ott¹, Carter D. Minson¹, Mason Engnell¹, Diego Lopez¹, Allison Blomme¹, Walter Friesen¹ ¹*Kansas State University, Grain Science and Industry, Manhattan, Kansas, United States;*

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Adequate niacin (NA) level in poultry diets is essential for improving turkey growth performance and health. Nicotinamide riboside (NR), an NA analog, is a novel feed additive which produces NAD⁺ through the Salvage Pathway. Hence, NR may serve comparable or complementary functions in turkey nutrition. Therefore, the objective of this study was to determine the effect of added NA or NR on commercial turkey hen growth performance. Day-old Hybrid poults ($N=900$) were randomly allocated to 30 pens located across 10 locations of a research barn ($N=30$ /pen). Within each location, pens were randomly assigned one of three dietary treatments consisting of a control diet (102 mg/kg NA, 0 mg/kg NR), or the control diet with either 70 mg/kg diet added NA or NR. Treatment additions were made through post pellet liquid application, where the treatment addition was mixed with 2% of the formulated soy oil and applied. Diets were fed for 42 days and data were analyzed using GLIMMIX procedure of SAS 9.4, with pen as experimental unit, diet as the fixed effect, and pen location as the random effect. For days 0–18 and 19–42 there was no evidence of differences in BWG, FI or FCR ($P > 0.05$). For days 0–42, there was no evidence of differences in BWG or FI ($P > 0.05$) between dietary treatments; however, turkeys fed added NA had improved ($P < 0.05$) FCR compared to those fed the control diet and turkeys fed added NR had a tendency for improved ($P < 0.07$) FCR compared to those fed the control diet. The NRC (1994) suggests the requirement for this age turkey poult is 60 mg/kg niacin; however, this research demonstrates an improvement in FCR when feeding up to 172 mg/kg of NA or 70 mg/kg NR.

Keywords: niacin, nicotinamide riboside, turkeys, vitamins

P394 Performance and eggshell quality of broiler breeders supplemented with enhanced levels of minerals and vitamins Eveline Berwanger¹, Fabrizio Oristanio^{*2}, Neyre N. Shiroma¹, Jumara Coelho³, Dausia Souza³, Rosana Vaz³, Edson Formighieri³, Felipe Cerri³, Hamilton Caixeta³, Soner Moreira³, Wesley Santos³ ¹*Salus Com. de Prod. de Saúde e Nutrição Animal S.A., Technical, Santo Antônio de Posse, São Paulo, Brazil;* ²*Salus Com. de Prod. de Saúde e Nutrição Animal S.A., Feed Additives, Santo Antônio de Posse, São Paulo, Brazil;* ³*Grupo Alvorada, Technical, Itapetininga, São Paulo, Brazil*

The aim of this study was to evaluate the effect of a nutritional supplement (Ovocap Plus) on broiler breeders' performance and egg quality. Ovocap Plus is composed of proline precursors, vitamin D3, vitamin K3, manganese, copper, iron, calcium, and magnesium. These nutrients are involved in calcium and bone metabolism. The trial was conducted on a commercial farm located in the state of São Paulo, Brazil. A total of 123,000 Cobb breeders, aged 53 and 59 weeks, from two flocks were used. Ovocap Plus was added to the diet at an inclusion of 1 kg per ton for a period of 28 days. Performance and eggshell quality were evaluated before and after the supplementation of Ovocap Plus. Data were subjected to paired T-test. Viable egg production (97.3% to 97.4%; p -value = 0.0898) and eggshell thickness (364.3 to 362.4 μ m; p -value = 0.3958) were the same before and after the supplementation of Ovocap Plus. In contrast, egg weight (70.5 to 71.7 g; p -value = 0.0040) and specific gravity (1074.8 to 1076.2; p -value < 0.0001) increased after the supplementation of Ovocap Plus, while the percentage of cracked eggs (0.79% to 0.68%; p -value = 0.0015) and the number of pores per cm² (111.9 to 99.1; p -value = 0.0105) decreased significantly. In conclusion, data from study indicates that the supplementation Ovocap Plus to broiler breeders improves performance and eggshell quality.

Keywords: specific gravity, number of pores, cracked eggs, eggshell thickness, egg production

P395 Calcium Pidolate (Calpid®) Enhances Carcass Traits, Bone Quality, and Mineral Digestibility in Broilers Fed Reduced Calcium and Phosphorus Diets AbdelRahman Y. Abdelhady*^{1,2}, Abdelhacib Kihal¹, Salah A. El-Safty², Ahmed Radwan¹, Sergio Merinero³, M. Puyalto¹, J. J. Mallo¹ ¹*Norel S.A., Madrid, Spain;* ²*Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt;* ³*Nuvel Agriservices S.L.U., Madrid, Spain*

This study examined the effects of adding calcium pidolate to diets reduced in Ca and available phosphorus (aP) on tibia bone quality, carcass traits, mineral digestibility, and footpad health in broilers. A total of 800-day-old chicks were allocated to 5 dietary treatments with 8 replicates each: a control diet (CONT) meeting FEDNA requirements; a diet with 15% reduced Ca and aP from CONT (N15); N15 supplemented with 0.03% Calpid® (CP15); a diet with 30% reduced Ca and aP (N30); and N30 supplemented with 0.03% Calpid® (CP30). Results for carcass analysis at 35 days showed that whole breast percentage of LBW was maintained in CP15 (31.98%), comparable to CONT (32.72%) and N15 (32.40%), and significantly higher than N30 (28.67%) and CP30 (28.63%) ($P < 0.01$). Other carcass components were unaffected by the treatments. Tibia ash content was significantly higher ($P <$

0.01) in CP15 (47.14%), matching CONT (47.43%), and exceeding N15 (44.15%) and N30 (43.51%). CP15 had the highest tibia breaking strength (29.01 kg), significantly exceeding N15 (25.31 kg, $P < 0.01$) and N30 (26.19 kg, $P < 0.01$), and slightly above CONT (28.33 kg, $P > 0.05$). Tibia redness (a* value) was lower in CP15 (1.918) compared to N15 (2.260) and N30 (2.607). Apparent digestibility coefficients for ash, Ca, and P were significantly enhanced in CP15 and CP30. Ash digestibility reached 63.95% in CP15 and 71.01% in CP30, surpassing CONT (59.86%) ($P < 0.01$). Ca digestibility increased to 70.04% in CP15 and 72.52% in CP30, compared to CONT (63.15%). P digestibility showed similar improvements among treatments. Footpad lesion evaluations indicated no significant differences among treatments, with most birds exhibiting healthy footpads. Supplementation with 0.03% calcium pidolate in broiler diets reduced in Ca and aP significantly improved tibia bone quality, evidenced by higher ash content and breaking strength, and maintained breast muscle similar to the CONT. Mineral digestibility of ash, Ca, and P was enhanced in Calpid®, surpassing the control. These findings highlight Calpid® as a valuable additive for improving bone quality, maintaining breast muscle yield, enhancing mineral absorption, and supporting overall broiler health without adverse effects.

Keywords: Broilers, Bone quality, Carcass Traits, mineral digestibility, Calcium pidolate

Physiology, Welfare and Behavior

P396 Differential responses of gut barrier function to heat stress in divergently selected broiler lines for water efficiency: insights from gene and protein expression profiles Maria Fernandez Cuadrado*¹, Brooklee Roach², Elizabeth S. Greene¹, Sara Orłowski¹, Sami Dridi¹ ¹*University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States;* ²*Har-Ber High School, Springdale, Arkansas, United States*

Through alteration of the structural and functional integrity of the gut, heat stress (HS) and water scarcity challenge poultry health, productivity, and welfare. Here, we examined the impact of HS on intestinal barrier function in two broiler lines divergently selected for high (HWE)- or low (LWE)-water efficiency. Day-old chicks from both lines were raised in environmentally controlled chambers, with half of the birds subjected to cyclic HS (36°C for 9 hours/day) from D29 to D49, while the remaining birds remained under thermoneutral (TN) conditions (25°C), resulting in a 2x2 factorial design. On D49, ileum was sampled for expression profiles of key genes and proteins involved in maintaining intestinal integrity. Data were analyzed by Two-Way ANOVA and Tukey's multiple range test using Graph Pad Prism. Data are expressed as mean \pm SEM and were considered significant at $P \leq 0.05$. Low water efficient birds showed greater gut permeability ($P < 0.05$), as indicated by elevated levels of fluorescein isothiocyanate-dextran (FITC-D), compared to HWE birds. HWE birds exhibited a significant upregulation of gap junction protein alpha 1 (GJA1) and tight junction proteins Claudin-1 and 4 (CLDN1/4), suggesting enhanced cell communication and barrier maintenance. HS selectively induced CLDN4 expression in HWE birds, which may contribute to their resilience to heat-induced permeability changes. Conversely, Claudin-5 (CLDN5) was upregulated ($P < 0.05$) in the ileum of HS-LWE birds, a response potentially indicative of infiltration of immune cells. In addition, lipocalin-2 (LCN2), an inflammatory marker, was markedly increased ($P < 0.05$) in HS-LWE birds. These findings highlight the molecular adaptations in HWE birds that indicate superior gut barrier function and resilience to HS, compared to the more vulnerable LWE line. Enhanced expression of these proteins in HWE birds under HS conditions suggests that

genetic selection for water efficiency may improve intestinal integrity, offering potential strategies for enhancing poultry health in challenging environments.

Keywords: broiler, heat stress, water efficiency, gut integrity

P397 Differential expression of genes in fatty acid metabolism and inflammation pathways in Pectoralis major of broilers with woody breast myopathy Linan Jia*¹, Chuan-Yu Hsu², Xue Zhang³, M. W. Schilling⁴, Kelley Wamsley¹, Pratima Adhikari¹, Li Zhang¹ ¹*Mississippi State University, Poultry Science, Mississippi State, Mississippi, United States;* ²*Mississippi State University, Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States;* ³*Mississippi State University, Department of Animal and Dairy Sciences, Mississippi State, Mississippi, United States;* ⁴*Mississippi State University, Department of Food Science, Nutrition and Health Promotion, Mississippi State, Mississippi, United States*

Woody breast (WB) muscles exhibit increased fat content and inflammation, indicating a potential etiology linked to dysregulation of fatty acid and inflammatory processes. This study examined gene expression related to fatty acid metabolism and inflammation response in WB muscle. A total of 112 Ross \times Ross 708 broilers were raised according to commercial husbandry standards in a floor-pen chicken house. At day 41, birds were assessed through palpation for WB condition and categorized into normal and WB phenotypes. Using a case-control study, birds showing moderate and severe WB conditions were placed in the woody group, while those with normal breast and slight WB conditions were assigned to the normal group. A total of 10 birds were euthanized, including five from normal group and five from WB group, followed by breast muscle RNA extraction and reverse transcription. The first-strand cDNA underwent analysis using the real-time RT² Profiler PCR Array, targeting 84 genes associated with fatty acid metabolism and 5 housekeeping genes. Additionally, this cDNA was subjected to RT-qPCR analysis for 10 inflammatory genes, utilizing the chicken *18S rRNA* as the

housekeeping gene. Fold changes were calculated using the $\Delta\Delta Ct$ method and were compared using the Student's *t*-test with a significant level set at $P \leq 0.05$. Eight of the 84 examined genes related to lipid metabolism showed differential expression (for all $P \leq 0.05$) between normal breast and WB samples. Specifically, 3 genes were upregulated (for all $P \leq 0.05$) in WB samples: *acetyl-Coenzyme A acetyltransferase 2*, *alcohol dehydrogenase 6*, and *glycerol kinase*. In contrast, 5 genes were downregulated (for all $P \leq 0.05$) in WB samples: *acyl-CoA dehydrogenase*, *acyl-CoA synthetase medium-chain family member 4*, *glycerol-3-phosphate dehydrogenase 2*, *5'-AMP-activated protein kinase subunit beta-2*, and *5'-AMP-activated protein kinase subunit gamma-3*. Compared to the normal group, the expression levels of *toll-like receptor 2A*, *toll-like receptor 4*, *interferon- γ* , *interleukin-1 β* , and *interleukin-6* in the woody group were increased (for all $P \leq 0.05$). In conclusion, gene expression in WB muscle indicated upregulation of lipid biosynthesis, downregulation of fatty acid catabolism, and an activated inflammation response.

Keywords: Woody breast, Fatty acid metabolism, Inflammatory response, Gene Expression, Broiler chicken

P398 Effect of different percentages of fertile eggs in setter trays on eggshell temperature and hatching parameters Ricardo J. Pereira^{*1}, Felipe L. Kroetz Neto^{2 1} *College of Veterinary Medicine and Animal Sciences - São Paulo University, Animal Reproduction, Pirassununga, São Paulo, Brazil;* ²*Aviagen America Latina Ltda, Campinas, São Paulo, Brazil*

Hatchery performance has a fundamental influence on fertility and hatchability of eggs. This study investigated the variations of eggshell temperature over the course of incubation under different fertility levels and assessed their impact on incubation parameters and chick quality. Eggs from a breeder flock (Ross 308AP®) were evaluated in three repetitions (34, 39, 41 wk of age), in which only eggs in the same specific gravity range (1.080-1.084 g/cm³) and under a maximum weight variation of 6g were used. 3,444 eggs/repetition were divided into three fertility groups (e.g. 60%, 80%, and 100% fertile eggs/setter tray); shell temperature during incubation, egg weight loss, hatching window, hatchability, chick yield and weight, neonatal quality, and embryonic mortality were assessed. Based on variance homogeneity and residual data normality, there was no need to transform the data. Student-Newman-Keuls (SNK) method was used for multiple comparisons, with significance at $P < 0.05$. Significant differences in egg weight loss were observed, especially in the 60% group. Hatchability was not significantly affected, but there were relevant differences in chick weight. The analysis of the hatching window did not reveal significant differences among treatments, and chick yield remained as expected. However, chick quality parameters (especially activity, residual membrane, and navel) were significantly reduced in the 60% and 100% groups. Embryonic mortality did not show significant differences between groups. There was a minimal difference of up to 0.2°F in temperatures during the last 3 days of embryonic development. In summary, the data revealed a reduction in chick quality when the setter trays had 60% and 100% fertile eggs, although there was no variation in eggshell temperatures during embryonic development. This decrease in quality was reflected in chick activity parameters, presence of residual membrane, and incomplete umbilical closure. In the 60%

Keywords: Hatchability, Incubation, Eggshell temperature, Fertility, Chick quality

