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INTERNATIONAL POULTRY SCIENTIFIC FORUM

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ABSTRACTS
2023 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 23-24, 2023

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ABSTRACTS

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Dendy Keynote Lecture

Update on the highly pathogenic avian influenza outbreak in the United States Mary Pantin-Jackwood* *Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, U.S. Dept. of Agriculture, Agricultural Research Service*

Highly pathogenic avian influenza (HPAI) H5 viruses of the A/Goose/Guangdong/96 (Gs/GD) lineage are a major global threat to poultry and are spread by wild birds. In December 2021, H5N1 HPAI viruses were detected in birds in Canada and subsequently in wild waterfowl the United States (US). These viruses were closely related to viruses from northern Europe. Once in the US, the virus spread in the wild bird population and spilled over to backyard birds and commercial poultry. The virus persisted through summer and as of October 31, HPAI had been confirmed in 250 commercial flocks and 328 backyard flocks across 43 states, and there have been more than 3100 wild bird detections. Phylogenetic analysis conducted with sequences from more than 1300 viruses from the outbreak corroborated virus spread via wild bird circulation, with most detections in poultry premises and non-poultry flocks consistent with independent wild bird introductions. The virus also mixed several times with North American lineage wild bird AI viruses and these reassortant viruses predominate among wild bird detections and have been identified in poultry across several states. Pathogenesis studies with one of the early H5N1 HPAI viruses from this outbreak showed that the virus is more infectious to poultry than the index H5N2 HPAI virus that caused the major 2015 outbreak in the US. Turkeys were also more susceptible and transmitted the virus better than chickens. Biosecurity, whether preventing virus entry or preventing secondary spread, paired with rapid detection and response remain the best tools to combat this virus.

Key Words: avian influenza

***Author presenting paper**

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Teaching, Pedagogy, Extension

M1 Current perceptions, use, and needs of precision livestock farming systems Tanner Thornton*[GS], Yang Zhao, Tom Tabler, Robert Burns, Shawn Hawkins *University of Tennessee-Knoxville*

Precision Livestock Farming (PLF) technologies offer a way to improve production and labor efficiency while also addressing welfare concerns that consumers express regarding how poultry are grown. There is an extreme lack of knowledge on the current perceptions and need of PLF within the U.S. poultry academia. The objective of this study was to design a survey to understand the perceptions and needs of PLF by academia. An online survey was designed via Qualtrics consisting of 25 questions. The survey was distributed via email to 276 poultry researchers in the U.S. acquired via search on university websites. The survey was made available for one month and yielded 68 total responses (N=68). The results were exported from Qualtrics and analyzed using SPSS. Respondents were separated into groups based on job type (extension specialist, researcher, both) and research type (animal science, engineer, vet). Results were analyzed using a chi-square test, frequency analysis, and the Monte Carlo feature to account for the relatively small sample size. Amongst researcher's results from this study show that 92% of researchers agree that cost influences a grower's decision to implement PLF technology and 86% of researchers agree usability influences a farmer's decision to implement. The results also show most poultry researchers are familiar with the term PLF (92%), however, only 55% of respondents have or currently use PLF technologies. Researchers also agree that PLF will be very important in improving poultry production (97%), and health and welfare (91%) when compared by research type there were no significant differences (p=0.360) found amongst groups regarding health and welfare, however, it was found that engineers agree more than animal scientists that PLF will be important to improve future poultry production with 61% of engineers strongly agreeing comparing to only 34% from animal scientists (p=0.017). Significant differences were also found when the researchers who already use PLF in which 57% strongly agree that PLF will allow them to expand their research compared to researchers who do not use PLF in which only 13% strongly agreed (p=0.001). It can be concluded that poultry researchers have realized the importance of PLF's role in poultry production, but applications of PLF technologies in research remain to grow. In addition, perceptions on PLF vary among researchers with different backgrounds.

Key Words: Precision Livestock Farming, Poultry Industry, Animal Welfare, Perception, Animal-based outcomes

M2 Using water usage modeling to generate alarm events related to poultry management Will Strickland*[GS], Michael Czarick, Brian Fairchild, Garret Ashabranner *University of Georgia*

Daily water consumption has been used as a management tool by poultry producers for decades. Since water consumption is closely correlated to feed consumption, decreases in daily water consumption are often an indicator of reduced feed consumption and/or health issue. With the emergence of ultrasonic water meters bird water usage can now be more accurately monitored on a minute-to-minute basis thus providing the opportunity to view bird drinking/feeding activity over the course of the day graphically.

Ultrasonic water meters were installed on two Free Range Layer Houses in Northeast Georgia and water usage was measured every 5 minutes for four weeks. The data were collected through a data acquisition system and analyzed.

From these data a daily water usage profile was established and shown to be highly repeatable. Preliminary data was analyzed graphically through Microsoft Excel. A 4th degree polynomial regression (R-squared = 0.93) was created to best match the daily water usage profile. The standard deviation was low showing that the data was very consistent. The Standard deviation ranged from .02 gallons to one gallon. This data was used to create a software program to model usage, flag uncommon usage, and generate an alert. Using known events that occurred during the 27-day collection period, the model was tuned to detect abnormally high or low flow rates during specific times of the day. Standard deviation of data from each sampling interval was calculated and used to set the upper and lower control limits through the day.

Many bird and house related management issues are missed unless the grower is constantly monitoring the data, which most do not have the time to do properly. It is hoped that after further testing this tool will provide a tool to the industry that will alert producers if there are deviations from the expected water usage profile.

Key Words: Water Consumption, Management, Precision Ag, Modeling

M3 Using ultrasonic water meters as management tool early in the flock Garret Ashabranner*[GS], Michael Czarick, Brian Fairchild *University of Georgia*

Water meters are used as a management tool to track daily water usage in poultry houses. Most mechanical water meters used in poultry housing can only measure water flow rates more than 0.25 gals/min which limits reliable measurement of water usage for birds that are less than four days of age. Producers can now accurately measure water usage within minutes of chicks being placed in the house by using ultrasonic water meters. Modern house controllers can collect, store, and graph water consumption when connected to ultrasonic meters allowing for identification of problems during early chick management. Over the last 2 years, a series of field trials were conducted involving multiple farms using ultrasonic water meters connected to a house controller and a data logger system. Using this combination of ultrasonic water meters and modern controllers, a number of common management related issues that can occur on broiler farms have been documented based on water usage.

In one field trial, a drinker leakage issue was discovered less than 24 hours after chicks were placed in the house. In the first 16 hours, water usage increased 133% more than the adjacent house adding 200 gallons of extra water to one area of the floor.

Birds typically do not consume water during dark periods. When water usage continued in one of the field trials, it was found that the house lights never turned off the previous night. This would not have been discovered unless the birds were checked between 12 and 4 am. These birds had a 18% longer day compared to the house light system that operated correctly.

Bird density at placement can be monitored by water usage. Data from a house indicated 20% more birds in the front of the house on Day 14. Despite the grower efforts to correct the issue, the difference continued throughout the flock resulting in bird weights that differed by over 0.5

lb between the front and back of the house. This body weight difference reduced the grower pay by 20%. The use of the ultrasonic water meter provides monitoring from day of placement through the end of the grow-out. This meter is a relatively low investment ($350) but can provide information that can yield large returns on investment through optimizing chicken management.

Key Words: Water consumption, brooding, house controller

Processing and Products

M4 Stunning and neck-cutting methods impact on the overall blood loss and rate of blood loss of broiler carcasses Rachel Osborne*[1GS], Caitlin Harris[1,2], Richard Buhr[2], Brian Kiepper[1] *[1]Department of Poultry Science, University of Georgia, [2]Poultry Microbiological Safety and Processing Research Unit, U.S. National Poultry Research Center, Richard B. Russell Agricultural Research Center, USDA-ARS*

With over 9 billion broilers processed each year in the U.S., blood is a significant by-product of poultry processing. Yet, there is little research on the effect of stunning method on blood loss in today's high breast meat yield broilers. The aim of this study was to determine blood loss and rate of blood loss for 3 stunning methods with 2 commonly used neck-cutting methods. Male broilers from 4 flocks at 62d (N=120; BW 5530g), 45d (N=120; BW 3561g), 43d (N=120; BW 3455g), and 43d (N=120; BW 3527g) of age were used. Broilers underwent an 8-hour feed withdrawal before being randomly assigned to 1 of 3 stunning methods: a 5s 120V AC electric stun from beak to vent (AC), a 10s pulsed 25V DC electrical stun from beak to vent (DC), and a 5-min CO_2 stun (gas charge 3 min, hold 2 min; CAS). After stunning, birds received either a 1-sided neck cut severing one carotid and jugular or 2-sided neck cut severing both carotids and jugulars. Carcass weights were then recorded at 15s intervals for 180s. Rate of blood loss and % blood loss were calculated based on BW loss, and data were analyzed by ANOVA in SAS JMP using Student's t-test (neck cut) and Tukey's HSD (stunning and neck-cutting x stunning) for means separation. Generally, DC stun resulted in significantly greater blood loss and rates of blood loss than either AC or CAS, while cutting method and treatment interactions were not significant for any time point or flock. At 90s of bleed-out (industry standard), DC (2.80%, 63d; 3.05%, 45d; 2.91%, 43d; 3.37%, 43d) showed significantly greater % blood loss than AC (1.54%, 63d; 1.88%, 45d; 1.68%, 43d; 1.97%, 43d) and CAS (1.33%, 63d 1.70%, 45d; 1.73%, 43d; 1.82%, 43d; $P \leq 0.0001$ in all trials). After 180s of bleed-out DC (2.98%, 62d; 3.25%, 45d; 3.14%, 43d; 3.57%, 43d) again showed significantly greater % blood loss than AC (1.83%, 62d; 2.23%, 45d; 2.26%, 43d; 2.51%, 43d) and CAS (1.18%, 62d; 2.25%, 45d; 2.52%, 43d; 2.49%, 43d; $P \leq 0.0001$ in all trials). At 90s of bleed-out DC stunned birds lost 42% more blood than AC and 46% more than CAS. At 180s of bleed-out DC stunned birds lost 32% more blood than AC and 35% more than CAS. These results highlight the need for potential modification of bleed-out and blood collection when changing stunning method in broilers.

Key Words: poultry processing, poultry by-products, blood loss, neck-cutting, stunning

M5 Analysis of broiler chicken blood parameters during carbon dioxide gas stunning Montana Riggs*[GS], Marco Reina, Andrea Urrutia, Juan Figueroa, Alexandra Jackson, Matthew Hughes, Brett True, Charlene Hanlon, Rüdiger Hauck, Bethany Baker-Cook, Dianna Bourassa *Auburn University*

Previous research indicates there is an increase in blood glucose concentrations in broiler chickens stunned by controlled atmosphere stunning (CAS) systems that utilize CO2. However, it is unknown whether this change occurs from CO2 triggered convulsions during either the conscious or unconscious phases of CAS, or if this change occurs due to a biochemi-cal reaction from respiration of CO2. Different conclusions may have vari-ous implications on the welfare of the broiler. To further define the time at which the change in glucose during CAS occurs, a trial was conducted evaluating blood parameters during CO2 stunning. Gas concentrations were 25-35% at 0-120 seconds, 35-45% at 120-180 seconds, and 65%at 180-300 seconds.

Blood samples from broilers 43 days of age were extracted with a pre-heparinized syringe from the brachial vein prior to stunning and every minute for five minutes. Blood glucose, pH, pCO2, BE (base excess), K, and HB (hemoglobin) were measured using an iSTAT Alinity v blood analyzer. Data were analyzed by GLM through SAS 9.4 university edition with a significance at $P \leq 0.05$ and means were separated by Tukey's HSD. Glucose concentrations (mg/dL) did not change between any timepoint (P=0.7177). pH values decreased over time from 7.38 to 7.09 (P<0.0001), while pCO2 (mmHg) increased consistently across all timepoints from 38 to 74 (P<0.0001). BE (mEq/L) decreased from 60 s to 120 s from -3.17 to -7.75 and remained unchanged for the remaining time (P=0.0348). K (mEq/L) did not change over time (P=0.0515) however a trend was noted as K increased at 300 seconds. HB (g/dL) increased be-tween 120 s to 300 s from 6.63 to 8.73 (P=0.0470). It Is important to note that that birds did not exhibit convulsions typically expected from CO2 stunning and were lightly restrained during stunning and blood collection. The lack of an observed increase in blood glucose concentrations in this study indicates that the single bird CO2 stunning method used was not representative of commercial CO2stunning outcomes. Inhalation of CO2 resulted in pH decreasing over time and an increase in pCO2 as was ex-pected and confirmed in this study. Other blood parameters of K, iCa, HB, and BE may be relevant in future studies for comparing and optimizing stunning methods. Key Words: stunning, broiler, glucose, ph, carbon dioxide

M6 Application of pressurized steam and forced hot air for cleaning broiler transport cage flooring Marco Reina*[1GS], Andrea Urrutia[1], Juan Figueroa[1], Montana Riggs[1], Kenneth Macklin[1], Richard Buhr[2], Dianna Bourassa[1] *[1]Auburn University, [2]US National Poultry Research Center, USDA-ARS*

This study evaluated the efficacy of pressurized steam and forced hot air for cleaning transport cage flooring and compared them to conventional cleaning procedures. Fiberglass and plastic flooring were assessed in this experiment. Flooring pieces were cut into squares and measured to offer a surface area of 5 x 5 cm per sample. For the intestinal contents, 100 g were extracted by manual expression from broiler viscera. A 108 CFU/mL Salmonella Infantis inoculum (1 mL) was added to 100 g of intestinal contents and stirred. One g of inoculated intestinal content was applied to each square. Samples were held at room temperature (20°C) for 60 min before any treatment. The treatments were pressurized steam for 15 s, forced hot air for 60 s, and the sequential application of both. The controls were pressure washing for 15 s, the application of a commercial disinfectant following pressure washing, and no cleaning. After treatment, samples were swabbed, and counts for Salmonella, E. coli (EC), coliforms, and aerobic bacteria (AC) were measured. Samples without Salmonella counts were enriched and later confirmed as positive or negative. Bacterial counts were log transformed and the effects of flooring type and treatment were analyzed by ANOVA with Tukeys HSD for mean separation. There was no difference (P>0.05) between fiberglass and plastic flooring in any of the treatments. When compared to the untreated control, forced hot air alone did not reduce any of the microorganisms measured (P>0.05). Pressurized steam alone reduced all counts by more than 2 log10 CFU/cm2. The combination of steam and hot air reduced all counts by 3 to 4 log10 CFU/cm2. Steam with hot air was as effective as using pressure washing and disinfectant (P>0.05) for Salmonella counts. Pressure washing with the application of disinfectant was the most effective reducing all counts (P<0.05) when compared to the untreated control, reducing 3 to 6 log10 CFU/cm2.

However, all treatments and controls had at least 3/5 samples confirmed as positive for Salmonella after enrichment. Processors may be able to adapt this process to clean transport cages with low water applications such as steam to reduce potential cross-contamination and lessen the presence of pathogens entering the processing plant.

Key Words: Transport, steam, salmonella, campylobacter

M7 Biofilm formation ability of Campylobacter jejuni isolated from commercial broiler processing plants. Diksha Pokhrel*[1GS], Hudson Thames[1], Hailey Fugate[1], Li Zhang[1], Thu Dinh[2], Wes Schilling[1], Shecoya White[1], Reshma Ramachandran[1], Anuraj Sukumaran[1]

[1]Mississippi State University, [2]Tyson Foods

Campylobacter jejuni is a microaerophilic pathogen and is a leading cause of food-borne gastroenteritis worldwide. During its transmission through the food chain, the organism survives stressful environments, particularly high oxygen levels and unfavorable temperatures. Biofilm formation is one of the suggested mechanisms used by this pathogen to survive such stress and is a food safety concern in poultry processing. Even though, the biofilm formation of C. jejuni has been investigated in polystyrene, data on common food contact surfaces is lacking. Additionally, C. jeuni's environmental survival and biofilm production are poorly understood. Therefore, the biofilm formation of three different aerotolerance groups of C. jejuni:5 aero-sensitive (AS), 4 intermediate aerotolerant (IAT), and 5 hyper aerotolerant (HAT), was evaluated on stainless-steel, a common surface material in poultry processing plants. Isolates were incubated overnight in BHI at 42°C under microaerophilic conditions (MA). Stainless steel coupons were immersed in working cultures of each isolate (6 log CFU/mL) and incubated under optimum (MA at 42°C) or environmental conditions (aerobic; room temperature) for 72 h. Coupons were rinsed to remove the planktonic cells and vortexed vigorously in BHI broth. Enumeration was performed by plating the broth onto Campylobacter agar base with 5% laked horse blood. Each isolate had triplicates and the entire experiment was repeated thrice. Data were analyzed by the GLIMMIX procedure of SAS 9.4 at a significance level of 0.05. Overall, the number of biofilm-attached C. jejuni cells on the coupons was affected by an interaction between isolate and incubation condition ($P < 0.001$). All isolates had a greater biofilm-formation ($P<0.001$) at optimum conditions than at environmental conditions. Notably, five isolates had over 5 log CFU/coupon at optimum conditions, whereas under environmental conditions, they had 3.4 log CFU/coupon. One exception was isolate #C344 (HAT), which had 6.8 and 5.9 log CFU/coupon at optimum and environmental conditions, respectively. These results indicate that biofilm formation might be a potential mechanism used by C. jejuni to persist in poultry processing and is influenced by environmental temperature and oxygen concentration.

Key Words: C. jejuni, Biofilm, Poultry Processing

M8 Isolation and identification of Salmonella and Campylobacter isolates from a commercial broiler complex through pullets to final raw product Yagya Adhikari*[1GS], Matthew Bailey[1], James Krehling[1], Kaicie Chasteen[1], Luis Munoz[1], Cesar Lobo[1], Leticia Galindo[1], Pankaj Gaonkar[2], Steven Kitchens[2], Dianna Bourassa[1], Stuart Price[2], Jeff Buhr[3], Kenneth Macklin[1] *[1]Department of Poultry Science, Auburn University, [2]Department of Pathobiology, Auburn University, [3]USDA ARS Poultry Microbiological Safety and Processing Research Unit*

Salmonella and Campylobacter, are leading bacterial pathogens of both economic and foodborne importance in the poultry industry. With the aim to isolate these pathogens, and to determine potential risk factors for introducing them to the poultry complex, samples were collected from two pullet farms, four breeder farms, nine broiler farms, hatchery, transport and processing plant. A total of 686 and 444 samples were collected for Salmonella and Campylobacter recovery respectively from inside and outside these facilities. All samples were analyzed with a 3M-Molecular Detection System (MDS) for rapid screening and suspected positive samples were further processed for confirmation of result and identification.iFrom the pullet farms, 5 MDS Salmonella positive and 6 MDS Campylobacter positive samples were identified. Among these, 1 sample for Salmonella and Campylobacter tested culture positive. From the breeder farms, there were 57 MDS Campylobacter positive samples, 7 of which tested positive on culture; none of the samples tested positive for Salmonella with the MDS. Broiler farms, had 13 MDS positive samples for both Salmonella and Campylobacter. Among these, 7 and 2 samples for Salmonella and Campylobacter respectively tested culture positive. The hatchery, had 13 MDS Salmonella positive and 2 MDS Campylobacter positive samples. From these, 5 samples tested culture positive for Salmonella while none tested culture positive for Campylobacter. Finally, from the processing plant, 3 and 11 samples for Salmonella and Campylobacter respectively tested positive with MDS and culture. Salmonella rough O: r:1,5 isolate was identified from pullet farm and serotypes S. Barranquilla, S. Kentucky, S. Liverpool and S. Luciana were identified from broiler farms. Similarly, the serogroups B and D1, and C2 and E were identified from the hatchery and the processing plant respectively. This study is survey-based and is focused on identifying potential risk factors. Thus, at this time, statistical analysis was not performed. With these findings, it can be inferred that the surroundings of poultry houses include various risk factors that can transmit these foodborne pathogens into the poultry complex, and hence, potentially to the consumers.

Key Words: foodborne pathogens, pullet, breeder, hatchery, processing

M9 Assessing Salmonella serovar dynamics through broiler processing Amber Richards*[1GS], Nikki Shariat[1], USDA-FSIS Eastern Laboratory[2] *[1]Department of Population Health, University of Georgia, [2]United States Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS)*

Salmonella is a leading bacterial cause of foodborne illness, with ~17% of salmonellosis cases attributed to broilers. Conventional Salmonella isolation procedures involve characterizing a single colony per positive sample. As such, these methods favor the most abundant serovar found in a sample, potentially allowing other serovars to remain undetected. CRISPR-SeroSeq is a novel, high-resolution sequencing approach that can detect and quantify the relative frequency of multiple serovars present in a sample. This study sought to determine how processing interventions influence serovar population dynamics by comparing Salmonella incidence between broiler carcasses at hot rehang and post-chill. We utilized deep serotyping by CRISPR-SeroSeq to analyze paired rehang and post-chill samples, and additional rehang samples without a Salmonella-positive post-chill pair. These samples were collected from broiler processing plants across the United States from August to November 2022. Multiple serovars were detected in 45.3% (24/53) of the samples. The most abundant serovars identified in rehangs (n=41) were Kentucky (78.0%; 32/41), Infantis (34.1%; 14/41), and Typhimurium (22.0%; 9/41). Alternatively, post-chill samples (n=12) contained predominantly Kentucky (91.7%; 11/12). These data demonstrate that processing interventions are effec-tive as sample complexity was reduced in nearly all post-chill samples. Serovars matched between rehang and post-chill carcasses in 50% (6/12) samples, plus they partially matched in an additional 25% (3/12) samples. Deep serotyping was performed on both RV and TT selective enrichment cultures. Statistical analysis comparing serovar populations between the culture types showed that 83.0% (44/53) and 86.8% (46/53) had good matches between the two media, using Bray-Curtis and Jaccard metrics, respectively. This analysis improves our understanding of Salmonella dy-namics during processing, which could guide the development of serovar-specific interventions.

Key Words: Salmonella, Food Safety, CRISPR-SeroSeq, Processing, Broiler

M10 Effect of chlorhexidine salts and cetylpyridinium chloride on Salmonella enterica Typhimurium (ST) recovery from an in vitro crop assay Lauren Laverty*[GS], Juan Latorre, Makenly Coles, Kristen Martin, Danielle Graham, Billy Hargis *University of Arkansas*

Previous research has indicated that a major source of carcass contamination post-scald within commercial poultry processing plants is related to leakage of crop contents during the evisceration process. Previous work from our laboratory has indicated that chlorhexidine has potential to replace potassium dichromate for *Eimeria* storage due to profound antimicrobial effects. Presently, we evaluated the ability of the common antimicrobial compounds in mouthwash and toothpaste, chlorhexidine (CHX) salts and cetylpyridinium chloride (CPC), to reduce ST in the simulated crop contents *in vitro* using a published model. CHX digluconate (Sigma), CHX gluconate (Spectrum), commercially available CHX gluconate (Durvet/VETone) and CPC (Sigma) were tested at 2%, 1%, 0.2% and 0.1% final concentrations (n=3/concentration). Borosilicate tubes, containing 2.5g of pasteurized mash chicken feed (80C for 16h) were combined with a final concentration of $2x10^5$ CFUs of ST in sterile saline with or without (negative control) treatments. Tubes were statically incubated at 37C for 2h and then serial diluted for CFU determination. Data were analyzed by ANOVA with significant differences further separated by Tukey's multiple range test. Significant differences are reported at $p<0.05$. Treatments that caused significant reduction (reported as CFUs (log10)/ml) from untreated controls were CHX digluconate (Sigma) at 2% (-1.83), 1% (-1.79) and 0.2% (-2.06), CHX gluconate (Spectrum) at 2% (-1.56), CHX gluconate (Durvet/VETone) at 2% (-5.26, no growth), and CPC (Sigma) at 2% (-3.3), 1% (-4.01), 0.2% (-1.55) and 0.1% (-1.46). In conclusion, these data suggest that this relatively short incubation (2h) caused meaningful reductions in *Salmonella* recovery in simulated crop contents. Ongoing results will further refine the potential additive or synergistic activity and required concentrations.

Key Words: Salmonella Typhimurium, Chlorhexidine, Cetylpyridinium chloride

M11 Field survey sampling strategies identifies flow patterns of Salmonella to the processing plant Jordon Gruber*[1], joshua Walker[2], Jennifer Freezeman[2], Connie Mou[1], Elizabeth Kim[1] *[1]IFF/Danisco Animal Nutrition and Health, [2]IFF Wilmington Microbiology Lab*

Salmonella transmission and critical control points within commercial agriculture production systems are poorly understood given the dynamics of the organism's biology. Current studies of Salmonella within production settings showcases a unique community lifestyle; however, control strategies do not appear to incorporate this organization of strains in a strategy. The results of many intervention programs fail to show significant reductions in Salmonella – especially field control strategies to create significant impact at the processing plant. Here we present findings related to an initial baseline of a live production complex measuring Salmonella density and serology during down-time, grow-out, and transfer of birds into the processing plant. These findings are based on meta data of perceived Salmonella risk (5 farms, 4 houses per farm, 2 complete flocks). Amongst the findings, trends were identified that include prevalence of positive results between different sampling methods (65% GITs, 98% boot swabs, 90% whole litter), trends in serogroup shifts during grow-out (0-100% serology shifts), and dynamics of Salmonella density at different stages in the processing plant (RANGE 0 to 1388CFU/ml). Other interesting findings include loading of Salmonella farm-to-farm (2.6 log ± 1.183) and the role individual bird density (2 log "normal" vs 5 log "Hot") may play on Salmonella-positives at the plant. Together, these findings provide a path for new study protocols that may help to improve field-based interventions against Salmonella.

Key Words: Salmonella, Field Sampling, Trial Design, Processing Plant, Control Points

Physiology, Endocrinology and Reproduction I Broilers, Turkeys

M12 CO2 Incubation for Woody Breast Mitigation Sameer Israni*[1], Frank Edens[2], Dellila Hodgson[2], Jenny Schlenker[2] *[1]Linde Inc., [2]North Carolina State University*

- Purpose: Investigate Woody Breast myopathy mitigation by subjecting embryos to various levels and profiles of CO2 gas during the incubation stage. The hypothesis is that subjecting embryos to CO2 mimics low oxygen environments, without the harmful effects of actual low oxygen. This forces embryos to adapt physiologically, resulting in changes that help overcome low supply of oxygen and other nutrients to breast tissue, thereby mitigating incidences of Woody Breast in later life. Woody Breast is estimated to cause more than $200 million in annual losses for the US poultry industry.
- Experimental Design: In phase 1 of the tests, Ross 708 eggs were subjected to different CO2 concentrations and profiles (how the concentrations change over the 21 days of incubation) by injecting CO2 gas into the incubator. Each treatment and control contained 300-400 eggs. The resulting male chicks (approximately 150 per treatment) from the hatch were grown to 28 days, after which they were evaluated for Woody Breast scores using trained panel. Hatch of fertiles, growth rates, mortality adjusted weight gains, food conversion ratios were also measured. In phase 2, the CO2 concentrations and profiles that showed promising results in phase 1 were repeated (600+ eggs each treatment or control) and the male chicken (approximately 300 per treatment) were grown to 6 weeks in floor pens. The hatch of fertiles, growth rates, mortality adjusted weigh gains, final weight, food conversion ratios, Woody Breast scores were all measured.
- Results: CO2 treatments resulted in up to 70% reduction ($p<0.05$) in severe Woody Breast scores compared to control (no external CO2 gas injected into the incubator) birds. This Woody Breast score reduction was accompanied by no significant change in final weights, mortality, adjusted daily weight gains, food conversion ratios. Additionally, up to a 5% increase in hatch of fertiles was noted for some CO2 treatments.
- Conclusion: CO2 incubator treatment represents a practical and economic method of reducing Woody Breast incidences by up to 70% and of increasing hatch of fertiles.

Key Words: Woody Breast Mitigation, Carbon Dioxide (CO2), Incubation, Hatch of Fertiles

M13 Effect of environmental temperature on the blood chemistry of young turkeys Rocio Crespo*, Jesse Grimes *North Carolina State University*

The purpose of climate control in poultry production is to produce a healthy bird, which enables it to grow more efficiently and live in the most desirable and least stressful environment available. It is known that blood analytes change to external stressors and health challenges, even before obvious behavioral or clinical signs are observed. We hypothesize that analysis of selected blood parameters could aid objective assessment of the effect of environmental manipulation and performance in young growing turkeys. For this study, a total of 2,240 female poults from a commercial hatchery were assigned to one of four treatments: T1 (85F-65%RH), T2

(90F-65%RH), T3 (95F-65%RH), and T1 (55F-80%RH). Ambient temperature and relative humidity were closely monitored and recorded every 30 minutes. Temperatures were lowered 5 degrees Fahrenheit each week. Blood from 12 birds, from each room, was collected on days 1, 3, 5, 7, 10, 14, 21, 28, 35. Whole heparinized blood was analyzed using an iSTAT 1 analyzer using the CG8+ cartridge. Additionally, ketone was measured using the Freestyle Optium Neo System. Statistics for all variables were performed by age by treatment. Confidence internals of 0.80 were calculated for each age and treatment group. Differences between the means were determined using Mann-Whitney. *Ketone* bodies were increased in the blood from treatments 3 and 4 birds, at 3 days of age. It is possible that these birds did not eat as much feed and relied on the fatty acids from the yolk sac as a source of energy. Throughout the experimental period, the blood *pH* was higher, and the *partial* CO_2 *was* lower in the two groups with the higher temperatures (T3 and T4). Furthermore, blood *glucose* was higher from 3 to 21 days of age in groups T3 and T4. The combination of high pH and lower pCO2 is recognized as respiratory alkalosis and associated with heat stress. Treatment also affected blood sodium. *Sodium* was depressed for treatments 3 and 4 for ages 3, 7 and 9 days. In chickens subjected to chronic heat stress, the addition of 0.5% $NaHCO_3$ in their feed enhanced body weight gain. It would be interesting to investigate whether NaHCO3 also helps turkeys to overcome some of the negative effects of heat stress.

Key Words: blood, temperature, relative humidity, point-of-care analyzer, turkey

M14 Comparison of standard and physiological cell culture temperatures on proliferation and differentiation of broiler chicken Pectoralis major primary muscle satellite cells Caroline Gregg*[GS], Brittany Wall, Joshua Flees, Charles Starkey, Jessica Starkey *Auburn University Department of Poultry Science*

Culture temperatures for broiler chicken cells are largely based on those optimized for mammalian species although normal broiler body temperature is typically more than 3 °C higher. The objective was to evaluate the effects of simulating broiler peripheral muscle temperature, 41 °C, compared with standard temperature, 38 °C, on *in vitro* proliferation and differentiation of primary muscle-specific stem cells (satellite cells; SC) from the *Pectoralis major* (PM) of broiler chickens. Immediately following euthanasia of 18-d-old Ross 708 × Yield Plus male broilers (n = 6), peripheral PM temperatures were recorded (41.14 °C +/- 0.08), and then primary SC were isolated. SC were plated on triplicate, 1.8-cm^2, gelatin-coated wells at 40,000 cells per well. Parallel plates were cultured at either 38 or 41 °C in separate incubators. At 48, 72, and 96 h post-plating, parallel cultures were immunofluorescence stained to determine expression of the myogenic regulatory factors, Pax7 and MyoD, as well as evaluated for apoptosis using a TUNEL assay. The remaining plates were switched to reduced-serum differentiation media at 96 h. After 168 h in culture, cells were immunofluorescence stained to determine myosin heavy chain and Pax7 expression, myotube characteristics, and assess SC fusion. The experiment was replicated 3 times with independent pools of cells. Data were analyzed using PROC GLIMMIX of SAS 9.4 and means were declared different at $P \leq 0.05$ and tendencies at $0.0501 \leq P \leq 0.10$. Population doubling times were not impacted by temperature ($P \geq 0.1148$), but culturing broiler SC at 41 °C for 96 h promoted more rapid progression through myogenesis while 38 °C maintained more primitive populations ($P \leq 0.0029$). The proportion of apoptotic cells was greater in primary SC cultured at 41 °C ($P \leq 0.0273$). Culturing at 41 °C reduced overall myotube fusion ($P < 0.0001$) and tended to result in thinner myotubes ($P = 0.061$) without impacting the total myonuclear density ($P = 0.7551$). These results indicate culture temperature alters primary broiler PM SC myogenic kinetics and has important implications for future in vitro work as well as improving our understanding of how thermal manipulation can alter myogenesis patterns during broiler embryonic and post-hatch muscle growth.

Key Words: broiler chicken, myogenic regulatory factors, muscle

M16 Assessment of late-stage thermal manipulation on broiler chicken pectoralis major muscle satellite cell heterogeneity at hatch John Rogers*[1UG], Jorge Banegas[1], Brittany Wall[1], Martha Rueda[2], Jeremiah Davis[2], Joseph Purswell[3], Charles Starkey[1], Jessica Starkey[1] *[1]Auburn University Department of Poultry Science, [2]Auburn University National Poultry Technology Center, [3]USDA ARS Poultry Research Unit*

Multi-stage hatching egg incubators are challenging to manage and still quite common throughout the US broiler industry. It is difficult to satisfy all embryonic temperature requirements in systems where late-stage embryos must be kept from overheating while ensuring early-stage embryos are not at sub-optimal temperatures. Skeletal muscle satellite cells (SC) are muscle-specific stem cells that play a critical role in post-hatch hypertrophic muscle growth. The aim of this study was to assess the effect of thermal manipulation (TM) during late-stage incubation (LSI) on pectoralis major (PM) muscle SC heterogeneity in broiler chicks at hatch. We hypothesized that decreased incubation temperature during LSI would alter SC population densities. Ross 708 × Yield Plus broiler eggs ranging

from 60 to 65 g were pre-warmed at 24 °C for 8 h and incubated at 37.5 °C from embryonic day (ED) 0 to 11 (4 90-egg trays per incubator; total n = 2,160). From ED 11 to 18, eggs were incubated at 1 of 3 temperatures: 37.5 °C (CTL), 36.4 °C (COLD), or 38.6 °C (HOT); n = 2 incubators per TM treatment. On ED 18, eggs were transferred to baskets in hatchers set to 36.7 °C. After 514 h of incubation, chicks from all incubators were removed simultaneously and PM muscle samples were collected from 6 chicks per treatment. Samples were cryosectioned and immunofluorescence stained to facilitate taxonomy of SC populations expressing the myogenic regulatory factors (MRF) and common SC markers, MyoD, MRF4, and Myf5, by fluorescence microscopy on a mm^2 basis and as a proportion of total and myogenic (MRF+) nuclei. Data were analyzed as a 1-way ANOVA with the GLIMMIX procedure of SAS and means were separated at $P \leq 0.05$ with the PDIFF option. Tendencies were declared when $0.0501 \leq P \leq 0.10$. Chicks from COLD incubators had the greatest density of MyoD+:MRF4+:Myf5+ SC (P =0.0201) as well as proportion of (MRF+) nuclei out of total nuclei (P =0.0264) and tended to have the highest proportion of MyoD+:MRF4+:Myf5+ SC (P =0.0644) in their PM muscles. Temperature gradients as little as 1.1 °C during LSI altered PM SC populations chicks at hatch, underscoring the importance of careful hatchery management, and warranting investigation into whether these perinatal changes result in altered meat yields.

Key Words: broiler chicken, incubation, satellite cell, thermal manipulation, myogenesis

M17 The effect of Campylobacter jejuni challenge on the ileal microbiota and short-chain fatty acids concentration in broilers Walid Al Hakeem*[1GS], Jeferson Lourenco[2], Emily Cason[1], Daniel Adams[1], Keila Acevedo Villanueva[1], Shahna Fathima[1], Bikas Shah[1], Revathi Shanmugasundaram[3], Ramesh Selvaraj[1] *[1]Department of Poultry Science, University of Georgia, [2]Department of Animal and Dairy Science, University of Georgia, [3]Toxicology and Mycotoxin Research Unit, USDA-ARS*

Acetate is a short-chain fatty acid that plays an important role in maintaining intestinal barrier integrity. *Campylobacter jejuni* infection decreases the relative abundance of ileal acetate-producing bacteria and ileal acetate concentration, leading to an impaired intestinal barrier. This study aimed to characterize the effect of the *C .jejuni* challenge on the ileal microbiota and ileal SCFAs concentration in broilers. Ninety broiler chicks were randomly divided into three treatments 0, 1 X 10^4, and 1 X 10^8 CFU/bird *C. jejuni* in six replications. At 21 days of age, birds were orally inoculated with PBS (control), 1 X 10^4, or 1 X 10^8 CFU/bird *C. jejuni*. Ileal mucus and contents from each treatment were collected on days 28 and 35 for microbiome and SCFA analysis. The bacterial compositions of the ileal contents were analyzed by sequencing the V1–V9 region of the 16S rRNA gene. *C. jejuni* decreased the alpha diversity (P=0.04) compared to the control group. On the class level, birds inoculated with 1 X 10^8 CFU *C. jejuni* had (P=0.06) a higher relative abundance of *Clostridia* on day 35 compared to the control group and to birds inoculated with 1 X 10^4 CFU *C. jejuni*. On the family level, birds inoculated with 1 X 10^8 CFU *C. jejuni* had (P=0.04) a lower relative abundance of *Lactobacillaceae* on day 35 compared with the control and birds inoculated with 1 X 10^4 CFU *C. jejuni*. On the genus level, birds inoculated with 1 X 10^8 CFU *C. jejuni* had a lower relative abundance of *Candidatus arthromitus* on day 35 compared to the control (P=0.06), and birds inoculated with 1 X 10^4 CFU *C. jejuni* (P=0.09). On the species level, birds inoculated with 1 X 10^8 CFU *C. jejuni* had a lower relative abundance of *Lactobacillus crispatus* on day 28 (P=0.06) and 35 (P=0.01) in comparison with the control group. Birds inoculated with 1 X 10^8 CFU *C. jejuni* had a lower (P=0.005) acetate concentration on day 35 compared with the control group and birds inoculated with 1 X 10^4 CFU *C. jejuni*. In conclusion, *C. jejuni* infection decreases acetate concentration and the relative abundance of beneficial bacteria, such as *Lactobacillus crispatu* and *Candidatus arthromitus,* leading to increased gut permeability.

Key Words: Campylobacter jejuni, Microbiome

M18 The Effect of Dietary Calcium and Phosphorus on Plasma Calcium and Phosphorus Levels Chelsea Phillips*, Carrie Walk, Aaron Cowieson *DSM Nutritional Products*

Birds attempt to maintain plasma calcium (Ca) and phosphorus (P) concentrations at physiological optima via hormonal regulation. However, the extent to which these mechanisms may be influenced by feeding diets with sub-optimal Ca and P supply is not well understood. We conducted a meta-analysis of four separate trials in which divergent Ca and P levels were fed. Plasma total Ca, ionized Ca, and P were measured in each of these studies. Birds were also evaluated for skeletal health issues such as rickets (RKT), bacterial chondronecrosis with osteomyelitis (BCO), and tibial dyschondroplasia (TD). The range of formulated Ca and available P represented by the contributing studies was 0.34-1.67% and 0.2-0.48% respectively. Increasing the formulated dietary Ca to available P ratio from 1 to 5 was found to be associated ($P < 0.05$) with a rise in the plasma Ca to P ratio from 1.3 to 6 mg/dl. Increasing dietary concentration of P resulted in an increase ($P < 0.05$) in plasma P concentration but had no effect ($P > 0.05$) on plasma Ca. Conversely, increasing dietary Ca concentration resulted in an increase ($P < 0.05$) in plasma Ca and a decrease ($P < 0.05$) in plasma P. Birds with a low concentration of plasma Ca had higher ($P < 0.05$) prevalence of BCO and RK. It can be concluded that dietary Ca and P supply influences plasma Ca and P concentration and optimal feeding strategies may be fine-tuned by monitoring plasma mineral concentration.