P399 Negative Impact of *Eimeria* spp. on the reproductive tract of Laying Hens with minimal influence of Mycotoxins

from contaminated corn DDGS Hamid Reza Rafieian-Naeini^{*1}, Deependra Paneru¹, Hemanth Reddy Katha¹, Silvia Carnaccini², Woo Kyun Kim¹ ¹*University of Georgia, Department of Poultry Science, Athens, Georgia, United States;* ²*University of Georgia, Population Health, Athens, Georgia, United States*

Understanding the interaction between mycotoxicosis and coccidiosis and their effects on the reproductive tract of laying hens is important for improving poultry health and productivity. This study investigated the combined effects of *Eimeria* mix (EM) infection (*E. maxima*, *E. tenella*, and *E. acervulina*, 50,000, 50,000, and 250,000 oocysts/bird respectively) and contaminated corn DDGS (deoxynivalenol, Zearalenone, T2-toxin, HT2-toxin, and Fumonisin) on the reproductive tract in laying hens. A total of forty-eight Hy-Line W-36 laying hens at 40 weeks of age were randomly allocated and kept for 21-day period in 4 treatments consisting of; 1) control diet without EM inoculation; 2) contaminated corn DDGS without EM challenge; 3) control diet with EM inoculation; and 4) contaminated corn DDGS with EM inoculation. Egg production and body weight were recorded daily. Blood and oviduct were collected on day 21. The data were analyzed using two-way ANOVA followed by Tukey's post hoc test, ($P < 0.05$). Egg production and body weight decreased in the combined exposure groups compared to the mock-inoculated control group. *Eimeria* infection alone showed its detrimental effects on oviduct weight, length, serum estradiol level, and dominant follicles. On the other hand, contaminated corn DDGS by itself did not change the aforementioned parameters. The height of primary and secondary folds in the magnum was reduced by about 61% in the EM challenge groups and 26% in the contaminated corn DDGS group, while magnum and uterus tunica muscularis thickness increased significantly in EM and contaminated corn DDGS respectively. mRNA expression of eggshell formation genes (*OC-116*, *OTOP2*, *CALCB*, *STC2*, *OSTN*) and albumen secretion genes (*RLN3*, *ACE*, *REN*, *AVD*, *GPX3*) in the uterus and magnum was significantly downregulated in EM challenge. However, none of the genes were affected by contaminated corn DDGS. Contaminated corn DDGS combined with EM may lead to increased damage to the reproductive tract, greater body weight loss, and a significant decrease in egg production. It was concluded that mycotoxins have a minimal effect on their interaction with *Eimeria* on the reproductive tract of birds. However, strict control of coccidiosis is crucial in laying hens to maintain egg production quality

Keywords: *Eimeria* infection, contaminated corn DDGS, reproductive tract, egg production

P400 The influence of egg weight on eggshell temperature during incubation and on hatching parameters Ricardo J. Pereira^{*1}, Felipe L. Kroetz Neto^{2 1} *College of Veterinary Medicine and Animal Sciences - São Paulo University, Animal Reproduction, Pirassununga, São Paulo, Brazil;* ²*Aviagen America Latina Ltda, Campinas, São Paulo, Brazil*

Consistency in egg weight during incubation is crucial as larger eggs may face challenges in gas and heat exchange. The incubation temperature is determined by the incubator, which uses the eggshell temperature to estimate the embryo's metabolic development rate in order to maximize hatchability and chick quality. However, thermal conductance varies with egg size, hindering heat dissipation in larger eggs. Our study investigated variations in shell temperature throughout incubation in eggs within the same specific gravity but with different weights, to verify what repercussions these differences in weight might have on temperature measurements and, consequently, on hatching parameters. Three repetitions were performed with eggs from the same parent flock at different ages (Ross 308AP® at 34, 39, and

41 weeks of age). In each repetition, a total of 16,128 eggs were tested for specific gravity (SG) with only 4,200 selected for the study (all within the same SG range - 1080 to 1084 g/cm³). Eggs were divided into three weight categories (light, medium and heavy) with a 6-gram difference between them, and subjected to incubation and temperature analyses. Variables were initially tested to determine variance homogeneity and residual data normality, and there was no need to transform the data. Student-Newman-Keuls (SNK) method was used for multiple comparisons and differences were considered to be significant when $P < 0.05$. We observed that egg weight had an effect on weight loss with lighter eggs being most affected. Different egg weight ranges did not alter hatchability when eggs were from the same batch, age, and SG, nor did they affect embryonic mortalities. Light eggs showed differences in chick yield and hatching window at 500 h (12 h), probably due to the precocity of chick hatching. Despite the small temperature difference between light and heavy eggs (up to 0.4°F in the last 3 days of incubation), this factor possibly influenced chick quality, as differences were observed in overall chick quality (including activity, abdomen, and navel). Understanding the relationship between egg size and temperature requirements is crucial for the incubation sector, especially considering the challenges faced by larger eggs in dissipating heat at the end of incubation.

Keywords: Egg weight, hatchery, broiler breeder, Chick yield, Hatching window

P401 Evaluation of skip-a-day feeding in Cobb 500 broiler breeder hens Alyssa M. Lyons^{*1}, Cristiane S. Araujo², Mário Henrique S. Lopes³, Giovana M. Longhini³, Raimundo G. Netto³, Melany Y. Lovon³, Lucio F. Araujo³, Mike E. Persia¹ ¹*Virginia Tech, School of Animal Sciences, Blacksburg, Virginia, United States;* ²*University of Sao Paulo, FMVZ-USP, Pirassununga, Brazil;* ³*University of São Paulo, Department of Animal Science, Pirassununga, São Paulo, Brazil*

Skip-a-day feeding is a common management tool that is used to control feed intake in breeding pullets so that they do not over consume feed resulting in reduced performance and animal welfare. This practice has been criticized due to the longer term of feed deprivation despite the fact that total feed intake over time is the same as everyday feeding. An experiment was conducted to determine the effects of skip-a-day (SaD) versus everyday (ED) feeding using broiler breeder hens as a model for SaD in pullets. There is speculation that SaD feeding or the lack of consistent feed in the intestinal system alters the intestinal lining and increases intestinal permeability (IP), but data are lacking. Although skip-a-day feeding is used in pullets, for this first experiment pullets were not available and broiler breeder hens were selected to model the effects of the pullets. Broiler breeder hens utilized in the current experiment were previously used in an experiment investigating the effects of hydrolyzed yeast feeding. Therefore, the experiment was a 2 x 2 factorial with diet (none v. 5,512 g/MT hydrolyzed yeast) and feeding regimen. Skip-a-day included a 48 h fast, feeding period with 2x feed and 24 hours of fasting while the ED period included a 24 h fast, feeding, 24 h fast, feeding and 24 h fast. A total of 36 Cobb 500 broiler breeder hens at 40 wks old were allocated to 4 treatments with 3 pens of 3 birds per treatment. Feed was provided in mash form and birds were fed according to Cobb breeder recommendations. Intestinal permeability was measured at 48 and 72 h by orally gavaging two birds per pen with 8.32 mg/kg of FITC-d dissolved in distilled water 1 hour before blood collection. Blood was stored in the dark and centrifuged to isolate serum for FITC-d determination. Data were analyzed in JMP Pro using ANOVA ($P \leq 0.05$). There were no interactions between diet or feeding regimen at 48 ($P = 0.22$) or 72 h ($P = 0.68$).

Feeding regimen did not affect intestinal permeability at 48 h as SaD and ED resulted in 170 and 168 ng/mL, respectively, or 72 h as SaD and ED resulted in 122 and 123 ng/mL, respectively. These data indicate that skip-a-day feeding may not alter intestinal permeability in laying hens but would need to be validated in pullets.

Keywords: skip-a-day, pullets, broiler breeders, intestinal permeability

P402 Factors affecting Leghorn breeder pullet hatchability from 2016 to 2023 Caleb M. Marshall^{*1}, Daniel Valbuena², Edgar O. Oviedo-Rondón¹ ¹*North Carolina State University, Prestage Department of Poultry Science, Raleigh, North Carolina, United States;* ²*Hy-Line International, Des Moines, Iowa, United States*

Quantifying factors impacting pullet hatchability (PH) is necessary to establish standards and determine opportunities for improvement. Using a commercial dataset, these analyses evaluated PH in Leghorn breeders over 8 years (2016-2023). The dataset included 51,245 observations from 240 breeder flocks across three hatcheries. These data represent 682.9 million eggs set and 275 million pullets hatched. Explanatory variables such as flock age (FA), egg storage duration (ES), and management practices such as short periods of incubation during egg storage (SPIDES) were evaluated. Eggs stored less than 7 d did not receive SPIDES, while eggs stored greater than 7 d did. The mean ES was 6.38 d (SD = 2.88), with averages of 4.83 d (SD = 1.16; non-SPIDES) and 9.65 d (SD = 2.67; SPIDES). Notably, ES was stable from 2016-2021 (~6 d; SD = 2.4), but ES increased in 2022 (9.18 d; SD = 4.64) and 2023 (8.94 d; SD = 4.39), marking a shift in storage practice in recent years. Mixed model analysis included FA and ES as response surface predictors, with Year and Treatment as fixed effects. Hatchery, flock, and FA nested within the flock were included as random effects to account for variability in management practice. An average reduction in PH ($R^2 = 0.61$) of 0.26% was observed ($P < 0.001$) over the lifecycle of flocks comparing SPIDES vs. non-SPIDES (42.01 and 41.75%, respectively). For non-SPIDES eggs ($R^2 = 0.58$), 2016 had the highest ($P < 0.001$) PH (42.57%), similar to all years but 2019 (40.55%) and 2022 (40.43%). The model ($P < 0.001$) for non-SPIDES $PH = 34.737 + 0.690*FA - 1.150*ES - 0.009*FA^2 + 0.001*FA*ES + 0.036*ES^2$ had a maximum PH of 43.86% at an optimal FA of 42 wk and ES of 3 d ($R^2 = 0.63$). Similar to the non-SPIDES eggs, PH was highest in 2016 (42.71%) for eggs receiving SPIDES, but 2016 was similar only to 2021 (41.47%) and 2023 (41.33%). The model ($P < 0.001$) for SPIDES $PH = 34.737 + 0.690*FA - 1.150*ES - 0.009*FA^2 + 0.001*FA*ES + 0.036*ES^2$ indicated a maximum PH of 44.39% at 41 wk FA and 8 d of ES ($R^2 = 0.60$). The average PH across the previous 8 years was not different, regardless SPIDES application. The PH of leghorn lines was described in response surfaces for both non-SPIDES and SPIDES eggs with quadratic effects of FA, ES, and their interaction.

Keywords: data analysis, pullet hatchability, leghorn, flock age, SPIDES

P403 Detection of Early Dead Embryos using Hyperspectral Imaging System Alin Khaliduzzaman^{*1}, Jason Lee Emmert², Mohammed Kamruzzaman¹ ¹*University of Illinois, Department of Agricultural and Biological Engineering, Urbana, Illinois, United States;* ²*University of Illinois, Department of Animal Sciences, Urbana, Illinois, United States*

In this research, we explored the application of hyperspectral imaging system for the early detection and removal of dead chick

embryos during incubation. Approximately 5-10% of embryos die due to thermal shock and inappropriate handling during the early incubation stages, posing significant challenges in hatchery practices, including contamination risks, space constraints, manpower and energy costs. To address this issue, we proposed a non-destructive and real-time approach using hyperspectral imaging combined with discriminant analysis to classify live and dead embryos. We used PIKA L hyperspectral camera (400-1000 nm) for spatial and spectral information of incubated eggs at day 4. We sorted 102 eggs (White Leghorn with 29 weeks flock age) collected from poultry farm of University of Illinois at Urbana-Champaign for incubation at 99.0 F and 60% RH. We maintained stage scanning speed of 0.06 cm/s with frame rate of 9.9 fps and exposure time of 100 ms. Hyperspectral imaging offers a non-invasive and high-resolution method to capture detailed spectral information from incubated hatching eggs. The spectral differences of hemoglobin absorbance in the range of 570-580 nm should be varied between live and dead chick embryos. Based on this hypothesis, we were able to classify the eggs non-destructively with an accuracy of 90.0% using discriminant analysis. The successful implementation of this methodology could significantly enhance hatchery efficiency, reducing contamination risks, conserving space, and optimizing energy usage. Ultimately, our research endeavors to contribute to the advancement of poultry hatchery industry practices by offering a proactive solution to improve hatchery operations and ensure the production of healthy chicks.

Keywords: Incubation management, early dead, computer vision, hatchery practices, animal welfare

P404 Effect of dietary tryptophan and photoperiod on the fear responses of broilers during the open field test Rosemary Whittle*¹, Chantel Pennicott¹, Angela Perretti¹, Victor Oyeniran¹, Jaalen Cherry¹, Shawna Weimer¹ ¹University of Arkansas, Poultry Science, Farmington, Arkansas, United States

Tryptophan (TRP), a precursor of serotonin and melatonin, plays a role in modulating fear responses. Photoperiod regulates the circadian rhythm of serotonergic-melatonergic pathways. Open field tests are used to assess fear in birds, where lower activity indicates increased fearfulness. In a 2x3 factorial design, 576 broilers were raised in 48 pens (N=12 birds/pen) within four houses (N=12 pens/house) under abrupt (ABT) or incremental (INC) photoperiods and fed 100%, 150%, or 200% dietary tryptophan. The INC photoperiod (N=2 houses) was 23h light (L) and 1h dark (D) on d0, increasing the dark period 0.5h daily until d16 when the photoperiod was on 16L:8D and the ABT photoperiod (N=2 houses) was 23L:1D from d0-d5, 20L:4D on d6-d13, 16L:8D from d14 until the end of the study. At D28, broilers (N=46) were individually placed in the center of an open field arena (2.4x3.7m) for 180s and weighed. Videos were analyzed in Noldus Ethovision XT to track distance travelled (m), speed (cm/s), and activity (%). Linear mixed effects models were fitted in R using diet and lighting interaction, body weight as a covariate and house as a random factor. Activity data were square root transformed to increase model fit. There tended to be an interaction between diet and lighting for distance, speed, and activity in the open field ($\chi^2=5.33$, $p=0.07$; $\chi^2=5.21$, $p=0.07$; $\chi^2=4.61$, $p=0.099$). Broilers from the 100%-INC treatment tended to have a greater distance and speed (11.88m, 6.61cm/s) than 150%-INC broilers (5.48m, 3.05cm/s; $t=2.754$, $p=0.09$; $t=3.56$, $p=0.08$). Diet tended to affect speed during the open field test ($\chi^2=5.08$, $p=0.08$), where broilers fed 100% tended to have greater speed (5.83cm/s) than 150% TRP (3.84cm/s). Diet did not affect distance ($\chi^2=4.50$, $p=0.11$) or activity ($\chi^2=1.92$, $p=0.38$). Lighting did not affect

distance ($\chi^2=0.10$, $p=0.76$), speed ($\chi^2=0.07$, $p=0.78$), or activity ($\chi^2=0.10$, $p=0.75$). Body weight did not affect activity in the open field test (distance $\chi^2=1.51$, $p=0.22$; speed $\chi^2=1.50$, $p=0.22$; activity $\chi^2=0.75$, $p=0.39$). There were no effects of TYP or photoperiod on broiler open field behavior. Adding TRP above the recommended levels may decrease activity, indicating increased fearfulness, but further exploration with a larger sample size is necessary to confirm.

Keywords: Broiler, Activity, Fear, Tryptophan, Lighting

P405 Predicting footpad condition and leg health in broiler chickens using Recurrent All Pairs Field Transforms for optical flow and advanced segmentation models Mahtab Saeidifar*^{1,2}, Guoming Li^{1,2,3}, Chongxiao Chen^{1,3}, Tongshuai Liu^{1,4}, Ehsan Asali^{5,1}, Venkat U. Bodempudi^{1,2} ¹University of Georgia, Department of Poultry Science, Athens, Georgia, United States; ²University of Georgia, Institute for Artificial Intelligence, Athens, Georgia, United States; ³University of Georgia, Institute for Integrative Precision Agriculture, Athens, Georgia, United States; ⁴Henan University of Animal Husbandry and Economy, College of Animal Science & Technology, Zhengzhou, Henan, China; ⁵University of Georgia, Computer Science, Athens, Georgia, United States

Monitoring and predicting the footpad condition and leg health of chickens are crucial to understand animal productivity and well-being. Computer vision-based methodologies provide automatic and non-invasive measures and minimize human error and effort. In this study, a novel approach was developed using Recurrent All Pairs Field Transforms (RAFT) for optical flow to capture the movement of chickens. Heatmaps from top-view videos of approximately 524 chickens walking on the platform were generated, with each assigned with scores 0 (no issues), 1 (moderate), and 2 (severe) based on walking ability and footpad dermatitis conditions. To address the challenge of background noise and interference of humans, an object detection model, You Only Look Once version 8 (YOLOv8), was first employed to detect chickens in the videos. The center coordinates of the obtained bounding boxes were then used as initial prompts for the Segment Anything Model2 (SAM2), allowing chickens to be accurately segmented across frames. By isolating the segmented chickens' heatmaps from the background, precise data for analysis were ensured and subsequently passed through pre-trained Convolution Neural Network (CNN) classifiers, such as the Residual Network (ResNet), for feature extraction. The features were then input into a Long Short-Term Memory (LSTM) network, where footpad dermatitis and gait scores were predicted. The segmentation models were evaluated based on segmentation success rate, Intersection over Union (IoU), precision, recall, and F1 score. It was found that SAM2 outperformed other models, achieving a segmentation success rate of 100%, IoU of 92.21% (± 0.012), precision of 93.87% (± 0.019), recall of 98.15% (± 0.011), and an F1 score of 95.94% (± 0.006) for isolating individual chickens. These metrics were calculated from 1,157 images. An accuracy of over 80% was achieved by the classification model for predicting gait scores. To enhance model interpretability, SHAP (SHapley Additive exPlanations) was used to identify the most influential regions of the heatmaps that contributed to the predictions. This comprehensive and accurate tool is provided to researchers as a reliable method for assessing poultry health, leveraging state-of-the-art segmentation and explainable AI techniques.

Keywords: poultry health, optical flow, segmentation models, explainable AI

Pathology

P406 Pathological and serological investigation of Newcastle Disease in poultry in certain areas of Jhenaidah District of Bangladesh Md Mustafijur Rahaman*²¹ ¹*Atlantis University, Miami, Florida, United States;* ²*Patuakhali Science and Technology University (PSTU), Pathology, Patuakhali, Barisal, Bangladesh*

Poultry diseases are among the major constraints of chicken production in Uganda of which Newcastle disease (ND) is still one of the most important devastating diseases of chicken. This study was conducted from June 2017 to May 2018 to identify and describe the lesions due to ND, determine its prevalence and relate the presence of the viral antigen in tissues to the lesions in the various organs of chicken presented for disease. Chickens for necropsy in the study period were received from selected upazilas of Jhenaidah district of Bangladesh. A total of 240 chickens (Layer, broiler and sonali; 80 birds in each group) were the sample population. Necropsy was done on chicken carcasses; samples for histopathology were obtained from various organs and fixed in 10% buffered formalin. The fixed tissue samples were then trimmed and processed for histopathology. Diagnosis based on clinical and pathological findings showed that 90 (38%) birds were positive for ND. Highest numbers of birds were in broiler group (44%) followed by layer (40%) and lowest was in sonali group (29%). In the present study, in 22% of chickens antibodies titers increases against Newcastle and the HI titer in infected flocks was 8.63±0.28 and in non-infected vaccinated flocks it was 5.36±0.2. In conclusion, Newcastle disease is still among the most prevalent diseases of chicken in the study area. Clinical – pathologic findings provided some bases for ND diagnosis but are less reliable method, therefore, a more sensitive and specific diagnostic tests such as IHC, RT-PCR, in situ hybridization and other definitive tests should be used in addition to histopathology to confirm ND, so as to provide accurate and reliable advice to poultry farmers.

Keywords: Newcastle diseases, poultry, Bangladesh, pathological investigation

P407 In vitro antimicrobial activity of essential oil solution (Sentinel Sol) against Enterococcus strains isolated from broilers Claire Carlu*¹, Claire Girard¹, Karine Fayolle², Reda Zeghadi¹, Sylvain Kerros¹ ¹*Phytosynthese, Mozac, France;* ²*VetAgro Sup, Lempdes, France*

Enterococcosis is a growing worldwide concern in broilers farming. *Enterococcus*, most frequently *E.faecalis* and *E.cecorum* are commensal opportunistic pathogens associated with locomotion disorders, septicaemia and omphalitis. The incidence of *Enterococcus* associated diseases in broilers increased from 1.4% in 2006 to 17.2% in 2020 in France. Essential oils are alternatives identified to reduce the use of antibiotics in farm. Sentinel Sol (EOMix) is a mixture of essential oils rich in phenols and terpenes active molecules with demonstrated broad-spectrum bacteriostatic activity and application widely used in broiler productions (Girard et al, 2017). The aim of this study was to compare the growth inhibition capacity of EOMix against 2 different strains of *Enterococcus* (*E.faecalis* and *E.cecorum*) compared to *E. coli* CIP 59.8T as a pre-validated control, using a high-throughput method of *in vitro* screening to determine MIC (minimum inhibitory concentration). The 3 selected strains were exposed to serial dilutions of ratio 2:1 from ½ (v/v) to 1/10240 of the Sentinel SOL. 10⁶ CFU/mL were incubated in triplicate in 100-wells microtiter plates with Mueller Hinton Broth (MHB) for 300 µl final volume, at 37 °C for 24 h in an automated turbidimeter (Bioscreen C). The 2 strains of *Enterococcus* (*E.faecalis* and *E.cecorum*) were inhibited at the same dilution of 1/2560 (=

0.04%) of EOMix. *E.coli* strain was inhibited at 1/1280 (=0.08%), showing a higher sensitivity of *Enterococcus* strains to EOMix than the *E.Coli*. In summary, *E.faecalis* and *E.cecorum* exhibited the same MIC against Sentinel SOL which was one additional dilution compared to *E.coli*. These results confirm the interest to further investigate the potential of Sentinel Sol to control enterococcus disease in broiler farms considering its efficacy against *E.coli*.

Keywords: essential oil, enterococcus, bacteriostatic, broiler

P408 Determining the Optimal Strain and Pass for Histomonas Feed Additive Trial Christina S. Sigmon*¹, Justin Lowery¹, Lin L. Walker¹ ¹*North Carolina State University, Poultry Science, Raleigh, North Carolina, United States*

Histomonas meleagridis is a protozoan parasite that causes blackhead disease in turkeys. All chemotherapeutics that were effective against *H. meleagridis*, have been banned in the US and EU. A good disease challenge model is required to test feed additives, reflecting the efficacy of disease prevention and control. To find an optimal strain and pass that causes relatively low mortality and moderate symptoms, a preliminary trial was conducted. Seven strains at various passes (1, 2,3,4,5,3,56,103,153 and 173) from commercial outbreaks and from in-house bird trials were inoculated into 10, 14 day-old turkey poults. A total of 11 cages were used, with 10 poults per cage. A pass 53 of a strain from a commercial farm in NC was selected for future trials due to its low mortality and moderate disease signs in the ceca and liver. The strain was used for a following feed additive trial. Fifteen feed additives were tested: 5 treatments had 6 cages per treatment, and 10 treatments had one cage per treatment. The infected control and the uninfected control had 4 cages each. All cages had 10 poults per cage that were cloacally challenged with the Pass 53 strain on day 14. The infection rate and % mortality for the infected control was 57.5 and 40%. The infection rate and % mortality for the 5 treatments that had 6 cages per treatment ranged from 55 to 73.3 and 26.7% to 48.3%. The remaining ten treatments had infection rates from 0 to 100 and % mortality from 0 to 90%. This variation has been seen in past histomonas feed additive trials. This feed additive trial has shown that having a good disease model is needed to find an additive that can prevent histomoniasis.

Keywords: *Histomonas meleagridis*, turkeys, feed additive, disease model

P409 Optimizing Poultry Health Management with AI: An Approach to Holistically Analyze Bird Health and Performance Igor Soares*¹, Bruna L. Belote¹, Otto Figueiró¹, Fernanda F. Rodrigues¹, Elizabeth Santin¹ ¹*ISI Institute, Curitiba, Parana, Brazil*

ISI Sys is a management tool that incorporates artificial intelligence (AI) to streamline multivariate analysis of poultry data, as demonstrated in this study. These analyses aim to identify and quantify the effect of various factors on bird health, and of bird health on performance. In ISI Sys, the overall bird health is represented by the ISI Total Score index (ISI TSc). A higher ISI TSc indicates poorer health status of individual birds or flocks, aggregating scores of alterations across 31 necropsy parameters combined with impact factors that reflect their impairment on tissue functionality. The AI analyzed a dataset of 357 flocks monitored from Dec 2021 to Feb 2024 in a poultry company in southern Brazil. Necropsies of 5 to 6 birds per flock for mean ISI TSc were conducted from 18 to 40 d of flock age (mean 25.75±3.44 d). The data were collected with the system's mobile

application, sent to a cloud database, and provided to the AI-powered platform. The AI executed multivariate general linear models (GLM) with Identity Link Function, setting flock as analysis unity and the most frequent category as the reference category for each qualitative variable. Only associations with $p \leq 0.05$ were considered significant (*exception mentioned), where positive regression coefficients indicate increase in ISI TSc or decline in overall bird health. The GLMs were run three times in separate AI sessions to ensure result repeatability, each run taking around 20 seconds. The AI revealed that 3 of 8 production regions and 9 of 59 barns had adverse effects on bird health relative to the references, raising ISI TSc. Housing birds in barns with "Isodark" structure tended to increase flock ISI TSc by 2.17 points. In

contrast, four regions and the month of December fostered healthier conditions, lowering ISI TSc. Each additional day of sanitary break reduced ISI TSc by 0.10 points, highlighting the benefits of longer flock intervals (* $p=0.096$). A 1-point rise in ISI TSc (evaluated around 25 d) led to a 0.07-day increase in slaughter age, a 1-gram increase in FCR, a 0.12 unit increase in mortality (%), and a 0.07-gram reduction in ADG. The integration of AI into farm management tools, such as ISI Sys, allows holistic and rapid data analysis for timely, data-driven decisions.

Keywords: Poultry Health Management, Artificial Intelligence, ISI Score, Multivariate Analysis, Necropsy

SCAD

P410 Antibody Response to Different 2-Way and 4-Way IBD, Reo, NDV, and IBV Killed Vaccines Brian Jordan*¹, Dale Gambrell¹ ¹Zoetis, Madison, Georgia, United States

Vaccination programs for broiler breeders can vary widely, but begin with live-attenuated vaccines to prime the immune system followed by inactivated vaccines to restimulate the immune system and boost circulatory IgY. IgY in chickens is known to help protect against viremia, thereby protecting internal organs such as the oviduct from damage. IgY is also the main portion of the maternal antibody passed to progeny and is critical for protection of young broiler chicks against certain infections. For these reasons, using a killed vaccine combination that adequately restimulates the immune system is critical for long-term health of the broiler breeder hen and the early health of the broiler progeny. Killed vaccines need to be injected and are often combined into a single product to reduce the number of shots given, with Infectious Bursal Disease Virus (IBDV) and Reovirus (Reo) being a common combination. Some manufacturers also add Newcastle Disease Virus (NDV) and Infectious Bronchitis Virus (IBV) to the mix. Success of the killed vaccine program can be measured by the antibody response post-vaccination. For this trial, a commercial broiler breeder complex used a 2-way and 4-way killed vaccine from one manufacturer for 5 months, a combination of a 2-way from one manufacturer and 4-way from another manufacturer for 10 months, and then both a 2-way and 4-way from the second manufacturer for 7 months. The live vaccine program and injection timepoints were the same throughout the trial. The complex measured antibody responses to IBDV, Reo, and IBV at 26 and 36 weeks of age. Using the 2- and 4-way program from manufacturer B as the baseline, results showed an 88% increase in IBDV antibody titers, a 93% increase in Reo antibody titers, and a 29% increase in IBV antibody titers when using the 2- and 4-way killed vaccine from manufacturer A at 36 weeks. When the 4-way from manufacturer A was used in combination with the 2-way from manufacturer B, there was also an increase in antibody titer though not as large as when both vaccines from manufacturer A were used. These data show that different killed vaccines can induce differential immune responses, which needs to be evaluated when designing a broiler breeder vaccine program for maximum protection.