Key Words: Calcium, Phosphorus, Blood Biomarkers, Skeletal Health

Physiology, Endocrinology and Reproduction II Layers, Breeders

M20 Physiological changes associated with regulation of calcium utilization at the onset of egg production in commercial laying hens. Alejandra Garcia*[1GS], Micaela Sinclair-Black[1], Roselina Angel[2], Bibiana Jaramillo[3], Xabier Arbe[4], David Cavero[4], Prafulla Regmi[1], Monika Proszkowiec-Weglarz[5], Dima White[1], Woo Kim[1], Laura Ellestad[1] *[1]Department of Poultry Science, University of Georgia, [2]Animal and Avian Sciences, University of Maryland, [3]Illuma Alliance, [4]H&N International, [5]Animal Biosciences and Biotechnology Laboratory, USDA-ARS*

The skeletal system is the primary reservoir of calcium (Ca) for eggshell formation in laying hens. At the onset of egg production, vitamin D_3 regu-lates changes in Ca utilization for shell mineralization and medullary bone formation. This study sought to determine 1) changes in intestinal and hepatic gene expression related to Ca regulation at the onset of lay, 2) changes in bone parameters at the onset of lay, and 3) impacts of dietary supplementation with 1α-hydroxycholecalciferol [1α(OH)D_3] on these changes. Ileum, liver, humerus, and tibia were collected from Nick Chick hens (H&N International) at 18 (n=8) and 31 (n=8/diet) wks. All hens were fed developer diet prior to lay (18 wks) followed by either a control or 1α(OH)D_3-supplemented diet through 31 wks. Levels of mRNA related to Ca utilization were measured in ileum and liver by qPCR. Bone mineral density (BMD) and bone mineral content (BMC) were determined for hu-merus and tibia by dual-X-ray absorptiometry, and tibial breaking strength (BS) was analyzed with a three-point bending test. Data were analyzed by one-way ANOVA and Fisher's LSD test when ANOVA indicated sig-nificance (P≤0.05). Changes in mRNA expression were influenced by age but not diet. In liver, expression of vitamin D receptor and its heterodi-meric partner, retinoid-X-receptor alpha, decreased between 18 and 31 wks (P≤0.05). In contrast, parathyroid hormone receptor (*PTH1R*) and calcium sensing receptor (*CASR*) were higher at 31 wks (P≤0.05), with *CASR* having a 40-fold increase. Ileal expression of *CASR* and *PTH1R* also increased at 31 wks (P≤0.05), with *PTH1R* showing a 64-fold in-crease. Likewise, ileal plasma membrane Ca ATPase and calbindin, both required for Ca uptake, increased at 31 wks (P≤0.05). Dietary 1α(OH)D_3 increased tibial BMD and BMC at 31 wks relative to control and 18 wks (P≤0.05). Moreover, control hens had reduced humeral BMD and tibial BS at 31 wks relative to 1α(OH)D_3-fed and 18 wks (P≤0.05). Changes in gene expression at the onset of lay indicate greater sensitivity to hormones regulating Ca homeostasis in ileum and liver and a greater capacity for ileal Ca absorption. Dietary 1α(OH)D_3 maintained bone mineralization after the onset of lay, which could reduce fracture incidences and improve welfare as the hens age.

Key Words: Vitamin D3, Calcium transport, Ileum, Liver, Bone mineralization

M21 Regulation of calcium and phosphorus homeostasis during peak egg production Micaela Sinclair-Black*[1GS], Alejandra Garcia[1], Roselina Angel[2], Bibiana Jaramillo[3], Xabier Arbe[4], David Cavero[4], Laura Ellestad[1] *[1]Department of Poultry Science, University of Georgia, [2]Animal and Avian Sciences, University of Maryland, [3]Iluma Alliance, [4]H&N International*

Within an egg-laying cycle during peak production, the hen maintains a finely orchestrated balance of calcium (Ca) and phosphorus (P) dynamics, which are regulated by vitamin D_3 (D_3). Over time, imbalances in Ca and P utilization lead to poor shell quality and compromised skeletal structure, negatively impacting hen welfare. To understand mechanisms regulating Ca and P homeostasis and how these processes can be optimally main-tained over an entire production cycle, the physiology in young, high-pro-ducing hens should be established. Therefore, the objective of this study was to elucidate circulating levels of D_3 metabolites and mRNA expression of genes regulating Ca and P dynamics during egg formation of hens entering peak production (98.6% hen day egg production). To this end, blood, shell gland (SG), kidney, and ileal mucosa were collected from 25-week-old Nick Chick hens (H&N International; n=12 per time point) at 1.5, 6, 15, and 21 hours post-oviposition (HPOP). Levels of mRNA were determined using real-time qPCR and D_3 metabolites were measured using liquid chromatography-tandem mass spectrometry. Data were analyzed by one-way ANOVA, and means were compared using Fisher's Least Significant Difference test when ANOVA indicated significance (P≤0.05). Plasma membrane Ca ATPase 1 (*ATP2B1*), which facilitates Ca transport, was elevated at 15 and 21 HPOP in the ileum and SG and 15 HPOP in the kidney (P≤0.05). The Ca chaperone protein, calbindin (*CALB1*), had elevated expression at 15 HPOP across all tissues (P≤0.05). Sodium-dependent P transporter 1 (*SLC20A1*) expression was elevated at 15 and 21 HPOP in SG and kidney (P≤0.05), while no differences were observed in the ileum. Levels of 25-D_3 and biologically inactive 24,25-D_3 remained unchanged across time points; however, bioactive 1,25-D_3 increased linearly from 1 to 15 HPOP before dropping at 21 HPOP (P≤0.05). Elevated levels of 1,25 D_3 at 15 HPOP may increase Ca availability to the shell gland and assist in the renal excretion of P generated during bone breakdown, in part elucidating mechanisms regulating Ca and P dynamics in young hens. Strategies aimed at managing 1,25 D_3 levels during shell calcification may maintain Ca and P homeostasis, thus positively impacting egg quality and welfare as hens age.

Key Words: calcium, phosphorus, laying hen, vitamin D3, homeostasis

M22 Recovery of Salmonella injected into the albumen or yolk of hatching eggs and then cold-stored and incubation to day 18. Caitlin Harris*[1,2GS], L. Nicole Bartenfeld Josselson[2], R. Jeff Buhr[2] *[1]University of Georgia, [2]USDA-ARS National Poultry Research Center*

Salmonella spp. are important foodborne pathogens and *S.* Enteritidis (SE) egg contamination is often a result of transovarian transmission during egg formation. SE may contaminate any component of forming eggs, but prior research indicates that the albumen on or the vitelline membrane are the most frequently contaminated. The objective of this study was to compare the recovery of SE from hatching eggs that were injection inoculated into the albumen (AL) or yolk (YO) then cold-stored (CS) and incubated. 240 SPF hatching eggs were collected and randomly divided into 2 trt (n=120 AL or YO). On d0, 10^2 CFU of SE marker strain (100ppm nalidixic acid resistant) was injected into the albumen or yolk of eggs that were then stored for 2d (6°C; 54%RH) then transferred to an incubator (37.5°C; 55% RH). On d0, CSd1, CSd2, and ed1, 15 eggs contents/trt were sampled, 1:1 diluted using BPW, and direct and enriched plated on Brilliant Green Sulfur (BGS) with 100ppm nalidixic acid in duplicate. On ed5, ed15, ed18 of incubation, viable embryos were aseptically removed and sampled separately from egg contents; SE recovery from egg contents and embryos was performed with microbiological methods and 3M™ Molecular Detection System. Direct plate counts were recorded as Log_{10} CFU/mL and analyzed using ANOVA; enrich results were analyzed with Kruskal-Wallis test to determine significance (p≤0.05). On d0, CSd1, and CSd2, all egg contents were 100%+ with enrichment. On all incubation d sampled, there was significantly higher direct recovery of egg contents from YO injected (7.89-8.68 Log_{10} CFU/mL) compared to AL injected (0-0.89 Log_{10} CFU/mL). On ed5 and ed15 no embryos were present for YO injected, and recovery from AL injected egg embryos was 13%+ on ed5 and 0%+ on ed15. On ed18, there was significantly higher direct recovery of egg contents between YO (7.79 Log_{10} CFU/mL) and AL (0.89 Log_{10} CFU/mL), but no significant differences with enrichment (89-100%+). One ed18 embryo that was YO injected was negative, however 56% of enriched embryos from AL injected were SE+. Results from this experiment indicate that hatching eggs injected with SE into the AL can result in

SE+ embryos on ed18, providing more evidence that the AL is the more likely location of SE contamination in forming eggs.

Key Words: Salmonella, egg inoculation, egg cold storage, incubation, vertical transmission

M23 Corticosterone binding globulin may regulate the stress response in broilers Coleman Hatmaker*[GS], Blayne Thomason, Adam Davis, Martha Freeman *University of Georgia*

In birds, free circulating corticosterone (CORT) is the primary glucocorticoid responsible for eliciting a stress response. Corticosterone binding globulin (CBG) is the major circulating carrier of CORT, and although it transports CORT to peripheral tissues, CBG's high affinity for CORT can limit the availability of free CORT to stimulate the stress response cascade in these tissues based on research in songbirds. The goal of current research was to determine if CBG mRNA is expressed in broilers and determine if its expression is altered by fasting. The mRNA expression of CBG was determined in a variety of tissue samples such as muscle, gastrointestinal, hepatic and kidney of 35-day-old broilers fed ad libitum, and in hepatic tissue collected from 44-day-old broilers that had been fasted for 36 hours or fed ad libitum. Theca and granulosa tissue from the preovulatory follicles of 45 to 52 week old broiler breeder hens was also collected at 6 or 72 hours post feeding for evaluation of CBG mRNA expression. Total RNA was extracted from tissue samples and DNase treated for two-step real-time RT-PCR analyses of CBG and GAPDH (control) mRNA expression. Taqman minor groove binding probes and primers were designed using Primer Express (Version 2.0, Applied Biosystems). Like mammalian species, broiler hepatic tissue had greater ($P < 0.05$) expression of CBG than all other tissues examined and is the likely source of circulating CBG. While CBG mRNA was detected in gastrointestinal samples it was not detected in cardiac, pectoralis major or gastrocnemius muscle tissue. The expression of CBG mRNA in liver samples of fasted birds was significantly less than fed broilers. Only the granulosa tissue of hierarchal preovulatory follicles expressed CBG mRNA, and expression was greater ($P < 0.05$) in fasted hens. The results indicate decreased hepatic CBG mRNA expression during metabolic stress could increase the level of free CORT available to elicit stress responses in peripheral tissues, but that local cytosolic production of CBG in peripheral tissues capable of producing it could bind entering free CORT to delay or mitigate the intended stress response.

Key Words: broiler breeder, feed restriction, follicles

M24 Graded levels of Eimeria infection temporarily ceased the egg production of Hy-Line W-36 laying hens at peak production Milan Sharma*[GS], Guanchen Liu, Doyun Goo, Dima White, Woo Kim *Department of Poultry Science, University of Georgia*

An experiment was conducted to investigate the effects of graded *Eimeria* challenge on production performance of Hy-Line W-36 laying hens at peak egg production. A total of 360, 25-wk old laying hens were randomly allocated into five treatment groups with six replicate cages, including a non-challenged control group. A mixed *Eimeria* species solution containing 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* sporulated oocysts per mL was prepared and inoculated to one group as a High-dose treatment at wk 25. The 2-fold serial dilution was done to prepare the Medium-High (25,000 *E. maxima*; 25,000 *E. tenella*; and 125,000 *E. acervulina*), the Medium-Low (12,500 *E. maxima*; 12,500 *E. tenella*; and 62,500 *E. acervulina*), and the Low (6,250 *E. maxima*; 6,250 *E. tenella*; and 31,250 *E. acervulina*) dose treatments and inoculated to three remaining groups, respectively. The body weight (BW) and body (BWG) of laying hens were measured from 0-14 days post-infection (DPI). Average daily feed intake (ADFI) and hen day egg production (HDEP) were measured from 0-15 and 0-28 DPI. Growth performance data were analyzed using one-way ANOVA, whereas split-plot in time for ADFI and HDEP. A significant level was set at $P \leq 0.05$, and means were separated using Fisher's LSD. A significant linear reduction in BW and BWG was observed with increased *Eimeria* challenge dosage on both 6 and 14 DPI ($P<0.001$, P-Lin<0.0001). An interaction between the *Eimeria* dosages and DPI was observed for ADFI. Feed intake started to drop in the challenged groups from 4 DPI and did not recover until 15 DPI. The most significant drop in feed intake was observed on 7 DPI in all the challenged groups. An interaction between the *Eimeria* challenge dosage and DPI was also observed for daily HDEP ($P<0.0001$). Overall, HDEP was lower in the challenged groups compared to the control. Daily HDEP in challenged groups started to drop from 8 DPI and became similar to the control birds only on 24 DPI. Egg production temporarily ceased in most of the laying hens challenged with the High and Medium-High groups. In conclusion, coccidiosis negatively affected the growth performance, reduced daily feed intake, and temporarily ceased the egg production of Hy-Line W-36 laying hens when infected at peak production.

Key Words: Laying hens, Eimeria, Coccidiosis, Hen-day egg production

M25 The effect of dietary energy level and feed restriction on energy intake, body fat percentage, and egg production in laying hens to 50 weeks of age. Jo Ann Chew*[GS], Thiago Noetzold, Martin Zuidhof *Department of Agricultural, Food and Nutritional Science, University of Alberta*

This study examined the effect of dietary energy level during rearing (DIET) and feed restriction (FR) on energy intake, body fat and egg production of laying hens. A 2 × 4 factorial arrangement of treatments with 2 FR levels (non-restricted (NR) and restricted (R)), and 3 DIET treatments (Low, Standard, or High dietary metabolizable energy (ME): 2,600, 2,800, or 3,000 kcal/kg) was used. The fourth DIET treatment (Choice) enabled birds to choose from the three diets. R birds were restricted fed using a precision feeding system to achieve the lower range of the breeder-recommended target BW. Lohmann Brown-Lite pullets were randomly assigned to either of 2 floor pens from 0 to 50 wk of age. All birds (n=184) were fed with multi-feeder feeding stations that allocated the correct amount of the correct diet to each individual bird. Thus, each bird was an experimental unit. All birds were photostimulated at 18 wk of age. Energy intake was summarized weekly. Body fat percentage was measured using Dual Energy X-ray Absorptiometry every 8 wk from 8 to 48 wk of age (n=80). Egg production was summarized weekly to 50 wk of age. Data were analyzed as a 3-way ANOVA with DIET, FR and age as main effects, and means were separated using Tukey's multiple range test. Differences were reported where $P<0.05$. DIET affected neither energy intake nor body fat percentage. NR birds had greater energy intake than R birds (225 vs 218 kcal/d, respectively; $P=0.009$), and greater body fat percentage than R birds (13.9 vs 11.7%; $P<0.001$). Neither FR nor DIET affected age at first egg. DIET did not affect total number of eggs to 50 wk of age. Birds fed NR tended to lay more eggs to 50 wk of age than birds fed R (194 versus 188 eggs; $P=0.078$). Overall, NR birds had a greater energy intake and body fat than R birds and tended to have greater egg production. DIET had no effect on any of the measured parameters.

Key Words: precision feeding, laying hens, feed restriction, body composition, egg production

M26 Impact of eggshell translucency and color intensity on egg quality parameters, moisture loss, and chick weight Leticia Orellana*[GS], James Krehling, Kaicie Chasteen, Marcela Quino, Carla Guardado, Luis Munoz, Yagya Adhikkari, Cesar Escobar, Matthew Bailey, Ken Macklin *Auburn University*

Previous research has correlated the effect of egg quality with hatchability and chick weight. This study aimed to evaluate the impact of translucency and color on egg quality parameters, moisture loss and chick weight of broiler eggs. A total of 4320 eggs from Ross 708 broiler breeder hens from 4 different flocks (35 to 65 weeks old) were collected for this study. An equal number of eggs from each flock were selected according to egg-

shell translucency level (1-3) and color (dark and light). For translucency classification, a 3-point subjective scoring system was used based on the amount and coverage of clear spots or mottling in the eggshell. Eggshell color was evaluated using an electronic colorimeter while egg thickness was taken using a noninvasive ultrasound gauge. Data for this experiment 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 when P≤0.05. Results show that translucency affected initial egg weight (IW)(P=0.0342) and transfer egg weight at day 18 (TW)(P=0.0001) where low translucent eggs had more weight (IW=68.12 g, TW=62.21 g) than high translucent eggs (IW=67.50 g, TW=61.08 g). Regarding chick weight, low translucent eggs were 1.44 g heavier than high translucent eggs. Moisture loss percent at hatch (P<0.0001), in contradiction to the above, was greater in high translucent eggs (9.5%) than in low translucent eggs (8.9%). Eggshell thickness (P<0.0001) was found to be thicker in high translucent eggs (468.6 μm) compared to low translucent eggs (432.2 μm). The color of the eggshell was found to affect IW (P=0.0018) and TW (P<0.0001) where dark-colored eggs had a higher weight (IW=68.10 g, TW=62.07 g) than light-colored eggs (IW=67.45 g, TW=61.07 g). For chick weight (P=0.0128), dark-colored eggs produced chicks that were 0.55 g heavier than light-colored eggs. Moisture loss (P<0.0001) was higher in light-colored eggs (9.9%) than in dark-colored eggs (8.8%). In conclusion, low translucent and dark-colored eggs had better egg quality through the parameters of IW, TW, and chick weight. The largest reduction was found for moisture loss and eggshell thickness when eggs are highly translucent or light-colored.

Key Words: Hatching eggs, translucency, color, chick weight, moisture loss

M27 The effects of dry hydrogen peroxide on hatchery performance when applied in single stage incubators Kylie Bruce*[1GS], Russ Stephens[2], Jeanna Wilson[1], Brian Jordan[1] *[1]University of Georgia, [2]Synexis*

Additional research has shown that using DHP in single stage incubators can achieve the same level of microbial reduction on eggshells as pre-fogging eggs with formaldehyde prior to set. The next step in the research process, and thus the purpose of this experiment, was to directly compare dry hydrogen peroxide to the traditional method of using formaldehyde for microbial reduction. The trial is set in a commercial broiler hatchery, where DHP units were installed throughout the egg room, inside incubators in one incubator hall, the transfer room, and in half of the hatchers. For the first three months of the trial, the DHP was used in addition to formaldehyde, then the formaldehyde will be turned off in the DHP treated hatchers for the next three months of the study. Eggs were followed throughout the hatchery and 3 & 7-day percent mortality, hatchability, HOF, fluff counts, egg and surface swabs, chick quality summaries, breakouts, and necropsy of 3-day mortality were parameters of interest, with hatchability and HOF tracked daily for analysis. After three months of data collection, a t-test was performed on daily hatchability and HOF, and summary results indicate a significant increase in percent hatchability when eggs were treated with DHP in incubators and hatchers (p=.0001). When broken out by treatment group, there was no significant difference in hatchability for eggs treated in the incubator and hatcher, treated in the incubator but not in the hatcher, or not treated at all, but there was a significant difference in hatchability for eggs not treated in the incubator but treated in the hatcher when compared to 2022 baseline averages prior to the beginning of the trial (p=.01, one-way ANOVA). In conclusion, DHP, when used in addition to formaldehyde, may improve hatch throughout the hatchery.

Key Words: dry hydrogen peroxide, hatchery sanitation, formaldehyde, microbes

M28 Utilizing the growing feather pulp as a cutaneous test system to monitor local cellular responses to autogenous Salmonella vaccines in sensitized and non-sensitized Light-brown Leghorn pullets Chrysta Beck*[GS], Jossie Santamaria, Marites Sales, Gisela Erf *University of Arkansas System, Division of Agriculture, Department of Poultry Science*

Salmonella vaccine administration aims to reduce chick morbidity and prevent poultry product contamination. These vaccines produce systemic humoral responses, but little is known about cellular responses at the vaccination site. By using the growing feather (GF) pulp as a cutaneous test system, we can noninvasively monitor inflammatory responses induced by bacterial antigens in a complex tissue. For this study, we investigated local cellular (GF pulp) and systemic humoral (blood) responses to intradermal (i.d.) injections of autogenous *Salmonella* bacterin vaccines (SV) in non-sensitized (NS) and sensitized (S) Light-brown Leghorn pullets. Treatments, i.e. SV1, SV2, *Salmonella* Enteritidis (SE) LPS, or oil-water emulsion (vehicle), were i.d.-injected into pulps of 15 week-old NS- & 19 week-old S-pullets (10μL/GF, 21 pulps/bird, ≥4 birds/treatment). NS- and S-pullet GF pulps were collected before (0d) and at 6h, 1d, 2d, and 3d post-injection (p.i.). For S-pullets, pulps were also collected at 5d and 7d-p.i. At each time point, pulp cell suspensions were immunofluorescent-stained for leukocyte population analysis by flow cytometry and one pulp was flash-frozen for cytokine gene-expression analysis by qRT-PCR. Blood plasma was isolated from NS- and S-pullets at 0, 3, 5, 7, 10, 14, 21, and 28d-p.i. to quantify SE-specific antibody levels by ELISA. Data were analyzed by two-way ANOVA (time, treatment, time by treatment interaction) with significance set at $P \leq 0.05$. Independent of treatment, heterophil and macrophage levels were greatest at 6h-p.i. and 3d-p.i. in NS- and S-pullets, respectively, while the vehicle also recruited CD4+ T cells in both NS- and S-pullets. Regardless of treatment, gene expression of IL-1β, IL-6, and IL-8 was highest at 6h-p.i. in NS-pullets, but steadily increased up to maximal levels by 2d-p.i. in S-pullets. Plasma levels of SE-specific IgM were elevated from 5d- to 10d-p.i. in NS- and S-SV-injected pullets. In contrast, IgG levels exhibited primary and secondary response profiles in NS- and S-SV-injected pullets, respectively. In summary, i.d. administration of *Salmonella* bacterin vaccines initiated local inflammation in NS- and S-pullets, with prolonged heterophil infiltration in S-pullets that was sustained by pro-inflammatory cytokines.

Key Words: Salmonella, Vaccine, Antibodies, Cytokines, Leukocytes

M29 Effect of fish meal on the semen quality of broiler breeders and Red Jungle Fowl Emmillie Boot*[GS], Rebecca Wysocky, Ramon Malheiros *North Carolina State University*

The hatchability of broiler chicks has decreased in recent years, resulting in both the broiler breeder hen and rooster needing evaluation for possible improvements in reproduction. Since the rooster is responsible for fertilizing many eggs a day, improving or sustaining sperm quality parameters is essential to improving hatchability. The objective of this study was to determine if the addition of 3.2% inclusion rate of fish meal to the diet of broiler breeder roosters and red jungle fowl would improve sperm quality parameters. Twelve broiler breeder roosters (6 replicates per treatment) and 10 red jungle fowl (5 replicated per treatment) were assigned to either the fish meal treatment or the control (industry standard). All broiler breeders used were fed 140 grams of their respective diets daily while all jungle fowl were fed *ad libitum*. Semen was collected and analyzed every 2 weeks from 39-44 weeks of age for a total of 60 samples. Immediately after collection, 100 microliters of each sample were diluted in 2mL of semen extender and kept at 40C until testing was completed with the SQA-Vt™ Automated Sperm Quality Analyzer. Semen volume, sperm concentration, total sperm count, and sperm motility were measured, and data was analyzed via one-way ANOVA in JMP Pro 15, with a significance level of P<0.05. While the data was not statistically significant, there were trends to be noted. Jungle fowl fed the fish meal diet had a higher total semen volume (P=0.0841) and the average sperm motility was higher than jungle fowl fed the control diet. Broilers that were fed the fish meal diet

had higher total sperm count, motility, and total semen volume than the control. Therefore, the results of this study suggest that the addition of fish meal to the diet of broiler breeder roosters and other chicken breeds is a viable additive to increase semen quality.

Key Words: Semen, Sperm, Rooster, Broiler Breeder, Jungle Fowl

Pathology I

M30 A comparative genomics approach to investigate virulence-associated genes in avian pathogenic E. coli during infection of broiler embryos Chalise Brown*[1GS], Grayson Walker[1], Marion Suyemoto[1], Heather Harbottle[2], Jeffrey Gilbert[2], Marilyn Martinez[2], Steven Foley[3], Luke Borst[1] *[1]Population Health and Pathobiology, NC State University College of Veterinary Medicine, [2]U.S. FDA, Center for Veterinary Medicine, Office of New Animal Drug Evaluation, [3]U.S. FDA, National Center for Toxicological Research*

Avian pathogenic *E. coli* (APEC) causes colibacillosis in poultry and is the leading cause of morbidity and mortality in the U.S. poultry industry. Moreover, the economic impact of APEC is profound; the U.S. broiler industry loses an estimated $40 million annually from carcass condemnations alone. This impact mandates further investigation of APEC virulence factors and their genetic basis. In this project, our goal was to correlate APEC genotypes with virulence phenotypes as determined by embryo lethality assay (ELA) to more clearly elucidate APEC virulence factors contributing to chick embryo mortality. Whole-genome sequencing of 25 APEC strains isolated from septic chickens and turkeys was done using an Illumina MiSeq. Sequencing reads were assembled in CLC genomics workbench and annotated using the RAST server (Rapid Annotation using Subsystems Technology). The ELA is a well-established tool for studying APEC virulence. Embryos were challenged with APEC strains via the intra-allantoic route at 12 d and candled every 24 hr for 5 days to determine viability. Embryo survivability was plotted as Kaplan-Meier survival curves and analyzed by the log rank test of significance ($P \leq 0.05$) relative to strains of known virulence. Strains were designated as virulent or nonvirulent using a cut-off point of 50% survivability based on previous assays. The genome annotations of the 3 most virulent strains were compared to the 3 least virulent strains using an 80% similarity threshold and the APEC O1 complete reference genome. In this analysis, we identified 12 predicted proteins in 3 virulent strains including the aerobactin siderophore biosynthesis proteins and a putative surface-exposed virulence protein homologous to the BigB adhesin of *Brucella abortus* which were absent in the 3 least virulent strains. The BigB adhesin is a known virulence factor in *Brucella abortus* but these proteins have not been previously described in APEC. This genetic screen provides insight into APEC colonization *in ovo*, which is important for research of APEC pathogenesis in poultry.

Key Words: E. coli, Genomics, Pathogenesis

M31 Evaluation of virulence characteristics of avian pathogenic Escherichia coli isolates from broiler breeders Jiddu Joseph*[GS], Linan Jia, Li Zhang, Pratima Adhikari, Reshma Ramachandran *Mississippi State University*

Avian pathogenic *Escherichia coli* (APEC) infections are widespread in the poultry industry and cause severe economic impacts on poultry production worldwide. Recent findings emphasize the importance of controlling APEC in broiler breeders due to the high degree of vertical transmission from breeders to chicks through contaminated eggs. In addition, the constant evolution of genetic diversity among APEC strains requires continuous monitoring in broiler breeders. Therefore, in this study, we evaluated the virulence characteristics of 28 APEC isolates collected from broiler breeders affected with colibacillosis in Mississippi using embryo lethality assay. Also, the relationship between phenotypic and genotypic virulence was determined. A total of 558 eggs obtained from a 38-week Ross708 x YPM breeder flock were divided into 31 groups (n=18/group), 28 isolates and 3 controls (PBS-injected, non-injected, and dry-punch), and incubated under standard conditions. On day 12, the allantoic sac of embryonated eggs was inoculated with 0.1mL of an overnight culture of APEC isolate reconstituted to a final concentration of 100-500 CFU/mL. The eggs were candled daily for 7d post-inoculation and embryo mortality was recorded. Pearson correlation analysis using SAS 9.4 was performed to determine the relationship of embryo mortality with the presence of virulence genes and day-old chick pathogenicity from a previous challenge study with these isolates. Embryo mortality among the 28 APEC isolates ranged from 6 to 78%. Based on embryo mortality, 23 (82.14%) isolates were categorized as highly virulent, 4 (14.28%) as moderately virulent, and 1 (3.58%) as avirulent. Further, embryo mortality was strongly correlated with day-old chick pathogenicity ($R = 0.73$, $P<0.01$). Positive correlations were observed for embryo mortality with the presence of virulence genes that predict APEC, *iroN, iss, ompT, hlyF,* and *iutA* ($R=0.53$, $P<0.01$). In conclusion, this study enabled us to characterize the APEC isolates from broiler breeders based on their phenotypic virulence as well as establish a relationship between phenotypic and genotypic virulence. Moreover, the virulent isolates identified could be used in future research to develop *E. coli* challenge model and vaccines for broiler breeders.

Key Words: avian pathogenic E.coli, broiler breeder, embryo lethality assay, virulence characterization, virulence genes

M32 Effects of virulence genotype, motility, and multi-locus sequence type of Escherichia coli on development, mortality, yolk retention, and weight loss of chicken embryo Fozol Ovi*[1GS], Linan Jia[1], Anuraj Sukumaran[1], Reshma Ramachandran[1], Douglas Cosby[2], Dan Wilson[3], Ishab Poudel[1], Pratima Adhikari[1] *[1]Mississippi State University, [2]USDA-Agriculture Research Service, [3]Wilson Veterinary Co.*

Previous research shows that, virulence-associated genes (VAGs) in avian pathogenic *Escherichia coli* (APEC) are correlated with embryo lethality (EL). Embryo lethality assay (ELA) was proposed to confirm the virulence of suspected APEC. Several other factors may compound the ELA. Motile bacteria may have advantages while infecting the embryos inside eggs. Besides, a unique lineage of *E. coli* might cause a higher EL. Therefore, our objective was to evaluate the effects of virulence genotype (VGt), motility and sequence type (ST) of *E. coli* on EL, relative embryo weight (REW), yolk retention (YR) and egg weight loss (EWL) between 12 to 18 days of incubation. We evaluated swarming motility (SM), twitching motility (TM) and VGt of 29 *E. coli* isolates. The VGt was determined based on the presence of 5 key VAGs. Isolates were classified as avirulent (A), moderately virulent (M) or virulent (V) based on VGt. SM and TM assay further classified those isolates as non-motile (n), motile (m) or hypermotile (h). Overall motility (OM) score was assigned by combining SM and TM. The ST of those isolates was determined by multi-locus sequence typing. Around 100 CFU of each isolate was injected into the allantoic cavity of 15 fertile eggs on day 12. EL was evaluated on day 18. REW, YR and EWL of the remaining live embryos were also recorded. These data were analyzed in a split-plot design by SAS 9.4 and $P<0.05$ was considered significant. The main factor was VGt, OM was the sub-factor, and ST was the covariance. Mortality data were evaluated in a binomial model. VGt and OM interaction affected EL ($P<0.001$). EL of non-motile isolates of virulent and moderately virulent class was lower than the motile and hypermotile isolates of the same class (V: n-10.0 vs h-70.7 and m-65.7%; M: n-13.3 vs h-26.7 and m-48.9). VGt affected the REW ($P=0.029$). Avirulent isolates caused the poorest REW (A-37.5 vs

M-41.5 and V-40.3%). ST did not cause a significant variation in the responses. Thus, we conclude that, SM may compound the effect of VGt on ELA. Avirulent *E. coli* hinders embryo development whereas virulent *E. coli* causes high EL. Regardless of isolate type, *E. coli* did not interfere with YR, which indicates maternal antibodies in the yolk could be passed down to the next generation.

Key Words: APEC, Embryo lethality assay, Sequence type, Motility, Virulence associated genes

M33 Microbial analysis of feed ingredients and manufactured animal feed Cesar Escobar Lobo*[GS], Luis R. Munoz, Matthew A. Bailey, James T. Krehling, Wilmer J. Pacheco, Ruediger Hauck, Kenneth S. Macklin *Auburn University*

Animal feed can be a source of *Salmonella* spp., *E. coli*, *Clostridium perfringens*, and other pathogens of human and animal health interest. Contamination of feed may occur at any time during growing, harvesting, processing, manufacturing, storage, and distribution of it. The aim of this project was to analyze and determine the microbial content of feed ingredients and manufactured feed from different feed mills in the United States focusing on *Salmonella* spp., *E. coli*, and *Clostridium* spp. A total of 269 samples (feed ingredients and manufactured feed) were collected from 6 feed mills (A, B, C, D, E, and F). Microbial isolation was performed using selective media and colony counts are presented as follows: *Clostridium* spp. counts (CSC), *E. coli* counts (ECC), *Enterobacteriaceae* counts (ENC), aerobic counts (AEC), and anaerobic counts (ANC). Colony forming units, were $\log_{10}$ transformed and analyzed using a GLM model, Proc Glimmix (significant $P \leq 0.05$), means were separated using Tukey's HSD in SAS® 9.4 software. Differences ($P<0.05$) were observed comparing manufactured feed with post mixing samples having higher ECC and ENC compared to post cooling and pellet loadout, however, pellet loadout had higher CSC. A comparison of feed ingredients showed that DDGS, poultry by-product meal and soybean meal were the samples with lower CSC, ECC, and ENC levels, while meat and bone meal had the higher CSC levels and wheat middling the higher ECC, ENC, AEC, and ANC. From the meat and bone meal samples, 4 *Salmonella* isolates were recovered using pre-enrichment/enrichment procedure, the serovars identified were *S.* Oranienburg, *S.* Senftenberg, *S.* Agona, and *S.* Infantis. Based on these results, the pelleting process is effective in decreasing ECC and ENC, however CSC can persist through this process. Meat and bone meal can be a source of CSC and *Salmonella*.

Key Words: Salmonella, E. coli, manufactured feed, feed ingredients

M34 Effect of Salmonella challenge doses and times on Salmonella colonization in turkey poults Hanseo Ko*[GS], Doyun Goo, Hanyi Shi, Woo Kyun Kim *University of Georgia*

The objective of this study was to investigate the effect of different *Salmonella Typhimurium* (ST) challenge doses (10^4 and 10^8 CFU/mL) and challenge ages (d 0, d 3, and double challenge at d 0 and d 7) on *Salmonella* colonization in turkey poults. A total of 420 turkey poults (male, as hatched) were randomly allocated into 35 cages in the completely randomized design (seven treatments with five replicates) for 21 d. Seven treatments included non-challenged control (NC), T1 (challenged with ST 10^4 CFU/mL at d 0), T2 (challenged with ST 10^8 CFU/mL at d 0), T3 (challenged with ST 10^4 CFU/mL at d 3), T4 (challenged with ST 10^8 CFU/mL at d 3), T5 (challenged with ST 10^4 CFU/mL at d 0 and d 7), and T6 (challenged with ST 10^8 CFU/mL at d 0 and d 7). All birds in NC and ST challenge groups orally received 0.5 ml of the sterilized PBS per bird and 0.5ml of ST 10^4 or 10^8 CFU/mL per bird, respectively. The growth performance and ST loads of total ceca, liver, and spleen were evaluated weekly. All the data from growth performance parameters and ST loads in different organs were analyzed by One-way ANOVA with Tukey's posthoc test, and the specific orthogonal contrasts were performed. A Chi-squared test was used for comparisons of ST-positive samples. The lowest body weight gain (BWG) and feed intake (FI) during 0 - 7d were found ($P < 0.05$) in T4. During 14 – 21 d, the BWG and FI in T6 were lower ($P < 0.05$) than those in NC. The cecal ST loads in T2 were higher ($P < 0.05$) than those in T1, T3, and T4. The cecal ST loads in T2 and T6 were higher ($P < 0.05$) than that in T3 at d 7. The increased cecal ST loads were found ($P < 0.05$) in T2 and T6 compared with T3. At d 21, the cecal ST loads in T2 were higher ($P < 0.05$) than that in T1, and the highest numbers of ST-positive ceca samples were found ($P < 0.05$) in T2 and T6. The ST-positive liver and spleen samples did not detect in T3 and T7 at d 7. This study indicated that the ST infection had adverse effects on growth performance, ST 10^8 CFU/mL challenge at d 0 promoted ST translocations and colonization in turkey poults.

Key Words: Salmonella Typhimurium, Turkey, Foodborne pathogen, growth performance, Salmonella challenge model

M35 Effect of a triple-strain Bacillus-based probiotic on in vitro inhibition of Enterococcus cecorum isolated from broilers raised in commercial farms Antoine Meuter*[1], Dorthe Sandvang[1], Line Skjoet-Rasmussen[1], Douglas Duane-Rhoads[2] *[1]Chr. Hansen, A/S, [2]Arkansas University*

Enterococcus cecorum is usually recognized as a harmless commensal of the gut of broiler chickens. However, dysbacteriosis conditions can allow this opportunistic pathogen to overgrow and benefit from loosening of tights junctions to translocate from a leaky gut to the bloodstream. *E.cecorum* can then be transported to specific tissues (e.g: joints) or organs (e.g: heart) where it can induce significant damages. The clinical picture of pathogenic *E. cecorum* infection in a flock is high mortality due to septicemia early in the growing period followed weeks later by lameness due to osteomyelitis of the femoral head.

E.cecorum currently causes a lot of damage on the profitability of broiler operations worldwide with increased mortality, poor production, and increased condemnation. In this context, 10 isolates of *E.cecorum* were collected from broiler chickens raised in US farms with the aim of evaluating the in vitro inhibition capability of a triple-strain *Bacillus*-based probiotic.

A pathogen inhibition assay was conducted to assess the capacity of the supernatant of the probiotic to inhibit the growth of those different *E.cecorum* isolates. Growth of the 10 isolates was measured with and without the probiotic supernatant added and optical density was utilized to assess growth. All experiments were conducted in triplicates, t-test was applied to the results and $P<0.05$ was considered significant.

Results revealed that the triple-strain *Bacillus*-based probiotic had a very high magnitude of inhibition against *E. cecorum*growth ranking from 87.5 to 100% for all 10 isolates ($P<0.05$).

This strong *in vitro* capability for direct inhibition of clinical *Enterococcus cecorum* isolates can be explained by the ability of the different *Bacillus* strains in the probiotic to release specific inhibitory lipopeptides like surfactins and fengycins to its nearby surroundings. *In vivo* studies are planned to confirm the efficacy of this triple-strain *Bacillus*-based technology in commercial broilers challenged with E.*cecorum*.