Keywords: Killed Vaccine, IBDV, Reo, IBV

P411 A Field Study of *Clostridium perfringens* Enumeration Methods in the Broiler House Environment Andrea Zedek*¹, David Smith¹, Charles Hofacre², Virginia Baxter², Matthew K. Jones², Roy Berghaus³ ¹Huvepharma, Inc., Peachtree City, Georgia, United States; ²Southern Poultry Research Group, Inc, Watkinsville, Georgia, United States; ³University of Georgia, Athens, Georgia, United States

Clostridium perfringens is an important pathogen of commercial chickens, most notably as the cause of Necrotic Enteritis (NE). The hypothesis of this study is that quantifying the *Clostridium* bacterial load present in the environment of a commercial broiler farm should be predictive of the risk that NE may occur. However, there is not a definitive sample type and laboratory procedure for *Clostridium perfringens* enumeration in the poultry house environment. This study collected ceca, boot sock, and litter samples from 14 different commercial broiler farms when birds were 41-45 days of age. Samples were cultured using both a direct Most Probable Number (MPN) assay and an enriched MPN assay. Results indicated there was no significant correlation between the enriched MPNs of ceca and boot socks ($r = 0.18$, $P = 0.53$), ceca and litter ($r = 0.18$, $P = 0.54$), or boot socks and litter ($r = -0.07$, $P = 0.80$). Future work will be done to try to establish a baseline for sampling for *Clostridium perfringens* enumeration that will be beneficial for broiler producers.

Keywords: *Clostridium perfringens*, Necrotic enteritis, boot socks, ceca

P412 Immune cellular and transcriptional changes in the mucosal tissues of broiler chickens during experimental *Clostridium perfringens*-induced necrotic enteritis Pok Man Chan*¹, Carissa Gaghan¹, Abigail Armwood¹, Raveendra Kulkarni¹ ¹North Carolina State University, Department of Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, North Carolina, United States

Virulent strains of *Clostridium perfringens* cause necrotic enteritis (NE) in chickens, and host immune responses during NE are poorly understood. The present work investigated the CD4+, TCR $\gamma\delta$ + T cells and IgM+ B cell responses along with evaluating the immune gene expression in the intestinal tissues (duodenum and jejunum) as well as the mucosal lymphoid organ, the cecal tonsil (CT) of broiler chickens infected with virulent *C. perfringens* (Str. CP64). Results showed that the infected chickens had reduced ($P=0.0524$) body weight gain and increased ($P<0.05$) NE severity as indicated by the gross pathology lesions in the small intestine. Immunophenotyping analysis of CT cells revealed a reduction ($P<0.05$) in the frequencies of CD4+CD25+ and TCR $\gamma\delta$ +CD25+ T cells, while IgM+ B cell frequencies were higher ($P<0.05$) in comparison to uninfected control. Gene expression analysis showed an increased ($P<0.05$) transcription of IL-1 β across all tissues from the infected group when compared to the uninfected control. In the CT and jejunum, infected birds exhibited elevated IL-6 expression and reduced CD25 expression. Additionally, an increased ($P<0.05$) transcription of IFN γ genes was observed exclusively in the CT of the infected group. In summary, these results suggested that virulent *C. perfringens* seem to induce an inflammatory lymphoid response via augmenting the expression of pro-inflammatory cytokine genes, while suppressing

the regulatory CD4+ and $\gamma\delta$ T cell responses, as well as the CD25 and FOXP3 molecular transcription. Further investigation is currently underway to design and develop NE control measures that can regulate *C. perfringens*-induced intestinal inflammation during NE in chickens.

Keywords: Chicken, Immuno-regulation, Clostridium perfringens, Regulatory T-cells, Necrotic Enteritis

P413 A multi-phase *Salmonella* vaccination program alters IgA levels in the ceca, bile, and peripheral blood of specific pathogen-free White Leghorns Chrysta N. Beck*¹, Jossie M. Santamaria¹, Ruvindu Perera¹, Gisela F. Erf¹ ¹University of Arkansas, Poultry Science, Fayetteville, Arkansas, United States

Multi-phase live- and killed-*Salmonella* vaccinations are administered to poultry flocks to decrease *Salmonella* load. *Salmonella* vaccines stimulate IgA antibody secretion in the gut, but concurrent assessment of temporal changes in peripheral blood- and secretory-IgA levels following administration of a vaccination program are scarce. The objective of this study was to assess IgA levels in peripheral blood, bile, and ceca contents of specific pathogen-free White Leghorn chicks during the administration of a commercial *Salmonella* vaccination program. The vaccination program was administered in three phases: P1) oral gavage (OG) with live attenuated *Salmonella* Typhimurium vaccine (LST) or sterile water (C) at day (d) of hatch, P2) OG with LST or C at 6 weeks of age, and P3) intramuscular injection of killed ST (KST) or C at 12 weeks of age. For each phase, all samples were collected at 0, 3, 7, and 10d post-vaccination (p.v.) with additional blood collections on 14, 21, and 28 d p.v. Total- and ST-specific IgA levels of samples were measured by ELISA. Data were analyzed by two-way ANOVA to determine the effects of time, vaccine treatment (trt), and their interactions for each vaccination phase, with significance established at $P \leq 0.05$. There was evidence of maternal IgA (total- and ST-specific IgA) in ceca contents at 3d of age ($P < 0.001$), independent of trt. However, following LST vaccination in P1, ST-specific IgA in blood was greater in LST-vaccinated birds (trt: $p = 0.04$) and reached maximal levels by 28d p.v. (time: $P = 0.004$). There were no effects on bile IgA in P1. In P2, LST vaccination did not result in differences among treatments for total- or ST-specific IgA in ceca contents, blood, or bile. In P3, KST vaccination greatly elevated ($P < 0.03$) ST-specific IgA levels in ceca and blood on 3d p.v. and in bile throughout the examination period (trt: $p = 0.03$). These results emphasize the importance of multi-phase *Salmonella* vaccinations in generating a strong systemic humoral response. Specifically, this study found that the third vaccination with killed *Salmonella* vaccine drastically increased IgA in the ceca, bile, and blood. Future studies should measure *Salmonella*-specific IgA levels in conjunction with assessing *Salmonella* load during a vaccination program.

Keywords: Salmonella, Antibodies, Immunology, Humoral Immunity, Layers

P414 Growth characterization and genetic validation of a genetically manipulated *pagP*-deficient avian pathogenic *Escherichia coli* strain Linan Jia*¹, Hailey Fugate¹, Chuan-Yu Hsu², Jeffrey Evans³, Kelsy Robinson³, Pratima Adhikari¹, Li Zhang¹ ¹Mississippi State University, Poultry Science, Starkville, Mississippi, United States; ²Mississippi State University, Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States; ³USDA-ARS, Poultry Research Unit, Mississippi State, Mississippi, United States

Avian pathogenic *Escherichia coli* (APEC) causes significant economic losses in the poultry industry worldwide, and effective antigens to protect poultry against APEC infection are needed. Our previous study identified antigen *pagP* as a potential vaccine candidate with noteworthy immunogenicity function in APEC. This study aimed to develop an APEC mutant strain through *pagP* gene deletion to study the function of *pagP* on growth characteristics of APEC. Using the CRISPR-Cas9 genome editing tool, a pEcCas/pEcgRNA system was employed to target the *pagP* gene in the APEC strain MS1657 (O2/O50:H1, ST429) with a homology donor DNA facilitating homology-directed repair to generate a deletion mutant MS1657 Δ *pagP*. PCR and whole genome sequencing (WGS) were conducted on both the wild-type (MS1657) and mutant (MS1657 Δ *pagP*) isolates to validate the deletion. Growth characteristics were assessed using a Citation1 Image plate reader, with optical density measurements (OD₆₀₀) recorded at 5-minute intervals over 24 hours. Five biological replicates were maintained for both wild-type and mutant strains, with sterile LB broth serving as a control. Statistical comparisons between wild-type and mutant strains were performed using unpaired Student's *t*-tests, with significance level set at $P \leq 0.05$. Successful deletion of the 638-base pair *pagP* gene was confirmed through both PCR amplification and WGS analysis, with no off-target effects observed. The cumulative bacterial growth over 24 hours was analyzed using the Area Under the Curve (AUC) method, and overall growth was higher in MS1657 Δ *pagP* compared to wild-type MS1657 (623.4 ± 15.2 OD₆₀₀ \times min vs. 589.7 ± 14.8 OD₆₀₀ \times min, $P = 0.042$). Compared to wild-type MS1657, mutant strain MS1657 Δ *pagP* exhibited a shorter doubling time (49.23 ± 2.15 min vs. 51.78 ± 2.43 min, $P = 0.048$). Growth curve analysis revealed MS1657 Δ *pagP* showing consistently enhanced growth parameters across all metrics. Further investigation on the immunogenicity and protective efficacy of MS1657 Δ *pagP* is needed to evaluate its potential as a vaccine candidate.

Keywords: APEC, *pagP*, growth curve, CRISPR-Cas9, gene deletion

P415 Development of a Taqman Multiplex Real-time qPCR Assay for Pro-inflammatory Gene Detection in Samples Infected with Avian Pathogenic *Escherichia coli* (APEC) Xin Ye¹, Chuan-Yu Hsu³, Linan Jia*¹, Christopher Magee², Stephanie Whitham², Spencer Leigh², Jeffrey Evans², Li Zhang¹, Kelsy Robinson² ¹Mississippi State University, Department of Poultry Science, Mississippi State, Mississippi, United States; ²United States Department of Agriculture, Poultry Research Unit, ARS, Mississippi State, Mississippi, United States; ³Mississippi State University, Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State, Mississippi, United States

Avian pathogenic *Escherichia coli* (APEC) is a primary cause of bacterial infections in poultry, leading in significant economic losses due to its impact on flock health and challenges to disease management. APEC infections activate complex immune responses, particularly pro-inflammatory pathways essential for host defense. Rapid detection of key pro-inflammatory genes linked to APEC infection provides insights for diagnostics and research, advancing our understanding of immune dynamics. In this study, we developed a TaqMan multiplex real-time qPCR assay targeting three genes involved in pro-inflammatory responses, *IFN- γ* , *IL-1 β* , and *IL-8L1*. Each probe was individually labeled with a distinct dye (FAM, JUN, or ABY) and paired with a QSY quencher for high specificity and sensitivity. Target sequences, amplified from cDNA of APEC-infected lung tissue, were cloned to create plasmid templates for assay standards. Serial dilutions of plasmid DNA established single and multiplex

standard curves, with Ct ranges for expected detection values: *IFN- γ* (19.2–33.5 single, 19.4–33.4 multiplex), *IL-1 β* (18.0–33.8 single, 18.0–34.1 multiplex), and *IL-8LI* (19.3–33.6 single, 19.7–33.3 multiplex). Reverse transcription qPCR (RT-qPCR) standard curves from RNA extracted from APEC-infected HD11 cells yielded Ct ranges of 28.0 – 32.4 for *IFN- γ* , 19.8 – 30.6 for *IL-1 β* , and 15.6 – 28.4 for *IL-8LI*. Statistical analysis involved calculating amplification efficiencies through linear regression of the standard curves, with each gene's performance evaluated across both plasmid DNA and RT samples. Results showed amplification efficiencies for plasmid DNA ranging from 90% to 110%: *IFN- γ* at 92.0% (single) and 94.0% (multiplex); *IL-1 β* at 104.7% (single) and 98.0% (multiplex); and *IL-8LI* at 90.5% (single) and 96.0% (multiplex). For RT-qPCR samples from APEC-infected HD11 cells (single assays only), *IFN- γ* showed 103.6% efficiency, *IL-1 β* 102.0%, and *IL-8LI* 99.5%. Each gene consistently yielded R² values above 0.98, confirming the assay's robustness and reproducibility across both plasmid and RT samples. This multiplex qPCR assay provides a sensitive tool for quantifying key pro-inflammatory responses in APEC-infected samples, offering a simplified, efficient approach to assess avian immune responses.

Keywords: Avian pathogenic *Escherichia coli*, Pro-inflammatory genes, Multiplex real-time PCR, Immune response, TaqMan probe

P416 Phylogenetic analysis of over 1,000 IBDV sequences of VP2 from commercial chicken flocks since 2009 and a comparison of key mutations shared with reference strains Po-Hsin Yu¹, Kalen Cookson*¹, Jon Schaeffer¹ ¹*Zoetis, Lawrenceville, Georgia, United States*

Since the mid-1980s when antigenic variants like Delaware-E displaced classical infectious bursal disease viruses (IBDVs) by overriding high classic maternal antibodies, the United States has seen a tremendous diversification of variant IBD viruses. While the adoption of bursal derived vaccines improved early passive immunity to Del-E and other similar variant IBDVs, by the late 1990s novel variants like AL2 and T1 had emerged that demonstrated the ability to infect progeny containing high levels of Del-E derived maternal immunity at an early age. Since the mid-2000s, there has been more antigenic diversity available in the commercial bursal derived vaccines and this has helped provide more consistent protection against early IBDV infections. Since 2009 our diagnostic lab has sequenced the variable region of VP2 of over 1,000 positive US flocks. This poster will present a phylogenetic tree of these IBDV sequences. Results will show that the IBDVs fall into 6 general clusters. While there were exceptions in every cluster, they generally split based on the following key mutations (unique amino acid and position) into the following groups: 1) Classic—16.6% (no key mutations), 2) AL2—28.9% (318N, 321-E), 3) Group-6—25.6% (322E), 4) Del-E—12% (254S, 323-E), Del-E—9% (254N, 323-E) and Variant Other—7.9%. The Classic cluster is made up mostly of the Faragher insert contained in most rHVT-IBD vaccines but also contains live vaccines Bursine-2 and W-2512. The Group-6 cluster, named after the many IBDVs sharing the same restriction enzyme pattern, contains the most extensive branching. The Del-E cluster includes the 89/03 vaccine, several VP-2 insert recoveries and wild type Del-E make up 5% of all US sequences. Over half of the Del-E cluster containing the mutation 254N also contain 299S—making them 98% identical to the China variant that has spread to much of Southeast Asia, Western Canada and, most recently, the Middle East. The Variant Other cluster notably includes viruses from Pennsylvania that are also most prevalent in Eastern Canada and named after the South Africa variant 05SA8. The poster will also list all the mutations in each of the 4 hydrophilic peaks of key

reference IBDVs found in each cluster to help people relate them to their field isolates.

Keywords: IBDV, VP2, AL2, Group-6, Del-E

P417 Generation of a Live Recombinant Thermostable Highly Attenuated Vaccine rK148/GVII-F and Protective Efficacy Evaluation using a Single Regimen Against Lethal NDV GVII.1.1. Amal A. Mouhamed*¹ ¹*University of Georgia, Population Health, Athens, Georgia, United States Minor Outlying Islands*

The ongoing global spread of Newcastle Disease underscores the crucial need for continued research on the efficacy of current vaccines against the various circulating strains of Newcastle Disease Virus (NDV). The Fusion gene of a representative Egyptian genotype VII.1.1. strain was used to substitute its corresponding gene in the K148/08 thermostable vaccinal strain after site directly mutating its cleavage site from 112RRQKRF117 to 112GKQGRL117 which was genetically stable over 10 consecutive passages of the rescued virus. Fusion gene exchange between GVII and GI did not affect the thermostability of GI K148/08. Attenuation of the chimeric rescued virus was confirmed with Mean Death Time (MDT) 144h and Intracerebral Pathogenicity index (ICPI) of 0.00. Animal Experiments of Egy-8_NDV_VII_Sohag_2018 determined its Chicken Lethal Dose as 106 EID50 which used to challenge 3 doses of rK148/GVII-F 105, 106, 107 EID50 and two vaccinal strains K148/08 and LaSota at the same dose of 107 EID50. Survival analysis after the challenge experiment confirmed that 107 EID50 is the protective dose of rK148/GVII-F and its immunoprotective efficacy is comparable to K148/08 and LaSota. Based on the fusion gene matching between challenging strain of GVII.1.1. (Egy-8_NDV_VII_Sohag_2018) and vaccine virus (rK148/GVII-F), rK148/GVII-F did not require high levels of antibodies to protect against mortality and morbidity as (>3.3log₂) was adequate for full protection in contrast to K148/08 and LaSota which strived with higher levels of antibodies (>4.1log₂) to reach same levels of protection. Viral shedding was significantly reduced oropharyngeally on 5th day post-challenge between rK148/GVII-F and LaSota and on 7th day post-challenge between rK148/GVII-F and LaSota-K148/08. Replication and tropism investigation confirmed the respirotropic nature of LaSota in contrast to enterotropic nature of K148/08 and the further attenuation of rK148/GVII-F as a lentogenic highly attenuated virus. Altogether, rK148/GVII-F is a thermostable, effective, and genetically stable vaccine candidate that could be adequate for use in countries that encounter GVII.1.1. and in those with tropical climate, such as Egypt and the Middle East neighboring regions.

Keywords: Newcastle disease virus- genotype VII, thermostability, recombinant vaccine, protective efficacy, viral shedding

P418 Impact of a Novel Granular Peracetic Acid Litter Treatment on Food Safety and Avian Pathogenic Bacteria *in situ*: Pilot Broiler Trials Suggest Utility as an Antibiotic-Free Litter Amendment K. S. McKenzie*¹, Charles Hofacre² ¹*EnviroTech, College Station, Texas, United States;* ²*Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States*

Peracetic acid (PAA) is a well-known antimicrobial chemistry that has gained widespread global acceptance for poultry production and processing, due to broad pathogen control performance, advantages for the environment, and safety as a poultry carcass sanitizer. A new, solid chemistry synthesis method was recently developed to produce a solid peracetic acid granule that has been shown effectiveness against both viral and bacterial pathogens. To

understand the potential impact of applying 40 lbs/1,000ft² (PG 40) or 80 lbs/1,000 ft² (PG80) PAA granules onto litter to reduce bacterial impact on broiler production, day-old chicks were exposed to *Salmonella* Enteritidis (SE), *E. coli*, and *Clostridium perfringens* in multifactorial pen study(s) with treated or control litter. *E. coli* and SE levels were quickly reduced in litter, although Reduction in environmental *C. perfringens* was not consistent throughout the trial. Most parameters were not improved using the higher application rate of PAA granule application, with the exception of moisture reduction of the litter, an alkaline shift of litter pH (9.83^a for PG 80 vs 7.93^b control) and 24h reduction of *C. perfringens* (3.71^blog₁₀ MPN control vs 2.29^alog₁₀ MPN PG80). The pH shift correlated to a numerically lower cecal enumeration for PG40 vs control at day 22 (P=0.12), suggesting larger *in vivo* commercial broiler trials to validate. Bootsock and litter microbial enumeration indicated the decrease of environmental *E.*

coli within 24h of application and some of these reductions persisted to day-of-processing, although reductions in cecal *E. coli* were not noted for PG40 or PG80 litter treatment vs the observed reduction in cecal *Salmonella*. SE levels were also significantly reduced in the litter through day 7 after PG40 treatment, and there were no negative impacts of PG40 on mortality, weight gains, or feed conversion. More evaluation is needed to confirm impact of PAA granule treatment on environmental pathogens in litter, that impact on mortality and feed efficiency, the alkaline shift followed by common acid shift of ammonia control treatments on pathogen survival, impact of PAA granule treatment on the gut microbiome and tissue colonization persistence, and economic return on investment for treatment of barn floors.

Keywords: Litter, *Salmonella*, Peracetic acid, *E. coli*, *Clostridium*

AUTHOR INDEX

A

A. El-Safty, Salah M133, P395
 Abascal-Ponciano, Gerardo A. M24, M94
 Abdelaziz, Khaled P236, P256
 Abdelmoez, Waleed T181
 Abigail Contreras Zelaya, Fanny T217
 Abo-Salem, R. A. T181
 abudabos, Ala E. P343
 Acosta Villafuerte, Francis Xavier P289
 Acuna, Patricia P369
 Adams, Eric S. P312
 Adams, Lincoln P379
 Adejumo, Reuben M139
 Adekoya, Abidemi M135
 Adeniyi, Desmond M36
 Adeola, Olayiwola M100, M111, M112, P257, P271
 Aderibigbe, Ayodeji P282
 Adesope, Seth O. M95
 Adewole, Deborah P269
 Adhikari, Pratima M11, M127, M143, M51, P237, P253, P318, P334, P397, P414
 Adhikari, Roshan M119, M120, M138, M143, M144, P334
 Adhikari, Yagya M26, T215, T222
 Afsharmanesh, Mohsen P339
 Aggrey, Samuel T213
 Ahmad, Dr. Tanveer M31
 Ahmed, Mostafa P236
 Ahmed, Waqas P367, P370, T207
 Ajao, Adeleye M. M98
 Ajayan, Amritha M58, P329
 Ajuwon, Kolapo M. P257
 Akshitha Reddy Kota, Sai M70, M71
 Al Hakeem, Walid G. P308, P322, P323, P324, P325, P326
 Alharbi, Khawla S. P258, P263, P357
 Ali, Ahmed M61
 Ali, Muhammad M116, M118, M138, M72
 Ali, Zubair T207
 Alkhtib, Ashraf P381, P386
 Allenspach, Karin T165
 Almendares, Kristian M17, P306, P307
 Alqahtani, Abdulaziz P258, P263
 Al-Qudah, Omar P336
 Alrubaye, Adnan M53, P258, P263, P357
 Alvarado, Emscho P306
 Alvarado, Ivan M54
 Alvarenga, Maria M61
 Alvarez-Narvaez, Sonsiray M57
 Aly, Faizan M54
 Amalaradjou, Mary Anne M79
 Amaral, Thaciane M37, P245, P292, P294, T160
 Amaz, Sadid Al P273, P275
 Amin, Nasiri M73
 Anderson, Annalise G. M144
 Anderson, Kenneth M21, P225, P303, P330, P331, P366, T190
 Andrade, Luane B. P350
 Andrea Amaya, Paola M12
 Angel, Roselina M115, M13

Angel-Isaza, Jaime A. M45, P338, T151
 Anthony, Amanda P258, P263, P357
 Applegate, Todd J P260
 Appleton, Stacie T216
 Arango, Marcela M59
 Araujo, Cristiane S. M122, P401
 Araujo, Lucio F. M122, P401
 Arbe, Xabier M13
 Archer, Gregory P302, P374
 Archibeque, Monique P392
 Arent, Susan M34
 Arguelles-Ramos, Mireille M61
 Arick II, Mark A P290
 Armwood, Abigail P412
 Arora, Sayantani P341
 Arsenault, Ryan T162
 Arsi, Komala P238
 Asaad, Rahaf P371
 Asad, Dr. Muhammad Javaid M31
 Asali, Ehsan M69, M70, M71, M72, M75, P405
 Asjad, Mehran T207
 Asnyanti, Andi P258, P263
 Atoo, Ashir F. M107
 Atungulu, Hannah T197
 Augspurger, Nathan M36
 Augusto, Karolina V. Z. P377
 Autret, Alain P309
 Avila-Reyes, Vicente A. P291
 Awonyemi, Olatunde I. M100
 Ayala, Diana P305
 Ayala-Velasteguí, David M82, P297
 Aydin, Ali P320
 Ayres, Grace M28

B

Babatunde, Olufemi O. M32
 Baca, Maria V. M80, T220
 Baek, Insuck M83
 Bailey, Christopher M125
 Bailey, Matthew M105, M117, M26, M88, T215, T222
 Baker-Cook, Bethany M20, P306
 Bakre, Abhijeet A. M42
 Balaña, Octavi C. P340
 Banegas, J. Enrique M7
 Baquero Cardenas, Maria Ines P289
 Barabara, Doupovec P260
 Barashkov, Nikolay M95
 Barbe, Florence P344, P345, P346
 Barberena, Juan J. M7
 Barbosa, Eder O. M2
 Barcelo, Colin A. P274
 Barnes, Sarah P262
 Barnett, Reagan P227
 Barrios, Miguel M32, P392
 Baughman, Scott M59
 Baumrucker, Craig R. M50
 Bautista, Daniel M49
 Baxter, Jennie T205
 Baxter, M M147, M34
 Baxter, Virginia P314, P316, P411, T168
 Baynes, Ronald T210
 Bazzi, Franco M41
 Bearson, Bradley T218
 Bearson, Shawn T218
 Becerra, Roel M60, T167
 Beck, Chrysta N. M45, M53, P300, P413, T166, T174
 Bédécarrats, Grégoy M20
 Bello, Abiodun M34
 Belote, Bruna L. P409
 BENARBIA, Mohammed el Amine P362, T204
 Benito, Carlos T149
 Ben Larbi, Manel P263
 Bennett, Grant G. P272
 Ben-Tal Cohen, Enbal T194
 Berger, Madison M17, M9, P306, P307
 Bergeron, Ariel P354, T153
 Berghaus, Roy P316, P411, T168
 Bergman, Melanie P301, P374
 Berhe, Michael Y. T198
 Bernal-Arango, Luis C. M103
 Bernardino, Michele M148
 Berto, Rafaela T185
 Bertoli, Francisco P358
 Berwanger, Eveline M40, P363, P394
 Betiku, Eniola M81, P244
 Beyer, Scott M102, M114, P393
 Bharathan, Greeshma M84, M85, P246
 Bhushan, Shanti P231
 Bianchi, Sophia M104, P250
 Biava, Adrieli P332
 Bielke, Lisa M37, M65, P245, P292, P294, T160
 Bien, Damian P362
 Bierly, Stephanie M128
 Bist, Ramesh P313
 Bist, Ramesh B. M74, T197
 Black, Micah T. M83, M93, P234
 Blair, Jennifer M126, P375
 Blair, Mike T158
 Blanvillain, Virginie M106
 Blomme, Allison M102, M114, P393
 Blue, Candice E. P293
 Bodempudi, Venkat U. M118, M64, M69, M70, M71, P405
 Bodempudi, Venkat Umesh Chandra M72, M75
 Bodle, Brooke C. M113
 Boeck, Jociane P355
 Boersma, Melissa D. M24
 Boltz, Timothy M110, M89, P253, P299
 Bonaspetti, Sandra P347
 Boney, John M104, M113, M128, M29, P250
 Bonner, Aliyah N. P282
 Boontarue, Darby R. M104, M48, P250
 Boot, Emmillie M16, P225, P270
 Borchardt, Thilo P287, P288
 Bortoluzzi, Cristiano M116, P255, P359
 Boukherroub, Kahina M3
 Bourassa, Dianna M8, M80, M88, M92, P239, P381, P386, T215, T217, T220, T221, T222
 Bowen, Kristina M. M123, M139, M141, M145, M25
 Bowerman, Allie K. P300
 Bowers, JoAnna P291