Key Words: bacillus, probiotic, broilers, Enteroccocus cecorum, inhibition

M36 Virulence of Enterococcus cecorum strains associated with pericarditis and early mortality in broilers Grayson Walker*[GS], M Suyemoto, Chalise Brown, Luke Borst *North Carolina State University College of Veterinary Medicine*

Virulent strains of *Enterococcus cecorum* (EC) are known to escape the gastrointestinal tract of broilers and cause septicemia followed by spinal lesions (enterococcal spondylitis), which result in paralysis and mortality late in the production cycle. In contrast to this traditional clinical presen-

tation, there are increasing reports of spikes in 2-week mortality due to EC pericarditis. To further investigate potential causes of this change in clinical disease course, the genetic relationships among 54 EC isolates from spinal lesions (SA), pericarditis lesions (PC) and hatchery residue were obtained from multiple locations in the southeastern United States and genotyped using pulsed-field gel electrophoresis. Band-based cluster analysis with an 80% identity threshold revealed 3 distinct groups that were predominantly associated with SA or PC lesion types or hatchery residue. A subset of 36 isolates representing each group was selected for whole-genome sequencing and SNP-based phylogenetic analysis. PC were genetically dissimilar to SA strains and clustered with hatchery residue isolates in the phylogenetic analysis. In an embryo lethality assay, 6 PC strains were moderately virulent (63-73% survival) relative to SA3, a well-characterized strain of known virulence isolated from a spinal lesion (50% survival). In our experimental bird infection model, 3 groups of 120, 1-day-old broiler chicks were randomly distributed among 6 replicate pens per group and orally inoculated with either pericarditis-associated strains or SA3. Spleen cultures were collected at 14 and 35 days to monitor septicemia, and pericardial fluid was cultured for all dead or euthanized broilers with gross pericarditis. Surprisingly, challenge with SA3 resulted in either similar or significantly greater ($P \leq 0.05$) prevalence of sepsis and pericarditis than did challenge with PC strains. These data indicate that strains of pathogenic EC associated with pericarditis and early mortality are genetically distinct from strains isolated from spinal lesions, yet predisposing factors and alterations in virulence mechanisms resulting in acute septicemia remain unclear.

Key Words: broiler, bacterial infections, pericarditis, Enterococcus

M37 Evaluation protection of H5 vaccination regimes against early challenge with HPAI-H5N8 Clade 2.3.4.4 Wael Elfeil*[1], Mohamed Rady[2], Magdy Elkady[3], Walid Kilany[2], Mohamed Hamoud[4] *[1]Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Suez Canal University, [2]Animal Health Research Institute, Agriculture Research Center, [3]Department of Poultry Diseases, Faculty of Veterinary Medicine, Beni-Suef University, [4]Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Cairo University*

The aim of this work was to evaluate effectiveness of inactivated H5 vaccine in different vaccination regime either as single vaccine dose at 7-day of age, two-dose regime (1/10-day of age) or using as one and half manufacture recommended dose at 7-day of age against early challenge at 28-day of age with HPAI-H5N8 clade 2.3.4.4 virus. 75,000 one-day old chicks obtained from commercial hatchery placed in three commercial broiler station "25,000 birds/station" (G-1 two-dose regime, G-2 one and half dose regime and G-3 single-dose regime) and 40 one-day old chicks moved to BSL-3 as control groups (non-vaccinated group challenged "G-4" and Non-vaccinated non challenge group "G-5"). On weekly basis blood samples, cloacal swabs and oropharyngeal swabs collected check develop of Humoral immune response and exposure to any life-threatening Respiratory virus (Avian influenza" AIV", Newcastle disease "NDV" and infectious Bronchitis "IB" virus). At 26-day of age 25 birds from each station (G1-3) moved to BSL-3 and kept under observation for 36 hours; cloacal and oropharyngeal swabs collected and three birds from each group euthanized, and internal organs examined for three repeated times with 12 hours interval to ensure that birds free from any live threating viral respiratory pathogen (AIV, NDV, IB). Birds in G1-4 challenged with HPAI-H5N8 clade 2.3.4.4 (10^6 EID_{50}) in 0.5 ml/ bird PSB via intranasal route and birds in G-5 received 0.5 ml PBS via intranasal route. Birds in G1-5 kept in BSL-3 for 10 days under observation and oropharyngeal swabs collected on 3,6,9 days post challenge (dpc). Regarding protection virus against mortalities following challenge with HPAI-H5; were 100% (12/12), 91.7% (11/12), 91.1% (11/12), 0% (0/12) and 100% (12/12) in groups 1-5 respectively. Regarding virus shedding birds in G-1 showed significant lower shedding virus (amount of virus shedding and number of shedding in comparison to G2/3. In conclusion, using homologous H5 inactivated vaccine in two-dose regime can provide protection to commercial broiler chicken against early challenge with HPAI-H5N8 clade 2.3.4.4 virus as early as 28 days of age, with significant lower shedding rate: the application of such regime can be an effective tool to control HPAI under a vaccination strategy.

Key Words: HPAI clade 2.3.4.4., field challenge, H5N8 early challenge, commercial broilers, maternal immunity

Pathology II

M39 Genotypic characterization of infectious bursal disease viruses (IBDV) detected in the Americas between 2019 to 2022. Hugo Ramirez*[1GS], Fernando Vargas[2], Lucas Colvero[3], Francisco Rios[4], Jesus Cabriales[4], Rosmar Marcano[4], Cesar Reyes[5], Pablo Nervi[6], Martha Pulido - Landinez[1], Alejandro Banda[1] *[1]Mississippi State University, [2]Merck AH US, [3]MSD AH Brazil, [4]MSD AH México, [5]MSD AH Perú, [6]MSD AH Argentina*

Infectious bursal disease (IBD) is caused by the genus *Avibirnavirus,* and is characterized by important immunosuppression. In addition, very virulent strains can induce high mortality. Due to the clinical and pathological effects of different IBDV subtypes and their impact on the chickens' performance, it is necessary to implement frequent monitoring to determine the subtypes that are present in the poultry-producing areas.

This study aimed to identify the IBDV genotypes present in the US and four Latin American countries. Bursa samples were collected during technical visits to broiler farms presenting immunosuppression problems in the Southern U.S. and South American countries, including Argentina, Colombia, Mexico, and Peru. Fresh bursal samples were collected from farms in the U.S. Samples of bursa from Latin American countries were spotted on FTA cards. Extracted RNA was reverse transcribed and amplified by PCR using primers targeting the hypervariable region of the VP2 gene, followed by nucleotide sequencing. Phylogenetic analyses were conducted using the Neighbor-Joining algorithm with 1000 bootstrap replicates. Genotypes were determined as per the nomenclature system proposed by Dr. D.J. Jackwood.

In the Southern U.S., variant strains classified within genotype G2b were predominant. One sequence shared a common ancestor with genotype 6 (ITA02 strain). In Argentina, most strains grouped in the same branch with viruses of genotype 3a (UK661), 3b, and 3c. The remaining sequences were associated with genotype 4 (dIBDV), Winterfield, Faragher, STC, and genotype 2b (T1). In Colombia, most of the strains were classified as genotype 2b, a couple of sequences as G2a(Delaware-E), and one as Winterfield. In Mexico, most of the strains belonged to genotype 2a (Delaware-E). Finally, in Peru, most of the strains were classified as genotype2b (T1).

Identifying these IBDV variants must be considered important in terms of poultry health and because it can affect the broilers' productive performance when they get susceptible to other diseases. These results can help decide vaccination programs to control infectious bursal disease.

Key Words: IBDV, Americas, Variant

M40 Assessing the effect of time of infection with Cochlosoma anatis in turkey poults on flock uniformity and gut health. Justin Lowery*[1GS], Catherine Fudge[2], Christina Sigmon[1], Zohreh Mehdipour[1], Jasmine Wiitala[1], Chongxiao (Sean) Chen[2], Lin Walker[1] *[1]North Carolina State University, [2]University of Georgia*

The flagellated protozoan parasite, *Cochlosoma anatis*, is a widespread turkey pathogen in the eastern United States. Infection commonly results in flock non-uniformity, runting, diarrhea, and depression with high morbidity and relatively low flock mortality. The cause of non-uniformity in affected flocks is suspected to be a construct of individual responses to infection. The objective of this study was to understand the impact of infection time on growth performance and uniformity in turkey poults. In this study, 200 day-old poults were evenly placed in 20 isolation cages (10 birds/cage) with 5 cages assigned to each treatment: 0, 7, and 14 days of age infection with a non-infected control (NC). At the time of challenge, each bird was orally gavaged with ~500,000 *C. anatis* cells except the NC group, then grown to 28 days of age. Individual bodyweights were recorded throughout the trial at D0, D14, D21, and D28, with feed intake (FI) measured at D28. Also, at D28, 2 birds/cage were orally gavaged with 4mg/kg FITC-d and their blood collected one hour later to assess intestinal permeability via FITC-d blood concentration. All data analysis used SAS 9.4 with a statistically significant p-value<0.05. This study showed elevated mortality associated with earlier infection, evidenced by significantly higher mortality throughout the study in D0- and D7-infected poults compared to the D14-infected and NC groups (P=0.0007). Bodyweight gains between all groups revealed significantly lower values in poults infected at D0 compared to those infected at D7 and D14, with all infected poults having significantly lower gains than NC poults (P=0.0008). Uniformity trended toward a decrease in uniformity relating to earlier time of infection (P=0.0648). Overall, feed conversion ratio (FCR) and gut permeability were not significantly altered between any of the treatment groups. In conclusion, the time of infection with *C. anatis* in turkey poults appears to have severe impacts on growth performance in turkey poults. Furthermore, the time of infection may also be a contributing factor in non-uniformity in addition to individual effects of the disease as growth seems to be stunted soon after infection onset.

Key Words: Cochlosoma anatis, Uniformity, Mortality, Time of infection, Bodyweight gain

M41 The cytology of resting and reactive NK cells of chickens; implications in H/L computation Paul Cotter*, Kathleen Cotter *Cotter Laboratory*

Avian lymphocytes are differentiated as to source as B-cells originating in the bursa of Fabricius, and T-cells originating in the thymus. A third category, the NK (natural killer) cells are thought to originate in the embryonic spleen. NK cells are found at low frequency in peripheral blood occurring between 0.5 – 1% of TWBC. They are believed to provide an important source of resistance to viral disease and can kill tumor cells directly, a feat likely important in the control of Marek's disease (MD). Here the purpose is to provide examples of NK cells as they occurred in the blood of control, MD vaccinated, vaccinated/challenged SPF chickens. Method: blood samples were collected on-site and smears were immediately air-dried and post-fixed in 100% MeOH. Afterward, they were sent to Cotter Laboratory where they were stained by Wright-Giemsa and representative cells were photographed. The results: the presentation begins with a comparison of lymphocyte types then focuses on how NK cells are distinct from B and T cells and concludes with examples illustrating NK variation. Emphasis is given to how NK cells contribute to the interpretation of the H/L ratio in determining homeostasis. Resting NK cell areas (A_C) are ~ 30-50 μm^2; with nuclei, $A_N \leq 30$ μm^2 and have nuclear/cytoplasmic ratios N/C of ~ 0.6-0.8. The nucleus is typically eccentric with patchy cobble-stone chromatin. Resting NK are diploids (ploidy ratios, PR ~ 1). Granules are small and few (6-12) per cell. NK cells in transition to a reactive state are larger, A_C ~ 100 μm^2; A_N ~ 50 μm^2, with higher ploidy (PR ≥ 1.6). Further activation results in growth via elaboration of the ER with an increased number of cytoplasmic granules. The nucleus may display a reniform (kidney) shape, and some are clearly polyploid. NK cells are signatures of infection and inflammation; and as reactive cells, they would be inappropriate components of the H/L denominator. Conclusion: An increase in the circulating NK complicates the interpretation of the hemogram and can disqualify the H/L value as an indicator of homeostasis.

Key Words: lymphocyte, natural killer cell, NK, H/L ratio, hemogram

M42 Campylobacter jejuni infection induces immune responses in HD11 avian macrophage cells Sabin Poudel*[1GS], Linan Jia[1], Chuan-Yu HSu[2], Cheng Wen-Hsing[3], Pratima Adhikari[1], Aaron Kiess[4], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, [3]Department of Food Science, Nutrition, and Health Promotion, Mississippi State University, [4]Prestage Department of Poultry Science, North Carolina State University*

Campylobacter jejuni (*C. jejuni*) is a leading food-borne pathogen present in poultry, which causes human gastroenteritis. However, infected birds remain asymptomatic even though the birds were colonized with *C. jejuni* at the concentration ≥8 $\log_{10}$ CFU/g. To understand the birds-immune responses during *C. jejuni* challenge, this study was conducted to evaluate the effects of *C. jejuni* infection on the expression of immune genes by an avian macrophage cell line (HD11). For the immune gene expression HD11 cells were infected with *C. jejuni* isolates from four different sources (cloacal swabs, chicken meat, bovine feces, and human feces) at a 1:100 multiplicity of infection for 3 h. The RNA extracted from non-infected HD11 cells was utilized as the control. The pro-inflammatory genes (*IL-1β, IFN-γ, IL-6, IL-8*), anti-inflammatory gene (*IL*-10), toll-like receptor genes (*TLR-2A*, *TLR-4*), and *iNOS*$_2$ gene expression were analyzed using RT-qPCR assays with gene-specific primers. The expression differences between control and treatments were analyzed with the ΔΔCt methods using the avian-specific *18S rRNA* gene as a reference for normalization. Fold change data were analyzed using an unpaired student's t-test, with a significant level set at $p \leq 0.05$. The tested pro-inflammatory genes (*IL-1β, IFN-γ, IL-6, IL-8*), anti-inflammatory gene (*IL-10*), and *iNOS2* gene were significantly upregulated in infected HD11 cells irrespective of source isolation with the fold-change expression ranging between 4.56 to 4047 compared to the control. Similarly, *TLR-4* was significantly upregulated in infected HD11 cells irrespective of the isolated sources of *C. jejuni* strains with the fold-change expression between 2.27 to 9.2. Interestingly, the *TLR-2A* was significantly downregulated in infected HD11 cells with fold-change ranging from 1.33 to 15.97 compared to the control. In conclusion, even though colonization of *C. jejuni* are asymptomatic to the birds, our results indicate that *C. jejuni* can interacts with the chicken macrophage cells and stimulates an immune response via activation of immune related genes. Future experiment can be done to understand birds' immune response during parental routes of infection.

Key Words: Campylobacter jejuni, immune, gene expression, RT-qPCR, poultry

M43 Characterizing the proteome of bacterial chondronecrosis with osteomyelitis (BCO) lesions in broiler's proximal tibiae – implications for novel molecular pathways in lameness Alison Ramser*[1], Sami Dridi[1], Elizabeth Greene[1], Garrett Mullenix[1], Jalila Dridi[2], Rohana Liyanage[1], jennifer cook[1], Robert Wideman[1] *[1]University of Arkansas, Center of Excellence for Poultry Science, [2]École Universitaire de Kinésithérapie*

The modern broiler remains first choice among animal protein consumers due to its health benefits and affordability. In order to remain desirable and competitive, the modern broiler must continue to improve performance while maintaining high standards of welfare and sustainability. Lameness in the broiler industry can dismantle both production and animal welfare and yet its exact etiology is still being determined. Our lab utilized

shotgun proteomics analysis to characterize the entire proteome in tibia affected by a leading cause of lameness, bacterial chondronecrosis with osteomyelitis (BCO). Liquid Chromatography with Tandem Mass Spectrometry was conducted, and samples were analyzed using Mascot. The functions and relevant networks of these proteins were mapped using Ingenuity Pathways (IPA) by Qiagen core analysis. Results showed a total of 547 proteins identified, 222 were differentially expressed (DE) with 158 up- and 64 down-regulated proteins in tibia of BCO *vs.* normal chickens. Pathways related to cell death, organismal injury, skeletal and muscular disorder, and immunological and inflammatory diseases being implicated in BCO tibia based on differentially expressed proteins. Understandably, networks revolving around stress response, bone structural integrity, and bone resorption were implicated in BCO. Novel networks such as unfolded protein response and ribosomal protein dysfunction were also linked to BCO indicating disruption of cellular functions at levels not previously identified. These insights are beneficial to understanding the mechanistic underpinnings of BCO etiology and its effects on bone cell function in the modern broiler.

Key Words: femur head necrosis, BCO, lameness, proteomics, broilers

M44 Curcumin reduces enteric isoprostane 8-iso-PGF2α and prostaglandin GF2α in specific pathogen-free Leghorn chickens challenged with Eimeria maxima Victor Petrone-Garcia[1], Raquel Lopez Arellano[1], Juan Latorre[2], Inkar Castellanos-Huerta[1], Billy Hargis[2], Guillermo Tellez-Isaias*[2] *[1]National Autonomous University of Mexico, [2]University of Arkansas*

The purpose of this pilot study was to evaluate and determine the concentration of prostaglandin GF2α (PGF2α) and isoprostane 8-iso-PGF2α in the plasma and intestine of specific pathogen-free (SPF) Leghorn chickens challenged with *Eimeria maxima*, with or without dietary supplementation of curcumin using solid-phase microextraction and ultra-performance liquid chromatography/tandem mass spectrometry. Eighty one-day-old male SPF chickens were randomly allocated to one of four groups with four replicates (n=5 chickens/replicate). Groups consisted of: 1) Control (no challenge), 2) Curcumin (no challenge), 3) *Eimeria maxima* (challenge), and 4) *Eimeria maxima* (challenge) + curcumin. At day 28 of age, all chickens in the challenge groups were orally gavaged with 40,000 sporulated *E. maxima* oocysts. No significant differences ($P > 0.05$) were observed in the groups regardless of the treatment or challenge with *E. maxima.* Enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days and 9 days post-challenge were significantly increased ($P < 0.01$) compared to the non-challenge control chickens. Interestingly, the enteric levels of both isoprostane 8-iso-PGF2α and PGF2α at 7 days post-challenge were significantly reduced in chickens fed curcumin, compared to control chickens challenged with *E. maxima.* At 9 days post-challenge, only levels of isoprostane 8-iso-PGF2α in the enteric samples were significantly reduced in chickens challenged with *E. maxima* supplemented with curcumin, compared with *E. maxima* challenge chickens. There were no differences in isoprostane 8-iso-PGF2α or PGF2α in plasma on both evaluation days. Similarly, no significant differences were observed between the challenge control or chickens challenge with *E. maxima* supplemented with curcumin at both evaluation times. The results of this pilot study suggest that curcumin's antioxidant and anti-inflammatory properties reduced oxidative damage and subsequent intestinal mucosal overproduction of lipid oxidation products. Further studies to confirm and extend these results in broiler chickens are required.

Key Words: coccidia, PGF2α, isoprostane 8-iso-PGF2α, mass spectrometry, chickens

M45 Saponin-rich plants mixture (Norponin® XO2) supports coccidiosis vaccination program in broiler chickens Iraida Rosario Huamancoli[1], Susana Fribourg[2], Robert Saavedra[2], Juan Manuel Garcia[3], Mohammed el Amine BENARBIA*[3,4] *[1]UNIVERSIDAD NACIONAL AGRARIA LA MOLINA, [2]ADISENS / Los Sauces, [3]NOR FEED SAS, [4]LABCOM FEED IN TECH: Université d'Angers*

Vaccination against coccidiosis is on the rise. It is supported by a growing societal demand for antibiotic-free meat products. However, vaccination may be accompanied by some adverse effects, such as a negative impact on growth and/or the development of necrotic enteritis. Moreover, during the time of the establishment of immunity, the birds are only moderately protected. In this study, we investigated different strategies of associating cocci vaccination and a mixture of saponin plants (Norponin XO2) in managing coccidiosis.

200 day-old male Cobb 500 broiler chickens were distributed in 20 experimental units, each experimental unit of 10 chickens. Chickens were randomly divided into 5 groups:

UUC: Uninfested Untreated control, IUC: infested untreated control, VACC: infested and vaccinated group at d1, VACC/NPXO: infested, vaccinated, and supplemented with Norponin XO from d1 to d42, NPXO: infested, non vaccinated and supplemented with Norponin XO from d1 to d42. The birds were orally inoculated on day 14 of age by live strain vaccine at 15 times the dose. Growth performances (live weight, feed intake, and FCR) were monitored at d42, 5 birds from each group were randomly selected, and intestinal samples were collected (jejunum) for morphometric. Statistical analyses were performed by analysis of variance (ANOVA) using GraphPad software.

The experimental infestation model was successful. It was evidenced by the significant reduction in live weight and increase of FCR of chickens in the IUC group compared to the UUC group. All treatments compensated this loss. Villi length and area were both degraded by *Eimeria spp*. infestation. Whereas all treatments, except vaccination, compensated for the loss of villi length, only VACC/NPXO and NPXO treatments were able to compensate villi area significantly.

This study evidenced that NPXO2 supplementation is a valuable tool to support vaccination programs. Moreover, the obtained results demonstrate that Norponin XO2 supplementation is a "stand-alone" solution to manage coccidiosis.

Key Words: Coccidiosis, vaccination, Saponins, Gut health, Broiler chickens

SCAD I

M46 Prophylactic efficacy of paromomycin (liquid form) against Histomonas meleagridis challenge in turkeys Vijay Durairaj*, Andrea Brake, Ryan Vander Veen *Huvepharma, Inc.,*

Histomonas meleagridis causes histomoniasis (blackhead disease) in gallinaceous birds. Histomoniasis is a fatal disease of turkeys resulting in mortalities up to 80-100%. Currently, there are no commercial vaccines or prophylactic/therapeutic measures to combat histomoniasis. In an attempt to find a solution for histomoniasis, multiple approaches have been evaluated. Several drugs have been identified as efficacious against *H. meleagridis* in *in vitro* studies but failed in *in vivo* studies. In this experimental study, the efficacy of paromomycin-oral solution (Huvepharma NV, Belgium), an aminoglycoside antibiotic, was evaluated against *H. meleagridis* infection in turkeys. One ml of paromomycin liquid presentation has 200 mg of paromomycin sulfate which is equivalent to 140 mg paromomycin base or 140, 000 IU of paromomycin activity. Poults (n=80) were

randomly divided into four groups. Groups 1 and 2 served as negative and challenge controls, respectively. Two days before challenge (21 days-of-age), groups 3 and 4 were treated with 0.2 ml/kg BW and 1 ml/kg BW of paromomycin until final day of the study. At 23 DOA, groups 2-4 were challenged by the cloacal route at the dose of $1x10^4$ *H. meleagridis*/0.5 ml per bird. Birds were necropsied at 15 days post-challenge (38 DOA). Group 2 challenge controls had 55% mortality and 80% gross lesions associated with histomoniasis. Group 3 birds treated with 0.2 ml/kg BW had only 5% mortality and 20% gross lesions, while group 4 birds treated with 1 ml/kg BW had no mortalities and only 5% gross lesions. The mortality and gross lesions were statistically different between the challenged control birds and paromomycin-treated birds. The reduced mortality and gross lesions observed in this study support the prophylactic efficacy of paromomycin administered before *H. meleagridis* challenge.

Key Words: Histomonas meleagridis, paromomycin, prophylactic treatment, histomoniasis prevention, blackhead disease

M47 Characterizing the o-serogroups of avian pathogenic E. coli (APEC) associated with colibacillosis in Georgia poultry production and their relationship with production types. Klao Runcharoon*[GS], Meaghan Young, Bellanirys Garcia, Nicolle Barbieri, Catherine Logue *Department of Population Health, College of Veterinary Medicine, University of Georgia*

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis a significant disease of poultry worldwide. Colibacillosis presents as systemic or localized infection such as airsacculitis, septicemia, pericarditis, perihepatitis, salpingitis, and cellulitis in chickens. Serotyping based on somatic O-antigen detection is a useful tool to classify *E. coli* into different serogroups related to disease. Currently, there are approximately 180 O- serogroups described with O1, O2, and O78 being the most common linked with disease. However, there is limited information on APEC O-serogroups in Georgia poultry associated with disease. A total of 568 isolates collected between March 2021 – 2022 from diagnostic cases were analyzed and screened for O-serogroups using multiplex polymerase chain reaction (PCR). The isolates were recovered from different bird types including broiler, broiler breeder, broiler breeder pullet/cockerel, commercial layers, and pet/hobby birds. PCR products were subjected to gel electrophoresis in a 2% agarose gel and stained with 1 mg/ml ethidium bromide and visualized under UV light using an imager. Chi-square analysis was performed using Stata/BE (V 17.0) and statistical significance was accepted when $p < 0.05$. Overall, 309 isolates were identified into the serogroups which included O78 (41%), O2 (19%), O25 (16%), O8 (9%), O1 (9%), O86, O18, and O15 (5%). The data revealed that there was no significant differences ($p > 0.05$) between the serogroups detected and bird type. However, when an odds ratio (OR) analysis with 95% confidence interval (95% CI) were applied, the odds of O25 were 1.16 higher in broiler than any other bird type (OR 1.16, 95% CI: 0.57-4.57), while in the broiler breeder the odds of O1 was 9.31 (OR 9.31, 95% CI: 0.57-4.57) compared to other bird types. Broiler pullet and cockerel had an odd ratio for O78 of 1.81 (OR 1.81, 95% CI: 1.08-3.03), and pet/hobby birds were more likely to harbor O8 (OR 2.94, 95% CI: 1.27-6.82). Collectively, different poultry production types are likely to be linked with different APEC O-groups. This data provides useful knowledge that should allow us to develop advanced diagnostic tools and further mitigation strategies specific to each bird production type in Georgia.

Key Words: Escherichia coli, poultry, colibacillosis, Georgia, APEC

M48 A novel presentation of focal ulcerative dermatitis syndrome (FUDS) in broiler breeder hens Gunnar Dunnam*[1GS], Andrew Bishop[2], Martha Frances Dalton[3], Jay Kay Thornton[1], Rebecca Mackey[1], Alejandro Banda[1], Martha Pulido-Landinez[1] *[1]Mississippi State University Poultry Research and Diagnostic Laboratory, [2]Amick Farms, [3]Mississippi State Veterinary Research and Diagnostic Laboratory*

Focal Ulcerative Dermatitis Syndrome (FUDS) is a severe bacterial dermatitis that has been described in white and brown cage free layers in the Midwest. The causative agent is suspected to be either *Staphylococcus hyicus* or *Staphylococcus agnetis;* however, no published reports are available in the literature. The disease is characterized by progressive focal ulcers on the backs of hens with mortality usually seen from secondary *Escherichia coli* infections. This report describes a case of suspect FUDS in a flock of 58-week-old broiler breeder hens in South Mississippi, which presented to Mississippi State University Poultry Research and Diagnostic Laboratory for workup of the suspected condition. 7 live broiler breeder hens presented with focal, featherless, round to ovoid, 7cm x 8cm, severely ulcerated lesions localized on the back, on midline cranial to the pygostyle, on each examined breeder. *Staphylococcus hyicus* was isolated in pure heavy growth from 6/11 samples and identified by spectrophotometry (Vitek-MS MALDI-TOF). Given that *Staphylococcus hyicus* (the causative agent of greasy pig disease) and *Staphylococcus agnetis* are highly similar genetically, further testing using 16r DNA sequencing was performed to determine the identity of the isolates. However, no conclusion could be made from this analysis. Finally, polymerase chain reaction amplifying the pfbA gene was performed to differentiate between the two *Staphylococcus* species. Additional bacterial isolates include *Staphylococcus xylosus* from both livers sampled for aerobic culture and *Paeniclostridium sordellii* (formerly *Clostridium sordellii*) isolated by anaerobic culture from the back lesions. No fungal growth was present. Histopathology confirmed moderate to severe chronic ulcerative dermatitis with intralesional gram positive cocci. This case report describes the clinical and gross findings of a suspect FUDS flock as well as the diagnostic testing used to identify the causative agent.

Key Words: Focal Ulcerative Dermatitis, Broiler Breeders, Staphylococcus agnetis

M49 Parenteral application of live E. coli and Salmonella Typhimurium vaccines as a tool to improve immune response Manuel Da Costa*, Kalen Cookson, John Dickson, Jon Schaeffer *Zoetis - U.S. Poultry*

Live *Salmonella typhimurium* (LVST) and E. coli (LVEC) vaccines have been widely used across the poultry industry as successful measures of controlling colibacillosis and salmonella, respectively. Both products are applied by mass administration, either by spray or water. This results in high mucosal exposure to the vaccine, which induces immunity mostly driven by cellular and IgA immunity. A sequence of challenge studies was conducted with the objective of evaluating if, when these vaccines are given parenterally, immunity would be enhanced when compared with the conventional route. Across studies, live bacterial vaccines were administered to SPF leghorns either alone or in combination with an inactivated *Salmonella Enteritidis*, Infectious Bronchitis, and Newcastle Disease vaccine between 10 and 12 weeks of age. Results from the LVST studies showed that LVST given parenterally induced greater Salmonella spp. load and prevalence reductions against Salmonella groups B, C, and D challenges. This effect was also reflected by improved serology GMT in the parenteral LVST treatments. Results from the LVEC trials showed that parenteral administration of the vaccine consistently gave the best protection results across various APEC O78 and O2 challenge loads. In addition, combining LVEC with the inactivated vaccine further improved colibacillosis protection and reduction of clinical signs when compared with LVEC given parenterally by itself. In conclusion, both LVST and LVEC, when

given parenterally, have shown the potential to broaden and further enhance their cross-protective features.

Key Words: Parenteral, Live vaccines, Salmonella, E. coli, Immunology

M50 The impact of Salmonella Typhimurium and coccidiosis vaccine on the cecal transcriptome in broiler chickens Andrea Pietruska*[1GS], Steven Kitchens[1], James Krehling[2], Ken Macklin[2], Stuart Price[1], Zubair Khalid[1], Maria Terra-Long[2], Teresa Dormitorio[2], Ruediger Hauck[1,2] *[1]Department of Pathobiology, Auburn University, [2]Department of Poultry Science, Auburn University*

In antibiotic free and organic poultry production the use of attenuated life vaccines against pathogens like *Salmonella* and coccidia has increased to reduce the risk of consumers health and economic losses. Previous research indicated that *Salmonella* Typhimurium (ST) infections in broilers were more severe after vaccination against coccidiosis and the expression of tight junction genes changed significantly after administration of both vaccines compared to use of either vaccine alone. In this study, we further investigated the interaction between life vaccines against ST and coccidiosis and the host transcriptome. We used a 2 x 3 experimental design. Factors were no ST vaccination, ST vaccination on day 14 or on day 0 and 14, as well as no coccidiosis vaccination or coccidiosis vaccination on day 0. Samples of the cecal wall were taken from 10 birds per group on day 28 and transcriptome analysis of ceca was performed based on RNA sequencing. Reads were trimmed with Trimmomatic and aligned to a chicken reference genome by TopHat2 and Bowtie2. The count of reads overlapping with exons was evaluated with HTSeq. The differential expression was calculated by fitting a quasi-likelihood negative binomial generalized log-linear model to the count data with edgeR. Only few genes were differentially expressed in the groups vaccinated with coccidia vs the control group and vs each other. Significant differences were observed between the groups only vaccinated against ST vs groups not vaccinated against ST. The highest difference was found between the groups vaccinated against ST vs the group vaccinated against coccidia only. The GO analysis revealed that the most significant pathways of differentially expressed genes in the group vaccinated on day 14 against ST were related to cardiac development and morphogenesis, and cellular responses. The pathways of the group vaccinated twice against ST contributed mostly to protein modification, cytoskeleton organization, and extracellular structure and matrix organization. Our study revealed that the vaccination against ST had a larger impact on the transcriptome in broiler chickens then vaccination against coccidia. However, combination of the ST vaccination with the coccidiosis vaccine can reduce that effect of the ST vaccine.

Key Words: chicken, transcriptome, gene ontology, Salmonella, Eimeria

M51 Evaluation of a blend of organic acids and essential oil in the drinking water during a Salmonella Heidelberg challenge in broilers Jonathan Broomhead*[1], Charles Hofacre[2], Matthew Jones[2] *[1]Perstorp Animal Nutrition, [2]Southern Poultry Research Group, Inc.*

Acidification of drinking water of broilers prior to processing is commonly used to reduce *Salmonella* entering into the plant, via crop contamination. This study evaluated an organic acid and essential oil product (OEP), when provided in the drinking water (3 ml/gallon) early and/or late in production, for reduction in *S. heidelberg* (SH) colonization in broilers. Eight hundred Ross x Ross day-old male chicks were assigned to 4 treatments in 32 floor pens, with 8 replicates per treatment and 25 chicks per pen. The treatments were: control, none (T1); OEP from 0-14 days of age (T2); OEP from 014 days of age plus the last 96 hours before end of trial (T3); OEP during last 96 hours before end of trial (T4). On day 7, a nalidixic acid-resistant SH was orally gavaged at 4.3 x 10^7 CFU/chick to 13 marked seeder chicks in each pen. Environmental bootsocks collected on days 14 and 42 and cloacal swabs collected from 5 non-seeder (horizontal exposed; HE) chicks on day 36 were tested for *Salmonella*. Feed withdrawal was performed 8-10 hours prior to termination. On day 42, 10 birds per pen (5 seeders and 5 HE) were euthanized and cecal contents were collect, as well as, crop contents from 5 HE birds. Birds were weighed and feed intake recorded on days 14, 35 and 42. All statistical testing assumed a two-sided alternative hypothesis, and $P < 0.05$ was considered significant. Birds in T2 had numerically larger 14 day ($P = 0.22$) and 42 day ($P < 0.1$) body weight gain than T1, with other two treatments being intermediate. Birds in T2 also had the lowest feed conversion through day 14 ($P < 0.05$). A reduction ($P = 0.05$) in crop *Salmonella* prevalence was observed in birds from T2 vs. T1, with T3 and T4 being intermediate. No treatment differences were observed in *Salmonella* most probable number (MPN) in bootsock and cloaca samples. In seeder chicks, a 40% and 35% reduction in cecal SH MPN was observed in T2 and T3 birds as compared to T1. When a Tobit regression was applied to crop data (0 MPN are censored to -0.5 $\log_{10}$ MPN/g), T2 and T4 had a numerical reduction of SH vs. T1 ($P = 0.16$). Acidifying the water with the product containing organic acid and essential oil may improve animal performance and reduce SH colonization.

Key Words: Salmonella Heidelberg, organic acid, essential oil, drinking water

M52 The Effect of Salmonella autogenous vaccination on serotype surveillance, recovery, and phenotypic characteristics Emily Robinett*, Marshall Putnam, Robert Beckstead *Ceva Animal Health*

Salmonella enterica is a major cause of foodborne illnesses around the world and is commonly caused by contaminated poultry food products. Currently, the poultry industry utilizes numerous protocols to mitigate the control of *Salmonella* transmission within various operations. Autogenous vaccines allow for the customized vaccination of poultry in different locations, but the selection of an appropriate vaccine formulation can be difficult to narrow down based on the isolates derived from each location. *Salmonella* surveillance studies can aid in the identification of *Salmonella* serotypes that are prevalent throughout a company or farm, and the use of various phenotyping methods can aid in determining which isolates should be included in the autogenous vaccine formulation. For this study, two large poultry operations in the United States were analyzed for changes in the recovery and prevalence of *Salmonella* over a four-year period following the implementation of autogenous vaccines. The bacterial phenotypic characteristics arginine dihydrolase, citrate utilization, acetoin production, d-sorbitol fermentation, inositol utilization, and biofilm formation were used as the basis for the selection of the autogenous vaccine formulations. Over the four-year study period, the overall prevalence of *Salmonella* decreased in both the hatcheries and the processing plants of the study sites. *Salmonella* Enteritidis and *S.* Typhimurium showed an overall decrease between 2017 and 2020 with *S.* Kentucky increasing during the same timeframe. Since *S.* Kentucky was not included in the autogenous vaccine formulations during this study, this could explain the increased prevalence of this serotype. *Salmonella* Infantis increased between 2017 and 2018, but steadily declined after an isolate was included in the autogenous vaccine formulation. Future studies should be conducted to determine whether the phenotypic characteristics of autogenous *Salmonella* vaccines are related to the vaccine efficacy.

Key Words: Salmonella, Autogenous Vaccination, Surveillance, Broiler

M53 Campylobacter hepaticus in Georgia layers – what have we learned so far Catherine Logue*, Roel Becerra, Julia Lima *University of Georgia*

Campylobacter hepaticus has emerged as a causative agent of spotty liver disease (SLD) in laying birds in the US and worldwide. SLD was recognized in the 1950s and then disappeared with modern poultry housing, however, its re-emergence may be linked with current farming practices. This study and review stems from a case report of SLD found in pasture raised laying hens in Georgia. In follow up studies, we examined the epidemiology of *C. hepaticus* in organic layers by assessing the prevalence

of the organism in the poultry environment, using culture and molecular analysis of samples of water, feces, feed, litter and other samples collected. We then developed a challenge model using SPF layers to replicate SLD and demonstrate how it could be transmitted to naive birds. We also assessed potential interventions to control transmission in the SLD model using approved agents for organic growers (organic acids and oregano). We also developed a model of infection based on wild birds to identify a potential transmission pathway for *C. hepaticus* in the organic layer environment. Finally, we compared *C. hepaticus* to other *Campylobacter* species by sequencing the genomes of multiple strains to better understand the pathogen and its pathogenic potential.

Our studies found the outdoor environment is a potential source of exposure and disease for organic pasture raised laying birds where *C. hepaticus* was detected in stagnant water and feces and could easily spread within a flock when present. This result was confirmed in our challenge model using SPF laying birds which showed liver lesions consistent with *C. hepaticus* that were confirmed by PCR. Control strategies using acids showed a slight reduction pathogen prevalence but the effect was transient at best. Genomic sequencing of four *C. hepaticus* genomes found *C. hepaticus* shares similarities with *C. fetus* and *C. bilis*. In addition, the number of genes linked with virulence and iron acquisition were different in *C. hepaticus* from other *Campylobacter* species and *C. hepaticus* harbored genes associated with bacteriocin production, the multidrug efflux pump *cmeABC* and resistance to heavy metals and antibiotics. Our study provides insight into the emergence of *C. hepaticus* in Georgia and how the disease can be transmitted.

Key Words: Campylobacter hepaticus, layers, SLD, epidemiology, genomics

M54 Understanding the transmission of Spotty Liver Disease (SLD) associated with Campylobacter hepaticus from wild birds using finches as a model. Roel Becerra*[GS], Catherine M. Logue *University of Georgia*

Spotty liver disease has emerged as an important cause of loss in egg-producing hens. The organism implicated, *Campylobacter hepaticus*, causes focal lesions on the livers of infected birds, reduced egg production, and increased mortality in layers. The transmission route is not well understood, but researchers suggest it is the fecal-oral route. It is also unknown how *C. hepaticus* can be transmitted from farm to farm. It is speculated that wild animals, such as wild birds, may potentially transmit *C. hepaticus* to production birds such pasture raised birds that are allowed external access where there is potential exposure to stagnant water contaminated by wildlife. Moreover, it is not known if *C. hepaticus* also causes mortality in wild birds or if it is part of their normal flora. The aim of this study was to assess the use of a wild bird model using commercially available finches to determine their potential role in the transmission of *C. hepaticus* to chickens via drinking water. Eight finches were orally challenged with *C. hepaticus* (10^6 cfu/ml), and one negative control group was not challenged (n = 4). The challenged finches were placed in a cage hanging from the ceiling directly above a water drinker. The floor of the finches' cage was removed allowing feces to fall directly on the drinker. A group of 25 chickens of mixed genders of 19 and 60 weeks of age were placed in the room with the orally challenged finches and 17 chickens of mixed gender, 19 and 60 weeks of age were placed with the non-challenge finch group. At 20, 28, 35 days post oral challenge, a sub-population of finches and chickens were euthanized and necropsied to record gross lesions and samples were collected from both finches and chickens for bacteriological and PCR analysis. Results found that direct bacteriological analysis failed to detect *C. hepaticus* from the finches and chickens in non-challenged and challenged finches. However, 5/25 chickens were positive by PCR for *C. hepaticus* from liver samples. Also, 3/8 finches that were orally challenged were *C. hepaticus* positive by liver PCR analysis. Overall, these results support that wild birds could harbor *C. hepaticus* and transmit it to chickens via fecal contamination of stagnant water, in outdoor areas where layer hens have access.