- Bowker, Brian M146
 Bragg, Ari P236
 Brannan, Kelly E. T190
 Bressan, Rodrigo P355
 Brister, Roy M35
 Brito, Luiz P284
 Brito Vieira, Rita P. M148
 Brizuela, Maria J. M88
 Broderick, Timothy P227
 Brown, Joseph T170
 Browning, Matthew P295
 Bruwiere, Stijn P361
 Bruzual, Jose J. T209
 Bryan, Dervan M113, M50
 Bugybayeva, Dina P245
 Buhr, Richard M146, M80, M84, M85,
 P240, P246, T215, T220
 BUI, Hoa T204
 Buiatte, Vinicius M48, M50
 Burbarelli, Maria Fernanda d. P373
 Burden, Yemi M125
 Buresh, Bob M130
 Burgreen, Greg W. P231
 Burns, Robert M22
 Burton, Emily P381, P386
 Buscheck, Dawn M132
 Byrnes, Jake P228
- C**
 C.H., Justice-Alucho T219
 Caceres, Jean C. M96, P248, P331
 Caixeta, Hamilton P394
 Calasans, Max W. P384
 Caliari, Carla T149
 Calik, Ali P265, P293
 Calson, Mark M95
 Camara, Ali M36
 Campbell, Joy M122
 Campbell, Yan L. M23, M96, P247,
 P248, P330, P331
 Cantley, Staci M25
 Cardoso, Camila d. P335
 Cardoso, Gabriela M135
 Carlu, Claire P407
 Carnaccini, Silvia P399, T165
 Carvalho, Lizia C. P251
 Casco, Karla V. M80, M92, T220, T222
 Casella, Enrico M29
 Cason, Emily E. P235
 Castilho, Ricardo P356, T185
 Castilho Heiss, Vivian A. P373
 Castillo, Elis O. P350
 Castillo Najera, Chelsea S. P226
 Castle, Jordan M13, P272
 Casto, Crosby P291
 Castro, Luis M10
 Catucci, Michael T165
 Caverro, David M13
 Cerri, Felipe P394
 Cevallos Almeida, Maria Belen P289
 Chai, Lilong M72, M74, P280, P281,
 P313, T197
 Chambers, Gabriella M28
 Chan, Diane M83
 Chan, Pok Man P295, P412
 Chaney, Evan T175
 Chang, Krista M39, M87
- Chao, Kevin M83
 Chapell, Olivia P258
 Charal, Jose M132
 Charriere, Maria Viviana P353, T150
 Cheeran, Maxim M58
 Chen, Chongxiao M116, M118, M138,
 M140, M68, M69, M71, M72, P278,
 P287, P288, P405
 Chen, Siya P302
 Cheng, Veronica T208
 Cherry, Jaelen M67, P283, P404, T195
 Chevaux, Eric P344
 Cho, Sohyun P308, P323, T213
 Cho, Sungeun P242
 Choi, Janghan M146
 Choppa, Venkata Sessa Reddy M116,
 P279, P286, P352
 Chopra, Surinder M48
 Christensen, Niels T189
 Chung, Taejung P308, P324
 Ciborowska, Patrycja P362
 Cinnamon, Yuval T194
 Cisneros Tamayo, Marco P289
 Cissé, Sekhou P362
 Clark, Alexis M61
 Clemons, Courtney T164
 Cloft, Sara M15, M18, T212
 Cockshott, Ian M131
 Codina, Raquel P341
 Coelho, Jumara P394
 Cohen, Izel T194
 Conrad, Steven M57
 Cookson, Kalen M49, P299, P319, P416,
 T171
 Coon, Craig P333, P387, P388, T202,
 T203
 Corban, James P232, P243
 Corbett, Megan P. T165
 Cordeiro, Lais P350
 Cordero, Jason M10
 Corray, Shelby M113, P385
 Cortes, Diego M54
 Corzo, Alex M119, M138
 Coufal, Craig M109, P264, P316, P327,
 P348, T168
 Cowieson, Aaron J. P257
 Crandall, Philip G. M81
 Crenshaw, Joe M122
 Crespo, Rocio T196, T210
 Cribillero Chuquiuhaccha, Nelly G. P253
 Cristobal, Minoy P375
 Croom, Mazette M66, P262
 Crowder, Stacie T162
 Cummings, Timothy P299
 Cunningham, Morgan L. P291
 Curry, Jordan M28
- D**
 Da Costa, Manuel P364, P365, T160,
 T161, T175
 Dahal, Samin M74, P280, P281
 Daigle, Courtney M66
 Dalloul, Rami A. M55, P265, P293
 Daniel Wagner, Tirado Agreda P289
 Das, Razib P254
 da Silva, Edney P. M148
 Dasireddy, Joseph Rishitha P260
- Davis, Adam J. M137, M140, M4, P316
 Davis, Ellen T162
 Davis, Jeremiah D. M9, P229, P276
 Davis, Jeremiah D. M6, M7
 de Castro Caldas, Silvana P332, P384
 Deda, Guilherme F. P268
 Deeker, Matthew M132
 De Laet, Manu T161
 Delago, Firmin P372
 Delago, Jodi M43
 De Leon, Daniel M125
 Della Badia, Antonella P341
 Demey, Vanessa P346
 de Moraes, Mariana L. M32
 Denness, Ellen T170
 de Oliveira, Jean E. P305
 de Paula Dorigam, Juliano P249
 de Souza, Jacqueline C. P335
 Desrousseaux, Guillaume P309
 Detzler, Derek M32, P392
 Dexter-Boone, Leah P330
 Dharanivasan, Shanthi T194
 Dhungana, Anjan M74, P280, P281
 Dias, Isabella d. P268
 Dickson, John P319, T171
 Diehl, Kristin M17, M9, P307
 Díez, David T157
 Dilger, Ryan M124, P249
 Dipta, Prantho Malakar P244
 DIRKS, BRIAN M121, M125, P372
 Do, Anh P258, P263
 Doi, Ana Carolina B. P268
 Dolatyabi, Sara P245
 Domer, Randy M97
 Donaldson, Caroline T161
 Dong, Bingqi P293
 Donoghue, Annie P238
 Dorton, Kristy P368, T158, T205
 dos Santos, Brenda Carolina P. P268
 Doster, Jakob P242
 Douglass, Eugene T165
 Dozier, William A. M105, M117
 Drewry, Jessica P231, T211, T214
 Dreyer, Maigel P356
 Dridi, Sami M147, M5, P305, P396
 Druyan, Shelly T194
 Drysdale, Bekah P375
 Drysdale, Rebekah L. P252
 Dumkliang, Ekachai P245
 Dundur, Nicole P306
 Dunham, Cierra M56
- E**
 Edge, Carson M. M9
 Edwards, Joseph T170
 Elbadway, Mohamed T165
 El-Ghoul, Abdelkawy A. T181
 Elizabeth Rodriguez Chinchilla, Karoll
 M84, M85
 Ellestad, Laura E. M13, P272, P274
 Elliot, Michael M143, P334
 Elliott, Katie E. M127, M6, M7, M9,
 T214
 Elrod, Charlie P237, P318, T152
 Elstner, Addison M125, M130, P259,
 P360
 Emami, Nima T154

Emmert, Brittny M18
 Emmert, Jason Lee P403
 Encinosa, Maya T218
 Engler, Paul P362
 Engnell, Mason M102, M114, P393
 Erasmus, Marisa P284
 Erb, Logan M136, T155, T183
 Erf, Gisela F. M45, M53, P300, P413,
 T166, T174
 Escobar, Jeffery M38
 Estanich, Emily B. M123, M139, M141,
 M145, M25
 Etherton, Joshua A. P229
 Ety, Umme S. P383, T223
 Ety, US T177, T224
 Evans, Ceinwen M34
 Evans, Jackson P225
 Evans, Jeffrey P310, P414, P415
 Evans, Nicholas P. P354, T153
 Ewuola, Muslim K. M1

F

Fain Binda, Virginia P353, T150
 Fan, Peixin M127
 Faruque, S T177
 Faruque, Shakila T223
 Fascina, Vitor B. P335
 Fayolle, Karine P407
 Feket, Peter P392
 Fenster, Davis A. P265
 Ferguson-Noel, Naola T172
 Fernandes, Jovanir P363, T149
 Fernandes, Melvy P231
 Fernandez, Arley M10
 Fernandez, Pia M41
 Fernandez Cuadrado, Maria M5, P396
 Fernando Soler, Rigo T216
 Ferrel, Jon M123
 Fickler, A. M147, M148
 Figueiró, Otto P409
 Flack, Brenda M17, M9, P307
 Flores, Abigail M130, P259
 Flores, Andressa P356
 Flores, Cody T158
 Flores, Karlinton T159
 Fonseca, Ana M128, M48
 Fontaine, Jean P392
 Foote, Jessica M43
 Forga, Aaron M54, P292, T160, T166
 Formigheri, Edson P394
 Fourie, Jamie M115, M134
 Fournis, Yann T161, T164
 Fraley, Gregory S. M14, M15, M27,
 M28, M63, P301, P374
 Frank, Kelsey M132
 Freeland, Skye M16, P330
 Freeman, Martha E. M4
 Friesen, Walter M102, M114, P393
 Froebel, Laney M124, P249
 Frye, Jonathan P312
 Fudge, Catherine M116, M118, M138,
 M72, P278, P287, P288
 Fugate, Hailey M127, M51, P414
 Fulton, Janet E. M55

G

Gabarrou, Jean François P309
 Gaghan, Carissa P294, P295, P298, P412
 Galbraith, Elizabeth P372
 Gall, Mayara P356
 Gallardo, Connie T157
 Gallissot, Marie T156
 Gambrill, Dale P410
 Gan, Hao M73
 Ganda, Erika M128
 Gang, Yang T219
 Gangaiah, Dharanesh M36
 Gaonkar, Pankaj P. M26
 Garcia, Javier M92
 Garcia, Leticia C. P373
 Garcia, Rodrigo G. P373
 García, Maricarmen M46
 Garcia-Mejia, R. Alejandra M13
 Garcia-Morales, Arturo M141
 Garner, Laura M83, P242
 Genin, Olga T194
 Gerardo Apolo, Vallejo Xavier P289
 Gharaibeh, Mohammad P371
 Giambrone, Joseph K419
 Gibbs, Kirsty M115, M34, P375, T189
 Gibson, Sally P344
 Giovagnoni, Giulia T184
 Girard, Claire P407
 Girard, Ivan P309, P361
 Giron, Luis M104, M29, P250
 Glenn, Anthony P312, P379
 Godoy, Alecia M36
 Gold, Scott P. P379
 Golden, Reed M26
 Gomes, Gilson M33, P337
 Gomes, Renata M37, P292
 Gomez, Carlos P289
 Gomez, Luis B. P347
 Gomez-Osorio, Luis-Miguel M41
 Gonzalez, John M114, P393, T192
 Gonzalez, Juan Diego M10
 González, Ignacio M41
 Goossens, Tim T153
 Gordillo, Alvaro T180
 Gore, Aaliyah T196
 Gott, Paige N. P385
 Gourapura, Renukaradhya J. P245
 Gowda, Nanje P327
 Graham, Danielle M54, P292, T160,
 T166
 Granghelli, Carlos A. M122
 Gray, Dan P287, P288
 Gray, Latasha P292
 Greene, Annel K. T219
 Greene, Elizabeth S. M108, M5, P396
 Greenwald, Charles P259, P360
 Gregg, Autumn M52
 Grey, JD M83
 Grider, Zachary M67
 Griffith, Nathan P284
 Grilli, Ester T184
 Grimes, Jesse L. M23, M96, P247
 Grisham, William C. P299
 Grum, Daniel P305
 Guardado, Carlos A. P237, P318
 Guato Guaman, Cesar M12
 Gudidoddi, Seshidhar Reddy P286

Guele, Clément T187
 Guimarães, Matheus G. P350
 Gulizia, Joseph P. M101, M117, M88,
 P264
 Guzman, Eva G. M101, M117, M88,
 P239, P264, T217
 Gyawali, Ishwari M129

H

Haldar, Sudipto M33, P341
 Halm, Regina M20
 Halowell, Ashley P347
 Hambrecht, Ellen P342
 Hammel Sobreira, Camila M96, P247
 Hamzat, Rasheed A. M1
 Hanlon, Charlene M17, M20, M6, M7,
 M9, P306, P307
 Hannay, Isabella M52
 Hanson, Andrea M132
 Haque, Shariful M91, P315
 Harding, Kari P225
 Hargis, Billy M45
 Haron, Amit T194
 Harrington, David P376, T186
 Harris, Caitlin M146, P240, P312, P317
 Harrison, Jonny P345
 Hassan, Hosni P256
 Hassan, Uzair T207
 Hatmaker, Coleman M140
 Hatmaker, Coleman J. M4
 Hauck, Ruediger M101, M105, M117,
 M144, M88, P239, T217
 Hawkins, Jaci P312, P379
 Hawkins, Shawn M22
 Hayat, Zafar T179
 Hayden, Michelle M84, M85
 He-DeMontaron, Athena D. M23
 Heil, Caiti M39
 Heim, Giseli P377, T188
 Heist, Christopher P317
 Helmes, Emily M36
 Herberger, Bailey P258
 Hernandez, Jose R. M101, M117, M88
 Hernandez Tapia, Brenda P285
 Herrera, Josselyn M10
 Heskett, Eric T216
 Hickman, Jason M132
 Hicks, Kennedy P258
 Hicks, Michael M36
 Higgins, Brendan P276
 Higgins, Courtney M26
 Higuaita, James M54, P292
 Hirai, Rosana M125, M130, P259, P360
 Hite, Jessica P233
 Hofacre, Charles P314, P316, P411,
 P418, T168, T170, T175, T205
 Hoover, Kara P258
 Hoque, Md Raihanul P342
 Hossain, MT T177
 Hsu, Chuan-Yu M127, P290, P397,
 P414, P415
 Huber, Laura M26
 Huffer, Samantha T178
 Hughes, Jay T183
 Hughes, Matthew M8, M92
 Hughes, Michael D. P312
 Hughes, Neketa M97

Humayoon, Sulthana M79
 Huppert, Sophie P345
 Hurley, David J. M46
 Hutson, Brittany L. M6, M7

I

Ibiwoye, Demilade I. P245, P292, P294
 Ibiwoye, Demilade M37, M87, T160
 Ienes-Lima, Julia M47, M56, T167
 Iglesias, Bernardo F. P353, T150
 Ilemobayo, Justus P276
 Ionescu, Catherine M132
 Iqbal, Muhammad Farooq M31
 Ishaq, Waqas M86, P241
 Islam, A T177
 Islam, Aminul P383, T223
 Ivy, Beau T211

J

J. Mallo, J M133, P395
 Jackson, Emily P391
 Jacobs, Kean G. M115
 Jacobs, Leonie M77
 Jankowski, Jan P346
 Jansen Van Rensburg, Christine M115
 Javaid, Hamza M58, P329
 Jaworski, Slawomir P362
 Jendza, Joshua P233
 Jenkins, Mark M48, M50
 Jennings, Madalyn M8
 Jensen, Tine V. P335
 Jespersen, Julianna M109, P249, P264, P316, P348
 Jesudhasan, Palmy M53, P238, P357, T174
 Jha, Rajesh P254, P273, P275, P277
 Jia, Linan M51, P290, P397, P414, P415
 Jiang, Xiuping T219
 Jimenez, Laura M10
 Jiral, Emily P374
 Johnson, Alison P344
 Johnson, Casey T162
 Johnson, Corey A. M136, T183
 Johnson, Natalie K. M76
 Johnson, Sarah T203
 Johnson, Tim M78, P305, P329
 Johnny, Anup K. M58, M78, P328, P329
 Jolayemi, Kazeem O. M1
 Jones, Carl T152
 Jones, Daniel M132
 Jones, Jodie P242
 Jones, Matthew K. P314, P316, P411, T168, T170, T175
 Jones, Thomas M137, M140
 Jordan, Brian M49, P410

K

K. Dhara, Amrita M33, P341, P349
 Kabir, Zobaidul M91, P315
 Kadardar, Housseem P340
 Kalapala, Tanmaie P238
 Kamruzzaman, Mohammed P403
 Kanike, Eswari M79
 Kappari, Laharika P260
 Karcher, Darrin M15, M18, P301, T212
 Kasireddy, Bhargavi M99, P255, P337