Key Words: Campylobacter hepaticus, wild bird, model, finch, layers

M55 Complete genome sequencing and comparison of Campylobacter hepaticus isolated from organic pasture-raised chickens Julia Ienes-Lima*[GS], Roel Becerra, Jenny Nicholds, Catherine Logue *Poultry Diagnostic and Research Center, College of Veterinary Medicine, University of Georgia*

Campylobacter hepaticus has been identified as a causative agent of Spotty Liver Disease (SLD), characterized by multiple hepatic lesions, mortality, and reduction in egg production. *C. hepaticus* has many properties of the other members of the genus, which allow it to colonize any tissue besides the liver and gall bladder. However, since *C. hepaticus* was recently identified, there remains a lack of information. The present study reports the first four genomes of *C. hepaticus* isolated in Georgia, USA. The study aims to characterize the genomes of *C. hepaticus* isolated from hens of organic pasture-raised laying chickens and compare them with other *C. hepaticus* genomes available (n = 13), as well as different *Campylobacter* species (*C. coli* = 6, *C. fetus* = 3, *C. bilis* = 1, and *C. jejuni* = 7). The strains were isolated from bile samples of birds with symptoms of SLD and confirmed as *C. hepaticus* through culture and Polymerase Chain Reaction (PCR). Genomic sequencing was performed using PacBio Sequel II (Pacific Biosciences) technology. One genome sequence (RBCL71delta) was closed into a chromosome, and the three other genomes were organized into contigs (RBCL76delta, RBCL81delta, and RBCL91delta). The genome sequences were annotated using NCBI's Prokaryotic Annotation Pipeline and Rapid Annotation using Subsystem Technology (RAST). The chromosome of *C. hepaticus* RBCL71delta has a GC content of 28.0% and consists of 1,516,079 base pairs. The pangenome analysis of the 17 *C. hepaticus* genomes identified 1,329 core genes conserved across all genomes. Genes related to bacteriocin production and multidrug resistance efflux pump (CmeABC operon) were identified, as well as genes involved in resistance to antibiotics and heavy metals. In comparison with other *Campylobacter* species, *C. hepaticus* has a significant decrease in the number of genes related to iron acquisition and virulence. The phylogenetic analysis indicated that *C. hepaticus* is closely related to *C. fetus* and *C. bilis.* Additional analyses are ongoing to understand this phylogenetic relationship better. Our results may provide more information regarding *C. hepaticus* pathogenicity and help to build an efficient control and prevention strategy for this emerging pathogen.

Key Words: Campylobacter hepaticus, Spotty Liver Disease, genome comparison, phylogenetic analysis

M56 Influence of avian reovirus infection on the intestinal microbiome Zubair Khalid*[1GS], Andrea Pietruska[1], Erfan Chowdhury[2], Rüdiger Hauck[1] *[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, [2]Thompson Bishop Sparks State Diagnostic Laboratory*

Avian reovirus (ARV) has been associated with runting-stunting/malabsorption syndrome. It is likely that the disturbance of the intestinal function affects the bacterial microbiota, potentially aggravating the disease. However, the impact of an ARV infection on the host microbiome composition has not been investigated. Herein, we report for the first time how an experimental ARV infection influences the intestinal microbiome of chickens in a dose-, strain-, and time-dependent manner.

Seven-day-old specific-pathogen-free chickens were inoculated with 10^4 or 10^6 $TCID_{50}$ of either an ARV field isolate or S1133 strain. Jejunal content was sampled from ten individuals before inoculation and seven individuals per group 7, 21, and 35days post-inoculation. The intestinal microbiome were investigated by 16S rRNA next-generation amplicon sequencing and analyzed using the Qiime2 pipeline.

The alpha-diversity metrics including Shannon and Faith's phylogenic diversity indices revealed a significant reduction in the richness of microbial communities among the ARV-inoculated groups compared to the negative control. Moreover, strain- and time-dependent distinct clustering patterns were observed on the emperor plots generated using Principal Co-ordinate Analysis. Similarly, Bray-Curtis and UniFrac distances revealed significantly differing beta-diversity among the groups at different sampling time points. Taxonomic Analysis of Composition of Microbes indicated a differential abundance of genera including *Lactobacillus*, *Ruminococcus*, *Clostridium*, and *Flavobacterium* among groups.

Comparison of time points demonstrated reduced Pileou's evenness and temporally increasing abundance of certain genera such as *Lactobacillus* in the groups inoculated with higher doses. Concordantly, a significant reduction in body weights observed among chickens inoculated with 10^6 $TCID_{50}$ of field isolate could be correlated with their intestinal microbiome composition. Conclusively, ARV inoculation induced strain- and dose-dependent alterations in the alpha- and beta-diversity of microbiota in the host intestine.

Key Words: Avian reovirus, microbial diversity, microbiome, chicken intestine, runting-stunting syndrome

M57 Enhanced cross protection after simultaneous vaccination with live infectious bronchitis virus (IBV) Massachusetts and recombinant Newcastle disease virus expressing Arkansas-type IBV spike Raimundo Espejo*[GS], Cassandra Breedlove, Kellye Joiner, Haroldo Toro *Auburn University*

We previously demonstrated that a prime-boost regime with recombinant Newcastle disease virus LaSota (rLS) co-expressing infectious bronchitis virus (IBV) Arkansas (Ark)-type trimeric spike ectodomain (Se) and chicken granulocyte-macrophage colony-stimulating factor (GMCSF), and a Massachusetts (Mass)-type vaccine virus enhances cross protection. We now evaluated protection against Ark-type challenge conferred by simultaneous vaccination with the recombinant rLS/ArkSe.GMCSF and the widely used live Mass vaccine. Chickens were vaccinated at hatch and challenged at 21 days of age with virulent Ark. Protection conferred by vaccination was evaluated by respiratory signs, tracheal virus isolation as well as IBV RNA quantitation, and tracheal histomorphometry. As anticipated, the control using live Ark vaccine showed the most optimal protection. On the other hand, vaccination with rLS/ArkSe.GMCSF and Mass provided enhanced protection against Ark challenge compared to the Mass-only control; i.e. the recombinant virus enhanced cross protection against the heterologous challenge.

Key Words: Infectious bronchitis virus, Newcastle disease virus, vaccine, recombinant, chicken

Metabolism and Nutrition I General Nutrition

M58 Effects of unused restaurant and bakery food products as protein supplements on laying hen production parameters and USDA egg size Alea Belflowers*[GS], Benjamin Alig, Kari Harding, Dimitri Malheiros, Emmillie Boot, Bhavisha Gulabrai, Madison Spangle, Kenneth Anderson, Ramon Malheiros *Prestage Department of Poultry Science, North Carolina State University*

As the human population grows, so does demand for high-protein food products. Animal agriculture supplies three of the largest sources of protein food products consumed worldwide: meat, milk, and eggs. However, animal protein comes at an environmental cost, so it is vital to explore eco-friendly alternative feed ingredients derived from unused restaurant and bakery food. In this study, the effects of adding two feed additives made entirely of unused restaurant and bakery food at varying inclusion rates were analyzed in laying hen diets from 81 to 97 weeks of age. Seven treatment diets were divided into 42 replicates with 6 replicates per treatment and 32 hens per replicate. The study consisted of four, 4-week periods totaling 16 weeks. The hens underwent a washout period before data collection to achieve a baseline starting point. Egg production was recorded daily by replicate. USDA egg size was measured using all eggs produced in 24 hours before the end of each period. Statistical analysis was performed using JMP PRO 16 and the data analyzed via standard least squares. Egg production was measured as percent of Hen-Day production (%) and USDA size was measured as extra-large (XL), large (LG), medium (MD), small (SM), and peewee (PW). Production and egg size were analyzed by treatment, period, and their interaction. A Tukey's honest significant difference test was performed for each and a P-value <0.05 was considered significant. Hen-Day production showed significant differences between treatments (P<0.01) and period (P=0.04), but only one treatment was significantly less than the control diet; no periods were significantly different from the control. Significant differences were seen in egg size by treatment for XL (P<0.01), LG (P<0.01), and MD (P<0.01) eggs; one treatment showed no significant difference from the control. Egg size was significantly different by period in MED eggs (P=0.01). In conclusion, supplementing a restaurant or bakery food product in low amounts in laying hen feed may not have a significant effect on Hen-Day production or USDA egg size. This is promising as it could decrease the inclusion of soybean meal in laying hen diets, which is the most common protein source in poultry diets and has a large environmental impact from production.

Key Words: laying hens, egg production, USDA egg size, alternative feed ingredients, bakery food

M59 Interactive effects of feeding naturally-contaminated corn distillers dried grains with solubles and Eimeria infection on egg production and gut health in laying hens Deependra Paneru*[GS], Milan Sharma, Doyun Goo, Janghan Choi, Woo Kim *University of Georgia*

Multiple mycotoxins are frequently present in poultry diet containing corn distillers dried grains with solubles (DDGS), which might compromise gut health and exacerbate the enteric diseases in poultry. An experiment was conducted to investigate the interactive effects of feeding 20% corn DDGS naturally-contaminated with deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin, and fumonisins on gut health and egg production of laying hens challenged with *Eimeria spp*. A total of 48 Hy-Line W-36 laying hens (47-week-old) were randomly allocated to four treatments in a 2x2 factorial arrangement of feeding corn DDGS (contaminated or non-contaminated DDGS) and *Eimeria* infection (challenge or non-challenge) with 6 replicated cages, each containing 2 birds. On 7th day of feeding corn DDGS, birds were orally inoculated with *Eimeria maxima*, *E. tenella*, and *E. acervulina* (25,000, 25,000, and 125,000 oocysts/dose, respectively) to the challenge groups. Growth performance, feed intake (FI), hen day egg production (HDEP), and mortality were measured from week 1 to 3. Intestinal lesions were scored on 6 days post-infection (DPI). Data were subjected to two-way analysis of variance (ANOVA) for the main and interaction effects. Results were considered statistically significant at $P \leq 0.05$. FI was reduced in the mycotoxin-contaminated groups during the first week ($P=0.0325$) compared to the non-contaminated groups. Following the *Eimeria* challenge, birds infected with *Eimeria* showed a significant decrease in body weight (BW; $P<0.0001$), body weight gain (BWG; $P<0.0001$), FI ($P<0.0001$) and HDEP ($P=0.0012$) during the second week, compared to non-challenged birds. Similarly, the mycotoxin-contaminated diet reduced the BWG ($P<0.0001$) and HDEP ($P=0.0012$) during the second week. Mycotoxin and *Eimeria* interactions further reduced the BWG ($P<0.05$) of birds during the second week and caused a 25%

mortality. Intestinal lesions of challenged birds were more severe in the birds fed mycotoxin-contaminated feed than those fed non-contaminated feed at 6 DPI. In conclusion, naturally-contaminated corn DDGS itself reduced the egg production and further exaggerated the *Eimeria* infections worsening the lesion score and limiting the egg production of laying hens.

Key Words: DDGS, Eimeria, Mycotoxins, Coccidiosis, Laying Hens

M60 Evaluation of corn particle size on the growth performance, gut permeability, pellet quality, and litter moisture of turkey hens raised without antibiotics Alyssa Lyons*[1GS], Joe Moritz[2], Tim Boltz[3], John Boney[4], Michael Persia[1] *[1]Virginia Tech, [2]West Virginia University, [3]Mississippi State University, [4]The Pennsylvania State University*

An experiment was conducted to determine the effects of corn particle size (CPS) on turkey hen performance, intestinal permeability (IP), and litter dry matter. Hybrid turkey hens were housed in floor pens from 0 to 42d. Hens were fed a crumbled starter 1 (0 to 21d) and pelleted starter 2 (22 to 42d) diet. The experiment was a 2 x 2 factorial with diet phase (starter 1 and starter 2) and CPS (fine or coarse), resulting in 4 treatments of 18 replicates of 22 turkeys. Corn was manufactured using a hammermill with a 4.76 mm screen (581 µm; fine) or a 6.35 mm screen (964 µm; coarse). Pellet quality was determined using pellet durability index (PDI), modified pellet durability index (MPDI), and New Holmen Pellet Tester (NHPT). Body weight (BW) and feed offered and refused were measured on D0 and D42. Mortality corrected feed conversion ratio (FCRm) was calculated from 0 to 42 d of age. The IP was measured on D42 by orally gavaging one turkey per pen with 8.32 mg/kg of FITC-d dissolved in distilled water 1 h before blood collection. Blood was centrifuged to isolate serum. Litter dry matter content was measured on D42. Data were analyzed using JMP Pro 16.0 ($P \leq 0.05$) and means were separated using Student's t-test. Corn particle size did not impact the measured pellet quality variables (PDI, MPDI, and NHPT) in the starter 2 diets ($P > 0.05$). There were no interactions between dietary phase and CPS over the experiment. There was a main effect of BW over the starter 1 phase as hens fed fine corn resulted in increased FI (3.28 vs 3.22 kg/bd; $P \leq 0.01$) and BW (2.49 vs 2.44 kg/bd; $P \leq 0.01$) compared to those fed coarse corn. Corn particle size did not alter FCRm ($P > 0.05$). The IP was not influenced by CPS in the starter 1 ($P = 0.59$) or starter 2 ($P = 0.31$) dietary phases when measured on D42. Litter dry matter content was not influenced by CPS of starter 1 ($P = 0.13$) or starter 2 ($P = 0.62$). These data indicate that fine corn increases both FI and BW of turkey hens when fed in the starter 1 diet, but had no effects on FCRm, gut permeability and litter dry matter content, or when fed in the starter 2 diets. Although critical for both feed intake and body weight in the starter 1 diets, fine corn may not be needed in the starter 2 diet to maximize turkey hen performance.

Key Words: corn particle size, turkey hens, gut permeability, dry matter

M61 Sample size, selection criteria, and personnel impact on processing metric variance in nutrition research Caleb Marshall*[1GS], Nickki Tillman[2], Kelley Wamsley[1] *[1]Department of Poultry Science, Mississippi State University, [2]Nutritional Statistics LLC*

Research from our lab has shown that using processing personnel (PPL) as a covariant can reduce processing metric variation (VAR). Additionally, our lab and others have found sample size (SS) and selection criteria (SC) for SS to influence VAR; however, these data were for sampling within the growout, rather than processing. Ideally, all birds (ALL; 100% of experimental unit (pen); EU) are sampled, but this is rarely done due to time, labor, and expense. Therefore, our objective was to identify the impact of utilizing various combinations of SC (Random within pens;RAND or ± 1 SD of Pen Mean; PM) and SS (7, 21, 36 or 100% of EU, either RAND or PM birds divided by total number of birds/pen) on the statistical power (PWR) and VAR in processing metric, with or without PPL (wPPL andwoPPL, respectively) as a covariant. Data were obtained from a RCBD protease corn/SBM diet study using male broilers, 4 treatments with 13 replicated pens (REP) each (14 birds/pen). On d 42, ALL (n=728) were individually weighed/tagged, then on d 43, processed, chilled, and cut-up. At cut-up, PPL were randomly assigned carcasses; resulting parts and PPL were recorded to determine VAR. ALL, were analyzed, utilizing the fit model platform of JMP 16, as individual (n=681, with outlier removal using boxplots) or pen mean (n=52). Each SC were replicated independently 5 times (REP) and analyzed wPPL and woPPL. PWR analysis was used to determine the ability to detect a 50g difference (DIFF) in Live Weight (LW) and 20g of Total Breast (TB); processing ALL provided PWR of >0.99 for LW and TB metrics. Overall, for SC-PM, PWR was >0.80 for LW and TB until 7% EU was utilized; wherein no DIFF were detected in 5 out of 5 REP. For SC-RAND, PWR was <0.80 for LW as SS decreased; wherein detecting DIFF in LW failed for 1/5 REP for 36% EU, and 5/5 REP for 21 and 7% EU. Detecting DIFF in TB failed for SC-RAND 0/5, 4/5, 5/5 REP for 36, 21, and 7% EU, respectively. While increasing SS (regardless of SC) decreased TB SEM, SC-PM had consistently lower SEM vs. SC-RAND. Also, regardless of SS and SC, wPPL further reduced SEM. When processing ALL is not possible, data suggest SC-PM at the largest SS feasible. Utilizing final LW could also assist in choosing SC and SS; wPPL can further reduce SEM and thus, SS.

Key Words: nutrition research, processing, statistical analysis, sample size, variance

M62 Phosphorus utilization of faba bean and field pea for broiler chickens using a regression method Abidemi Adekoya*[GS], Olayiwola Adeola *Purdue University*

Faba bean (FB) and field pea (FP) are legume seeds that can be used as feed ingredients in diets for broiler chickens. Due to the storage form of phosphorus (P) in plants, P in these ingredients is not entirely available to broilers. Hence an accurate evaluation of P utilization of FB and FP for broilers is necessary for adequate diet formulation. Therefore, 4 experiments were conducted with broiler chickens to determine the true ileal digestibility (TID) and true total tract utilization (TTTU) of P in FB (Exp. 1), FP DS-Admiral (FPD) (Exp. 2), FP Hampton (FPH) (Exp. 3), and FP 4010 (FP4) (Exp. 4) by regression analysis. A total of 162 birds in Exp. 1 and 2, and 192 birds in Exp. 3 and 4 were allotted to 3 diets in a randomized complete block design with BW as a blocking factor on d 19 post-hatch. Each diet consisted of 6 replicate cages with 9 birds per cage in Exp. 1 and 2, and 8 replicate cages with 8 birds per cage in Exp. 3 and 4. Faba bean and FPD were included in the diets at 21, 42, or 63%, and 16, 32, or 48% respectively, whereas FPH and FP4 were included at 20, 40, or 60%, and 21.5, 43, or 64.5%, respectively. Birds were fed experimental diets for 3 d with free access to water, and excreta collection was conducted for 2 d. On d 22 birds were euthanized by CO_2 asphyxiation and ileal digesta collected from the distal two-thirds of the ileum. Data were analyzed by ANOVA using the GLM procedure. In Exp. 1, there was no significant effect on apparent ileal digestibility (AID) and apparent total tract utilization (ATTU) of P in the diets. There was a linear effect ($P < 0.05$) on AID of P in Exp. 2, whereas a quadratic effect ($P < 0.05$) was seen in Exp. 4. Both linear and quadratic effects ($P < 0.05$) was observed on ATTU of P in Exp. 2 and 3. In Exp. 1, 2, and 3 the AID and ATTU of Ca were linearly decreased ($P < 0.05$) whereas ATTU of Ca linearly increased ($P < 0.05$) in Exp. 4. The TID and TTTU of P in FB, FPD, FPH, and FP4 determined by a regression method were 66.5 and 66.7%, 73.4 and 73.8%, 74.6 and 68.3%, and 74.3 and 61.7% respectively. In conclusion, the information on the availability of P in FB and FP provided could enhance proper diet formulation, thereby meeting the P requirement of broiler chickens and reducing waste in the environment.

Key Words: broiler, faba bean, field pea, phosphorus, regression

M63 Relationship between viscosity of digesta and development of the gastrointestinal tract of broiler chickens Emmanuel Alagbe*[1GS], Patricia Jaynes[1], Chansol Park[2], Olayiwola Adeola[1] *[1]Purdue University, [2]Pathway Intermediates*

The objective of this study was to determine the effects of digesta viscosity on growth performance, apparent ileal digestibility (AID) of nutrients, metabolizable energy (ME) and N-corrected ME (MEn) of diets, organ parameters and intestinal modulation in broiler chickens. The experiment was designed as a randomized complete block design with body weight (BW) used as a blocking factor. On d 8, a total of 168 broiler chickens (186.8 ± 20.5 g) were allocated to 3 corn-soybean meal-based diets with increasing concentrations of carboxymethyl cellulose (CMC) – 0, 10, or 20 g/kg added at the expense of cornstarch from the basal diet. There were 8 replicate cages per dietary treatment with 7 birds per pen. Birds were euthanized on d 22 for sample collection. Data were analyzed using the GLM procedure of SAS. Orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of CMC. The ileal and jejunal digesta viscosity linearly increased ($P < 0.01$) with dietary CMC addition. There was a linear reduction ($P < 0.01$) in the BW of birds on d 15 and 22 with CMC. Moreover, the BW gain and the gain-to-feed ratio of birds linearly reduced ($P < 0.01$) with CMC addition throughout the experimental period. With CMC inclusion, the duodenal, jejunal, and ileal length linearly increased ($P < 0.05$). The addition of CMC also linearly increased ($P < 0.05$) the relative weight of the ileum and proventriculus in birds. The AID of dry matter, nitrogen, ME, and MEn linearly decreased ($P < 0.01$) with CMC inclusion. In the jejunum, the digesta crude mucin quadratically reduced ($P < 0.05$). However, there was a tendency for a quadratic increase ($P < 0.1$) in the jejunal villus height and villus height to crypt depth ratio with CMC addition. In summary, elevated viscosity of digesta reduced nutrient digestibility, leading to a reduction in the growth performance of birds. Moreover, the intestinal morphology, crude mucin production and the weight of organs were regulated by increasing digesta viscosity due to dietary CMC addition.

Key Words: carboxymethyl cellulose, ileal digestibility, goblet cells, histomorphology, intestinal modulation

M64 Nutrient, starch and energy utilization along the digestive tract and their impacts on cecal short chain fatty acid production in broiler chickens receiving different types and graded dietary levels of resistant starches Iyabo Oluseyifunmi*[1GS], Jeferson Lourenco[2], Oluyinka Olukosi[1] *[1]Department of Poultry Science, University of Georgia, [2]Department of Animal and Dairy Science, University of Georgia*

A total of 480 Cobb 500 male broiler chicks were used in a 21-day study to investigate the site and extent of nutrient, starch, and energy utilization and their effects on cecal short-chain fatty acids (SCFA) profile in broiler chickens receiving different types and graded levels of resistant starches (RS).The birds were allocated to 10 treatments in a 3×3+1 factorial arrangement comprising a corn-soybean meal control diet and the factors: 3 RS types (RST): banana starch (BS), raw potato starch (RPS) and high-amylose corn starch (HCS); each at 3 levels (RSL) 25, 50 or 100 g/kg. On d 21, jejunal and ileal digesta, excreta, and cecal content were collected for digestibility, total tract nutrient, starch, energy utilization, and cecal SCFA profile. RST × RSL was significant ($P < 0.01$) for total tract DM and N retention, AME, and AMEn. Overall, DM, N and starch retention, AME, and AMEn were greater ($P < 0.05$) for the control than other diets. Increasing RSL decreased ($P < 0.05$) DM, N and starch retention, AME and AMEn for BS and RPS, but increased all these for HCS except for starch retention which was not different at all HCS levels. There was significant ($P < 0.01$) RST × RSL for ileal N and starch digestibility. Birds receiving RS diets had ileal N digestibility comparable to the control diet whereas birds receiving 50 g/kg BS had lower ($P < 0.01$) N digestibility. Ileal starch digestibility was lower ($P < 0.01$) for birds receiving RS diets compared with the control. Increasing RSL decreased ($P < 0.01$) ileal starch digestibility for BS and RPS but had no effect on HCS. RST × RSL was significant ($P < 0.01$) for jejunal starch digestibility. Birds receiving RS diets had ($P < 0.01$) lower starch digestibility than control except for HCS. Jejunal starch digestibility decreased ($P < 0.01$) with increased RSL except for HCS. There was no RST × RSL nor significant RST effect for any SCFA measured. Birds receiving 100 g/kg RSL had greater ($P < 0.05$) acetate and total SCFA ($P < 0.05$) than those receiving 50 g/kg but comparable to other treatments and the control. In conclusion, increasing fermentable starch fraction in diets had the overall effect of increasing cecal SCFA, irrespective of starch type and this may have implications on gut health in broiler chickens.

Key Words: resistant starches, starch digestibility, apparent metabolizable energy, short-chain fatty-acids

M65 Energy utilization and ileal amino acids digestibility of cereal grains for broiler chickens June Hyeok Yoon*[GS], Changsu Kong *Kyungpook National University*

This study was conducted to determine the energy and ileal amino acids (AA) digestibility values of cereal grains (corn, extrusion-processed [EP] corn, barley, wheat, and wheat flour) for broilers. A nitrogen (N)-free diet was formulated to estimate the basal endogenous losses (BEL) of AA. Five semi-purified diets were prepared to contain each cereal grain (946.6 g/kg corn and EP corn, 949.5 g/kg barley, 948.6 g/kg wheat and wheat flour) as the sole source of energy and AA. All diets had 5 g/kg Cr2O3 as an indigestible index. All birds were fed a standard broiler diet until day 17. On day 17, a total of 336 Ross 308 male broilers was assigned to 6 dietary treatments with 8 replicates (7 birds/cage) in a randomized complete block design. Experimental period consisted of 2 days of adaptation and 2 days of excreta collection periods. On day 21, birds were euthanized with CO2 and ileal digesta were collected. Dry matter (DM) retention, N retention, apparent metabolizable energy (AME), AME:gross energy (GE), and N-corrected AME (AMEn) were calculated using both total excreta collection and index methods. Data for energy utilization were analyzed by two-way ANOVA using GLM procedure for main effects of ingredients and collection methods, and the interaction. Data for ileal AA digestibility were analyzed by one-way ANOVA. There was no interaction on DM retention and AME:GE, and the values obtained from the total collection method were greater ($P < 0.05$) than the index method. The DM retention and AME:GE did not differ between corn and EP corn, which were greater ($P < 0.05$) than the other ingredients. The N retention, AME, and AMEn showed interaction ($P < 0.05$), and energy values of EP corn, barley, wheat, and wheat flour determined by the total collection method were greater ($P < 0.05$) than the index method. The AME and AMEn of corn and EP corn were greater ($P < 0.05$) than the others. The AID and SID of AA were greatest ($P < 0.05$) in wheat flour followed by corn, EP corn, wheat, and barley. The results showed that corn and EP corn showed higher energy utilization, and wheat flour had the greatest AA digestibility. Determination using the index method might underestimate energy values of cereal grains, thus the methodology should be considered in feed evaluation.

Key Words: energy utilization, cereal grain, amino acid digestibility, broiler

M66 Field traits, fumonisins concentration, nutrient composition and digestible amino acids of different types of corn for poultry Cristina Simoes*[1GS], Juliano Vidal[1], Janine Sarturi[1], Isadora Laber[1], Cristiane da Silva[1], Denize Tyska[2], Tiago Madalosso[3], Carlos Mallmann[1] *[1]Federal University of Santa Maria, [2]Pegasus Science, [3]Cooperativa Agroindustrial Consolata*

This study aimed to evaluate field traits, fumonisin contamination, and nutrient composition of four different types of corn intended for poultry feeding. A total of 213 corn samples from the same experimental fields in Brazil were separated according to the endosperm texture into dent (n=30), flint (n=51), semi-dent (n=60) and semi-flint (n=72) types. Crop

yield, thousand grains weight (TGW) and damaged grains were measured. Analyses of nutrient composition were obtained via NIRS, using the calibration curves from the AMINONRG® and AMINONir® programs. The following nutritional values were predicted: dry matter (DM), crude protein (CP), starch, total P, phytic P, total and digestible amino acids (AA) as well as gross energy (GE) and metabolizable energy (AME_n) for poultry. These values were then corrected for an 88% DM basis. Fumonisins (FB_1 and FB_2) were quantified by HPLC-MS/MS. Data were submitted to analysis of variance using the mixed GLM procedure of the Statgraphics Centurion XVI. Fumonisin means were transformed by $\log_{10}(x+1)$ prior to analysis of variance. Different means were separated by Tukey's test at 5% significance. Dent corn presented higher percentage of damaged grains ($P < 0.01$) than other types, whereas flint corn had the lowest crop yield ($P < 0.001$). There were no significant effects of corn types on TGW. Flint corn presented the highest CP content ($P < 0.0001$). Dent corn presented higher starch concentration than flint corn ($P < 0.01$). Flint corn had higher total Cys, Thr, Arg, Ile, Leu, Val, His, Gly, Ser, Pro, Ala and Glu than the remaining three types ($P < 0.001$). Digestible Cys, Thr, Arg, Ile, Leu, Val, His, Phe, Gly, Ser, Pro, Ala, and Glu were higher ($P < 0.0001$) in flint corn compared to dent, semi-dent and semi-flint. Also, digestible Met and Lys were higher in dent and flint ($P < 0.0001$) compared to semi-dent and semi-flint corns. Energy values, total P and phytic P were not different among corn types ($P > 0.05$). Flint corn had the lowest concentration of FB_1, whilst corns classified as dent had the highest FB_1 ($P < 0.0001$) and FB_2 ($P < 0.05$) concentration. In conclusion, nutrient composition and fumonisins were affected by the endosperm texture of corn and such differences should be considered for poultry feed formulation.

Key Words: Endosperm texture, Fumonisin, NIRS, Poultry nutrition

M67 Effect of sorghum-based conventional diets with or without phytase superdosing on broiler performance Santiago Sasia*[1GS], Brett Lumpkins[2], Mireille Arguelles-Ramos[1] *[1]Clemson University, [2]Southern Poultry Feed and Research, Inc.*

Modern American tannin-free sorghum is a potential alternative ingredient to corn. However, there is a dearth of updated information about the use of these new sorghum hybrids in poultry diets. This research aimed to assess the growth performance of chickens fed sorghum-based diets with or without phytase superdosing. A 42-d grow-out trial was conducted during the summer of 2022 at Southern Poultry Feed and Research (SPFR), Inc., in Athens, GA. A total of 1500 male broiler chicks (Ross 708) were housed in a floor pen house with built-up wood shavings as bedding and curtain sidewalls. This experiment consisted of 30 pens of 50 birds (10 replicate pens per treatment). Three treatment diets were replicated in 10 blocks and randomized within each block. The dietary treatments evaluated were: T1 – corn-based diet with 550 FTU/kg (standard), T2 – sorghum-based diet with 550 FTU/kg, and T3 – sorghum-based diet with 2000 FTU/kg. A crumbled starter and a pelletized grower and finisher diet phases were formulated for each treatment. Birds were fed ad-libitum for each diet phase (starter: 0-14; grower: 14-28; finisher: 28-42 d). Cumulative body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio adjusted to mortality (AdjFCR) were calculated. Each pen was considered the experimental unit. One-way ANOVA followed by Fisher LSD with statistical significance considered at p-value ≤ 0.05 was used for the statistical analysis. The result of the statistical analysis showed no differences in performance at 7 d. At 14 d-of-age, birds fed T3 tended to gain more weight and be more efficient than birds fed T1 (P<0.10). Similarly, by 21 d, birds fed the T3 diet were more efficient (P<0.05) and tended to have higher BWG than T1 birds (P<0.10). Also, at 28 d, birds fed T3 tended to be more efficient than T1 (P<0.10). No significant differences in FI were observed during the trial (P>0.05). After 28 d, treatments were similar in all performance parameters. Therefore, this study confirmed that full replacement of corn with American sorghum is possible without negatively affecting bird performance. Also, more research about phytase superdosing in sorghum-based diets seems warranted.

Key Words: American sorghum, Broiler, Phytase superdosing, Growth performance

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M68 Comparative impact of incorporation of specialty protein feedstuffs and antibiotic growth promoters in broiler chicken starter feeding program on growth performance through to 49 days of age Anastasia Tsementzis*[1GS], Anderson Maina[1], Colin De Cloet[1], David Trott[2], Lee-Anne Huber[1], Elijah Kiarie[1] *[1]University of Guelph, [2]Wallenstein Feed & Supply Ltd.*

Specialty highly digestible protein feedstuffs (SPF) and antibiotic growth promoters (AGP) are often added to partially replace regular soybean meal (SBM) in starter phase. However, there is limited comparative data on the effectiveness of these approaches on lifetime growth performance (GP). We conducted two experiments (Exp.) to assess the impact of inclusion of SPF or AGP in the starter on GP through to 49 d of age. Six iso-caloric and -nitrogenous starter (d 0-10) diets were formulated to meet or exceed specifications. Diets were: 1) NC, corn and SBM, 2) PC, NC+ AGP (Bacitracin Methylene Disalicylate and Narasin), 3) FSBM, NC+ further processed SBM, 4) SPC, NC+ soy protein concentrate, 5) PM, NC+ pork meal and 6) BSFLM, NC+ black soldier fly larvae meal. Day old Ross x Ross 708 males were placed in 48 cages (6 birds/cage) for Exp. 1 and 48 floor pens (35 birds/pen) in four rooms (block) for Exp. 2. Test diets were fed for 10 days and birds in Exp. 2 transitioned to a common diet. The BW, feed intake (FI) and mortality were monitored for calculation of BWG and FCR. A sample of birds were necropsied on d 10, 28 and 49 in Exp. 2 for visceral organs and breast weight. For statistical analyses, diet was the fixed effect in Exp. 1 whereas diet and block were fixed effects in Exp. 2 and LSmeans were separated using Tukey method. Crude protein in SBM, FSBM, SPC, PM and BSFLM was 48.4, 52.6, 71.6, 57.2 and 54.5% as fed, respectively. Birds fed PC, SPC and BSFLM tended (P= 0.09) to be heavier than NC birds at the end of Exp. 1 (d 10). In Exp. 2, birds fed PC and SPF had higher (P<0.001) starter BWG relative to NC birds. In the overall (d 0-49), birds fed PC had similar (P>0.05) d 49 BW and FI to birds fed SPF but higher (P≤0.02) than for birds fed NC diets. Day 49 BW was 3,759, 3,479, 3,664, 3,649, 3,739, and 3,735 for PC, NC, FSBM, SPC, PM and BSFLM, respectively (P=0.003). The FCR and breast yield was not affected by the diet. On d 10, birds fed SPC and PM had heavier d 10 liver than NC birds whereas birds fed FSBM had heavier proventriculus than PC, SPC, and BSFLM birds (P=0.01). In conclusion, partial replacement of SBM with SPF was commensurate to AGP in bolstering growth in starter phase resulting in heavier birds at market age.

Key Words: broiler chick, starter feeding program, growth performance

M69 Evaluation of dietary nutrient reduction on broiler chicken growth performance, carcass characteristics, and breast meat quality defects Brittany Wall*[GS], Caroline Gregg, Joshua Flees, Cristopher Almendares, Orlando Fiallos, Charles Starkey, Jessica Starkey *Auburn University Poultry Science Department*

Numerous nutritional intervention strategies have been investigated with the aim of eliminating the breast meat quality defects, Wooden Breast

(WB) and White Striping (WS). Yet, the etiology of these defects is still unknown. Previously, feeding diets in meal form with a 30% reduction in metabolizable energy (ME), digestible Lys (dLys), and digestible Met (dMet) during the starter diet phase decreased WB incidence, BW, BWG in birds fed to d 21. Thus, the objective here was to develop a feeding model resulting in market-weight broilers with a variety of WB and WS scores. A corn and soybean meal-based commercial diet served as the control (C) and the second diet was formulated with a 30% reduction in ME, dLys, and dMet (R). The C diets were fed as crumbles in the starter (d 0 to 15) and grower (d 16 to 29) phases and were pelleted for the finisher (d 29 to 40) phase, whereas R diets were fed in meal form in all feeding phases. Feeding the C and R diets during the 3 feeding phases produced the following 4 treatments: CCC, RCC, RRC, and RRR. Male, Ross 708 ′ Yield Plus chicks (n = 1,440) were randomly allotted to 1 of 4 treatments, blocked by location, and reared in floor pens (30 birds per pen) until processing on d 42 when carcass weights and part yields, and incidence and severity of WB and WS were assessed. Data were analyzed as a 1-way ANOVA using SAS PROC GLIMMIX and a complete pairwise mean comparison analysis was done using the PDIFF option at $P \leq 0.05$. As expected, BWG and FCR worsened the more phases the broilers were fed the R diet ($P < 0.0001$). Carcass, breast, tender, wing, thigh, and drum weights and yields were lowest in the RRR-fed broilers, highest in CCC-fed broilers, with the RRC and RCC-fed broilers being intermediate ($P < 0.0001$). The incidence and severity of both WB and WS were lowest in the RRR-fed broilers compared with all other treatments ($P < 0.0001$); however, it is important to note that this feeding strategy did not eliminate either breast meat quality defect. Overall, the feeding strategy employed in this experiment using the R diet with 30% targeted reductions in ME, dLys, and dMet successfully produced a population of market-weight broilers with breast filets exhibiting a variety of WB and WS severities.

Key Words: Broiler Chicken, Diet Nutrient Restriction, Digestible Amino Acids, Growth Performance, Carcass Characteristics

M70 Impact of moisture at harvest and drying temperature on quality parameters, and nutrient content of corn varieties Joaquin Cabanas-Ojeda*[1GS], Nicolas Mejia-Abaunza[1], Paula Lozano-Cruz[1], Valmiro Lima Aragão Neto[1], Muhammad Ali[1], Maria Camila Alfaro-Wisaquillo[1], Gustavo Quintana-Ospina[1], Gilson Gomez[2], Edgar Oviedo-Rondon[1] *[1]Prestage Department of Poultry Science, North Carolina State University, [2]AB Vista*

Conditions at harvest time may modify corn nutrient properties for feed manufacturing and broilers' nutrient utilization. The moisture content at harvest (MH) can interact with drying temperatures (DT) when seeking the desired 13% moisture for storage. Interaction effects between MH and DT on nutrient profile have not been evaluated. The study aimed to assess the effects of MH on nutrient composition and quality parameters of four corn varieties (CV) differing in vitreousness and dried at two temperatures. Four yellow dent CV Dekalb 6869, 6205, 6357, and 6208, were planted under similar agronomic conditions. They were harvested with 34, 28, 22, and 16% MH. The kernels were dried in an air-forced oven at 35 or 120°C to obtain grains with moisture near 13% for storage. Kernel moisture was measured before and after drying using a portable moisture tester device. Pre- and the post-drying kernel were analyzed with NIRS using AB Vista calibration curves for proximate composition, protein solubility index (PSI), vitreousness, and AME. In a completely randomized design, data were analyzed in a 4x4x2 factorial arrangement of treatments with CV, MH, and DT as main effects. Three-way interaction effects were detected for nutrient content ($P<0.001$). Higher protein was observed in CV 6208 and 6869 with 28% MH and 120°C DT, while CV 6357 had the lowest protein content at every MH. Fat content was higher in 28% MH and 120°C DT for all CV, except 6357. CV 6208 with 28% MH had the highest fiber content. In contrast, CV 6869 with 34% MH and 120°C DT had the lowest fiber content. There was a quadratic effect of MH on AME and the highest AME was observed for all CV harvested at 20.06% MH and dried at 35°C DT, except for CV 6357 dried at 120°C. The lowest AME were found for all CV at 34% MH and dried at 120°C. No three-way interaction effects were observed ($P>0.05$) for PSI or vitreousness. However, a two-way interaction was detected ($P<0.05$) between MH and DT. The highest DT had a lower PSI for all MH. Furthermore, PSI decreased linearly as MH increased. In conclusion, CV, MH, and DT affected protein, fat, fiber, AA content, and corn quality parameters. Drying at 35°C increased AME for every CV up to 126 kcal/kg and MH and lowered the PSI between 20 and 33% points in all MH.

Key Words: Corn hardness, moisture at harvest, drying temperature, broiler

M71 Nitrogen correction of true metabolizable energy values for poultry feed ingredients Reed Dillard*[GS], Nicholas Dale, Adam Davis *The University of Georgia*

The apparent metabolizable energy (AME) and true metabolizable energy (TME) assays of poultry feed ingredients have traditionally been nitrogen (N) corrected to yield AMEn or TMEn. By correcting to zero N retention in the AMEn bioassay all feed ingredient results are directly comparable to one another. Roosters for the TMEn bioassay are fasted for 24 hours prior to precision feeding of the test ingredient which places them in a negative N balance. The N correction for the TMEn assay was utilized to avoid an overestimation of the endogenous energy correction. In the original published research conducted over 40 years ago, roosters remained in a negative N balance when fed test ingredients and the N correction resulted in TMEn values that were 1 to 14% lower than their corresponding TME values. In the current research N retention, TME and TMEn were determined on over 700 ingredient samples to determine if the original findings on N retention and correction were still appropriate. For all ingredients tested, a positive correlation ($P < 0.0001$) existed between N balance and the TME minus TMEn value. Unlike the original research, over 15% of the samples tested had a positive N balance. These samples were almost exclusively animal by-products (ABP) such as feather and meat and bone meals. About 45% of the tested ABP had a negative N balance. There was a positive ($P < 0.0001$) correlation between total digestible amino acid content and N balance in the ABP samples. For 68 of the ABP samples the TMEn value was decreased by 10% or greater from the TME value. However, unlike the original research, N correction increased the TMEn value relative to its TME value in 10% of the samples. Roosters fed these samples had a negative N balance that exceeded that of the unfed endogenous control roosters, and the samples such as soy and rice hulls, were low in protein and/or high in fiber. For some of these samples the N correction inflated the TMEn value by over 20%. These results suggest that for the vast majority of ingredient samples, the N correction established by the original research is appropriate, but for samples with a very positive N balance or with a negative N balance that exceeds the endogenous controls, a change in the degree of the N correction may be necessary.

Key Words: nitrogen balance, ingredient quality, apparent metabolizable energy

M72 Effect of dietary metabolizable energy levels and conditioning temperature on broiler performance, processing yield and footpad dermatitis from 1 to 42 days of age Jose Hernandez*[1GS], Joseph Gulizia[1], Jose Vargas[1], Susan Bonilla[1], Sureerat Thuekeaw[2], Wilmer Pacheco[1] *[1]Auburn University, [2]Chulalongkorn University*

This study evaluated the main effects and interactions of two metabolizable energy levels and three conditioning temperatures on broiler performance, processing yield, and footpad dermatitis (FPD) from 1 to 42 d of age. A total of 1,350 d-old YPM x Ross 708 male broilers were randomly distributed in 54 floor pens and assigned to six treatments (9 replicates/treatment). Diets were formulated to contain two metabolizable energy levels (standard energy (SE) and -130kcal/kg reduced energy (RE)) in all feeding phases. Both basal diets were manufactured using three condition-

ing temperatures (80, 84, and 88°C). Body weight (BW) and feed intake (FI) were determined at 14, 28, 35, and 42 d of age and feed conversion ratio (FCR) was adjusted for mortality. All birds were assessed for FPD on d 42. Processing and parts yields were determined on d 43. Data were analyzed as a 2 x 3 factorial arrangement (energy level x conditioning temperature) using the GLM procedure of SAS to evaluate main effects and interactions. Tukey's HSD test was used to separate means and statistical significance was considered at $P \leq 0.05$. Birds fed SE diets had higher ($P < 0.05$) BW at 14 (478 vs. 468 g) and 28 (1,730 vs. 1,689 g) d than broilers fed RE diets. Birds fed RE had higher ($P < 0.05$) FI at 35 (3637 vs. 3548 g) and 42 (5082 vs. 4941 g) d compared to birds fed SE. Birds fed RE diets exhibited higher FCR ($P < 0.05$) compared to birds fed SE diets in every evaluated period and a 7-point higher FCR was observed on d 1-42. Birds fed diets conditioned at 80°C had lower d 1-14 FCR ($P < 0.05$) compared to birds fed diets conditioned at 84°C (1.14 vs. 1.16 g:g). Birds fed SE diets had lower ($P < 0.05$) breast yield (29.11 vs 29.61%), but higher tender weight (272 vs. 268 g) than birds fed SE diets. In addition, broilers fed SE diets had lower incidence of FPD with a higher proportion of score 0 and a lower proportion of scores 2 and 3 compared to birds fed RE diets ($P < 0.05$). Overall, birds fed SE diets had improved growth performance and less incidence of FPD, but bird performance and processing yields were essentially unaffected by conditioning temperatures.

Key Words: energy, conditioning temperature, broilers, growth performance, processing

M73 Nitrogen corrected apparent metabolizable energy (AMEn) and apparent ileal digestibility (AID) of amino acids (AA) of thermally processed food waste for broilers Nelsa Beckman*[GS], Haley Otott, Allison Blomme, Patrick Badger, Liberty Thompson, Phillip Lancaster, Yi Zheng, Raghavendra Amachawadi, Melissa Schrader, Charles Stark, Chad Paulk *Kansas State University*

To determine the economic viability of utilizing food waste in feed, the nutrient availability and variability needs to be determined. The objective of this study was to determine the nutrient variability, AMEn, and AID of food waste when fed to broilers. Food waste was collected for 30 consecutive days at Kramer Dining Center, Kansas State University. Food waste products were created by dividing the 30-day collections into weekly intervals resulting in product 1 (FW1; days 1-7), 2 (FW2; days 8-14), 3 (FW3; days 15-22), and 4 (FW4; days 23-30). Each product was mixed with soybean meal (SBM) and extruded (InstaPro 2000-10 RTT, Des Moines, IA) to a target barrel temperature of 140°C. At hatch, 420 and 360 one-day old male broilers (Ross 708, initial BW 38.5 g) were used in an 18-day study to determine AMEn and AID AA, respectively. Treatments were randomly assigned to cages, resulting in 10 cages per treatment with 6 broilers per cage. Treatments for both studies consisted of conventional SBM, extruded SBM (ESBM), FW1, FW2, FW3, and FW4. A common basal corn/SBM treatment was added to the AMEn study as a reference control. For AMEn determination, broilers were fed treatment diets from d 8-18 with fecal samples collected on d 16-18. For AID AA determination, broilers were fed treatment diets d 10-18, and euthanized via CO_2 for ileal sample collection on day 18. Data were analyzed using the PROC GLIMMIX in SAS v. 9.4 (Cary, NC). Broilers fed ESBM had increased ($P < 0.05$) AID of indispensable AA, except Met, when compared to SBM. Broilers fed ESBM had increased ($P < 0.05$) AID of indispensable AA, when compared to FW products. Of the FW products, broilers fed FW1 and FW3 had increased ($P < 0.05$) AID of indispensable AA, except Leu, when compared to FW2 withFW4 AID AA being intermediate. The FW3 had increased ($P<0.05$) AMEn when compared to SBM, ESBM, FW1, and FW2, with FW4 being intermediate. In conclusion, extruding food waste blended with SBM successfully served as a nutrient source for broilers. The food waste product's AMEn was similar or improved when compared to SBM. However, the AID AA value of food waste differed depending on collection period and was similar or less than that of SBM.

Key Words: Food waste, Broilers, Apparent metabolizable energy, Amino acid digestibility

M74 Influence of perennial wheat grass screenings and exogenous carbohydrase inclusion on broiler performance. Mason Engnell*[GS], Nelsa Beckman, Haley Otott, Charles Stark, Chad Paulk *Kansas State University*

Kernza® grain is a perennial grain that is harvested from intermediate wheatgrass (Thinopyrum Intermedium) that is currently found in niche markets in the food and beverage industry. Screenings from this grain are currently being utilized as cattle fodder. The objective of this study was to evaluate the effect of Kernza® grass screenings and exogenous carbohydrases inclusion on growth performance, gizzard weight, and cecal volatile fatty acid content of broilers. A total of 288 one-day old, male (Ross 308; initial BW 37.6 g) broiler chicks were housed in 3 batteries and used in a 21-d study. Birds were randomly assigned to battery cages balanced by BW with 6 birds per cage. Cages were then randomly allotted to 1 of 6 treatments balanced by BW with 8 replicates per treatment. Dietary treatments were arranged as a 2 × 3 factorial with factors consisting of carbohydrase enzyme (with or without xylanase and β-mannanase) and addition of Kernza® grain screenings (0, 1.5, or 3%). The control diet was corn and soybean meal-based with screenings and enzymes added in the place of corn. The Kernza® screenings consisted of 91.32% DM, 14.1% crude protein, 1.60% crude fat, 23.51% crude fiber, 53.21% NDF, 30.44% ADF, and 6.39% ash. Dietary treatments were fed as a starter crumble from day 0 to 21. On day 21, birds were euthanized and gizzard weights and cecal samples were collected. Data was analyzed using the PROC GLIMMIX procedure of SAS with pen as the experimental unit. There was no evidence ($P > 0.244$) of an enzyme × screenings interaction for growth performance or relative gizzard weights. There was no evidence ($P > 0.058$) of main effects for enzyme or screenings on growth performance of broilers. Increasing screenings in the diet from 0 to 3% increased (linear, $P < 0.001$) relative gizzard weight of broilers. In conclusion, Kernza® grain screenings were added to broiler starter diets at small inclusion levels without negatively impacting growth performance while increasing gizzard weight. However, exogenous carbohydrase did not impact broiler performance or gizzard development.

Key Words: perennial wheat grass, carbohydrase, xylanase, β-mannanase

M75 Effect of feed retention time in the conditioner and conditioning temperature on pellet mill throughput and pellet durability index (PDI) Carter Minson*[UG], Haley Otott, Charles Stark, Chad Paulk *Kansas State University*

This experiment evaluated the effects of feed retention time in the conditioner and conditioning temperature on pellet mill throughput and pellet durability index. Treatments were arranged as a 3 × 2 factorial of retention time (30 seconds, 60 seconds, and 90 seconds) and conditioning temperature (73.8°C and 85°C). All treatments were pelleted at 3 different time points to provide 3 replications per treatment. Diets were steam conditioned (254 × 1397 mm Wenger twin staff pre-conditioner, Model 150) and pelleted (CPM Model 1012-2) with a 4.3 x 22.2 mm (8 length:diameter [L:D]) pellet die at approximately 1042 kg/hr. Production rate was kept constant during the trail. Pellet samples were collected at 3 time points throughout each run and immediately placed in an experimental counterflow cooler for 10 min. Samples were analyzed for pellet durability index (PDI) using the modified tumble box (M-PDI) and Holmen 60 second (H-PDI) methods. Conditioning temperature, hot pellet temperature (HPT), and production rate were recorded throughout each pelleting run. Data were analyzed using PROC GLIMMIX in SAS (v. 9.4), with pelleting run as the experimental unit and time as the block. There was no evidence

($P > 0.20$) for a conditioning retention time × conditioning temperature interaction for H-PDI. There was no evidence of difference ($P = 0.600$) in H-PDI when increasing conditioning temperature from 73.8°C to 85°C. Increasing condition retention time from 30 to 90 sec increased (linear, $P < 0.001$) PDI (59.6, 61.7 and 67.9%, respectively). For M-PDI, there was a marginally significant ($P = 0.093$) conditioning retention time × conditioning temperature interaction. When conditioning diets at 73.8°C, increasing conditioning time from 30 to 90 sec increased M-PDI (59.4, 65.3, and 67.4% respectively). However, when conditioning diets at 85°C, there was no evidence of difference in M-PDI when increasing the conditioning retention time (65.8, 69.0, and 69.0%, respectively). When pelleting diets using the settings reported herein, conditioning retention from 30 to 90 sec improved pellet quality. However, when analyzing PDI using the modified tumble box method, conditioning retention time only improved pellet quality when conditioning at 73.8°C.

Key Words: conditioner retention time, conditioning temperature, pelleting, pellet quality

M76 Effects of grinding kernza® grain with different hammermill screen sizes on subsequent particle size and flowability Walter Friesen*[UG], Chad Paulk, Haley Otott, Charles Stark *Kansas State University*

Kernza® grain is a perennial grain that is harvested from intermediate wheatgrass (Thinopyrum Intermedium). The objectives of these experiments were to: 1) evaluate the effects of grinding Kernza® grain with different hammermill screen sizes on subsequent particle size and flowability and 2) determine the effects of Kernza® inclusion on the pelleting process and subsequent pellet durability index. For objective 1, three separate lots of Kernza® were ground with a laboratory-scale 1.5 HP Bliss Hammermill using a 2.0 mm, 2.8 mm, and 3.6 mm screen. Each treatment was ground at three separate time points to provide three replications per treatment. For each replication, three samples were collected and analyzed for particle size geometric mean diameter (d_{gw}), standard deviation (S_{gw}), and angle of repose (AoR). For objective 2, Kernza® ground using the 2.0 mm screen was used. Treatments were arranged in a completely randomized design with increasing Kernza® concentration in a corn soybean meal-based diet (0, 2.5, 5, 10, and 30% Kernza®). Diets were steam conditioned and pelleted with a 4.3 x 22.2 mm pellet die. Samples were analyzed for pellet durability index using the Holmen 60 second method. Data for the pellet experiment is currently being analyzed and will be presented at the conference. For objective 1, increasing screen size from 2.0 to 3.6 mm resulted in increased (linear, $P < 0.001$) d_{gw} of Kernza® (323, 478, and 672 µm, respectively). However, increasing screen size from 2.0 to 3.6 mm resulted in decreased (linear, $P < 0.021$) S_{gw} of Kernza (3.15, 3.10, and 2.78 respectively). Reducing the particle size of Kernza® by reducing the screen size from 3.6 to 2.0 mm resulted in an increase (linear, $P < 0.001$) in AoR (43.0, 46.7, and 50.1°, respectively). Therefore, grinding Kernza® with a 2.0 mm, 2.8 mm, and 3.6 mm screen resulted in dgw of 323, 478, and 672 µm and flowability characterized as poor (46-55), poor, and passable (41-45). Further research is needed to determine how Kernza® influences growth performance of livestock and poultry when included in diets. However, based on the AoR data collected herein, grinding Kernza® using 3.6 mm screen resulted in improved flowability compared to using a 2.0 and 2.6 mm screen.

Key Words: Kernza®

M77 Effects of AZOMITE (AZO) and distiller's dried grains with solubles (DDGS) inclusion on pellet mill electrical efficiency and subsequent pellet quality Patrick Badger*[1GS], Charles Stark[1], Jerry Shepherd[2], Michael Sodak[2], Kyle Coble[2], Maks Kapetanovich[2], Chad Paulk[1] *[1]Kansas State University, [2]JBS Live Pork*

The objective of this experiment was to determine the effects of AZO and DDGS inclusion on pellet mill (PM) electrical efficiency and subsequent pellet quality. This experiment was designed as 2 × 2 × 2 factorial with main effects of AZO inclusion (0 vs 0.25%), diet type (0 or 30% DDGS), and pellet mill (PM1 and PM2). For this experiment, two 500-horsepower pellet mills (Model 32-700, Andritz) equipped with a 4.4 × 39.0 mm (L:D 8.9) or 4.4 x 35.8 mm (L:D 8.2) die were used. For each replicate, 48 tons were mixed and stored above their prospective PM before pelleting. Diets were steam conditioned at 82°C and pellet mill throughput was held constant at 35 tons per hour. All treatments were pelleted once per day per mill, yielding 4 replications per treatment. Electrical data was collected every 15 seconds throughout all replicates with data analyzed over 25 minutes at production parameters. Pellets were collected 10 minutes after pelleting parameters were achieved with subsequent samples collected every 10 minutes and analyzed for pellet durability index (PDI) with the Holmen NHP 100 (TekPro) 30-second air agitation. For kilowatts per hour per ton (kWh/ton), there was a DDGS × PM interaction ($P = 0.040$) with DDGS diets decreasing kWh/ton in PM1 while kWh/ton was similar between the control and DDGS diet when using PM2. There was a DDGS × PM interaction ($P = 0.019$) for kWh/ton standard deviation, with inclusion of DDGS increasing the standard deviation of kWh/ton for PM2 while the standard deviation was similar between the control and DDGS diets when using PM1. There was an AZO x DDGS interaction ($P = 0.026$) for kWh/ton standard deviation with standard deviation decreasing when AZO was added to DDGS diets; however, there was no evidence of difference in control diets. For PDI, inclusion of AZO decreased ($P < 0.05$) PDI and DDGS increased ($P < 0.05$) PDI compared to control diets. In conclusion, when pelleting diets at a constant production rate the influence of DDGS on PM energy consumption was dependent on the PM but adding DDGS to the diet improved PDI. The electrical consumption was decreased when pelleting diets containing 0.25% AZO; however, this resulted in poorer PDI.

Key Words: AZOMITE, DDGS, kWh/ton, pellet quality

Metabolism and Nutrition III Vitimins, Minerals

M78 Phosphorus digestibility responses of broiler chickens to autoclaving of feed ingredients Vitor Santos Haetinger*[GS], Olayiwola Adeola *Purdue University*

Regression-derived ileal digestibility and utilization coefficients of phosphorus (P) responses to autoclaving soybean meal (SBM) or poultry meal (PM) were investigated in two studies. On day 19 post hatching in respective Experiment (Exp.) 1 or 2, 384 or 320 male Cobb 500 broiler chickens were individually weighed and allotted into 6 or 5 treatments. Both experiments consisted of 8 replicate cages with 8 birds per cage in a randomized complete block design. In Exp. 1, 6 diets were formulated with either non-autoclaved or autoclaved soybean meal at 380, 480 or 580 g/kg in a 2 × 3 factorial arrangement. One corn-soybean meal and cornstarch based basal diet and 4 diets with 40 or 80 g/kg of non-autoclaved PM or autoclaved PM for a total of 5 diets were used in Exp. 2. Chromic oxide was added as an indigestible index marker to determine the ileal digestibility and retention of nutrients. Birds received the experimental diets for 3 days and excreta collection was conducted during the last 2 days. All birds were euthanized by CO_2 asphyxiation and ileal digesta samples were collected. Data were analyzed using the GLM procedure. In both studies autoclaving decreased ($P < 0.05$) DM digestibility and retention. Increasing the inclusion level of test ingredients linearly increased ($P < 0.05$) intake of digestible and utilizable P in both studies, and linearly reduced the digestibility and retention of DM and P in the soybean meal study. Autoclaving SBM resulted in higher ($P < 0.01$) ileal digestibility of P and retention of P and Ca. The estimated ileal digestibility of P in SBM, autoclaved SBM,

PM and autoclaved PM were 45, 53.6, 61.2 and 61.2%, respectively, the corresponding retention were 40.6, 45, 51.7 and 59.2%, respectively. Autoclaved SBM tended ($P = 0.058$) to have higher P digestibility than non-autoclaved but no effect was noted with PM. In conclusion, autoclaving feed ingredients reduced digestibility and retention of dry matter and the digestibility of phosphorus of soybean meal may be improved by heat treatment but not that of poultry meal.

Key Words: broiler, digestibility, heat treatment, phosphorus, soybean meal

M79 Growth performance, bone characteristics, and nutrient utilization of broiler chickens fed graded levels of calcium and phytase during the starter phase Opeadura Osunbami*[1GS], Olayiwola Adeola[1], Carrie Walk[2] *[1]Purdue University, [2]DSM Nutritional Products*

A total of 1152 one-day-old male broiler chickens were used in a 21-d experiment to investigate the effectiveness of a new phytase (HiPhorius) and graded levels of Ca supplied with limestone (LM) on growth performance, bone mineralization, apparent ileal digestibility (AID) and apparent total tract retention (ATTR) of dry matter (DM), crude protein (CP), Ca, and P. Dietary treatments were based on a corn-soybean meal negative control diet (NC) containing 5.1 g of Ca and 5.1 g of total P per kg. Treatments consisted of the NC; the NC + 1.3, 2.6 or 3.9 g of Ca from LM per kg; and the NC + 500, 1000, 2000 or 4000 phytase units per kg. There were 12 replicate cages for each diet. Birds were fed the experimental diets either for 3 d (7 to 10 d of age) or 14 d (7 to 21 d of age). In the 10 d old birds, increasing the levels of LM decreased gain, feed intake (FI), tibia P, AID of P, and Ca (linear, $P < 0.05$). Increasing the levels of phytase linearly improved ($P < 0.05$) gain and gain:feed (G:F), a linear and quadratic effect ($P < 0.05$) was also observed with tibia ash and P, AID of Ca and P. The AID of DM and CP in the younger birds quadratically increased ($P < 0.05$) as LM levels increased. There was a linear and quadratic effect ($P < 0.05$) of increasing phytase level for the AID of DM and a linear effect ($P < 0.05$) for the AID of CP. Similar patterns were observed in the 21 d old birds as the increase in LM or phytase levels resulted in the respective linear decrease or linear increase ($P < 0.05$) in gain, FI and G:F. A linear effect ($P < 0.05$) of increasing LM was observed on tibia ash and P, whereas there were linear and quadratic effects ($P < 0.05$) of increasing phytase on tibia ash and P. In addition, increasing LM levels resulted in a linear decrease ($P < 0.05$) in the AID of CP and P, and a quadratic decrease ($P < 0.05$) in the ATTR of CP. Increasing phytase levels linearly and quadratically ($P < 0.05$) improved the AID of CP and P, and ATTR of CP, P and Ca. The results showed that the detrimental effect of increasing Ca in P-deficient diets and the benefit of phytase inclusion are consistent between the older and younger birds. Also, the results showed the extra-phosphoric effects of phytase on the utilization of other essential nutrients such as protein and Ca.

Key Words: broiler chickens, calcium, phosphorus, phytase, limestone

M80 Effects of phytase and 25-OHD fed to calcium and phosphorous-reduced diet on growth performance, body composition, and bone quality of broilers challenged with Eimeria spp. Hanyi Shi*[GS], Woo Kyun Kim *University of Georgia*

An experiment was conducted to evaluate effects of phytase and 25-OHD in a calcium (Ca) and available phosphorous (avP)-reduced diet of broilers challenged with *Eimeria* spp. A total of 840 14-day-old male broilers were allocated to a 2 × 5 factorial arrangement based on coccidia challenge and dietary treatments: 1) a positive control (PC; 0.84% Ca/0.42% avP); 2) a negative control (NC; 0.64% Ca/0.22% avP); 3) NC+1,500 FTU/kg of phytase (NC+phytase); 4) NC+5,000 IU/kg of 25-OHD (NC+25-OHD); and 5) NC+1500 FTU/kg of phytase+5,000 IU/kg of 25-OHD (NC+phytase+25-OHD). Data were analyzed by SAS software. During 0-6 days post infection (DPI), the challenged groups showed lower BW and BWG compared to the unchallenged groups, and the unchallenged NC and NC+Phytase+25-OHD groups showed lower BW and BWG compared to unchallenged PC, whereas the challenged NC+Phytase+25-OHD group showed a higher BW and BWG than the challenged PC group. During 0-6 DPI, coccidia challenge compromised the femur metaphysis BMD of birds fed with PC or NC+25-OHD diet. Unchallenged birds fed with a NC diet showed a lower BMD compared to PC, and adding 25-OHD or phytase+25-OHD improved BMD to the same level of PC. For the challenged groups, the birds fed with the NC+25-OHD diet showed a lower BMD compared to PC, but supplementing phytase+25-OHD increased the BMD to the same level of PC. From 4 to 8 DPI, coccidia challenge compromised bone growth rate of birds fed with PC or NC+25-OHD diet. Birds fed with a NC diet showed a lower BMD compared to PC, while adding phytase or 25-OHD or both improved bone growth rate to the same level of PC, despite *Eimeria* challenge. Additionally, during 0-6 DPI, the *Eimeria* challenge decreased bone area, fat percentage, total tissue, and fat mass ($P<0.05$) of birds. Birds fed a NC diet showed lower BMD, BMC, bone area and bone ash compared to the PC group, but supplementing phytase alone or combine with 25-OHD was able to reach the same level of the PC ($P<0.0001$). In conclusion, *Eimeria* challenge and Ca and avP-reduced diet compromised growth performance, body composition, and bone quality; adding phytase or 25-OHD, or both improved growth performance, but supplementing 25-OHD alone did not improve body composition or bone quality regardless of coccidia challenge.

Key Words: phytase, 25-OHD, Eimeria challenge, growth performance, bone quality

M81 Determination of ileal digestible phosphorous from several inorganic phosphate sources and two meat and bone meals in 3 week old broilers Jay Hampton*[3GS], Wenting Li[1], Franco Mussini[1], Katie Hilton[1], Janet Remus[1], Samuel Rochell[2] *[1]Danisco Animal Nutrition and Health, IFF Nutrition & Biosciences, [2]Auburn University, [3]University of Arkansas*

The limitations of relative bioavailability assays for generating P availability values that can be directly incorporated into feed formulations has increased interest in determining ileal P digestibility values for feed ingredients. An experiment was conducted to determine the apparent (AID) and standardized ileal digestibility (SID) of P in monosodium phosphate (MSP), monocalcium phosphate (MCP), dicalcium phosphate (DCP), defluorinated phosphate (DFP), monodicalcium phosphate (MDCP), and bovine (B-MBM) and porcine (P-MBM) meat and bone meal. A total of 576 male chicks from a Cobb 500 female line were reared in 72 battery cages (8 birds/pen) and provided a common diet from 0 to 19 d post hatch. On 20 d post hatch, birds were re-allotted to 6 per cage and experimental diets were provided in a randomized block design. A semi-purified N and P free diet (NPF) based on dextrose and corn starch containing Celite was used for determination of endogenous P flow in 6 replicate cages. Test ingredients were included in the NPF basal as the sole source of P at the expense of Celite. Diets containing MCP, DCP, DFP, MDCP, B-MBM, and P-MBM were formulated to contain 0.31% P and fed to 7 replicate cages. To establish a linear regression of ileal P digestibility from MSP, 3 levels of MSP were used to provide 0.13, 0.23, and 0.33% P with each diet fed to 6 replicate cages. Limestone was used to balance all diets at 0.65% Ca. Diets were fed ad libitum for 48 hours and birds were euthanized by CO_2 for ileal digesta sampling. The AID of P values were separated on two levels of statistical significance ($P<0.01$) with all dietary concentrations of MSP (92.57, 91.44, and 88.53%), MCP (89.55%), DCP (90.04%), and B-MBM (92.24%) having similar and higher values than DFP (73.59%), MDCP (76.58%), and P-MBM (77.31%). Endogenous P flow was determined to be 75 mg/kg and SID correction had minimal influence on AID values. Although all sources were highly digestible (all >73%), no P source, including MSP, was totally available. Thus, relative bioavailability assays may overestimate P availability if the P from the standard is considered

100% available. Additional work is needed to establish the factors that influence ileal P digestibility among inorganic and animal protein sources.

Key Words: phosphorous, digestibility, broiler, bone meal, direct method

M82 The effects of feeding different zinc, manganese, and copper sources and phytase levels on the growth performance and sustainability of 42-day-old broilers administered a coccidiosis vaccine Macey Randig-Biar*[1GS], Rosana Hirai[1], Daniel De Leon[1], Austin Silva[1], Raquel Konrad Burin[2], Marco Rebollo[2], Duarte Neves[2], Leonardo Linares[2], Audrey McElroy[1] *[1]Texas A&M University, [2]Zinpro Corporation*

Research has indicated modifying mineral (MIN) source and dietary level, can aid in lowering nutrient loss and carbon footprint, while improving environmental sustainability. This study investigated the effects of zinc (Zn), manganese (Mn), and copper (Cu) sources with 2 phytase (PHY) levels on sustainability and performance of Cobb 500 males. A total of 1,872 chicks were randomly allocated to 6 treatments (T), with 12 pens/T, for a 3×2 arrangement of 3 MIN premixes (PMX) (A: Inorganic Zn 80ppm, Mn 80ppm, and Cu 120ppm; B: ProPath® Zn 50ppm, Inorganic Mn 80ppm, and ProPath® Cu 20ppm; C: ProPath® Zn 50ppm, Mn 30ppm, and Cu 20ppm) at 500 or 1500 FTU/kg PHY. Chicks received 2X coccidiosis vaccine (Advent®). Data was analyzed using SAS, where means were determined by LSMEANS and adjusted by Tukey's HSD and Fisher's LSD with P-value≤0.05. Feeding phases were starter (d0-15), grower (d16-30), and finisher (d31-42), and growth parameters were evaluated for each phase and cumulatively. On d42, fecal and litter samples were collected for MIN analysis. MIN×PHY interactions were seen in FCR for all phases and cumulatively. C+1500 had the lowest FCR with A+500 having the highest on d0-15 (P<.0001). C+1500 had the lowest FCR with C+500 the highest on d0-42 (P=0.0236). MIN×PHY interactions were seen in d15 BW (P=0.0108) and d0-15 BWG (P=0.0104), where A+1500, B+500, and PMX C had a greater performance than A+500, with B+1500 performing similarly. A PHY effect was seen in d30 BW (P=0.0021), d42 BW (P<.0001), and d0-42 BWG (P<.0001), where 1500 FTU/kg PHY performed better than 500 FTU/kg. A MIN effect was observed for Mn, Zn and Cu in fecal and litter samples (P<.0001). PMX A and B had the greatest Mn excretion in those samples, where PMX A had the greatest Zn and Cu excretion. A PHY effect was seen in fecal samples, where 500 FTU/kg had a greater moisture % (P=0.0442), as well as a greater excretion of phosphorus (P) (P=0.0074) and Zn (P=0.0167) when compared to 1500 FTU/kg. A PHY effect was seen in litter as 500 FTU/kg had a lower excretion of Ca (P=0.0301) and P (P=0.0494) when compared to 1500 FTU/kg. Results from this trial indicate the importance of better understanding the interactions of PHY level and MIN source for performance and sustainability benefits in broilers.

Key Words: minerals, phytase, broiler performance, sustainability

M83 Does Zn source and dietary level influence the phytase effect on growth performance, blood minerals and bone ash in broiler chickens? Hanna Philippi*[1,2GS], Vera Sommerfeld[1], Alessandra Monteiro[3], Oluyinka Olukosi[2], Markus Rodehutscord[1] *[1]University of Hohenheim, [2]University of Georgia, [3]Animine*

The aim of this study was to investigate whether Zn source or Zn dietary level or both influence phytase effect on growth performance, bone mineralization, and blood minerals in broilers. The factors were phytase level (0 or 750 FTU/kg, Quantum®Blue, AB Vista, UK), Zn source (none or $ZnSO_4 \cdot 7H_2O$, Sigma Aldrich, USA or ZnO, HiZox®, Animine, FR), and Zn supplementation level (0, 30, 90 ppm). A total of 640 male broilers (Ross 308) were allocated to 64 floor pens of 8 treatments with 8 replicates each. From d0 to d7 broilers were fed a nutrient adequate starter diet ac-cording to Gesellschaft für Ernährungsphysiologie (GfE, 1999). Pelleted experimental diets were fed ad libitum from d7 to d28 and were based on corn, soybean meal, rapeseed meal, and rice bran with native Zn concentration of 30 ppm and a non-phytate P level of 3.6 g/kg.

Data were analyzed in a 2x3+2 factorial nested design using the MIXED procedure of SAS (version 9.4). The factors phytase and Zn source were included in the full factorial design. The 2 additional treatments, both of which included high Zn levels (90 ppm) and phytase supplementation, were added to test the effect of Zn levels.

Performance traits were not affected by interaction (phytase x Zn source). Phytase supplementation significantly increased ADG and reduced FCR of broilers (P < 0.01). Phytase supplementation significantly increased tibia and foot ash concentration and serum inorganic P and significantly decreased serum Ca (P < 0.01). There was no significant difference between treatments supplemented with 30 ppm vs. 90 ppm Zn. A significant interaction (phytase x Zn source) was found for serum Zn concentration (P = 0.014). Significantly lowest Zn concentration was observed for serum of broilers fed without supplementation of both Zn and phytase. The addition of either Zn source, phytase or both significantly increased serum Zn concentration (Zn source, phytase: P < 0.01).

In conclusion, all measured traits were increased by phytase supplementation, but neither Zn source nor Zn supplementation level negatively affected the phytase effect on growth performance and bone ash concentration under the conditions of this study. Hence, Zn does not seem to be a relevant influencing factor for phytase efficacy.

Key Words: zinc, phytase, bone ash, growth performance, broiler

M84 Determining the bioavailability of Vitamin A acetate concentrates mixed in a vitamin-mineral premix after a 56-day temperature and humidity storage stress Daniel De Leon*[1GS], Rosana Hirai[1], Austin Silva[1], Ervey Sanchez[1], Macey Biar[1], Audrey McElroy[1], Christopher Bailey[1], Nicolas Martinez[2], Adebayor Sokale[2], Lawrie Music[2] *[1]Texas A&M AgriLife Research, [2]BASF Corporation*

The current study was conducted to determine the availability of Lutavit® A 1000 NXT (A-1000), a vitamin (Vit) A acetate product, after a 56-d incubation period. Four Vit A sources (A-1000, B, C, and D) were blended into Vit-trace mineral premix designed to put oxidation stress on the Vit A beadles. These Vit premixes along with a negative control (NC), without Vit A, were stored in two different conditions, Cold Stress (CS) at 4°C or Heat Stress (HS) at 35°C and ≥60% Relative humidity. Day-old Cobb 500 chicks were placed in battery cages for a 28-d grow out study. The birds were fed a sorghum-soybean meal diet formulated to meet the breeder's recommendations. A Vit A devoid diet was fed for the initial 14 days to deplete the broiler chicks stores of retinol. On d14, all birds were allocated 6/cage (±1 body weight SD). After 56 days of Vit A storage, 10 test diets containing the CS or HS premixes were pelleted and provided to the birds from d14-28 (8 cages/NC and 10 cages/Vit A diets). Liver and blood samples were collected on d21 and 28 for Vit A analysis. A 2×4 factorial design was used with main effects of storage temperature and Vit A sources. The NC treatment (trt) was statistically analyzed as an ANOVA contrast with each of the 4 Vit A sources. Given a significant interaction between CS or HS, data were analyzed as a one-way ANOVA. P-value was set as ≤0.05 and the means were separated by Duncan's test. Results showed that hepatic concentrations of retinol were significantly lower in NC and Source D diets in comparison to other trts for both CS and HS at d21 and 28 (P=0.000). However, there was no statistical difference between A-1000 and Source B in levels of hepatic retinol (P=0.000). In addition, circulating plasma retinol concentrations at d28 was significantly lower in the NC trt in comparison to A-1000 and Source B for both CS and HS, but no significant difference was observed between A-1000 and Source B. In the current study, no classic Vit A deficiency symptoms were observed in the NC trt potentially due to the presence of maternal stores of Vit A. In conclusion, based on liver and plasma retinol concentrations, the A-1000

has better availability than Source D but there was no observed difference in comparison to Sources B and C.

Key Words: Vitamin A acetate, Bioavailability, Heat stress, Broiler, Butylated hydroxy toluene

M85 Hatching egg quality of broiler breeders fed diets supplemented with conventional free or lipid microencapsulated premix forms of trace minerals at standard or high levels Dimitri Malheiros*[1GS], Ramon Malheiros[1], Kenneth Anderson[1], Ondulla Toomer[2], Peter Ferket[1] *[1]North Carolina State University, [2]USDA*

Lipid matrix microencapsulation of trace minerals (TM) is hypothesized to improve bioavailability and reduce toxicity to broiler breeders fed diets supplemented with commercial levels of inorganic trace mineral salts. The objective of the study was to evaluate the effect of microencapsulated trace mineral premix on internal and external quality and mineral composition of eggs. Twelve pens containing 9 females and 2 males were randomly assigned to one of 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and microencapsulated) and 2 mineral premix supplementation dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100%); 2) Microencapsulated 100% (MI100%); 3) Free 200% (FR200%); and 4) Microencapsulated 200% (MI200%). Eighteen eggs per treatment were collected from each pen at weeks, 6, 10, 13, 21, and 27 of lay for analysis. Egg quality parameters evaluated were: egg weight (EW), Haugh unit (HU), yolk color, shell strength, shell elasticity, vitelline membrane strength, and vitelline membrane elasticity. Mineral composition of the eggshell and whole eggs was also analyzed. Microencapsulation improved HU at the 10th week of lay, while EW and yolk color were reduced at week 21 and week 1 of lay, respectively. Higher TM dose improved EW during week 1 of lay, yolk color at week 6 and 21 of lay, and shell strength at week 21 of lay. Where treatment MI100% presented improvements in EW, vitelline membrane strength and elasticity during week 13 of lay. In contrast, treatment MI100% adversely affected shell strength at week 6 of lay (P=.03). Lastly, treatment FR200% most significantly enhanced shell elasticity during week 13 of lay (P=.0287). Taken together, these data may suggest that microencapsulation of trace minerals may have marginal effects on external and internal egg quality parameters, and on mineral composition of broiler breeder eggs.

Key Words: Mineral, Broiler Breeder, Microencapsulated

Metabolism and Nutrition IV Amino Acids, Enzymes

M86 Delineating the phase-specific effects of functional amino acids during infection and recovery of broiler chickens challenged with a mixed coccidian infection Adeleye Ajao*[1GS], Guanchen Liu[1], James Taylor[2], Todd Applegate[1], Ramesh Selvaraj[1], M.E.E. Ball[2], Ilias Kyriazakis[2,3], Woo Kim[1], Oluyinka Olukosi[1] *[1]University of Georgia, [2]Agri-Food & Biosciences Institute (AFBI), [3]Institute for Global Food Security, Queen's University*

A 35-day experiment was conducted to investigate the phase-specific effects of the functional amino acids, Arg and branched chain amino acids (BCAA), in broilers receiving marginally low-protein diet, and challenged with *Eimeria*. All birds received the same starter (d 0-10) and finisher (d 28-35) diets that met Cobb 500 nutrient requirements. Four grower diets were formulated as: positive control (PC) with adequate protein; low protein (NC) (18.5 and 16.5% CP respectively); or NC supplemented with either Arg or BCAA. All supplemental amino acids (AA) were added at 50% above requirement, but the ratio of individual BCAA were kept the same in all diets. A total of 8 treatments in a 4×2 factorial arrangement (4 diets each with or without a mixed *Eimeria* challenge at d14) were used; each treatment had 8 replicate pens with 20birds. Birds and feed were weighed at different phases: prepatent (14-17d), acute (18-21d), recovery (22-28d), and compensatory phase (29-35d). Pen average body weight at the start of each phase was used as covariate in the statistical analysis. Data were considered significantly different at $P<0.05$, and trends ($0.05<P<0.10$) were also presented. There was no diet × *Eimeria* interaction during any phase; *Eimeria*-challenged birds had lower weight gain and higher FCR ($P < 0.05$) compared with non-challenged birds in all phases. During the prepatent and acute phases, PC fed birds outperformed ($P < 0.05$) NC fed birds. In the prepatent phase, Arg tended to increase ($P < 0.10$) body weight gain (BWG) relative to NC. During the acute phase, Arg increased BWG and reduced FCR ($P < 0.05$) whereas BCAA tended to increase BWG and reduce ($P < 0.10$) FCR. There were no significant ($P>0.05$) dietary treatment effects on BWG and FCR during the recovery phase. During the compensatory phase there were no dietary treatment effects on WG or FCR, but BCAA tended ($P < 0.10$) to reduce FI of birds challenged with *Eimeria*. BW on d 35 was greater for the PC fed birds ($P < 0.05$), but similar for NC and AA-supplemented diets. It can be concluded that all the supplemental AA reduced the negative effect of *Eimeria* challenge during the infection phases, but the effect of Arg was more pronounced.