Kasu, Praveen Reddy M86, P241
 Keel, A. Jake M24
 Keller, Lorraine H. M46
 Kemenova, Olga P317
 Kenney, Sophia M128
 Kerros, Sylvain P407
 Kers, Jannigje G. P305
 Keshavareddy, Venkata Prathap Reddy M19, P321
 Kewan, Ahmed T181
 Key, Jen M43
 Khadka, Vedbar P277
 Khaleduzzaman, A.B.M. M91, P315
 Khaliduzzaman, Alin M30, P403
 Kiarie, Elijah T208
 Kidd, Michael T. M108, M142, P258
 Kiess, Aaron P330
 Kihal, Abdelhacib M133, P395
 Kim, Elizabeth P375
 Kim, In H. P342, P378
 Kim, Minh P322, P323, P324, P325, P326
 Kim, Moon M83
 Kim, Woo Kyun M116, M129, M143, M19, M72, P279, P286, P321, P334, P351, P352, P365, P399
 Kindlein, Liris P332, P384
 King, Cindy M59
 King, Michael P372
 Kizil, Abdurrahman P320
 Knarr, Lucas E. M123, M139, M141, M145, M25
 Knoper, Audrey P274
 Ko, Hanseo M129, P321, P365
 Kock, Marlien de P342
 Kogut, Michael P305
 Kolan, Sierra M132
 Kolawole, Usman P378
 Kolli, Rama Devi P255
 Komiyama, Claudia M. P373
 Kong, Byungwhi M146
 Koppen, Megan P364, T160
 Korada, Mohan Naidu M86, P241
 Kosonsiriluk, Sunantha M3
 Kosuri, Praveen M79
 Kota, Sai M72
 Kota, Sai Akshitha Reddy M68, M75
 Krabbe, Everton L. P268
 Kragh, Karsten M. M34
 Kriseldi, Ruben M119, M138, M143, P334
 Krishna, Venkatramana D. M58
 Krishnan, Hari B. M139
 Kroetz Neto, Felipe L. M2, P398, P400
 Kulbacki, Stephanie P258, P284
 Kulkarni, Raveendra P294, P412
 Kulkarni, Ravi P298
 Kumar, Arvind M36
 Kuritza, Leandro T199
 Kushnir, Tatyana T194
 Kuttappan, Vivek P365, T164, T175
 Kwon, Young M. M62

L

L. F. V. Assumpcao, Anna P238
 Lahaye, Ludovic M32, P392
 Lambert, Alyssa M26

Lambert, William P363, T187
 Lamed, Eva T194
 Lancini, João B. P373
 Lang, Jhessica T149
 Lange, Agata P362
 Latham, Rocky M120
 Lavergne, Theresia P237, P318, T152
 LaVorgna, Mark P368, T158, T205
 Lawal, Abiola S. P257
 Lawrence, Jodie P308, T213
 Lawson, Tyler M15, T212
 Lebreton, Pierre P344, P345, P346
 LeComte, Abigail P258
 Lection, Jennine M128
 Lee, BD M91
 Lee, Chang-Won M44
 Lee, Jason T. M120, M143, M144, P334
 Lee, Jason M119
 Lee, Jiho M44
 Lee, Jihwan M129
 Lee, Kwang Young M129
 Lee, Kye j. P378
 Lee, Scott M44
 Leeds, Payten P242
 Leigh, Spencer P415
 Lemâle, Olga T153
 León, Eva P341
 Leonard, Suzanne M. M65
 Lerner, Steve T154
 Le Roux, Jean Francois P376, T186
 Lesko, Tyler M48
 Lever, Yann P376, T186
 Levesque, Crystal M107
 Leyva-Jimenez, Hector M121, M125
 Li, Guoming M118, M64, M68, M69, M70, M71, M72, M75, P405
 Li, Xiang P308, P323, T213
 Lim, Chae Bin P342
 Lima, Gabrieli A. P350
 Lin, Yang M105
 Linhoss, Anna P229
 Linhoss, John P229, P276
 Lisa, NN T177, T224
 Lisa, Nushrat N. T223
 liu, Tongshuai M68
 Liu, Tianming M64
 Liu, Tongshuai M118, M69, M70, M71, M72, M75, P405
 Livingston, Kimberly P294
 Logue, Catherine M. M47, M56, M60, T167
 Longhini, Giovana M. M122, P401
 Lopes, Júlia d. P350
 Lopes, Mário Henrique S. M122, P401
 Lopez, Diego M102, M114, P393
 Lorenzoni, Alberto Gino M48, M50
 Loughmiller, Joe T155
 Lovon, Melany Y. M122, P401
 Lowery, Justin M39, M87, P327, P408
 Lozano-Poveda, Carlos P258
 Lum, Jacob M34
 Lumpkins, Brett M35, P347, T169, T219
 Luna, Lexie M62
 Lynch, Elizabeth A. M123, M141
 Lynch, Elizabeth A. M139, M145, M25
 Lyons, Alyssa M. M121, P401, T178
 Lyte, Joshua M. P305

M

- Ma, Huihui M116, M118
 Maaskant, Sam M20
 Maccio, Laura M41
 Macklin, Kenneth M10, M11, M110, M127, M26, M88, P239, P277, T215, T217
 Madi, Aisha M84, M85, P246
 Madkour, Mahmoud T181
 Magee, Christopher M110, M51, P415
 Magee, Emily M89, P299
 Maguregui, Ekaitz P340
 Mahato, Prem Lal L. P277
 Mahdavi, Fatemeh S. M19
 Mahmoud, Kamel P336
 Mahmoud, Mahmoud A. M48, M50
 Maia, Rosana M148
 Maimon, Michal T194
 Maiorka, Alex P268
 Malheiros, Dimitri P392
 Malheiros, Ramon M16, P225, P331, P366
 Malheiros, Ramon D. M65, P251, P270
 Malinak, Chad P347
 Mallavarapu, Bharath T221
 Manaig, Yron M131
 Mandiga, Aravind M64, M70, M71, M72, M75
 Manginsay, Natalie M59
 Mani, Tatyany S. M16, P251
 Manjankattil, Shijina P328, T222
 Manjunatha, Vishal T219
 Manuja, Simmi M36
 Maqueda, Maricela M76
 Marasini, Hari M62
 Marchioro, Alessandro T201
 Marcon, Raul M45
 Marecaille, Clemence T150
 Mariani, Maria Leticia B. P268
 Marshall, Caleb M. M106, M12, P311, P402
 Martin, Keonjah P. T206
 Martinez, Blanca C. P338, T151
 Martinez, Diego A. P333, P369, P387, P388, P389, P390, T202, T203
 Martinez, Jibely M88
 Martinez-Bernal, Gustavo M103
 Masadeh, Mahmoud P368, T158, T205
 Masner, Martin M41
 Massei, Arthur d. T201
 Mathis, Greg T169
 Mathis, Greg F. M35
 Matiak, Emily P232, P233, P243
 Matias, Christiane P258
 Matloub, Ali M90
 Matter, Kevin T180
 Matusik, Kasey M54
 Matuszewski, Arkadiusz P362
 Mayer, André N. M40, P363
 McCafferty, Klinton W. M105, M110, M117, P253
 McCamy, Jason P364
 McCarver, Hannah P258
 McConnell, Abigail M8, M80
 mccormick, katherine M125
 McCrea, Brigid M92
 McCutchen, Megan P228, P243
 McElroy, Audrey M125, M130, P227, P259, P360
 McGinley, Casey P228
 McGovern, Kate P245
 McGuire, Olivia M17, M20, P306, P307
 McKenzie, K. S. P418
 McKinley, Enid T. M43
 McKinnon, Nicholas M83
 McLendon, Beverly M16
 McMillan, Amy M36
 McMillan, Elizabeth P240, P312
 McNaughton, James T192
 Mears, Megan M42
 Medina, Bertrand P361
 Mehdi, Manisha P245
 Mehmood, Sajid P370
 Meinersmann, Richard P312
 Mejia Abaunza, Nicolas P278
 Mejia-Abaunza, Nicolas M116, M118, M138, M72
 Melo, Julian E. T150
 Melo Neta, Suzete d. P268
 Melvin, Bryan J. T165
 Mendoza, Mary M87, P327
 Mendoza-Reilley, Alexandra M54
 Merinero, Sergio M133, P395
 Messias, Rodrigo K. M148
 Messina, Antonino T184
 Meuter, Antoine P263, P321, P357, T154
 Michalczuk, Monika P362
 Mikulski, Dariusz P346
 Millan, Jose P304
 Miller, Edgar T192
 Miller, Sharon H. P368
 Miller, Elizabeth J. M136, T183
 Minson, Carter D. M102, M114, P393
 Miquilino, Cátia T185
 Mishra, Birendra P254, P273, P275, P277
 Mishra, Pravin P254
 Miska, Katarzyna M48
 Mitchell, Trevor M146, P312, P379
 Mochel, Jonathan P. T165
 Mohammadi-Aragh, Maryam P310, T214
 Mohammadilalabadi, hosna M70
 Molina Villarraga, Edgar Leonardo M12
 Molins, Mireia M104, M29
 Montanhini Neto, Roberto T161
 Monteiro, Alessandra R. M134
 Moon, Jonathan T211
 Moore, Dan M120, P392, T159
 Moreau, Romain T187
 Moreira, Soner P394
 Morey, Amit M83, M93, P234, P242
 Moritz, Joseph S. M123, M139, M141, M145, M25, P230
 Morris, Cassidy A. M10, M11
 Morris, Colwayne M97
 Mortada, Mohamad M132
 Moss, Dylan M137
 Mouhamed, Amal A. P417
 Moyo, Margaret Taona P380
 Mubeen, Sara P367, P370, T207
 Muchiri, Felix T208
 Muentes Buenaventura, Natalia Naidelyn P289
 Muhammad, Muhammad Ali P258
 Munmun, Afsana Rahaman M96, P248, P331
 Muñoz, Luis M10, P299
 Muringattu Prabhakaran, Dhananjai M58, M78, P328, P329
 Murugesan, Raj P260
 Musa, Abubakar M1
 Myers, Eric M135

N

- Nacer-Khodja, Elise P349
 Naeem, Muhammad P381, P386
 Naguib, Mostafa P256
 Nalla, Sravan Sai Rahul M75
 Naqvi, S. Ali T164
 Nascimento dos Santos, Midian T182
 Nawaz, Akhtar T207
 Neeno-Eckwall, Eric P233
 Nelson, Alexander M67
 Nelson, Kenneth B. M108
 Nelson, Nathan T196
 Netto, Raimundo G. M122, P401
 Neupane, Durga T218
 Neupane, Indira P321
 Neves, Duarte M12
 Neves Tavares, Mariel P349
 Nicholds, Jenny A. T210
 Nicholls, Catalina P341
 Nicholson, Hannah T165
 Niraula, Abhisek M55
 Nkosi, Siphon P380
 Nolan, Lauren M35
 Nolin, Shelly P294
 Noll, Sally M141
 Nourin, Nushrat P383
 Novak, Curtis M35
 Nozeran, Anaís P344, P345
 Nungester, Eric M36
 Nusairat, Basheer P336, P371

O

- O'Flaherty, Sarah P298
 O'Lear Reid, Taylor M21, P303, P330
 Obe, Tomi M81, M95, P238, P244
 Oelschlager, Maci P249, P364, T160, T175
 Ogory, Roseline O. P269
 Oguey, Clémentine T163
 Oladeinde, Adelumola M98, M99, P308, P323, P324, T213
 Oladokun, Samson P262
 Olatunji, Folanike M13
 Oliveria, Jean T175
 Olowe, Olumide S. P271
 Olson, Elena P232
 Olson, Elena G. P228, P233, P243, P261, T191
 Olukosi, Oluyinka M98, M99, P255, P266, P337, P382
 Oluseyifunmi, Iyabo W. M99, P382
 Oluwagbenga, Esther M. P301
 Oluwayinka, Eniope B. T172
 Oluwayinka, Olatunde M57
 Onah-kachiside, Chinelo M83
 Onyema, Reginald P357
 Orellana Galindo, Leticia A. M10

Oristanio, Fabrizio M40, P394
 Orlowski, Sara M65, M76, P396, T195
 Ornaghi, Mariana P355, P356, P358,
 T149, T185
 Ortiz, Romel P278, P288
 Oso, Oluwadamilola M. M70, M71,
 M72, M75
 Osunbami, Opeadura T. M112
 Ott, Haley M114, P393
 Ovi, Fozol P237, P318
 Oviedo-Rondón, Edgar O. M103, M106,
 M12, P311, P402
 Owens, Casey M. M95
 Oyeniran, Victor M67, P404, T195

P

Pacheco, Wilmer J. M101, M105, M110,
 M117, M88, P239, P264, T217
 Pain, Victor P344, P345
 Pairis-Garcia, Monique T196
 Palacin, Roger T157
 Panek, Bryan M48
 Paneru, Bidur M74, P280, P281
 Paneru, Deependra M143, M19, P334,
 P351, P352, P365, P399
 Parada Roa, Viviana P338
 Parasuraman, Ramviyas Nattanmai M64
 Parra-Mendez, Loufrantz P338, T151
 Parra-Suescun, Jaime E. T151
 Parsons, Benjamin W. P252, T160
 Parsons, Carl M. P375
 Patel, Krutik T200
 Patil, Pranita T221
 Patil, Veeru P245
 Patterson, Rob T208
 Paudel, Roshan M46
 Paul, Pradyut P294
 Paulk, Chad M102, M114, P393
 Payling, Laura M. T200
 Peckenschneider, Maxim A. P228
 Peichel, Claire P328
 Pekel, Ahmet Y. P320
 Pelletier, Wolf T180
 Pender, Chasity P260, P359, P385
 Pennicott, Chantel M67, M76, P404,
 T195, T197
 Pereira, Raquel P349
 Pereira, Ricardo J. M2, P398, P400
 Perera, Ruvindu M53, P263, P357, P413,
 T174
 Perez-Calvo, Estefania M116, P258
 Perez-Palencia, Jorge Y. M107
 Perretti, Angela M67, P404, T195
 Perry, Famatta T162
 Persia, Mike E. M121, M126, P372,
 P401, T178
 Peterson, Brynn M28
 Petritz, Olivia T210
 Pfann, Michael T194
 Philips, Geetha P238
 Piantino Ferreira, Antonio J. P377
 Pidaparti, Ramana M M64
 Pietruska, Andrea P239
 Pilevar, Mohammad P337
 Pineda, Lane P342, T188
 Pinto, Silvina P353, T150
 Pio, Andres M41
 Piva, Andrea T184
 Plocher, Samantha S. M109
 Plumstead, Peter W. M115, M134
 Podvin, Claire M43
 Pokoo-Aikins, Anthony P312, P379
 Ponce-de-Leon, Carol P369
 Porta, Earnie P314
 Porto, Regina C. M2
 Poudel, Ishab M21, P303, P331, P366
 Poudel, Sabin T215, T217, T220, T221,
 T222
 Poudel, Suman P275
 Poulson, Rebecca L. T165
 Proaño Pérez, Freddy P289
 Proctor, Rachel M132
 Proszkowiec-Weglarz, Monika M48,
 P305
 Pulido-Landinez, Martha M52, T176
 Pullin, Allison N. M23, M65, M96, P247,
 T196
 Purswell, Joseph P229, P231, P310, T211
 Puyalto, M. M133, P395