Key Words: Low protein, Amino acids, Coccidiosis, Arginine, Branched chain AA

M87 The effects of arginine and branched chain amino acids supplementation in low protein diet on intestinal permeability, histology and tight junction protein expression in broiler chickens challenged with Eimeria spp. Guanchen Liu*[1GS], Adeleye Ajao[1], James Taylor[2], Todd Applegate[1], Ramesh Selvaraj[1], Elizabeth Ball[3], Ilias Kyriazakis[2], Oluyinka Olukosi[1], Woo Kim[1] *[1]Department of Poultry Science, University of Georgia, [2]Institute for Global Food Security, Queen's University, [3]Agri-Food & Biosciences Institute (AFBI)*

We aimed to investigate the effects of supplementing arginine (Arg) and branched chain amino acids (BCAA) in broilers receiving a low protein diet and challenged with *Eimeria spp*. All birds were fed the same starter (d 0-10) that met Cobb 500 nutrient requirements. Four grower diets were formulated as: positive control (PC) with 18.5% crude protein (CP); low protein (NC) with 16.5% CP; or NC supplemented with Arg or BCAA. All supplemental amino acids were added at 50% above requirement. Birds were randomly allocated in a 4×2 factorial arrangement (4 diets, each with or without challenge) with 8 replicate pens per treatment. The challenged and non-challenged birds were orally gavaged with mixed *Eimeria spp.* (12,500 sporulated oocysts of *E. maxima*, 12,500 sporulated oocysts of *E. tenella*, and 62,500 sporulated oocysts of *E. acervulina*) or distilled water respectively on d 14. Intestinal permeability to a 4 kDa FITC dextran was measured on d 21. Ileal and jejunal tissues were collected on d 21 and 28 for gene expression and morphometric analysis. There were no diet by challenge interactions at either time point for any parameter. *Eimeria* challenge increased ($P < 0.01$) intestinal permeability. NC fed birds had higher permeability than PC ($P < 0.05$) whereas the permeability of Arg and BCAA groups did not differ significantly from PC. *Eimeria* challenge decreased villus height (VH) on d 21 ($P <0.01$) as well as increased crypt depth (CD) and decreased VH: CD on d 21 ($P < 0.01$) and d 28 ($P < 0.05$). On d 21, ileal expression levels of claudin 1 and occludin were increased ($P < 0.01$) by *Eimeria* challenge. However, on d 28, claudin 3 and occludin were expressed less ($P < 0.01$) in challenged birds. No significant dietary treatment effects were observed on jejunal histology and ileal tight junction protein expressions. In conclusion, *Eimeria* challenge affected tight

junction protein expressions and negatively impacted intestinal integrity. Feeding low CP diets to challenged birds exacerbated the impact but this was partially compensated by Arg and BCAA supplementation.

Key Words: Arginine, BCAA, Broiler, Low protein, Eimeria

M88 Interactive effects of dietary isoleucine and valine ratios to lysine in response to varying leucine to lysine ratios on jejunal protein expression in commercial broilers Diego Ventura*[1GS], Cristopher Almendares[1], Ruben Kriseldi[2], Alejandro Corzo[2], Roshan Adhikari[3], Jason Lee[3], Chance Williams[4], Charles Starkey[1], Jessica Starkey[1] *[1]Department of Poultry Science, Auburn University, [2]Aviagen, [3]C. J. Bio America, [4]Wayne-Sanderson Farms*

A central composite design (CCD) study was conducted to understand the relationship among dietary branched chain amino acid (BCAA) ratios and expression of proteins related to glucose and energy metabolism in the jejunum of broilers. A total of 2,592 d-old Ross 344 × 708 male broilers were randomly placed in 144 floor pens. Each pen received 1 of 15 dietary treatments in a 2^3 CCD with 6 center points from 20 to 35 d of age, varying in digestible ratios of isoleucine:lysine (Ile:Lys; 52 to 75), valine:lysine (Val:Lys; 64 to 87), and leucine:lysine (Leu:Lys; 110 to 185). On d 35, one bird per pen was selected, euthanized, and jejunum tissue samples were collected for protein extraction and proteomic analysis via data independent acquisition protein sequencing with a timsTOF Pro 2 LC/MS/MS and Spectronaut 15 software. Glycogen synthase kinase-3 beta (GSK3A), dihydrolipoamide acetyltransferase (PDHX), AMP deaminase (AMPD3), succinyl-CoA-Ketoacid-coenzyme A transferase (OXCT1), adipocyte-type fatty acid-binding protein (FABP4), and O-N-acetylglucosamine transferase subunit p110 (OGT) were identified and quantified. Protein quantification data were analyzed as a CCD using the RSREG procedure of SAS v. 9.4 with significance declared at $P \leq 0.10$. The surface response model for OGT expression was significant ($P = 0.028$; $R^2 = 0.20$). However, the models for GSK3A, PDHX, AMPD3, OXCT1, and FABP4 were not significant ($P \geq 0.1356$) and the R^2 values ≤ 0.15 did not allow for prediction of protein expression means. The coefficients for the Val:Lys ratio effect on PDHX ($P = 0.09$), the Leu:Lys ratio effect on PDHX ($P = 0.0716$) and OGT ($P = 0.0763$) protein expression were significant. In addition, the Ile:Lys × Leu:Lys interaction for PDHX ($P = 0.0415$) and the cross-product coefficient for Ile:Lys ratio effect on OGT ($P = 0.0535$) protein expression were also significant. The expression of OGT was affected by the Leu:Lys ratio ($P = 0.0511$) and the Ile:Lys ratio altered GSK3A ($P = 0.0633$) expression. Varying concentrations of dietary BCAA and their interactions seem to alter jejunal protein expression and may help explain the mechanisms underlying their influence on growth.

Key Words: branched chain amino-acids, lysine, isoleucine, valine, jejunum

M89 Interactive effects of dietary isoleucine and valine ratios to lysine in response to varying leucine to lysine ratios on Pectoralis major protein expression in commercial broilers Cristopher Almendares*[1GS], Diego Ventura[1], Ruben Kriseldi[2], Alex Corzo[2], Roshan Adhikari[3], Jason Lee[3], Chance Williams[4], Charles Starkey[1], Jessica Starkey[1] *[1]Department of Poultry Science, Auburn University, [2]Aviagen, [3]C.J. Bio America, [4]Wayne-Sanderson Farms*

Optimizing concentrations of dietary branched-chain amino acids (BCAA) can improve broiler chicken growth performance and carcass yields. A central composite design (CCD) study was conducted to understand the impact of dietary BCAA concentrations on *Pectoralis major* (PM) muscle protein expression and investigate the mechanisms behind how BCAA ratios affect broiler growth and muscle deposition. A total of 2,592 d-old Ross 344 × 708 male broilers were randomly placed in 144 floor pens. Each pen received 1 of 15 dietary treatments in the 2^3 CCD with 6 center points from 20 to 35 d of age, varying in digestible ratios of isoleucine:lysine (Ile:Lys; 52 to 75), valine:lysine (Val:Lys; 64 to 87), and leucine:lysine (Leu:Lys; 110 to 185). On d 35, 1 bird per pen was randomly selected for PM protein extraction and proteomic analysis via data independent acquisition protein sequencing with a timsTOF Pro 2 LC/MS/MS and Spectronaut 15 software. Branched-chain-amino-acid aminotransferase (BCAT1), adipocyte-type fatty acid-binding protein (FABP4), phosphoserine aminotransferase (PSAT1), O-N-acetylglucosamine transferase subunit p110 (OGT), and large neutral amino acids transporter small subunit 1 (SLC7A5) were identified, quantified, and analyzed as a CCD using the RSREG procedure of SAS ver. 9.4 with significance set at $P \leq 0.10$. The surface response model for PSAT1 expression was significant ($P = 0.0149$; $R^2 = 0.24$). However, the models for the other proteins were not significant ($P \geq 0.9592$; R^2 values ≤ 0.0336). A linear model effect ($P = 0.0023$) was observed for PSAT1 protein expression, while no quadratic ($P \geq 0.2085$) or cross product ($P \geq 0.5886$) regression effects were observed for any other proteins. A minimum stationary point was observed for BCAT1 protein. However, optimal values for the other proteins could not be obtained due to stationary saddle points. Still, the coefficients for the Ile:Lys cross-product ratio effect on PSAT1 ($P = 0.0957$) protein expression was significant. Overall, the results indicate that varying concentrations of dietary BCAA may impact expression of proteins related to broiler skeletal muscle growth; however, further exploration will be required to explain the mechanisms behind how BCAA impact broiler growth and muscle development.

Key Words: branched chain amino-acids, lysine, isoleucine, valine, pectoralis major muscle

M90 The effects of hygienic pelleting in diets that differ in amino acid density on Ross 708 broiler performance, amino acid apparent ileal digestibility, and requirement elizabeth lynch*[GS], kristina bowen, tim boltz, victoria ayres, joseph moritz *west virginia university*

To reduce pathogenic bacteria, such as Salmonella, in feed and ensure a high-quality pellet, diets for broiler and turkey breeders may be pelleted using high conditioning temperatures and retention times. However, increased conditioning temperature and time may alter amino acid conformation, decreasing digestibility, and subsequent bird performance. Therefore, increasing formulation density of amino acids may be necessary to maintain performance. The objective of this study was to evaluate high conditioning temperature and retention time within the pelleting process using a hygieniser (Hygienic Pelleting) in diets that vary in digestible lysine and corresponding amino acid ratio on Ross 708 starter broiler performance, amino acid apparent ileal digestibility (AAAID), and requirement. A 5 x 2 factorial arrangement of treatments with 5 levels of digestible lysine (-20%, -10%, 2019 Ross Broiler Starter Nutrition recommendation, +10%, and +20%) and 2 degrees of pelleting (Standard and Hygienic) was utilized in a randomized complete block design. Standard pelleting utilized 77˚C conditioning for 30 sec and Hygienic pelleting utilized 88˚C conditioning for 60 sec and 6 min retention time in a hygienizer. Each treatment was fed to 12 replicate pens of 10 male broiler Hubbard x Ross 708 chicks for a 21-day period. On the morning following day 7, 14 and 21, birds and feed were weighed by pen for performance calculations and ileal contents were collected on d22 for AAAID calculations. Standard Pelleting and Hygienic Pelleting demonstrated a 1.19 and 1.37% Lys requirement, respectively, using the two-straight line, one break point broken-line regression model. Performance data and the broken-line regression model suggest an approximate 10% increase in Lys and corresponding amino acid ratio relative to 2019 Ross Broiler Starter Nutrition Specifications for Hygienic Pelleting to provide optimal live weight gain. Hygienic Pelleting decreased AAAID ($P=0.0001$) and increased subsequent FCR ($P<0.05$) when diets were formulated to decreased amino acid density. Broiler and turkey breeder producers should be cognizant that nutritional detriment may accompany procedures used to create hygienic pelleted feed.

Key Words: lysine, Amino Acid Digestibility, Hygieniser, thermal processing, feed conversion ratio

M91 The influence of dietary glycine concentration in reduced crude protein diets with different Met to Cys ratios fed to broilers Trevor Lee*[1GS], Kentu Lassiter[1], Walter Bottje[1], Samuel Rochell[2] [1]*Center of Excellence for Poultry Science, University of Arkansas System Division of Agriculture,* [2]*Auburn University*

Two experiments (EXP) investigated broiler growth (0-21 d) and tissue glutathione (EXP 2) when fed reduced CP (RCP) diets with different dietary Gly levels and Met:Cys ratios. Ross 708 male off-sex chicks reared in floor pens (12 birds/pen) were used in EXP 1 (1,716 chicks) and 2 (1,344 chicks). EXP 1 included 13 diets: a control (CTL) diet or RCP diets (~3.6 unit CP reduction) formulated to either a high (74:26) or low (60:40) Met:Cys ratio and 1.75, 2.01, 2.27, 2.53, 2.79 or 3.05% total Gly+Ser levels (tGly+Ser). EXP 2 included 6 diets: a CTL diet or RCP diets (~2.6 unit CP reduction) formulated to have either a high (72:28) or low (55:45) Met:Cys ratio and 1.60, 2.08 or 2.56% tGly+Ser. An additional factor in EXP 2 was *Eimeria* vaccination status [without (UNVAC) or with (VAC)]. Both EXP used one feeding phase. Treatments were replicated in 11 (EXP 1) or 16 (EXP 2) pens. Body weight gain (BWG), feed intake (FI), and FCR were evaluated, and in EXP 2, liver, blood and jejunum mucosa from 2 birds/pen of 70 pens were sampled for glutathione analysis. Data from EXP 1 were analyzed by a 2-way ANOVA with linear and quadratic contrasts across Gly levels, while EXP 2 data used a 2- and 3-way ANOVA ($P \leq 0.05$). In EXP 1, birds fed the RCP diet had lower ($P<0.05$) 0-21 d BWG and FI than CTL-fed birds, and increasing tGly+Ser linearly reduced ($P<0.001$) BWG and FI, but did not affect FCR. In EXP 2, 0-21 d bird BWG, FI and FCR were negatively affected ($P<0.001$) by VAC, and increasing tGly+Ser improved ($P \leq 0.043$) BWG, FI and FCR independently of VAC status. Birds fed RCP diet had inferior performance ($P<0.05$) than CTL-fed birds. Increasing tGly+Ser lowered FCR of UNVAC, but did not benefit the FCR of VAC birds. The Met:Cys ratio did not interact ($P>0.05$) with Gly levels or affect bird performance in either EXP. In EXP 2, liver glutathione levels were increased ($P<0.05$) by VAC, and VAC×Met:Cys interactions were observed for mucosa glutathione. In conclusion, birds fed RCP diets in both EXP had lower performance than CTL-fed birds, with added Gly only having a positive effect on performance in diets with a relatively lower CP reduction in EXP 2. The Met:Cys ratio did not influence bird performance or responses to tGly+Ser in either trial, including in *Eimeria*-vaccinated birds.

Key Words: glycine, TSAA, reduced protein, live Eimeria vaccine, broiler

M92 Interactive effects of stimbiotic supplementation and wheat bran inclusion in corn or wheat-based diets on glucose and amino acids transporters, ileal oligosaccharides profile, and ceca protein content Shravani Veluri*[1GS], Oluyinka Olukosi[1], Mike Bedford[2], Gemma Gonzalez-ortiz[2] [1]*University of Georgia,* [2]*AB Vista and Feed Ingredients*

Stimbiotic (SB), a combined product of xylanase and xylo-oligosaccharides, supplementation in broilers increases the fiber fermentation in the ceca of broilers and promotes gut health with the released short-chain fatty acids. It was hypothesized that adding wheat bran (WB) at a 5% inclusion level provides additional fiber for fermentation, and adding a stimbiotic would further increase fiber fermentation in the ceca. A 42-day study investigated the influence of stimbiotic supplementation and WB inclusion in corn- or wheat-based diets. Treatments were arranged in a 2×2×2 factorial, the factors being: diet (corn-SBM or wheat-SBM); SB (- or +); and WB (0 or 5%). A total of 960 day-old Cobb 500 broiler chicks were allocated to 64-floor pens with 8 treatments and 8 replicates/treatments. Jejunal tissue, ileal, and ceca contents were collected on d18 and 42 to determine the expression of nutrient transporter genes, oligosaccharides profile, and ceca protein content, respectively. Data were analyzed as a 2×2×2 factorial using the mixed model of JMP. There was no significant three-way interaction between factors for the expression of nutrient transporter genes at d18 and d42. There was significant diet × SB ($P < 0.05$) for GLUT2 measured on d18. There was an upward expression of GLUT2 with SB supplementation ($P < 0.05$) in corn- but not wheat-based diets. On d42, there was an upward expression ($P < 0.05$) of jejunum SGLT1 with SB supplementation. There was neither significant interaction nor significant main effects of factors on amino acid transporter genes analyzed. Ceca protein on d18 was greater for birds receiving corn-based than those receiving wheat-based diets ($P <0.05$). There was a significant diet × SB, where SB supplementation decreased ($P < 0.05$) ceca protein in corn-based diets but not in wheat-based diets on d42. Ileal pentose oligosaccharides, $Pent_4$ and $Pent_5$, were reduced ($P < 0.05$) with SB supplementation. In addition, SB decreased ($P < 0.05$) $Pent_3$ concentration in corn but not wheat diets. In conclusion, SB supplementation increased the expression of glucose transporters, reduced ileal oligosaccharides profile, and decreased ceca protein content; these are expected to contribute positively to the gut health of broiler chickens.

Key Words: stimbiotic, wheat bran, ceca protein, oligosaccharides, nutrient transporters

M93 Dietary supplementation of monosodium phosphate and exogenous phytase effects on growth, ileal phosphorus digestibility, and bone characteristics in broiler chickens Jung Yeol Sung*[1GS], Brittney Emmert[1], Darrin Karcher[1], Carrie Walk[2], Olayiwola Adeola[1] [1]*Purdue University,* [2]*DSM Nutritional Products*

The objective of this study was to evaluate effects of dietary monosodium phosphate (MSP) and exogenous phytase on growth performance, ileal digestibility of phosphorus (P), and bone characteristics in broiler chickens. Six experimental diets consisting of a P-deficient basal diet based on corn and soybean meal, basal diet plus 0.9 or 1.8 g/kg of inorganic P from MSP, and basal diet plus 500, 1,000, or 2,000 FYT/kg of exogenous phytase. Calcium to phosphorus ratio in all diets was maintained at 1.5:1. A total of 576 male broiler chickens (Cobb 500; initial body weight = 190 ± 17 g) at d 8 post hatching were allotted to the 6 dietary treatments in a randomized complete block design using body weight as a blocking factor. Each dietary treatment contained 8 replicate cages with 12 birds per cage. On d 11 post hatching, 7 birds from each cage were euthanized by CO_2 asphyxiation and dissected for the collection of ileal digesta. On d 18, ileal digesta were also collected from the remaining 5 birds in each cage. Left femur and tibia were collected from the bird with median BW on d 11 and 18 and analyzed for breaking strength and bone ash. Weight gain and gain to feed ratio linearly or quadratically increased ($P < 0.05$) in every period as the inclusion rate of MSP or phytase increased. Ileal digestibility of P increased ($P < 0.05$) on d 11 with increasing MSP, but there was no significant effect on d 18. Increasing phytase concentration linearly increased ($P < 0.05$) ileal digestibility of P on d 11 and 18. Bone-breaking strength and bone ash linearly or quadratically increased ($P < 0.05$) with increasing inclusion rate of MSP or phytase on d 11 and 18. In conclusion, adequate supplementation of MSP or phytase can increase growth, ileal P digestibility, and bone characteristics in broiler chickens.

Key Words: broiler, monosodium phosphate, phosphorus, phytase

M94 Impact of a cellulase-xylanase enzyme supplementation on performance and intestinal health in pullets fed almond hulls Nelly Cribillero*[1GS], Craig Wyatt[2], Kelley Wamsley[1], Timothy Boltz[1], Pratima Adhikari[1] [1]*Poultry Science Department, Mississippi State University,* [2]*AB Vista Feed Ingredients*

The objective of this study was to determine the effect of the supplementation of ground almond hulls (AH) and a cellulase-xylanase enzyme (EZ) inclusions on performance, gizzard and intestine weight, and measurements of pullets from 4 to 16 weeks (wks) of age. A total of 900, 4-week-old Lohmann LSL-Lite pullets were randomly distributed into six treatment groups in a 3 x 2 factorial arrangement. The factorial treatments comprised 3 levels of AH: 3%, 6%, and 9%, and 2 levels of EZ: yes (0.015%) or no. A standard breeder recommended diet was used as control. The pullets were raised in cages and fed mash diet ad libitum. From

4 to 8 wks of age, pullets were housed in 3 replicates/treatment (45 birds/pen). Then, the pullets were kept in rearing cages from 8 to 16 wks of age in 35 cages (25 birds/pen, 5 replicates/treatment). Data were analyzed using PROC GLM SAS 9.4. In the grower phase (4 – 8 wks), there was an interaction effect between the AH and the EZ inclusion regarding feed intake (FI; P=0.0216); FI was significantly higher in the 9% AH without EZ group in contrast to the 3% AH without EZ. A main effect of AH was found for feed conversion ratio (FCR; P=0.0348); the lowest FCR was observed in the 6% and 9% AH. At the developer phase (8 – 16 wks), there was a main effect in the EZ inclusion regarding FI (P=0.0054); FI was significantly lower in the 9% AH with EZ compared to the 6% AH without EZ. As for BW, there was a main effect in AH and EZ (P=0.0407; P=0.0388 respectively), with a significantly higher BW in the 6% AH without EZ than in 3% AH with EZ. There was a main effect regarding average daily gain (ADG; P=0.0343), the groups without EZ had significant higher ADG in relation to the groups with EZ. For ceca weight, there was a main effect of EZ with a significant higher weight in the 9% AH with EZ compared to 6% AH without EZ (P=0.0268). The results showed that at 6 and 9 % AH resulted in lower FCR without negative effect on FI and BW in grower phase. The AH inclusion at 6% without EZ showed higher BW and a positive effect in ADG in the developer phase. Further studies could assess the performance, egg quality and microbiota of the use of almond hulls and enzymes in early and post-peak production.

Key Words: almond hull, enzyme, performance, pullet

M95 Role of phytase and limestone particle size ratios on performance, egg breaking strength and gizzard pH and inositol levels of late-lay phase of single cycled laying hens Charis Waters*[1GS], Kelley Wamsley[1], Michael Elliot[2], Mike Bedford[3], Craig Wyatt[3], Pratima Adhikari[1] *[1]Mississippi State University, [2]A&E Nutrition Services, LLC, [3]AB Vista Feed Ingredients*

The objective of this study was to investigate the role of the ratio of fine (F) and coarse (C) limestone and exogenous phytase at standard and superdosing levels on performance, egg quality and bone quality of late-lay Hy-Line W-36 hens from 60 to 80 weeks of age. A total of 420 hens (20 replicates of 3 hens per treatment) were randomly assigned into 7 experimental diets in a 2 x 3 + 1 factorial arrangement. The positive control (PC) was an industry standard that consisted of a 40F:60C limestone ratio without phytase and had 4.4% calcium (Ca) and 0.44% available phosphorus (avP). A common negative control (NC) diet was created by reducing 0.19% Ca and 0.17% avP of the PC. The factorial treatments consisted of two limestone ratios (40F:60C and 15F:85C) and three phytase levels (0, 400, and 1500 FTU) formulated from the NC. Hens were housed in A-frame cages and feed was given at 100g per bird per day. Data were analyzed using PROC GLM SAS 9.4. There was an interaction effect with respect to hen day egg production (HDEP) (P <0.0001), a significant increase in HDEP was observed in 40F:60C compared to 15F:85C but only at 0 FTU. In contrast, there was an interaction regarding feed intake (FI) (P = 0.0006), where a higher intake was observed in 15F:85C with 1500 FTU groups compared to 15F:85C with 400 FTU. A significant increase in eggshell breaking strength (EBS) (P = 0.0016) was observed in 40F:60C with 1500 FTU groups and 15F:85C with 0 FTU groups produced significantly higher EBS compared to 400 and 1500 FTU. A main effect was observed in limestone ratios, in which 15F:85C had significantly more unsaleable eggs (P <0.0001) as compared to 40F:60C. Regarding phytase, 400 FTU had a significantly lower gizzard pH (P = 0.0372) and significantly higher gizzard inositol breakdown (P = 0.0224) compared to 0 FTU. Additionally, 400 FTU and 1500 FTU had a significant increase in gizzard IP3 breakdown (P = 0.0493) compared to 0 FTU. This study revealed that 40F:60C with 1500 FTU had benefits on EBS and 15F:85C with 0 FTU caused a decrease in HDEP. Independent of phytase, 40F:60C groups resulted in a decrease in unsaleable eggs. Future studies could involve the digestibility of total mineral content influenced by calcium and phosphorus due to added phytase levels.

Key Words: egg breaking strength, inositol, laying hens, limestone particle size, phytase

Metabolism and Nutrition V Feed Additives

M97 Butyrate affects chicken monocyte-like cell cycle progression. Famatta Perry*[1GS], Cristiano Bortoluzzi[2], Jeyashree Nathan Elango[1], Ayiana James[1], Emma Jones[3], Cinthia Eyng[4], Mike Kogut[4], Ryan Arsenault[1] *[1]University of Delaware, [2]Texas A&M University, [3]University of Maryland Eastern Shore, [4]USDA-ARS, Southern Plains Agricultural Research Center*

Butyrate is an important short chain fatty acid to the poultry industry because of its potential as a suitable antibiotic alternative. However, there are concerns about the toxicity of sodium butyrate (SB) within its therapeu-tic concentration range. The scientific literature is full of reports of many different concentrations of SB from 1mM to 150mM. Some papers even

referred to 45mM SB as a sub inhibitory concentration. Interestingly, in many of these studies the exposure time of cells or model organisms to butyrate differ. Using the kinome peptide array, our group examined the proteomic effects of 0.1% SB supplementation in the jejunum of Cobb 500 broiler chickens at day 21 and 35. Using cell viability assays, we assessed the effects of treating HD11 cells with 1mM to 16mM SB at different time points (2, 6, 18, 50 hours). Trypan blue cell counts showed no significant differences ($p>0.05$) in live cell counts between control (ctrl) and treated groups at 2 hrs, and between the number of dead cells in the ctrl versus treated groups for 2, 6, 18 and 50 hrs. At 6 hrs, only 16mM treated cells were significantly lower than the ctrl. At 18 hrs, there were more live cells in the ctrl group than the 8mM and 16mM SB groups and a similar statistical trend for the 12mM group ($p=0.08$). At 50 hrs, there were more live cells in the ctrl group than the 4 ,8 and 16mM SB groups. Alamar blue cell viability assay showed no statistical differences between the ctrl group and cells treated with 4 or 16mM SB at 6 or 24 hrs. The kinome peptide array results showed more changes in phosphorylation for SB supplemented jejunum at day 35 (366 peptides) than day 21 (261 peptides). There were also differences in the number of proteins observed in cell growth regulatory pathways like mTOR and cellular senescence. The phosphorylation status of cell cycle proteins were assessed, activation of CDK2, Cyclin-D1, CDK9 and CDK6, phosphorylation of Cyclin E; deactivation of mTOR, CDK1 and CDK7 indicates active G1 phase but not M or S phase in day 21 groups. CDK2 and mTOR were active in day 35 groups. These results suggest that SB does not decrease cell viability but may lead to reduction in immune cell growth and/or halt cell proliferation overtime by inducing changes in the cell cycle regulation.

Key Words: Butyrate, Toxicity, Cell proliferation, Cell cycle regulation, HD11 macrophages

M98 Effects of red osier dogwood extract and grape pomace on growth performance, blood biochemistry, immune response, and antioxidant capacity of broiler chickens challenged with heat stress. Fisayo Akinyemi*[GS], Deborah Adewole, *Dalhousie University.*

The ban of antibiotics in poultry farms has raised substantial concerns, especially with the increase in global temperature, which has resulted in huge losses in the industry. Much attention has been shifted to using polyphenols in attenuating heat stress (HS) due to their antioxidant ability. Considering the high polyphenol concentration of red osier dogwood extract (ROD) and grape pomace (GP), the current study aimed to determine their efficacy on growth performance, blood biochemistry, immunoglobulins (IgG and IgM), and total antioxidant capacity (TAC) of broiler chickens challenged with HS. 288 one-day-old broiler chicks were randomly allotted into 4 dietary treatments; 1) Negative control (NC; corn-wheat-soybean based diet), 2) NC + 0.05% bacitracin methylene disalicylate (BMD), 3) NC + 0.3% ROD, and 4) NC + 2.5% GP in 2 different rooms: 1) Thermoneutral (TN; 24oC±1 on d 21-27) and 2) Heat stress (HS; 32-34oC for 8 hr/day on d 21-27). Rectal temperature (RT) of 2 birds per cage was measured to confirm induced HS on d 21, 24, and 27. On d 28, two male birds per cage were euthanized for measuring blood biochemical, antioxidant, and immune status. Data were analyzed as a 4 × 2 factorial ANOVA using GLM of Minitab LLC (2019) software. on d 21, 0.05% BMD and 0.3% ROD inclusion improved ($P \leq 0.05$) the ABWG and FCR, compared to NC. HS reduced ($P < 0.05$) AFI, bursa and spleen weights. HS also increased ($P < 0.05$) the birds' RT, plasma Ca and activity of alanine phosphate and gamma-glutamyl transferase but reduced ($P < 0.05$) urea, aspartate and alanine transferase, lipase, and CK concentrations. Irrespective of HS, 0.3% ROD improved ($P=0.046$) plasma CK compared to the NC diet. HS further increased serum IgM ($P<0.001$) and IgG ($P=0.035$) compared to TN birds, while plasma CORT and glutathione peroxidase were not affected by HS and dietary treatments. 0.05% BMD and 2.5% GP inclusion increased ($P=0.002$) the plasma TAC of birds in the TN room, compared to NC and 0.3% ROD. Our results suggest that 0.3% ROD extract may function as an alternative to antibiotics in improving broiler growth performance parameters. Dietary supplementation of 2.5% GP improved the antioxidant capacity of broiler chickens in the absence of HS challenge.

Key Words: red osier dogwood, grape pomace, heat stress, growth performance, immunoglobulins, broiler chickens.

M99 Effects of in ovo injection of xylobiose and xylotriose on growth performance, carcass traits, and immune-related gene expression of broilers Razib Das*[GS], Pravin Mishra, Birendra Mishra, Rajesh Jha *Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa*

Xylooligosaccharides, namely xylobiose (XOS2) and xylotriose (XOS3) are prebiotics when supplemented with poultry diets could promote bene-ficial gut microbes and health. *In ovo* injection could deliver the prebiotics to the embryo and help study the posthatch effects. This study investigated the effects of *in ovo* injection of XOS2 and XOS3 on growth performance, carcass traits, and immune-related gene expression in broilers. A total of 145 fertile eggs of Cobb 500 broilers were divided into three groups: (a) non-injected control (CON), (b) XOS2, and (c) XOS3. The eggs from XOS2 and XOS3 groups received 0.5ml 0.85% normal saline solution containing 3mg XOS2 and 3mg XOS3, respectively, into their amniotic sac on the 17th embryonic day. A total of 131 hatched chicks were raised equally in 18 replicate cages following standard management practices and fed a corn-soybean meal-based diet for 21 days. The feed intake and body weight (BW) data were recorded weekly. The chickens were eutha-nized on d7 and d21 to collect ileum sections, and the carcass traits were recorded on only d21. Total RNA was extracted from ileum tissues, and qPCR was performed to measure the gene expression. All the statistical analyses considered the level of significance at a P value <0.05. The result showed that *in ovo* treatments did not affect the hatchability. The XOS2 group chickens had a higher BW at hatch day and the average daily feed intake was high for them during the first week. The XOS3 group showed an increased BW at d7 and improved FCR in the first week. There was no statistically significant difference ($P > 0.05$) among the groups for the relative weight of the breast muscle, drumsticks, gizzard, proventriculus, and carcass weight. On d7, the expressions of *IL10* and *CD56* were significantly higher ($P < 0.05$) in the XOS2 group, but showed no difference for the *IFNγ*, *TLR4*, and *IL4*. On d21, the XOS2 and XOS3 had signifi-cantly downregulated *IFNγ*, whereas there was no difference for the *IL10*, *TLR4*, *CD56*, and *IL4*. The XOS2 and XOS3 group performed better than the CON group to improve growth and immunity. In conclusion, *in ovo* XOS2 has the potential to increase the body weight of chicks at hatch and modulate the immunity in the chicken during the post-hatch days.

Key Words: in ovo, prebiotic, xylooligosaccharides, immunity, growth performance

M100 Individual and combined effects of phytase and butyric acid on performance and processing yield of YPM x Ross 708 male broilers reared to 42 days of age Joseph P. Gulizia*[1GS], Jose I. Vargas[1], Susan M. Bonilla[1], Jose R. Hernandez[1], Sureerat Thuekeaw[2], Ruediger Hauck[1,3], Ken Macklin[1], William A. Dozier, III[1], Klint W. McCafferty[4], Wilmer J. Pacheco[1] *[1]Department of Poultry Science, Auburn University, [2]Department of Animal Husbandry, Faculty Veterinary Science, Chulalongkorn University, [3]Department of Pathobiology, Auburn University, [4]USDA-ARS Poultry Research Unit*

The U.S. poultry industry has reduced the use of antibiotic growth promoters (AGP) in broiler production. This has led to increased incidence of disease and reduced broiler performance, which emphasized a need for AGP alternatives. The objective of this study was to evaluate the effects of phytase, butyric acid (BA), and their combination as AGP alternatives on broiler performance and processing yield from 1 to 42 d of age. A total of 2,560 d-old YPM x Ross 708 male broilers were distributed randomly in

64 floor pens (40 birds/pen) and assigned to 1 of 8 treatments with 8 replicates per treatment. This experiment was a 2 x 2 x 2 factorial arrangement of phytase (500 or 1,500 FTU/kg), BA (0 or 0.5 g supplement/kg diet), and BMD 50 (0 or 55 ppm). Feed intake (FI) and BW were determined at 14, 28, and 42 d of age, and FCR corrected for mortality. On d 43, 10 birds per pen were processed to determine carcass yields. Data were analyzed as a 2 x 2 x 2 factorial arrangement (phytase x BA x BMD) and as independent orthogonal contrasts. Means were separated using Tukey's HSD test and considered significant at $P \leq 0.05$. Phytase inclusion rate did not influence BW or FI of birds reared to 42 d of age ($P > 0.05$). Broilers fed a diet with 1,500 FTU/kg phytase had lower d 1 to 42 FCR (1.66 vs. 1.68 g:g) compared to 500 FTU/kg ($P < 0.05$). There were no differences in broiler performance when birds consumed a diet with or without BA ($P > 0.05$). Inclusion of BMD in broiler diets increased d 42 BW (3,251 vs. 3,166 g/bird), while reducing d 1 to 42 FCR (1.66 vs. 1.68 g:g) compared to diets without BMD ($P < 0.05$). There was no phytase by BA interaction for broiler performance ($P > 0.05$). Further, there were no main effect processing yield differences observed for phytase, BA, or BMD and no phytase by BA interaction ($P > 0.05$). Broilers fed a diet with BMD had improved d 1 to 28 performance compared to birds fed BA as indicated by the contrast analysis. However, broilers fed a diet with BA regardless of phytase inclusion had similar d 1 to 42 FCR compared to birds fed a diet with BMD ($P > 0.05$). Although broiler performance was primarily improved by BMD, further research is warranted on phytase and BA supplementation as an alternative to AGP.

Key Words: phytase, butyric acid, antibiotic growth promoters, broilers, phosphorus

M101 Effects of phytase in combination with a direct fed microbial (DFM) and a xylanase on broiler chicken performance, apparent metabolizable energy (AMEn), ileal P, Ca, and amino acid digestibility (AAD). Cooper Fritzlen*[1GS], Nathaniel Barrett[1], Brain Glover[2], Jose Charal[2], Milan Hruby[2], Michael Persia[1] *[1]Virginia Tech, [2]ADM Animal Nutrition*

An experiment was conducted to evaluate the effect of phytase, DFM and xylanase on the performance, AMEn, ileal P and Ca, and AAD of broilers. Straight run Cobb 500 broilers were housed in starter batteries (0.03 m^2) from 0 to 16 d. Positive (PC) and negative control (NC) diets were formulated with reductions of 0.16% nPP, 0.16% Ca and 110 kcal/kg AME resulting in 0.42% nPP, 0.90% Ca and 3065 kcal/kg and 0.26% nPP, 0.74% Ca and 2955 kcal/kg, respectively. Phytase (750 FTU/kg) was added to the NC diet alone (PY) or in combination with DFM (min: 3.2 x 10^9 CFU/g: PD), xylanase (2 xu/kg: PX), or DFM and xylanase (PDX) resulting in 6 treatments with 18 replicates of 9 birds. Body weight (BW) and feed offered and refused were measured at 0 and 16 d. Feed intake and mortality corrected feed conversion ratio (FCRm) were calculated over the 0 to 16 d period. Ileal P, Ca, AAD and AMEn were determined on d16. Data were analyzed using SAS ($P \leq 0.05$) and means were separated using Fishers LSD test. Overall, NC resulted in reduced BW in comparison to the PC (437 vs. 503 g; $P \leq 0.01$). The PY, PD, PX, and PDX diets resulted in increased BW over the NC ($P \leq 0.05$) similar to the PC (490, 488, 506, and 494 g, respectively; $P > 0.05$). The NC fed birds resulted in worsened FCRm of 1.369 in comparison to PC (1.313; $P \leq 0.05$). Inclusion of PY, PD, PX, and PDX to the NC resulted in improved FCRm over the NC ($P \leq 0.05$) and similar to PC (1.307, 1.317, 1.310, and 1.328, respectively; $P > 0.05$). The NC resulted in reduced AMEn (2,753 kcal/kg) compared to the PC (3,072 kcal/kg; $P \leq 0.01$). Addition of PY to the NC diets resulted in AMEn (2,950 kcal/kg), intermediate to NC and PC. Addition of PD, PX, and PDX to the NC resulted in AMEn that was similar to the PC (3,014, 3,002, and 3,031 kcal/kg, respectively; $P > 0.05$). Addition of PY, PD, PX, and PDX to the NC resulted in increased P digestibility over the PC and NC ($P \leq 0.01$) but did not alter Ca digestibility ($P > 0.05$). Generally, the feed additives did not affect the AAD, but the addition of xylanase seemed to worsen AAD of some AA. Phytase was able to restore performance and increase both ileal P digestibly and AMEn in reduced nutrient diets, but additional feed additive supplementation was required to fully restore dietary AMEn.