Q

Qin, Jianwei M83
 Quant, Anthony D. M136, T183
 Qudsieh, Rasha P265
 Quino, Marcela P306
 Quino, Patricia M17, P306, P307
 Quintana, Gustavo M103

R

Radwan, Ahmed M133, P395
 Rafeian Naeini, Hamid Reza P279
 Rafeian-Naeini, Hamid Reza P286, P399
 Ragyari, Sai Kumar Reddy M19
 Rahaman, Md Mustafijur P406
 Rahman, MS T224
 Raines, Tracy M38
 Rajan, Shijinaraj Manjankattil P381,
 P386
 Ramalingam, Divyashree M86, P241
 Raman, Srivatsan P232, P243
 Ramaswamy, Lakshmi M. M69
 Ramos Provin, Mirian Leticia P384
 Ramzy, Ahmed T181
 Rangel, Luis M122
 Rasheed, Shameer P368, T158, T205
 Rashid, MA T177
 Rashid, Muhammad A. T223
 Rauber, Ricardo H. T201
 Read, Quentin M42, P312, P379
 Reddy Katha, Hemanth M129, P279,
 P286, P399
 Reddyvari, Ragini M79
 Reed, Kent M3
 Regmi, Prafulla M62, M72, P258, P284
 Rehberger, Joshua P305
 Reichelt, Sara P357, T196
 Reina, Marco M82
 Remus, Janet P375
 Ren, Yuying M79
 Renner, Alexis M123, M139, M25
 Rentsch, Ana M20
 Reynolds, David M142
 Reznik, Gary P368, T158, T205

Riahi, Insaf P341
 Riaz, Mian P333
 Riaz, Tuba T179
 Riboty, Rodolfo P389, P390
 Richards, Amber P240
 Richter, Stephanie P317
 Ricke, Steve P228, P232, P233, P243,
 P261, T191
 Riggi, Alain T155
 Riggs, Anna Kathryn P267
 Rigo Monteiro, Alessandra M131
 Rincker, Mike J. M124
 Roach, Brooklee P396
 Roberts, Delaney F. T166
 Robinson, Kelsy M51, P290, P310, P414,
 P415
 Rocha Calvette, Facundo M41
 Rocheford, Evan M15, P301, T212
 Rocheford, Torbert M15, P301, T212
 Rochell, Samuel J. M101, M105, M117,
 M144, P381, P386, T222
 Rodenburg, T. Bas T193
 Rodrigues, Carla P358
 Rodrigues, Fernanda F. P409
 Rodrigues, Paola A. P350
 Rodriguez, Maria Angeles P349, T156
 Roge, Erica R. M27
 Rogers, J. Wesley M6, M7, M94
 Roman, Tania M10
 Romero, Luis F. T200
 Romines, Lillie P287
 Rosero, Esteban P264
 Rossi, Aldo T183
 Rosso, Diogo P335
 Rostagno, Marcos T159
 Rothrock, Michael P308, P322, P323,
 P324, P325, P326, T213
 Rousseau, Xaviere M33, P337
 Roux, Melanie M130
 Rowland, Matthew T211
 Rueda, Martha S. M6, M7
 Ruiz-Ramirez, Juan R. M103
 Runcharoon, Klao M47
 Runcharoon, Klao M. M56, T167
 Rysdon, Katherine M39, M87

S

Sabo, Daniel P317
 Sacy, Audrey P344, P345
 Sadiq, Iram P367, P370
 Saeidifar, Mahtab M69, M70, M71, M72,
 M75, P405
 Salako, Florence A. M1
 Salazar, Tatiana M10
 Salim, H.M M91
 Salim, H.M. P315
 Sanchez-Plata, Marcos T216
 Sandoval, Jorge L. M94
 Sands, Jason M89
 Sandu, Dulmelis M35
 Santamaria, Jossie M. M45, M53, P300,
 P413, T166, T174
 Santana-Pereira, Alinne M26
 Santativongchai, Pitchaya M3
 Santin, Elizabeth P373, P409
 Santos, Regiane T156
 Santos, Wesley P394

Santos Haetinger, Vitor M111
 Saqui-Salces, Milena M3
 Sarkar, Anirvid P341
 Sarker, Md. Sazedul Karim P383
 Sarker, Md Sazedul Karim T177, T223, T224
 Sartori, José R. P350
 Schaeffer, Jon P319, P416, T171
 Schal, Coby T210
 Scharch, Christian T180
 Scharlau, Carson P227
 Scheaffer, Abe P261, T191
 Schilling, M. W. P397
 Schleifer, John P368, T205

Sharma, Shreeya P236, P256
 Sharmin, F T224
 Sharmin, Farhana P383, T223
 Sharmin, MF T177
 Shastak, Yauheni T180
 Sheffield, Hunter M84, M85, P246
 Shehata, Abdelrazeq M. T181
 Sheikhsamani, Ehsan P333, T203
 Sher, Usma P367, P370
 Sheth, Parimal M86, P241
 Shields, Jacob M36
 Shinder, Dmitry Dima T194
 Shirley, Rob P354, T153
 Shiroma, Neyre N. M40, P394
 Shoaib, Muhammad P367, P370
 Shoeb, Safat M132
 Shohail, AM T224
 Shrestha, Sunita P290
 Sibbel, Richard M38
 Sicheloff, Amy T. P296, P297
 Sierra, Katherine M83, P234
 Sigmon, Christina S. M39, M87, M96, P247, P248, P327, P331, P408
 Silva, Austin O. M125, M130, P259, P360
 Silva, Isabela E. M122
 Silva, Joyce A. P350
 Silva, Laya T196
 Silva, Marcelo M119
 Simões, Cristina T. M117
 Simon, Shamar T. M97
 Sinclair-Black, Micaela M13
 Singh, Amit K. T221
 Sirmon, Madison M83, P242
 Skipper, April L. M46
 Slick, Virginia G. M108, M142
 Smith, Alexandra H. M43, P305
 Smith, Cameron M83, M93, P242
 Smith, David P411
 Smith, Kaeden T212
 Soares, Igor P409
 Sokale, Adebayo M147
 Sokolov, Stanislav M83
 Sommer, Kaitlyn P249
 Son, Sona P372
 Soto, Jose A. M35
 Souza, Dausia P394
 Spackman, Erica M44
 Srikumar, Shabarinath M84, M85, P246
 Sroda, Lauren R. M9
 Stabler, Lisa J. T165
 Stanev, Vasil P347

Schneider, Abigail P236
 Schober, Jenna M. M14, M27, M28, P301
 Schrock, Jennifer P245
 Schulz, Mike P391
 Schwartz, Meghan P321, T154, T162
 Scott, Abdullah P333
 Seekatz, Anna P256
 Seets, Dylan W. M18
 Seibel, Samantha M128
 Selby, Callie P333
 Sellers, Holly M57, P291
 Selvaraj, Gokul P284
 Selvaraj, Ramesh M86, P241, T216
 Stanton, James P291
 Stark, Charles M102
 Starkey, Charles W. M24, M6, M7, M94
 Starkey, Jessica D. M24, M6, M7, M94, P229
 Stayer, Phil P299
 Stein, Andressa S. M45
 Stevens, Iyanni P274
 Stewart, Michelle P337
 Stuard, Osmair P356
 Stuart, Emma M28
 Studniski, Marissa M3
 Stumpff, Jolie A. P239
 Suarez, Juan P316, T168
 Subbiah, Jeyam P327
 Subedi, Deepak T221
 Subedi, Sachin P313, T197
 Sudom, Nathan T178
 Suesuttajet, Nawin P333
 Suesuttajit, Nawin P387, P388
 Suyemoto, M M. T168

T

Tabish, Rana Waqar M105, M117, P239
 Tabler, Tom M22, M73
 Tactacan, Glenmer M32
 Taguchi Kawaoku, Allison Jun M118
 Tahir, Yabaiz M87, P248, P327
 Tamatey, Patrick M113
 Taniguchi, Masahiko P330
 Tarcin, Ashley A. P261, T191
 Tarcin, Haley P243
 Tarrant, Katy J. P226, P285, P304
 Tashiguan, Vianca M83, P242
 Taylor, Robert L. M55
 Techer, Clarisse M40, P363, T187
 Teevan, Neil P314
 Tejada, Oscar P351
 Telg, Blair P347
 Tellez-Isaias, Guillermo M45
 Tellini, Caio P355, P358, T149, T185
 Terra-Long, Maria T. T155
 Tesouro, Anna T157
 Thames, Hudson M109, M110
 Thea Sullivan, Toni P245
 Thippareddi, Harshavardhan T221
 Thoele, Taylor M132
 Thomas, Alexa P258
 Thornton, Jay Kay M52
 Thornton, Tanner M22, M73
 Tian, Yang T197
 Tillman, Nickki M115, M136, P267
 Timmons, Jennifer P283

Serna, William M41
 Serrano, Dulman M10
 Setler, McKenzie P230
 Seyoum, Mitiku P305
 Sganzerla, Juliano P358
 Shad, Aqeel Ahmed M31
 Shafer, Kirsten M76
 Shah, Sanjay P225
 Shahid, Md Ahsanul P273
 Shakeri, Majid M146
 Shamugasundaram, Revathi M98, M99, P260, P379
 Shariat, Nikki M82, P235, P240, P296, P297
 Todorovic, Nemanja P376, T186
 Toomer, Ondulla T. M87, P248, P331, P366
 Torrent, Joan T199
 Torrey, Stephanie T182
 Troian, Bruna P355
 Tsai, Yu Yang M47, T167
 Tugnoli, Benedetta T184
 Turner, Bradley P348
 Turpin, Liz M90, T173

U

Ulcak, Caren P258
 Ullah, Sana T179
 Umberson, Cole P333, P387, P388, T202, T203
 Urban, Jakub P362
 Uribe-Diaz, santiago M45
 Uribe-Serrano, Alvaro M45, P338, T151
 Urrutia, Jorge M110
 Utterback, Pam P375

V

V.T. Nair, Divek M78
 Vagedes, Christian P353
 Vahjen, Wilfried M131
 Valbuena, Daniel P311, P402
 Valenzuela, Fabio P264
 van der Klein, Sasha P375, T189
 Van Diepen, Janjoris T163
 Vargas, Jose I. M101, M117, M88, P264
 Varnadoe, Kloie P272
 Vasefi, Fartash M83
 Vaughn, Mathew A. T192
 Vaught, Wesley P258
 Vaz, Rosana P394
 Veeraganti, Sujitha P266
 Venter, Kyle M. M115, M134
 Vicente, Gonzalo P353
 Vieira, Vivian I. P268
 Vilchez, Carlos P389, P390
 Villegas, Ana P347
 Vitek, Samantha M77
 von Seggern, Isabella M27, M63
 Vu, Thien M87, P248, P331, P366

W

Wajeed, Muhammad M37
 Walk, Carrie L. M112
 Walker, Lin L. M23, M39, M87, M96, P248, P327, P408
 Wallrich, Colin P. P232, P243

Walsh, Maria C. T200
 Walzem, Rosemary M66, P262
 Wamsley, Kelley M109, M110, M143,
 P253, P267, P334, P397
 Wang, Anhao T208
 Wang, Dongyi T197
 Wang, Jeng-Jie P265, P371
 Wang, Jingxue T219
 Wang, Jinquan M118, T221, T222
 Wang, Manhong M127, M51
 Wang, Weishan T204
 Wang, Yu P302
 Waters, Charis A. M11, M143, P334
 Watkins, Kevin T216
 Watson, James M59
 Watson, Wes T210
 Waugerman, Taylor M139, P230
 Weaver, Allison D. M65
 Weaver, Lanny P314
 Weimer, Shawna M67, M76, P283, P404,
 T195, T197
 Weli, Jordan P333
 Wells-Crafton, Savannah M108, M142
 Welsh, Justin M54
 Wesley, Serena M15, P301
 Wesson, Jacob C. M16
 Whelan, Rose P249
 Whelan, Samuel T218
 White, Skylar P259, P360
 Whitham, Stephanie P310, P415
 Whittle, Rosemary M76, P404, T195

Wilder, Camryn P259
 Wileman, Ben M3
 Wilkes, Joshua T202
 Wilson, Doreon M97
 Wilson, Jeanna M72
 Wilson, Nina M14
 Wilson, Victoria M102, M114, P393
 Wise, Tanner M136
 Wolc, Anna M55
 Wolff, Joshua M128
 Woodward, Adrienne P372
 Wyatt, Craig P253
 Wysocky, Becca P303, P331, T190
 Wysocky, Rebecca M16, M21, P366
 Wythe, Lindsey A. P261, T191

X

Xavier, William d. P350
 Xu, Ziteng P302

Y

Y. Abdelhady, AbdelRahman M133,
 P395
 Yadaigiri, Ganesh P245
 Yan, Frances M135, P391
 Yang, Xiao M73, M74, P280, P281,
 P313, T197
 Ye, Xin M127, M51, P415
 Yeboah, Philip M37, P245, P292, P294,
 T160

Yildiz, Alp E. P320
 Yoon, June Hyeok M98
 Young, Kasey E. M130, P259
 Young, Kelsey T. P291
 Yu, Po-Hsin M49, P416

Z

Zalewska, Anna P362
 Zamora, Ana C. M46
 Zandonai, Jéssica P355
 Zawada, Zachary M43
 Zdyrski, Christopher T165
 Zedek, Andrea P411
 Zeghadi, Reda P407
 Zhang, Guolong P305
 Zhang, Li M127, M143, M51, P253,
 P290, P334, P397, P414, P415
 Zhang, Xue M109, M127, M51, P397
 Zhao, Yang M22, M73
 Zheng, Haotian P330
 Zheng, Lan P385
 Zhou, Shengyu M73
 Zhuang, Hong M146
 Ziabtchenko, Elizabeth M146
 Zihan, Sadman S. P383, T223
 Zihan, SS T177, T224
 Zock, Gregory P308
 Zulfiqar, Zeshan P367
 Zumwalt, Mallory P262

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