Key Words: Broiler Performance, Phytase, DFM, Xylanase, Apparent Metabolizable Energy

M102 The effect of a dacitic (rhyolitic) tuff breccia (Azomite®) in corn, soybean, and DDGS based diets across pellet die size on production rate and pellet quality under a constant mill load. Lucas Knarr*[1GS], Kristina Bowen[1], Jon Ferrel[2], Joe Moritz[1] *[1]West Virginia University, [2]Azomite Mineral Products*

Azomite (AZM) has been shown to increase pellet production rate (PPR) when included in corn and soybean meal-based diets containing dicalcium phosphate (DCP) and dried distillers grains with solubles (DDGS). It is presumed that these increases in PPR are due to the scouring and lubrication properties associated with the coarse angularity and fine fractions of AZM, respectively. The authors hypothesized that if pellet mill motor load were held constant, then PPR would increase with the inclusion of AZM across various pellet die thicknesses (PDT) in corn, soybean meal, and DGGS-based poultry diets. The objective was to determine the effect of AZM (0.25%) on PPR, hot pellet temperature (HPT), and pellet durability index (PDI) while utilizing three different pellet die thicknesses (4.5mm x 32mm (7.1 L:D), 4.5mm x 38mm (8.4 L:D), and 4.5mm x 45mm (10.0 L:D)). Pellet dies were newly purchased prior to the start of the study. Feed was manufactured in a 2 (AZM) x 3 (PDT) factorial in a latin square design, utilizing a California Pellet Mill and conditioner (80°C for 30sec), with each bach totaling 653kg. Main effect interactions of AZM and PDT were significant for HPT ($P = 0.007$). As PDT increased (32mm to 38mm/45mm) PPR decreased by 28.5% (0.897 to 0.642 Mt/hr) ($P < 0.0001$), and PDI increased by 10.0 percentage points (83.1 to 93.1%) ($P < 0.0001$). The inclusion of AZM increased PPR by 7.9% (0.699 to 0.754 Mt/hr, ($P = 0.0002$) and decreased PDI by 1.6 percentage points (90.6 to 89.0%, $P < 0.001$). Contrast analysis showed that PPR increased by 5.0, 7.9, and 11.8% when comparing AZM treatments to their respective PDT controls (32mm, $P = 0.012$; 38mm, $P = 0.003$; 45mm, $P = 0.004$). Contrasts also showed that PDI decreased by 2.26 percentage points with the addition of AZM for the 32mm PDT (84.27 vs. 82.00, $P < 0.001$), and HPT decreased (82.23 to 80.29°C) for the 38mm PDT ($P < 0.001$). In conclusion, AZM increased pellet production rate across pellet die thickness and decrease pellet durability index, albeit to an extent that would likely not affect broiler performance.

Key Words: Azomite, Pellet Die Thickness, Pellet Production Rate, Pellet Durability

Metabolism and Nutrition VI Feed Additives

M103 The impact of antibiotic growth promoters on intestinal function during a subclinical necrotic enteritis challenge in broilers Shailes Bhattrai*[GS], Laura Ellestad *University of Georgia*

The ban on the use of dietary antibiotic growth promotors (AGPs) has increased the incidence of *Clostridium perfringens*-induced subclinical necrotic enteritis (SNE), which leads to poor growth performance in broilers. Though the mechanisms are unclear, AGPs may enhance production efficiency, in part, by improving intestinal function. This study investigated how bacitracin methylene disalicylate (BMD) fed at AGP levels (55 mg/Kg) impacted intestinal function in SNE-challenged broilers. Day (D)-old male Ross broilers were grouped into three treatments (n=5 pens/

treatment): unchallenged control, challenged without BMD (SNE), and challenged with BMD (SNE+BMD). Whole tissue and mucosal scrapings from jejunum and ileum were collected from one bird/pen at 0 (D21), 24 (D22), 72 (D24), and 168 (D28) hours post final infection (HPFI) for intestinal histomorphometry and metabolite analysis. Data were analyzed by one-way ANOVA followed by Fisher's LSD test when ANOVA indicated significance (P<0.05). SNE+BMD birds had shorter jejunal villi height (VH) compared to control and SNE birds at 0 HPFI (P<0.05). SNE-challenge, regardless of BMD inclusion, increased crypt depth (CD) and decreased VH:CD in both jejunum and ileum at 0 HPFI (P<0.05). At 0 HPFI, SNE+BMD birds had higher concentration of threonine compared to control birds in jejunum and compared to control and SNE birds in ileum (P<0.05). SNE challenge, regardless of BMD inclusion, increased the concentration of threonine in ileum at 72 HPFI (P<0.05). Ileal concentration of phenylalanine decreased in SNE birds at 0 HPFI, but in this case, BMD supplementation prevented this effect (P<0.05). In ileum, SNE+BMD birds had higher concentration of α-ketoglutarate compared to control birds at 0 HPFI, while the concentration of pyruvate was higher in SNE birds at 24 HPFI (P<0.05). Elevated threonine in BMD+SNE birds at earlier HPFI could support protective mucin production, while reduced VH in these birds at this time could reduce intestinal maintenance energy requirements. BMD inclusion could further alleviate negative effects of SNE by altering levels of metabolites that indirectly (phenylalanine) or directly (pyruvate and α-ketoglutarate) support energy production through the citric acid cycle.

Key Words: Antibiotic growth promoters, crypt depth, histomorphometry, subclinical necrotic enteritis, villi height

M104 Effect of synbiotic supplementation on production performance and necrotic enteritis severity in broilers under an experimental necrotic enteritis challenge Bikas Shah*[1GS], Walid Al Hakeem[1], Shahna Fathima[1], Revathi Shanmugasundaram[2], Ramesh Selvaraj[1] *[1]Department of Poultry Science, The Unviersity of Georgia, [2]Toxicology and Mycotoxin Research Unit, USDA-ARS*

Synbiotic supplementation in broilers is beneficial for growth, performance, maintaining gut health and induce mucosal immunity against bacterial diseases by production of antimicrobial compound, bacteriocins. To evaluate the efficacy of synbiotic, a total of 360-day-old chicks were randomly assigned into four experimental groups in 2x2 factorial setup: control, challenge, synbiotic and challenge + synbiotic, with 6 replicates. Necrotic enteritis (NE) in birds were induced by gavaging $1x10^4$ *E. maxima* oocysts and $1x10^8$ CFU/ml of *C. perfringens*on day 14 (D14) and D19, 20 and 21 respectively. The data were analyzed using 2x2 factorial ANOVA by Jmp software. At D35, NE challenge decreased body wt. by 200 gm, increased FCR and feed intake by 0.22 points and 70 gm respectively, while synbiotic supplementation during NE challenge decreased body wt. by 190 gm *(p<0.01)*, increased FCR by 0.04 points *(p=0.04)*, and decreased feed intake by 220 gm *(p=0.04)*, when compared to control group. At D21, NE challenge increased rank score means for mid-gut lesions, increased FITC-d concentration in blood serum, increased anti *C. perfringens* IgA in bile, decreased percentage of CD4+:CD8+ T cells in cecal tonsil (CT), and decreased percentage of regulatory T cells (Tregs) in CT, while synbiotic supplementation during NE challenge increased rank score means for mid-gut lesions *(p<0.01)*, increased FITC-d concentration in blood serum *(p<0.01)*, decreased anti *C. perfringens* IgA in bile *(p<0.01)*, increased percentage of CD4+:CD8+ T cells in cecal tonsil CT *(p<0.01)*, and decreased percentage of Tregs in CT *(p=0.02)*, when compared to control group. At D28, NE challenge decreased percentage of CD4+:CD8+ T cells in CT and increased percentage of Tregs in CT while synbiotic supplementation during NE challenge decreased percentage of CD4+:CD8+ T cells in CT *(p<0.01)* and decreased percentage of Tregs in CT *(p<0.01)*, when compared to control group. At D35, NE challenge decreased anti *C. perfringens IgA* in bile while synbiotic supplementation during NE challenge decreased anti *C. perfringens IgA* in bile *(p=0.02)*, when compared to control group. It can be concluded, the synbiotic supplementation increases production performance, decrease mid-gut lesions and enhance protective immunity against NE.

Key Words: Clostridium perfringens, immunity, necrotic enteritis, synbiotic

M105 Cecal microbiota profile of broiler chickens raised under different antimicrobial feeding frograms Philip Mak*[1,2GS], Lisa Bean-Hodgins[3], Elijah Kiarie[2], Dion Lepp[1], Moussa Diarra[1] *[1]Guelph Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), [2]Department of Animal Biosciences, University of Guelph, [3]New-Life Mills, A division of Parrish & Heimbecker*

The cecal microbiota plays an important role in fermentation, metabolism, and immunity in broilers. This study analyzed the cecal digesta bacterial community diversity and composition in male and female broilers under different feeding programs. A total of 2,304 Ross 708 broilers (equal ♀ and ♂) were placed in 48 floor pens (48 birds/pen; 24 pen/sex). Each sex group was allocated to one of three feeding programs: Conventional (CON: program with bacitracin); raised without medically important antibiotics (RWMIA: program with avilamycin) and raised without antibiotics (RWA). Two birds/pen were necropsied on D28 and 41 to access cecal digesta and genomic DNA extracted for downstream 16S rRNA sequencing using Illumina MiSeq. Data were analyzed with QIIME 2; pairwise statistical tests were used for α (Kruskal-Wallis) and β diversities (PERMANOVA) and analysis of composition of microbiomes (ANCOM) reported differences among bacterial abundance. On D28, there were no (P>0.05) differences between sex and feeding program for the α-diversity (Chao, Simpson, and Shannon). Pairwise statistical PERMANOVA of b-diversity showed differences between treatment groups (P<0.001). ANCOM showed that at the phylum level, Cyanobacteria were more abundant in female birds under CON and RWA programs, while at the genus level, *Enterococcus* were more predominant in RWA birds, with female birds exhibiting higher abundances. Using PERMANOVA, the cecal microbiota showed significant differences in b-diversity between treatments and sex except for when comparing RWA males to RWA females (P<0.05). From ANCOM's statistical analyses, a significant difference in *Enterococcaceae* (g_*Enterococcus*) abundances was confirmed, which were higher in RWA birds. Overall results of both sampling days revealed that abundances of families including *Bacillaceae*, *Enterococcaceae*, *Anaeroplasmataceae*, *Veillonellaceae* and *Clostridiaceae*, were significantly influenced by sex and feeding programs. In conclusion, the present study showed that in a specific feeding program such as RWA, the cecal microbiota of broilers can be significantly affected by sex. These results will contribute to understanding the influence of sex in broiler gut microbiota composition, which could define their functionality.

Key Words: broiler chickens, sex, cecal microbiota, antimicrobial feeding

M106 Gut heath of broiler fed enzymes treated or untreated cranberry pomace in a Eimeria spp. challenge model. Aline Pereira*[1GS], Bruna Belote[2], Moussa Diarra[3], Kely Ross[4], Qi Wang[3], Elijah Kiarie[1] *[1]University of Guelph, [2]ISI Institute, [3]Guelph Research and Development Centre, Agriculture and Agri-Food Canada, [4]Summerland Research and Development Centre, Agriculture and Agri-Food Canada*

Cranberry pomace (CBP), a residual of fruit processing is rich in phenolic compounds that may be beneficial to broiler chicken production. We evaluated the effects of inclusion of enzyme treated (ETCBP) and untreated (UTCBP) on gut health, organ weight and oocysts shedding in broiler challenged with *Eimeria spp*. For preparation of ETCBP, thawed CBP was mixed with multi-enzyme supplement (1 kg CBP:2.5 g), placed in an incubator shaker for 24 h at 40˚C, dried at 60˚C along with thawed UTCBP samples and ground for feed preparation. A total of 960 d-old male Ross 708 chicks were placed in floor pens (20 birds/pen) and allocated to 6 diets

(n=8) as follow: 1) NC; no additives, 2) PC; NC + bacitracin methylene disalicylate and narasin, NC + 0.5 and 1% ETCBP or UTCBP. Birds had free access to water and feed for 42 days. On d 14, birds orally received 1 mL of 100,000 *E. acervulina,* 15,000 *E. maxima,* and 10,000 *E. tenella* culture. On d 2, 6, 9, and 13 post-challenge, excreta samples were for oocyst count. On d 21, 28 and 42, two birds per pen were weighed and euthanized for intestinal lesion scorings and organs (liver, and bursa) weight. Histological analyses were performed on duodenum and ileum samples on d 21 and 28 using the I See Inside (ISI) methodology. The ISI translates macroscopic and microscopic histological alterations into score (ISI score). The statistical model had diet as a fixed factor and LSmeans were separated using Tukey test. Diets had no (P>0.05) effects on organs weight and gross lesion score. On d 21 and 28, birds fed PC had lower (P<0.01) duodenal ISI score than NC and CBP birds. Birds fed PC, 0.5% ETCBP or UTCBP exhibited lower (P<0.01) inflammatory cells on duodenal lamina propria (ICLP) than NC birds. Among CBP birds, 0.5% ETCBP lower duodenal ICLP than other birds on d 21 and higher than birds fed UTCBP on d 28 (P<0.01). On d 28, 1.0 %UTCBP had higher ileal ISI score than PC but lower (P<0.01) than NC or 0.5% UTCBP or ETCBP. Birds fed PC shed (P=0.017) less oocyst than NC and 0.5% ETCBP birds, however, birds fed other CBP diets were intermediate. In conclusion, narasin was effective in reducing oocysts shedding, however, enzyme treated or untreated CBP modulated indices of intestinal health and further investigations are warranted.

Key Words: Broiler chickens, gut health, coccidiosis, cranberry pomace

M107 In-feed bacitracin methylene disalicylate impaired microbial function and increased antibiotic resistance in cecal microbiota of turkey in a dose dependent manner Paul Oladele*[1GS], Carmen Wickware[1,2], Julian Trachsel[2], Torey Looft[2], Timothy Johnson[1] *[1]Department of Animal Sciences, Purdue University, [2]National Animal Disease center, Agricultural Research Service, United States Department of Agriculture*

Antibiotic growth promotors (AGP) have been widely used in turkey production, but the increasing occurrence of antibiotic resistance genes (ARG) has necessitated the need for viable alternatives. Previous studies have shown that bacitracin methylene disalicylate (BMD), a common AGP in poultry production, decreased taxonomic diversity of turkey microbiome but a clear understanding of its impact on the functional potential of the microbiome is limited. Such information can improve our understanding of the mechanism of growth promotion by BMD. In this study, we investigated the effect of subtherapeutic and therapeutic doses of BMD on the functional potential of the turkey cecal microbiota. Two-hundred and forty male turkey poults were assigned to three treatments: Control (no BMD), subtherapeutic BMD (50g BMD/ton feed) and therapeutic BMD (200g BMD/ton feed for 35 days before switching to the subtherapeutic dose from day 35 to 78). Ten turkeys/treatment were euthanized on day 7, 35 and 78, and cecal contents were collected for metagenomic sequencing. Open reading frames were annotated with TIGRFam database and ARG with comprehensive antibiotic resistance database (CARD). Kruskal-Wallis test was used to test difference between treatment groups and DESeq2 was used for differential abundance analysis in R. Both doses of BMD reduced functional diversity and impaired bacterial metabolism with the magnitude being higher in the therapeutic dose. The subtherapeutic dose of BMD altered the functional profile of the community on day 7 ($P < 0.05$) which was restored on days 35 and 78. The therapeutic dose of BMD decreased estimated bacterial growth rate on day 7 ($P < 0.001$) and induced a change in the functional profile of the community ($P < 0.05$) which remained different even after switching to the subtherapeutic dose on day 35 and 78 ($P < 0.05$). The therapeutic dose increased abundance ARGs ($P < 0.05$), conjugation-related genes belonging to type IV secretion system ($P < 0.05$), and transduction-related genes ($P < 0.05$). Overall, subtherapeutic BMD induced a transient change in functional potential of the turkey microbiome while therapeutic BMD caused a longer-term impairment in the functional potential, metabolism and abundance ARG in the turkey cecal microbiota.

Key Words: cecal microbiota, antibiotic growth promoter, antibiotic resistance, metabolism, turkey

M108 Evaluation of dietary plasma fed to turkeys during brooding on performance to market age. Ashley Gernat*[1GS], Joy Campbell[2], Adam Fahrenholz[1], Jesse Grimes[1] *[1]North Carolina State University, [2]APC Proteins*

Commercial turkey production can experience economic loss due to bird exposure to stress. Turkeys can experience various levels of stress including, but not limited to; hatching, brooding/growing, and transport. Temperature and climate are two important issues of concern for poultry producers. Both heat and cold stress are major livestock stressors accompanied by economic losses. Stress exposure also causes increased probability of infections and diseases that also have a negative economic impact in production. Nutritional products that could benefit or alleviate stress in animals are in need of evaluation. In this study, Large White commercial turkey hens were reared to 12 wk to evaluate their stress responses and performance alterations due to induced stress to mimic brooder house to grow-out transition. Parameters for performance, blood, bone, and meat yield were recorded. Spray Dried Bovine plasma (SDBP) (AP920, APC, LLC) was formulated iso-nutritionally into the diets. AP920 has shown to be an ingredient in animal diets that may help support immune health and have a positive effect on performance. This product was used for a total of 6 wk in starter 1 and starter 2 at different percentages of inclusion. Treatments included a control diet (0% plasma), a 1.0% (AP1) plasma inclusion and 2.0% (AP2) plasma inclusion. At 6 wk, common diets were fed (grower and finisher). Management stressors were applied for 24 h: feed and water restriction and reduced temperatures. Previously used pine shavings were used for bedding. No statistical differences due to feed treatments were observed for BW, BW gain, or feed intake. Feed conversion ratio (FCR) was improved 6 wk due to SDP, AP1(1.90) and AP2(1.97) were lower than for the control diet (2.04). No difference in FCR was found at 12 wk. No differences were observed for % of meat yield, mortality, bone ash or other bone parameters. Blood analysis resulted in no effects for immunoglobulin levels. A significant difference was observed for corticosterone levels post stress: AP1(23.81ng/mL) and AP2 (19.17ng/mL) were higher than control (16.40 ng/mL). Further research is needed to ascertain the effects of SDP on production and immune function in turkeys.

Key Words: turkeys, performance, stress, corticosterone, spray dried plasma

M109 Impact of feeding sources of α-linolenic acid and yeast bioactives on growth and development of egg-type Lohmann LSL-Lite pullets from hatch through to 16 weeks of age Junhyung Lee*[GS], Veronica Cheng, Elijah Kiarie *University of Guelph*

Egg-type pullets are characterized with rapid growth of visceral, skeletal, and lymphoid organs. There is limited research on the role of functional nutrients and feed additives on pullet growth and development. Therefore, we investigated the effects of feeding sources of α-linolenic acid (ALA) and yeast bioactives (YB) on growth and organ development in Lohmann LSL-Lite pullets from hatch through 16 weeks of age (woa). The ALA was from co-extruded full fat flaxseed and pulse mixture (FFF; 1:1 wt/wt), and YB was derived from hydrolysis of whole yeast by β-1,3-glucan hydrolase. A total of 1,064 d-old pullets were placed in conventional cages (19 birds/cage) based on body weight (BW) and allocated to 7 diets in a completely randomized design (n=8). The diets were: corn-SBM control, control+1, 3 or 5% FFF, and control+0.025, 0.05 or 0.1% YB. The birds had *ad libitum* access to feed and water. Body weight (BW), body weight gain (BWG), and feed intake (FI) was monitored bi-weekly. Feed conversion ratio (FCR) was calculated using BWG and FI data. Six wing-

banded birds per cage were individually weighed at 4, 8, 12, 16 woa for calculation of BW uniformity. One bird/ cage was sampled at 4, 8, 12, 16 woa for bursa, spleen, and thymus weight. Statistical model used diet as a fixed factor, LSmeans were separated using Tukey method and dose responses of FFF and YB were evaluated. Quadratic effect (P≤0.06) was observed for 16 woa BW; for FFF, 1% FFF birds were heavier than 3 and 5% FFF and for YB birds, 0.05 and 0.1% YB had similar (P>0.05) BW, but heavier than 0.025% YB. FFF linearly (P=0.01) reduced BWG, but YB quadratically (P<0.05) increased BWG at 8 and 16 woa. Inclusion of YB linearly (P=0.02) increased FCR between 1 to 4 woa and quadratically (P=0.01) decreased FCR between 5 to 8 woa. Feeding FFF linearly (P=0.02) reduced bursa and thymus weight at 4 and 8 woa, respectively and quadratically (P=0.045) increased spleen weight at 16 woa. Feeding YB quadratically increased thymus weight (P=0.07) at 4 woa and spleen (P=0.05) at 8 woa. In conclusion, dietary provision of ALA and YB influenced pullet BW at sexual maturity, and development of lymphoid organs.

Key Words: α-linolenic acid, Yeast bioactives, Growth and development, Pullet

M110 Effect of a glycan microbial metabolic modulator on fecal shedding and tissue translocation of Salmonella Enteritidis in laying hen Ishab Poudel*[1GS], Murtala Umar-Faruk[2], Fernando Cisneros-Gonzalez[2], Aaron Kiess[3], Li Zhang[1], Pratima Adhikari[1] *[1]Department of Poultry Science, Mississippi State University, [2]DSM Nutritional Products AG, [3]Prestage Department of Poultry Science, North Carolina State University*

A study was conducted to evaluate the efficacy of a glycan microbial metabolic modulator in controlling *Salmonella* Enteritidis (SE) infection in laying hens. A total of 72 Hy-Line W-36 hens were orally challenged for two consecutive days with approximately 10^8 colony forming units (CFU) of nalidixic resistant SE at 35-weeks of age. After the challenge, three treatments were applied: 1) Control diet without test product (CON), 2) CON + 450mg/kg feed test product, and 3) CON + 900mg/kg feed test product. Each cage had two birds with an individual feeder and fecal collection tray. Feces were enumerated at 0, 3- 7-, and 14-days post inoculation (dpi). Fecal samples were pre-enriched in buffer peptone water at 37°C for 24 h. One ml of pre-enriched sample was transferred for enrichment in 10 ml of tetrathionate enrichment (TT) broth and 0.1 ml of sample was inoculated in 9.9 ml of Rappaport Vassiliadis (RV) broth and incubated at 42°C for 24 h. The enriched samples were serially diluted and 100 µl of the sample was further inoculated in xylose lysine tergitol 4 agar plates with 200 ppm nalidixic acid and incubated at 37°C for 24 h. Blood was collected at day 0, 7 and 14 dpi. Liver with gall bladder, spleen, ovaries, and ceca were collected at 7 and 14 dpi. Tissue samples were triturated using a rubber mallet and transferred to TT broth in a ratio of 1:10. Samples were incubated at 37°C for 24 h and processed with same procedure as fecal samples. The data was log-transformed and analyzed using PROC GLIMMIX procedure of SAS 9.4. Levels of inclusion of the test product had a linear reduction of SE in fecal shedding at 7 dpi (P=0.042). Higher fecal shedding of 0.55 $\log_{10}$ CFU/g was observed in CON as compared to 0.21 and 0.18 $\log_{10}$ CFU/g in 450mg/kg and 900mg/kg respectively. A trend was observed in cecal samples at 7 dpi (P=0.07) with 0.77 $\log_{10}$ CFU/g in CON as compared to 0.29 from 450mg/kg. Anti-*Salmonella* IgY significantly increased over time (P<0.001), it was observed that day 0 and 7 had 0.74 and 0.73 (ng/ml) as compared to 1.39 (ng/ml) at day 14. Indicating that oral SE challenge produced a detectible immune response at 14 dpi. In conclusion, addition of a glycan microbial metabolic modulator in feed may reduce *Salmonella* in fecal shedding in laying hen.

Key Words: Laying hen, microbial metabolic modulators, glycan, Salmonella Enteritidis

M111 A microencapsulated blend of thymol and organic acids can control necrotic enteritis caused by Clostridium perfringens in broiler chickens Benedetta Tugnoli*[1], Andrea Piva[1,2], Brett Lumpkins[3], Ester Grilli[2,4] *[1]Vetagro S.p.A., [2]University of Bologna DIMEVET, [3]Southern Poultry Feed and Research, Inc, [4]Vetagro, Inc*

Thymol is a botanical well-known for its various biological properties (antimicrobial, anti-oxidant, antiinflammatory), while organic acids are widely used in poultry nutrition as antimicrobials. The aim of the study was to evaluate the effects of a microencapsulated blend of thymol and organic acids (mTOA) in broilers exposed to a necrotic enteritis (NE) challenge caused by *Eimeria* cocci and *Clostridium perfringens* (CP). A total of 3,000 day-old male chicks (Cobb500) were vaccinated for coccidiosis (COCCIVAC®-B52), divided in floor pens (50 chicks/pen) and assigned to 5 groups (12 pens/group): negative control (NEG), unchallenged, fed a basal diet; positive control (POS), challenged, fed a basal diet; and three groups challenged and fed a basal diet supplemented with mTOA at either 100, 200 or 300 g/MT of feed (T1, T2, T3, respectively). The challenge consisted of an oral inoculation with *E. maxima* (5,000 oocysts) at day 14 and with a CP strain isolated from clinical case of NE (α toxin and netB toxin positive, 10^8 CFU) for 3 days (d19, 20, and 21). The study lasted 42 days with small intestine lesions scored at d21 (3 chicks/pen) and growth performance recorded throughout the study. Data were analyzed with ANOVA and differences considered significant at P<0.05. From d14 to d28, during the challenge, POS group showed an FCR significantly higher than NEG group (1.89 vs 1.62) while the product inclusion improved FCR in a dose-dependent manner (1.78, 1.73, 1.61 for T1, T2, T3, respectively; P<0.0001). From d28 to d36, all the groups receiving mTOA had intermediate FCR values between NEG and POS groups (P=0.03). In the overall period, the FCR value for POS group (1.75) was significantly higher than NEG group (1.61) and all the treated groups FCR values were comparable to NEG, unchallenged (1.66, 1.65, 1.63 for T1, T2, T3, respectively; P<0.0001). Moreover, treated groups showed reduced small intestine lesion score at d21 (P<0.0001) and lower overall mortality due to NE compared to POS control (10 % for POS vs 4.3 %, 2.5%, 1% for T1, T2, T3, respectively, P<0.0001).

To conclude, the microencapsulated blend of thymol and organic acids used in this study has the potential to contain loss of performance and lesions associated with NE challenge in broilers.

Key Words: Organic acids, thymol, microencapsulation, necrotic enteritis, broilers

M112 IMW50® association with essential oil and probiotic prevents negative effects of necrotic enteritis on broilers Ricardo Barbalho[1], Melina Bonato*[1], Fernando Souza[1], Lucio Araujo[2], Aaron Kiess[3], Claudia Castaneda[4], Caio Barbalho[2], Raquel Carvalho[2] *[1]ICC Industrial Comercio Exportacao Importacao S.A, [2]Faculty of Animal Science and Food Engineering, University of Sao Paulo, [3]North Caroline State University, [4]Mississippi State University*

The objective of this study was to evaluate the efficacy of IMW50® (yeast cell wall), essential oil, and a probiotic in non-AGP diets of broilers challenged with *Eimeria* spp. and *Clostridium perfringens*. Seven hundred 1-day-old chicks (male) Ross x Ross 708 from a commercial hatchery were allocated to 48 pens with 15 chicks per pen for ten days. On day ten, 672 birds were moved to a biosecurity level 2 room (BSL2 facility) to acclimate. They were allocated to 96 cages in a CRBD distributed in 4 treatments with seven birds per pen and 24 replicates. They were randomly distributed in the groups: (NC) Negative control – without coccidiostats; (PC) Positive control – with coccidiostats (Maxxiban® at 72 g/MT); (YCW1) NC + Yeast Cell Wall (IMW50®) at 500 g/MT + Essential Oil (blend of Carvacrol and Thymol) at 300 g/MT; (YCW2) NC + Yeast Cell Wall (IMW50®) at 500 g/MT + Essential Oil (blend of Carvacrol and Thymol) at 600 g/MT + *Saccharomyces Boulardi* 10ME (Levucell®) at 100g/MT. On day 14, all birds were individually challenged via oral

gavage with a 20-fold dose of Coccivac-B52® (Intervet Inc., Omaha, NE) and received 1 mL of inoculum containing a mixture of 5 *Eimeria* strains. On days 14 and 21, 1mL of *C. perfringens* (10^8 CFU/mL) was inoculated per bird. The performance was measured until 28 days. Some samples of Bursa of Fabricius, duodenum, jejunum, ileum, and cecum were collected at 21 and 28 days to calculate the lesion score of necrotic enteritis (NE). No statistical differences were observed (P>0.05) in the NE score. Body weight gain (BWG) was improved (p=0.002) in PC groups vs. the other treatments (387 g vs. NC 353 g, YCW1 361 g, and YCW2 358 g) on the first period until 14 days. However, regarding the total period (1-28 days) BWG was similar in groups PC (1185 g), YCW1 (1155 g), and YCW2 (1184 g) and differing (P=0.0001) from NC (1075 g). The feed conversion rate (FCR) was improved in treatments YCW1 (1.17) and PC 2 (1.15) (P=0.003) for the first 14 days, but on the accumulative period, PC (1.32), YCW1 (1.32) and YCW2 (1.31) showed statistical differences (P=0.001) from NC (1.43). The results of this study demonstrated the efficacy of the feed additives association in preventing the negative effects of NE on performance in broilers.

Key Words: Yeast Cell Wall, Essential Oil, Saccharomyces cereisiae, Saccharomyces Boulardi

M113 The impact of monoglyceride blend on improving broiler performance during necrotic enteritis challenge Jundi Liu*[1], Matthew Jones[2], Ross Wolfenden[1], Charles Hofacre[2] *[1]Eastman Chemical Company, [2]Southern Poultry Research Group, Inc.*

To further understand the beneficial impacts of monoglycerides in broiler chickens, a 42-day study was conducted to evaluate an Eastman monoglyceride blend on performance during a floor pen necrotic enteritis challenge model. A total of 1,400 male Ross broilers were placed on the floor pen into 4 groups with 25 broiler chickens per pen and 14 replicates per treatment: (1) non-challenged control (NCC); (2) challenged control (CC); (3) challenged control + 1,000 mg/kg monoglyceride blend (low-dose); (4) challenged control + 2,000 mg/kg monoglyceride blend (high-dose). Broilers were placed on the fresh pine shavings and the monoglyceride blend was supplemented only during the starter (d 1-14) and grower (d 14-28) phases. On d 14, challenged birds were inoculated in-feed with ~2500 oocysts/bird *Eimeria maxima*, followed by 1.0 x $10^{\wedge 8}$ CFU/bird *Clostridium perfringens* on d 19 and 20. On d 22, 1 broiler per pen was randomly selected to score intestinal lesions. Data were analyzed by ANOVA model with significance deemed at $p < 0.05$. Means were separated using Tukey's HSD test. Monoglyceride blend at 1,000 mg/kg (FCR = 1.308) significantly improved FCR compared to the CC (FCR = 1.471) on d 14. From d 1 to 28, birds from the CC (FCR = 1.540) had significantly higher FCR compared to the NCC (FCR = 1.451), and adding monoglyceride blend at low- (FCR = 1.500) and high-doses (FCR = 1.491) showed equivalent FCR compared to the NCC. There was a numerical improvement on the 42-d mortality-adjusted FCR when adding monoglyceride blend at low- (FCR = 1.550) and high-doses (FCR = 1.564) when compared to the CC (FCR = 1.580). For lesion score, numerical decreases were observed in the monoglyceride blend groups at low- (mean score = 0.64) and high-doses (mean score = 0.64) compared to the CC (mean score = 0.93). In conclusion, Eastman monoglyceride blend improved gut health and demonstrated improvement on FCR of broilers under necrotic enteritis challenge.

Key Words: monoglyceride blend, growth, coccidiosis, necrotic enteritis, broiler

M114 Evaluation of a monoglyceride blend on broiler performance during necrotic enteritis challenge in battery cages Danielle Graham*[1], Jundi Liu[2], Ross Wolfenden[2], Billy Hargis[1] *[1]Department of Poultry Science, University of Arkansas Division of Agriculture, [2]Animal Nutrition BU, Eastman Chemical Company*

The objective of this study was to evaluate the impact of an Eastman monoglyceride blend on broiler performance under a 25-day necrotic enteritis challenge model in battery cages. A total of 320 one-day old male Cobb broilers were obtained from a local hatchery and randomly distributed into 4 groups with 10 chicks per cage and 8 replicates per treatment: (1) non-challenged control (NCC); (2) challenged control (CC); (3) challenged control + 500 mg/kg monoglyceride blend (low-dose); (4) challenged control + 1,000 mg/kg monoglyceride blend (high-dose). Upon arrival, groups 2-4 were orally inoculated with 2.0 x 10^7 CFU/0.25 mL/bird *Salmonella* Typhimurium. On d 18, the challenged group birds were orally inoculated with ~2,000 *Eimeria maxima* oocysts/1 mL/bird, followed by *Clostridium perfringens* on d 22 (1.0 x 10^8 CFU/1 mL/bird) and d 23 (1.3 x 10^7 CFU/1 mL/bird). Total cage and feed weights were assessed on d 7, 14, 18, and 25. On d 25, 20 chicks per treatment (2-3 birds per cage) were randomly selected to evaluate intestinal lesion scores. Data were analyzed by ANOVA model with significance deemed at $p < 0.05$. Means were separated using Tukey's HSD test. On d 14, results showed that the monoglyceride blend at the high-dose (FCR = 1.179) numerically improved the FCR compared to the CC (FCR = 1.296). From d 1 to 25, chicks from the CC (FCR = 1.645) had significantly higher FCR than the NCC (FCR = 1.403), and monoglyceride blend at both low- (FCR = 1.546) and high-dose (FCR = 1.531) significantly improved the cumulative FCR compared to the CC (FCR = 1.645). Additionally, monoglyceride blend at high-dose also showed significantly higher BW gain compared to the CC group on d 25. However, no significant differences were observed for intestinal lesion scores between treatments. Adding the monoglyceride blend at both 500 mg/kg and 1,000 mg/kg significantly improved 25 d FCR and improved BW gain under the necrotic enteritis challenge used in the present study.

Key Words: monoglyceride blend, growth, coccidiosis, necrotic enteritis, broiler

Environment/Management I Animal Well-being

M115 The effects of varying methods of depopulation on laying hen gene expression Kari Harding*[1GS], Rebecca Wysocky[1], Shelly Nolin[1], Ramon Malheiros[1], Sanjay Shah[2], Kenneth Anderson[1] *[1]Prestage Department of Poultry Science, North Carolina State University, [2]Biological and Agricultural Engineering, North Carolina State University*

During 2015 and 2022, avian influenza outbreaks resulted in the loss of over 46 million and 50 million poultry production birds, respectively, illustrating the need for effective depopulation methods. This trial examined the effects of VSD+, VSD+ with humidity (VSD+ Rh), or VSD+CO_2 on laying hens measuring the expression of DNAJ1, HSP70, and BAG3. It was expected that there would be no differences in gene expression between treatments. High heat stress, triggers expression of specific genes to prevent cell death, one being heat shock protein 70 (HSP70). Three different phases were completed with 1 and 2 being in temperature-controlled chambers, and phase 3 scaled to industry conditions. Phase 1 was to determine average time of death (TOD); Phase 2, monitored the progression the birds HSP70 response from onset to TOD. Phase 3 utilized 150 hens for each treatment, with 6 hens used to establish baseline measurements with 10 birds sampled at TOD for each group. Half the brain of each hen was extracted and placed in RNA Later. RNA was then extracted from the brain using an RNeasy® Micro Kit. Quantitative PCR (qPCR) was used to measure the expression levels of each gene of interest. Statistical analysis was performed using JMP Pro 15 with a one-way ANOVA and a significance value of $\alpha<0.05$. Phase 1 showed no significant differences between treatments in either BAG3 or DNAJ1. However, HSP70 levels were significantly lower in the CO_2 (P=0.0002) treatment compared to the other treatments. When comparing the gene expression in phase 2,

there were no significant differences observed between either of the treatments. Much like phase 1, phase 3 showed no significant differences in treatments with the expression of BAG3 and DNAJ1, however, the CO_2 treatment was significantly lower (P=0.0004) than the other treatments. The significant reduction in HSP70 expression in the CO_2 treatments in phases 1 and 3 may be due to the earlier time to death compared to the VSD+ and VSD+ Rh. The lack of difference in Phase 2 may be the result of the different time intervals in which the birds were removed from the chamber. Based on gene expression alone, VSD+ CO_2 reduces the amount of HSP70 expressed during depopulation.

Key Words: depopulation, gene expression, laying hen, avian influenza

M116 Tracking cage-free laying hens on litter floor with machine vision Xiao Yang*[GS], Ramesh Bist, Sachin Subedi, Lilong Chai *Department of Poultry Science, University of Georgia*

Animal behaviors monitoring is critical to the evaluation of poultry production and welfare. Daily observation of animals is performed manually in laying hen and broiler houses manually, which is subject to human errors. Automatic detection of laying hens or broilers will enable the timely monitoring of poultry behaviors and health. The objectives of this study were to develop a deep learning model (YOLOv5x-hen) based on YOLOv5, an advanced convolutional neural network (CNN), to monitor hens' behaviors in cage-free facilities. More than 1200 imagines were used to train the model and test it in detecting hens on the litter floor, which is required for birds to perform natural behaviors. The one-way ANOVA and Tukey HSD analysis were conducted using JMP software to determine whether there are significant differences between predicted number of hens and actual number of hens under various ages, light intensities, and observational angles. The difference was considered significant at p<0.05. Results show that the precision of the YOLOv5x-hen model reached 0.96 in tracking hens on the litter floor. The newly developed YOLOv5x-hen was tested with stable performances in detecting birds under different lighting intensities, angles, and ages. The model was tested with 95% accuracy after birds were 8 weeks old. The uncertainty is high in detecting younger chicks that were one-week old birds due to smaller body size and the interference of equipment such as feeders, drink lines, and perches. This research provides basis for developing an automatic poultry welfare evaluation tool in a commercial cage-free house.

Key Words: Egg production, precision farming, cage-free house, animal activities, deep learning

M117 Investigation of stocking density, age, and time of day on behavior profiles and aggression of white egg layer hens housed in colony cages Benjamin Alig*[GS], Kenneth Anderson, Allison Pullin, Ramon Malheiros *Prestage Department of Poultry Science, NC State University*

The amount of space provided to laying hens has been an animal welfare topic of concern from consumers, special interest groups and lawmakers. The freedom to perform normal behaviors is one component of animal welfare; therefore, our objective was to assess behavior at different stocking densities. In this study, Shaver White laying hens were housed in colony cages at five stocking densities: 208, 139, 104, 83 and 69 in^2. Video recordings of 3 cages/treatment (15 cages total) were taken at 30, 46 and 62 weeks of age in the morning (7:30-7:50am), afternoon (3:00-3:20pm), and evening (9:00-9:20pm). At each age, a 20-min segment of video from each time of day was analyzed. Every minute was annotated to identify the behavior that hens spent the majority of their time performing during that minute: standing, crouching, preening, feeding, drinking, stretching, walking, feather pecking, pecking inanimate objects, sham dust bathing and sham foraging. Data were averaged to calculate the percentage of hens engaged in each behavior per cage. The total count of aggressive bouts were recorded across the 20-min, divided by the number of hens/cage, and expressed as acts per hen per cage. Data were analyzed in Rstudio 4.2.1 with a general linear model utilizing the variables time of day, density, age, and the full factorial of interactions. Tukeys HSD was performed for multiple comparisons and P-value of <0.05 was considered significant. Intermediate stocking densities displayed the lowest percentage of crouching (P=0.009) and aggressive acts per hen (P<0.0001). Hens stocked at 208 in^2 walked the most (p<0.0001). Hens were seen standing and preening (P<0.0001) more in the morning; crouching and sham dust bathing (P<0.0001) more in the afternoon and stretching less while feeding, drinking, and being more aggressive in the evening (P<0.0001). Finally, as hens aged, they began to stand and crouch more (P<0.0001) and preen (P=0.013), walk (P<0.0001), and demonstrate aggressive behaviors (P=0.007) less. In conclusion, the majority of behaviors assessed were not influenced by stocking density. However, the different levels of aggression could indicate higher stress and frustration which warrants more investigation.

Key Words: Egg Laying Hens, Hen Behavior, Welfare, Stocking Density, Colony Cages

M118 Keel bone tracking from rearing to laying phase for understanding bone mineralization and 3-D structural development Dima White*[GS], Woo Kim *University of Georgia*

Keel bone fractures and osteoporosis are crucial skeletal issues in the laying hen industry. There is interest in improving bone quality parameters to reduce/prevent these conditions. Understanding keel bone development from rearing to laying phase can be a strategy to mitigate/eliminate bone fractures. We hypothesized that dual energy x-ray absorptiometry (DEXA) and Micro-computed tomography (Micro-CT) scanning would provide high-resolution images and detailed observations on keel bone mineralization and 3-D structural development to better understand keel bone structure. The objectives were to evaluate keel bone development and mineralization using DEXA and Micro-CT and to track keel bone development throughout the layer phase. A total of 150-day old chicks were utilized from 0-60 weeks (wks). The pullets were fed a basal diet formulated according to the Hy-Line W36 guide (2020) from 0-17wks of age, and layer phased diets from 18-60wks of age. Keel bones were collected weekly during the rearing period and then at 18, 25, 32 & 52wks of age for DEXA and Micro-CT scanning & analysis. Femur bones were collected for Micro-CT analysis for rearing and laying periods. The data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significant at P<0.05. Results indicated significant bird weight changes weekly during the rearing period that corresponded to the keel bone weight. For DEXA results, there were significant increases in keel bone mineral density (BMD) and bone mineral content (BMC) from wks 6-10. For Micro-CT results, significant increases in tissue & bone volume (TV/BV) and BMC for keel bones were observed from wks 6-9. Substantial increase for BMD and a 2nd surge for BMC for keel bones were observed from wks 18-25. For femurs, there were significant differences for wks 6-10 and wks17-19 with substantial increase in TV/BV, BMD, and BMC. The bone parameters for the femur are fully developed at sexual maturity, however, for keel bone development, the bone parameters continuously increased after onset of egg production and still peaked after 52 wks of age. This result indicates that while the femur bone is developed at the onset of laying, the keel bone is still growing throughout the hens laying phase.

Key Words: keel bone, pullet, laying hens, DEXA, Micro-CT

M119 Effect of oxidative stress on broiler bone homeostasis Yuguo Tompkins*[GS], Woo Kim *University of Georgia*

The genetic selection for heavy muscle gain and fast growth in broilers has been linked to the increased prevalence of leg problems. Moreover, oxidative stress has been reported in several chicken bone disorder studies. However, understanding of the direct interaction between oxidative stress and bone homeostasis in broilers is limited. To further understand the potential role of oxidative stress in broiler bone homeostasis, coccid-

iosis, one of the most economically impactful enteric diseases in poultry production, was used as an *in vivo* challenge study model. The microCT method, histology analysis, mRNA expression, and ELISA assay was used for bone growth parameters. Oxidative stress status was measured by total antioxidant content assay and lipid peroxidation assay, and data were analyzed by one-way ANOVA. The results indicated that inflammation-mediated osteoclast activity and oxidative stress-correlated bone growth suppression attributed to the lower bone quality during *Eimeria* infection. Moreover, in vitro study by exposing chicken primary mesenchymal stem cells (MSCs) to hydrogen peroxide (H_2O_2), showed that oxidative stress negatively regulated osteogenic differentiation gene markers and suppressed cellular mineralization in the chicken MSCs. The in ovo study, by microinjecting H_2O_2 into chicken embryos, showed the inhibition of bone formation and embryo development by mRNA expression analysis. In conclusion, current results demonstrate that oxidative stress is a negative factor that suppresses bone formation and decreases bone quality during pathogenic or stressed conditions in broiler chickens.

Key Words: Oxidative stress, bone health, animal welfare, MSCs, bone homeostasis

M120 Body composition and reproductive differences between broiler breeders fed under everyday or skip-a-day rearing programs Luis Avila*[GS], Kelly Sweeney, Camille Evans, Dima White, Woo Kim, Prafulla Regmi, Jeanna Wilson *University of Georgia Department of Poultry Science*

Broiler breeder feed restriction practices have intensified as broiler feed efficiency has improved. Offering pullets feed on a skip-a-day regime (SAD) has controlled BW gain, but the reproductive benefits of this practice have become questionable for modern breeders. The objectives were to compare everyday (ED) and SAD programs and their impact on pullet growth and reproduction. At d 0, Ross 708 (Aviagen) pullet chicks (n = 1,778) were randomly assigned to 7 floor pens and full-fed a common crumbled starter diet through wk 3. Three pens were fed using the ED and 4 pens with SAD regime through wk 21 using chain feeder systems. Feed allowance was adjusted equally every wk. A 2-stage grower diet program was fed after wk 3. ED and SAD grower diets were formulated to be iso-nutritious, with the only difference that ED diets had 0.5% more crude fiber. Pullets were moved to hen pens by treatment at wk 21 (n = 16 pens total; n = 44 birds per pen) with 3 Yield Plus males (Aviagen), and daily fed common laying diets. Pullet and hen BW were recorded throughout the study. Sampled pullets and hens were scanned using dual energy X-ray absorptiometry (DEXA) to obtain whole body composition. Hen performance, eggshell quality, and hatchery metrics were recorded from wk 25 to 60. Data were analyzed using a GLM PROC with SAS v 9.4 at a significance of $P \leq 0.05$, and tendencies declared when $0.05 < P \leq 0.10$. ED birds were heavier from wk 10 to 45 ($P \leq 0.013$), and pullet uniformity was unaffected by regime ($P \geq 0.443$). SAD-birds had lower bone density at wk 7, 15, and 19 ($P \leq 0.026$). ED birds had higher fat contents at wk 19 (120 vs. 89 g per bird; $P = 0.017$). Rearing regime did not impact egg production, feed conversion, hatchability, or hen mortality ($P \geq 0.247$). Eggs from ED hens were heavier ($P = 0.015$) and tended to have increased eggshell quality ($P = 0.057$) and hatch of fertile % (94 vs. 93; $P = 0.088$). Fewer embryonic losses due to egg contamination ($P = 0.020$), dead pips ($P = 0.036$), and late mortalities ($P = 0.055$) were detected in incubated eggs from ED hens. Altogether, ED pullet feeding increased the bone mineral density and carcass fat contents of pullets as they approached sexual maturity and had a positive effect on eggshell quality and hatch of fertile during lay.

Key Words: broiler breeder pullet, feed restriction, reproduction, egg production, rearing

M121 Cecal microbiota transplantation: Modulation of aggressive behavior through the crosstalk between central serotonergic activity and gut microbiota in roosters Yuechi Fu*[1GS], Jiaying Hu[1], Marisa Erasmus[1], Timothy Johnson[1], Heng-wei Cheng[2] *[1]Department of Animal Sciences, Purdue University, [2]Livestock Behavior Research Unit, USDA-ARS*

Growing evidence has highlighted the linkage between the gut microbiota and host physiological and behavioral homeostasis via the gut-brain axis. To assess if the gut microbiota plays a role in regulating aggressive behavior in chickens, we transplanted cecal contents from the divergent chicken lines (donors) with nonaggressive or aggressive behavior to Dekalb XL, a commercial strain (recipients). Eighty-four one-day-old male chicks were randomly allocated to 1 of 3 treatments with 7 replicates per treatment and 4 birds per replicate (n = 7): saline (control), cecal content of line 6_3(6_3-CMT), and cecal content of line 7_2 (7_2-CMT). Transplantation was performed once daily from day 1 to 10, and then boosted once weekly from week 3 to 5. At weeks 5 and 16, behavior in home-cage and paired aggression tests were recorded. Samples of blood, brain, and cecal contents were collected from post-tested birds to detect transplantation-induced biological and microbiota changes. Behavioral and physiological data were analyzed using PROC MIXED repeated measures and one-way ANOVA, respectively. Correlations were determined by Spearman's rank test. Results indicate that 6_3-CMT birds displayed less aggressive behavior with a higher hypothalamic serotonergic activity at week 5 ($P < 0.05$). Correspondingly, two amplicon sequence variants (ASVs) belonging to *Lachnospiraceae* and one *Ruminococcaceae UCG-005* ASV were positively correlated with the levels of brain tryptophan (r = 0.87) and serotonin (r = 0.79), respectively. The 7_2-CMT birds had lower levels of brain norepinephrine and dopamine at week 5 with higher levels of plasma serotonin and tryptophan at week 16 ($P < 0.05$). ASVs belonging to *Mollicutes RF39* (r = -0.79) and *GCA-900066225* (r = -0.78) in 7_2-CMT birds were negatively correlated with the brain 5-hydroxyindoleacetic acid at week 5, and one *Bacteroides* ASV was negatively correlated with plasma serotonin at week 16 (r = -0.74). In conclusion, modifications of gut microbiota at an early age affect aggressive behavior via modulating the cecal microbial composition and central serotonergic activity in recipient birds. The selected transplantation could be a novel strategy for reducing aggressive behavior through regulating signaling along the gut-brain axis.

Key Words: aggressive behavior, cecal microbiota transplantation, microbiota-gut-brain axis, rooster, serotonergic activity

M122 Evaluating broiler welfare and behavior as affected by growth rate and stocking density via PLF technologies Shengyu Zhou*[GS], Pattarawan Watcharaanantapong, Xiao Yang, Tom Tabler, Jun Lin, Maria Prado, Hao Gan, Yang Zhao, Tanner Thornton *The University of Tennessee*

It's been claimed that slow-growing broilers have better welfare conditions than standard/fast-growing ones; however, no scientific data support such a claim. 1. Objective: The purpose of this study is to evaluate broiler welfare and behaviors as affected by growth rate (GR) and stocking density (SD). 2. Experimental design: Slow-growing (GR < 50 g/day) and medium-growing (GR = 50 – 60 g/day) broilers were produced via providing 60% and 80% of the feed intake of standard Cobb 700 broilers (GR > 60 g/day). The broilers at all three GRs were reared at two stocking densities of 30 and 40 kg/m2. Broiler welfare indicators, including gait score, tibia strength, feather coverage, and footpad condition were evaluated when birds reached 1, 2, and 3 kg of body weights. Activity index and feeding hours were determined by top camera, image processing and radio-frequency identification (RFID) systems. It took 45 days for standard, 52 days for medium-growing, and 62 days for slow-growing broilers to reach 3 kg body weight. 3. Statistical analysis: The effects of growth rate, stocking density, broiler body weight will be analyzed using the PROC GLM (generalized linear model) procedure in SAS. The rest of data will be analyzed as a completely randomized design using PROC MIXED procedure of SAS. A significant difference in multiple compari-

sons of group means will be defined as $p < 0.05$. 4 Results: Feed conversion ratios (FCR, kg feed per kg body weight) were 1.57 for standard, 1.67 for medium-growing, and 1.80 for slow-growing broilers ($P < 0.01$). Slow-growing broilers were more active ($P = 0.02$) and had better feather coverage ($P < 0.01$) and gait score ($P < 0.01$), medium-growing broilers were superior in bone strength ($P = 0.01$), and standard broilers showed best production performance ($P < 0.01$). Broilers at 30 kg/m2 showed better bone strength ($P = 0.04$), footpad condition ($P = 0.01$) and gait score ($P < 0.01$) compared to those at 40 kg/m2. 5. Conclusion: Lowered GR and SD improved certain broiler welfare conditions at a high expense of compromised production performance and prolonged production cycle.

Key Words: Broiler, Welfare, behavior, Stocking density, Growth rate

Environment/Management II Animal Well-being

M123 Comparison of skeletal and cardiac parameters in commercial broilers raised using a standard or slow growth rate Nabin Neupane*[GS], Laura Ellestad, Robson Giglio, Prafulla Regmi *University of Georgia*

Modern commercial broilers grow up to 3 kg and acquire heavier muscle mass in six weeks. Heavy body mass and faster growth, however, are associated with skeletal and cardiac issues in meat broilers. This study investigated the effect of growth rate on long bone biomechanical properties and cardiac ventricular measurements. Four hundred and eighty Ross 708 male chicks were used for the study. Chicks were sexed post-hatch and randomly assigned to two treatments: Control (C) and Treatment (T) in floor pens (10ft*4ft) in an environmentally controlled house. Both groups were fed ad-libitum for the first week then 100% and 80% of breeder's recommended feed allocation during wk2. After wk2, C was provided ad-libitum feed whereas T was pair-fed with 80% of the feed intake for C birds from the previous day. Each treatment consisted of 8 replicate pens with 30 birds/pen. Body weight was measured on a weekly basis. Right and left tibiotarsus and humerus and whole heart were collected from two birds per pen when C and T reached 1kg, 2.5kg and 4kg. Tibiotarsus and humeri biomechanical properties (peak force to fracture and area under the curve) were assessed using a 3-point bending test (TA. HDPlus, Stable Micro System). Heart weight was taken and left ventricular thickness was measured using a vernier caliper. Statistical analyses were conducted using a one-way ANOVA in R-3.6.1. Post feed restriction, C birds were heavier and grew faster (94.10 g/d vs. 79.92 g/d) than T birds. Bone and heart parameters were normalized to body weight at each target weight. Tibiotarsus peak force to fracture was greater in C than T at all target weights ($P<0.05$). Area under the curve (work-to-fracture) was higher in T at 1 kg and 4 kg ($P<0.001$). Humeri peak force was greater in C than T at 1 kg and 2.5 kg whereas at 4 kg, T was greater than C ($P<0.05$). There was no difference in heart weight at any target weight, however, C birds had thicker left ventricles than T at 2.5 kg ($P<0.01$). In conclusion, slowing growth rate in commercial broilers using feed reduction impacted the tibiotarsus strength negatively. On the other hand, slowing the growth rate might benefit the cardiac structure of broilers.

Key Words: Broilers, Slow growth, Bone strength, Cardiac measurement

M124 Environmental enrichments affect behavior and plasma corticosterone levels in broiler breeder pullets Camille Evans*[GS], Luis Avila, Lauren Vaccaro, Laura Ellestad, Jeanna Wilson, Prafulla Regmi *Department of Poultry Science, University of Georgia*

To control body weight for optimal reproduction, broiler breeders are subjected to feed restriction programs which can increase stress and compromise their welfare. Adding enrichments to the environment can alleviate stress and may improve welfare. This study was aimed at assessing the effectiveness of environmental enrichments to reduce stress and abnormal behaviors in broiler breeder pullets. A total of 2,540 Aviagen Ross 708 day-old pullet chicks were placed into 10 floor pens (254 birds/pen). At wk 4, pens were randomly assigned to either a traditional skip-a-day (SAD) or an every day (ED) feeding program. Environmental enrichments (2 PVC perches, 5 strings, and 5 compact discs per pen) were introduced to selected pens at wk 10. Final treatments were SAD with enrichment (SAD1, n=4), SAD without enrichment (SAD0, n=4), and ED without enrichment (ED0, n=2). Blood samples were collected at wk 10 and 22 for analysis of plasma corticosterone (CORT) concentrations. Pullet behavior was assessed at wk 18 and 21 using instantaneous scan sampling for 3 1-hour periods (every 15 min per h) on a feed day and a non-feed day. Data were analyzed using three-way ANOVA and Kruskal-Wallis test in SAS v 9.4 and *P*-value less than 0.05 was considered significant. CORT was not different between treatments prior to introduction of enrichments. At wk 22, pullets from SAD0 had lower CORT than SAD1 ($P = 0.04$) while neither SAD treatments were different from ED. At wk 18 and 21, a greater percentage of birds were observed foraging in SAD treatments compared to ED ($P \leq 0.02$). Stereotypic spot and feeder pecking behaviors were observed less frequently in SAD1 than in SAD0 at both ages ($P \leq 0.01$). A lower percentage of SAD1 pullets performed oral stereotypies compared to ED0 at both ages ($P \leq 0.01$). Feather licking behavior was observed more frequently among ED0 pullets compared to both SAD groups ($P \leq 0.01$). Furthermore, more SAD1 pullets were observed using perches on non-feed days than on feed days ($P \leq 0.01$). In conclusion, environmental enrichment resulted in reduced oral stereotypic behavior among pullets. Reduced corticosterone production and more frequent oral stereotypies in SAD0 pullets probably indicate a depression-like state, however, further research is warranted for confirmation.

Key Words: environmental enrichments, broiler breeder pullet, corticosterone, stereotypies

M125 Effect of ultraviolet radiation on reducing airborne Escherichia coli carried by poultry litter particles Xuan Dung Nguyen*[1], Yang Zhao[1], Jeffrey Evans[2], Jun Lin[1], Brynn Voy[1], Joseph Purswell[2] *[1]Department of Animal Science, The University of Tennessee, [2]Poultry Research Unit, Agriculture Research Service, United States Department of Agriculture (USDA)*

Airborne *Escherichia coli (E. coli)* originating in poultry houses can be transmitted outside poultry farms through the air, posing risks of barn-to-barn infection through airborne transmission. The objective of this study is to examine the effect of ultraviolet (UV) light on the inactivation of airborne *E. coli* carried by poultry dust particles under laboratory conditions. A system containing two chambers that were connected by a UV scrubber was designed in the study. In the upstream chamber of the system, airborne *E. coli* attached to dust particles were aerosolized by a dry aerosolization-based system. Two sets of air samplers were placed in the two chambers to collect the viable airborne *E. coli*. The system was tested with three doses of UV light which were zero, one, and two UV lamps. With each dose of UV light, there were 4 wind speed levels being tested at 0.11, 0.51, 1.74, and 2.61 m s^{-1} corresponding to the contact times of 5.62, 1.17, 0.34, and 0.23 s. Temperature and relative humidity were kept stable during experiments. With each wind speed level, the test was repeated three times which makes the total observations of 36 data points. Statistical Analysis System (SAS 9.4, SAS Institute Inc., Cary, NC, USA) was used in statistical analysis to assess the inactivation rate of airborne *E. coli* and the k-values as influenced by the airborne *E. coli* and initial bacterial concentrations. The significant level was applied as the *p*-value of 5%. The airborne *E. coli* inactivation rates were tested at different UV irradiance levels (1707 μW cm^{-2} and 3422 μW cm^{-2}). The inactivation rates varied from over 99.87% and 99.95% at 5.62 s of contact time to

72.90% and 86.60% at 0.23 s of contact time with 1707 μW cm^{-2} and 3422 μW cm^{-2} of UV irradiance, respectively. In addition, an increasing number of UV lamps would not proportionally increase the inactivation rates. The k-values were different (with *p*-value = 0.05) at different contact times which indicated turbulent flow might affect the inactivation efficiency of the UV system. The findings of this study may provide an understanding of the effect of UV light on the inactivation of airborne *E. coli*carried by dust particles and help to design an affordable mitigation system for poultry houses.

Key Words: airborne E. coli, barn-to-barn infection, dry aerosolization, poultry houses, ultraviolet radiation

M126 Effect of dimming wave characteristics on lamp performance Joseph Purswell*[1], Jeremiah Davis[2], Jesse Campbell[2], Matthew Rowland[1], Klinton McCafferty[1] *[1]USDA-ARS Poultry Research Unit, [2]Auburn University*

Use of LED lamps in broiler houses is widespread due to vastly improved energy use characteristics when compared to traditional incandescent lamps. Early adopters of LED lighting experienced significant challenges regarding dimmer compatibility with differing lamp designs. Recent concerns regarding lamp life and performance have highlighted the need for systematic evaluation of dimmer-lamp compatibility to ensure proper operation and performance. A test system was developed to evaluate lamp performance under commercial usage patterns. Each test system used 12 lamps, equivalent to 50% load of one half-house lighting circuit in a typical broiler house. A total of seven dimmers from four manufacturers were used in this test and were configured to provide power with a leading, trailing, or centered waveform. All lamps were of the same manufacturer, construction, power, and color temperature and obtained from the same vendor. Dimmer operation was controlled with a datalogger with an analog signal interface to allow for both photoperiod and intensity control. A typical commercial broiler lighting program was used as follows: 23L at 3 fc (placement-7 days), 20L at 10 lux (8-28 days), 18L at 5 lux (29-61 days); all dark periods were limited to one hour to reduce the overall test duration. Lamp output was measured at 100% power using an integrating sphere and spectrophotometer prior to each test flock. Lamp output data were analyzed as a completely randomized design with repeated measures using PROC MIXED in SAS and means were separated using Fisher's LSD; significance was assessed at $P \leq 0.05$. Initial mean output for all lamps was 1204 ± 1 lumen. Lamp output increased approximately 15% during the Flock 1 and remained elevated through Flock 2. Output decreased during the Flock 3 and has remained relatively stable through Flock 7 with a mean output of 1328 ± 3. No differences in lamp performance resulting from dimmer waveform have been observed, and no bulb failures have been recorded. Additional flock test cycles will be conducted to approximate two years of lamp operation.

Key Words: broiler, lighting, energy

M127 Potential epigenetic effects of heat stress on ACTH response in F1 offspring of breeder Pekin ducks. Esther Oluwagbenga[1], Jenna Schober[1], Victoria Tetel[1], Daniel Shafer[2], Steven Corbitt[2], Gregory Fraley*[1] *[1]Purdue University, [2]Maple Leaf Farms, Inc.*

Rising temperature due to global warming poses detrimental effects on poultry production leading to substantial economic losses. It is generally thought that prolonged stressors in breeders can elicit phenotypic changes in their offspring, potentially through deposition of glucocorticoids (GC) into the egg during periods of prolong stress. We set out to determine if heat stress (HS) would elicit an increase in glucocorticoids in egg, and whether the HS was related to phenotypic changes in the F_1's response to ACTH. Breeder hens (60/pen/treatment) and drakes (20/pen/treatment) were randomly allocated into 2 treatments: control and HS groups. The HS group were subjected to cyclic HS of 35°C for 10h/day and returned to 29.5°C for the remaining 14h/day for 3 weeks while the control room was maintained at 22°C. Eggs (N = 10/treatment) were collected weekly and tested for GC in albumen and yolk using mass spectrometry. Student's T-test analyses showed that eggs from HS hens showed significantly ($p < 0.05$) levels of cortisol in albumen compared to eggs from control hens. Corticosterone was not detected in albumen and neither GC was detected in yolk. These findings have been corroborated by other studies using MS. To determine effects of HS on the F_1, eggs from the last 3 days of the treatment period were incubated, and hatchlings placed into pens by treatment in a single room to minimize environmental differences. An ACTH (cosyntropin 0.06 mg/kg or saline vehicle) challenge was given to 10 ducks per treatment/sex as described previously by our lab. Serum samples were collected hourly for 5 hours following injections and analyzed by ELISA for GC levels. A repeated measures ANOVA showed that circulating levels of corticosterone were significantly ($p < 0.05$) elevated at hours 1 and 2 after ACTH injection in the HS F1 group compared to controls. Circulating levels of cortisol increased significantly at hours 1, 2, 3, and 4 ($p < 0.05$) in the control F_1 group following ACTH injection compared to controls. Our results suggest that there are epigenetic effects because of prolonged heat stress that is typically experienced by poultry due to climate change, and that these effects may be mediated through cortisol deposition in the egg.

Key Words: chronic stress, egg quality, fertility

M128 Effect of some phytogenics on growth performance, serum biochemistry, and antioxidant status in broilers during summer stress Mounika Thota[1], Srilatha Mangalam[1], Srinivas Gurram[1], Bhaskar Ganguly*[2] *[1]P. V. Narasimha Rao Telangana Veterinary University, [2]Ayurvet Limited*

This study was undertaken to evaluate the effect of some phytogenics on growth performance, serum biochemistry, and antioxidant status in broiler chicken during summer stress. 240 one-day old commercial broiler male chicks were randomly allocated to either of four dietary treatments, each having four replicates of 15 birds, and reared up to six weeks of age during summer season with average temperature range of 33.8 to 37.5°C and relative humidity of 46-75%. The treatments consisted of a control diet (CD), CD supplemented with AV/EIF/19 (M/s Ayurvet Limited, India) @ 250 ppm of feed, or Brand X @ 100 ppm of feed, or AV/HEP/18 (M/s Ayurvet Limited, India) @ 100 ppm of feed. Parameters pertaining to growth and feeding performance, antioxidative status (malondialdehyde, MDA), and serum biochemistry (glucose; alanine transaminase, ALT; aspartate transaminase, AST; and cortisol) were recorded.

At the end of six weeks, the results showed that body weight gain (BWG) was significantly ($P<0.05$) higher in the AV/EIF/19 group. Cumulative feed intake and FCR were significantly ($P<0.05$) improved in the phytogenic-supplemented groups over control. Carcass parameters revealed improved dressing weight percentage ($P<0.05$) in all the supplemented groups. Dietary supplementation with phytogenics reduced lipid peroxidation (MDA levels) significantly ($P<0.05$), the lowest value of lipid peroxidation being recorded in AV/EIF/19-supplemented group. Serum biochemical profile indicated that supplementation with phytogenics significantly ($P<0.05$) reduced ALT, AST, total glucose, and cortisol levels in the AV/EIF/19-supplemented group. Based on the results, it could be concluded that supplementation with phytogenics improved growth performance, feeding efficiency, antioxidant status, and serum biochemical profile (glucose, ALT, AST, and serum cortisol) in broiler chicken reared under summer stress.

Key Words: Heat stress, Physiology, Phytogenic, performance, antioxidant

M129 Environmental conditions shape the gut microbiome of broiler chickens Benjamin Zwirzitz[1], Adelumola Oladeinde*[2], Jasmine Johnson[3], Gregory Zock[3], Marie Milfort[3], Lorraine Fuller[3], Ahmed Ghareeb[3], James Foutz[4], Jose Teran[3], Reed Woyda[5], Zaid Abdo[5], Jodie Lawrence[2], Denice Cudnik[2], Samuel Aggrey[3] *[1]University of Natural Resources and Life Sciences, [2]US National Poultry Center, [3]University of Georgia, [4]Boehringer Ingelheim Animal Health (BIAH),, [5]Colorado State University*

The broiler house environment is one of the most important management factors that has been shown to significantly affect broiler performance, welfare, and health. However, there is limited data on how changes in house environmental factors affect the microbiome of broiler chickens. In this study, we investigated the effect of broiler house environmental conditions on the microbiome of chickens from post-hatch through pre-harvest. Six hundred 1-day old Cobb 500 broiler chicks were raised on floor pens for 49 days in two separate houses (House 1 and House 2). We performed short-read and full-length sequencing of the bacterial 16S rRNA gene present in the meconium and in cecal and litter samples collected over the duration of the study. We monitored the relative humidity, temperature, and ammonia in each house daily and the pH and moisture of litter samples weekly. The microbial community structure of the ceca and litter consistently changed throughout the course of the grow-out and was influenced by the environmental parameters measured (P<0.05). The litter microbiome was affected by the five parameters measured while the cecal microbiome was influenced by house temperature only. We found that the ceca and litter microbiome were similar in the two houses at the beginning of the experiment, but over time, the bacterial community separated and differed between the houses. When we compared the environmental parameters in the two houses, we found no significant differences in the first half of the growth cycle (day 0-21), but morning temperature, morning humidity, and ammonia significantly differed (P<0.05) between the two houses from day 22-49. Lastly, chickens in House 2 experienced higher mortality, lower body weight and were more likely to harbor pathogens including *Salmonella* compared to house 1. These results show that the environmental conditions in a broiler house can influence the microbiome of raised chickens.

Key Words: Broiler chickens, Environmental conditions, Microbiome, ceca and litter, Pre-harvest

Environment/Management III Animal Well-being

M130 Heating system and feed additive effects on foot pad quality, broiler performance, and immune status Victoria Ayres*[1], Timothy Boltz[2], Shawn Grushecky[3], Jingxin Wang[3], Joseph Moritz[3] *[1]Tennessee Tech University, [2]Mississippi State University, [3]West Virginia University*

Radiant propane brooders are commonly used to heat poultry houses in the United States. However, these brooders combust within the barn, releasing moisture into the grow-out environment. The objectives of this study were to investigate the effects of two feed additives (antibiotic and muramidase) provided to broilers reared using two heating systems (external combustion wood boiler heat exchanger or radiant propane brooders), on broiler performance, foot pad quality, and immune status. Two identical experiments were completed, using two identical rooms heated with either radiant propane brooders or a wood boiler heat exchanger. 1,472 Ross-308-AP straight-run broiler chicks were utilized for each experiment, for 35 days. Each room contained 32 floor pens. One of four dietary treatments (positive control (PC), negative control (NC) (15% reduction in digestible amino acids), NC + antibiotic, NC + muramidase) were randomly assigned to each pen within a block. A block consisted of four adjacent floor pens; eight blocks were utilized for each room per experiment. The use of a wood boiler heat exchanger reduced d21 litter moisture (P=0.1013), d23 serum interleukin-6 (P<0.0001), and d35 foot pad scores (P=0.0112), relative to radiant propane brooders. Diet influenced 0-35d live weight gain (LWG) and feed conversion ratio (FCR). The PC had the highest LWG and lowest FCR, NC had the lowest LWG and highest FCR, with antibiotic and muramidase being intermediate (P<0.05). Birds fed the PC had higher d35 litter moisture and FPS (P<0.05). Heating system did not affect overall performance (P>0.05). The wood boiler heat exchanger and both tested feed additives had positive influences on broiler production.

Key Words: broiler, wood-boiler-heat-exchanger, propane, muramidase, antibiotic

M131 Effect of environmental enrichments on behavior, leg and mental health of broilers in the commercial houses Seong Kang*[1], Karen Christenson[2], Michael Kidd Jr[1], Sara Orlowski[1], James Clark[2] *[1]University of Arkansas, [2]Tyson Foods, Inc.*

The environmental enrichments (EEs) were tested to evaluate the effects on natural behaviors, locomotor activity and mental health of commercial broilers. Day old broilers (Cobb 700) were housed in four broiler houses (12.8 m x 122 m, wood-shavings). Each quadrant (section) of the house was placed with 4,800 chicks with all source flocks equally represented in each section. Two lighting programs began on day 7 with 20 lux (lx) or variable light (VL, 40 lx over the feed lines and 2-5 lx at the sidewalls) using LED lights on a 16L:8D photoperiod. To select the most favorable broilers EE, three different EEs (ramp, perching board and hut, 3 /1000 sqft) were tested. Differences of data were analyzed using one-way ANOVA followed by mean separation using the Tukey's HSD test. Engagement of birds to enrichment huts (EHs) was highest compared to the ramp and board treated sections (p<0.05). Daily activity and numbers of dust-bathing holes (indicator of the natural behavior) were highest in the EH treated sections in VL house (p<0.05). The number of culled birds caused by leg-health issue was lowest in the EH treated birds of 20 lx and VL houses. These results indicate that EH is the most favorable broilers EE in the commercial broiler farm. In the succeeding trial, brains of birds were sampled at 14, 28, and 42 days of age in 20 lx and VL houses (n=8/section, male). Ventral tegmental area (VTA) of midbrain of birds were dissected as previously reported for the study of welfare indicators of chicken brain, tryptophan hydroxylase 2 (TPH2), tyrosine hydroxylase (TH), brain-derived neurotrophic factor (BDNF), and glucocorticoid receptor (GR) by qPCR. At the 42 days of age, expression of TPH2 in the VTA of VL-hut treated birds was significantly lower compared to VL-control (Con) and 20 lx-hut treated birds (p<0.05), suggesting the synergistic effect of EH with VL lighting program on the central serotonergic homeostasis of commercial broilers. Expressions of TH, BDNF, and GR in the VTA of the VL-hut treated birds were significantly lower than those of VL-Con and 20 lx-hut birds (p<0.05), suggesting lower stress-susceptibility and less chronic social stress of VL-hut treated birds. Taken together, results indicate the beneficial effects of EH on commercial broilers welfare.

Key Words: Broilers, Enrichiments, Welfare, Serotonin, BDNF

M132 Prevalence of antimicrobial resistant Escherichia coli in broiler litter decreases with consecutive reuse of litter Reed Woyda[1], Adelumola Oladeinde*[2], Dinku Endale[3], Timothy Strickland[3], Kimberly Cook[4], Jodie Lawrence[2], Denice Cudnik[2], Zaid Abdo[1] *[1]Colorado State University, [2]US National Poultry Center, [3]Southeast Watershed Research, USDA-ARS, [4]Nutrition, Food Safety/Quality, USDA-ARS-ONP*

Although, commensal *Escherichia coli (E. coli)* does not cause diseases, some strains can cause debilitating infections in broilers that can lead to increased mortality and severe economic loss. *E. coli* infection is commonly treated with antibiotics; however, the development of antimicrobial

resistance (AMR) can result in an ineffective treatment. In this study, a longitudinal sampling of the litter in four commercial broiler houses was conducted over three consecutive flocks to evaluate the prevalence of antibiotic resistant *E. coli*. Broiler chickens were raised under a newly adopted "No Antibiotics Ever" program and prior to the start of the study, a complete house clean-out was done, and fresh peanut hull was used as the bedding for the first flock of broilers. The second and third broiler flock were raised in succession on the same litter without any litter clean-out between each grow-out cycle. Pooled litter grab samples were collected at the beginning and the end of each grow-out (n = 288). Antimicrobial susceptibility testing and whole genome sequencing was performed on 217 randomly selected *E. coli* isolates. Our results revealed that 42% of *E. coli* isolates recovered from litter were susceptible to all antibiotics tested while 58% of isolates were resistant to at least one antibiotic. The most common resistance found was to tetracycline (46%). Additionally, 41% of isolates were found to be resistant to 1 or 2 antibiotics, 13% were resistant to 3 to 5 antibiotics and 12% were resistant to 6 to 9 antimicrobials There was no significant difference between houses ($P > 0.05$) in the number of antibiotic drugs *E. coli* isolates were resistant to, but AMR differed between flocks. *E. coli* isolates recovered from the litter of the first flock of broilers raised on fresh peanut hull-based litter were more likely to be resistant to at least one antibiotic drug (71%, n = 63) compared to isolates from flock 2 (53%, n = 94) and flock 3 (52%, n = 60) that were raised on reused litter ($P = 0.02$). These results suggests that antimicrobial resistant *E. coli* strains can persist even when antibiotics are not used during grow-out, however the risk for AMR development decreased as the number of flocks raised on litter increased.

Key Words: Broiler chickens, Escherichia coli, Antimicrobial resistance, Peanut hulls, Litter reuse

M134 Effects of Transportation on Broiler Blood Physiology Matthew Livingston*[1], Chelsea Phillips[2], Aaron Cowieson[3] *[1]DSM Animal Nutrition - Precision Services, [2]DSM Animal Nutrition - Bioscience and Process Innovation, [3]DSM Animal Nutrition - Nutritional Health Innovation*

Many parameters can be responsible for the variation detected in broiler blood physiology, including nutrition, environment, genetics, age and sex. It is known that transportation is stressful to broilers and can result in changes in blood physiology, however it is not always clear if those changes are due to limited access to water and feed or the transportation and environmental exposure. This study evaluated the effect of transportation on broiler blood physiology during the spring and summer using two separate events where unfasted birds were transported and blood measurements were immediately collected. Blood were evaluated using the VetScan VS2 with the avian/reptile cartridge and the iStat Alinity with the Chem8+ cartridge. Collectively, this data consists of 100 control and 42 birds that were transported for up to two hours at 28d and 30d of age in the Spring and Summer, respectively. Results demonstrated a detectable change in blood electrolytes and proteins, with transported birds exhibiting a physiological signature related to dehydration and fasting. These blood parameters included: uric acid ($p \leq 0.0001$), glucose ($p \leq 0.0001$), albumin ($p \leq 0.0219$), ionized calcium ($p \leq 0.0001$), potassium ($p \leq 0.0001$), chloride ($p \leq 0.0001$), sodium ($p \leq 0.0001$), anion gap ($p \leq 0.0035$), total CO2 ($p \leq 0.0001$), hemoglobin ($p \leq 0.0017$), and hematocrit ($p \leq 0.0016$). However, the detected changes associated with transportation were not great enough to be considered outside of normal, established baseline ranges. While we were unable to differentiate transportation stress and auxiliary effects such as the observed mild dehydration in this design, the results highlight the importance of stress, including food and water fasting times when interpreting blood physiology markers. Since certain health conditions will render birds unable to adequately consume food and water, fasting time should be considered when predicting, diagnosing, or treating disease in chickens based upon blood physiology data. Further work should be conducted to evaluate the specific baseline deviations in blood biomarkers as a result of incremental fasting times.

Key Words: Transportation, Blood physiology, Stess, Broilers

Environment/Management IV Environmental Impacts

M135 Repellency of Eastern Redcedar (Juniperus virginiana) to darkling beetles Catherine Fudge*[GS], Chongxiao Chen *University of Georgia*

Darkling beetles are a common pest in poultry housing and a known vector for *Salmonella, E. coli,* and *Campylobacter.* Darkling beetle management is imperative to reduce pathogenic bacteria loads within poultry production. Cedar shavings are used as bedding materials for pets and food animals. They have been shown to have insecticidal and repellent properties, but there is no data on their efficacy as a darkling beetle repellent. Two studies were conducted to evaluate the efficacy of Eastern Redcedar and Eastern Redcedar products (hydrosol) as repellents on adult darkling beetles and darkling beetle larvae. In study I, adult darkling beetles was exposed to treatments NC: pine shavings, T1: kiln dried cedar

shavings, T2: kiln dried cedar shavings reconstituted to 30% moisture, T3: pine shavings (PS)+ 1 mL hydrosol, T4: PS + 5 mL hydrosol, T5: PS+ 10 mL hydrosol. Bioassays were conducted in experimental arenas utilizing the choice test. 10 darkling beetles were placed in the experimental arena and provided a choice of 20 grams of pine shavings or the assigned treatment. Beetles were provided 10 minutes to interact with products within the arena and choose which side to remain on. Beetles were removed from the experimental arena and enumerated in the control and treatment sides. Observations were repeated 5 times for each treatment. For study II, treatments from study I were repeated with darkling beetle larvae between 6-8 weeks of age. Both studies were conducted under total darkness, and environmental temperatures were kept at 25 °C and relative humidity between 55-65%. All data were analyzed in JMP pro 14 using ANOVA with Tukey's multiple comparisons test with significance reported at $P<0.05$. In study I, cedar shavings with 30% moisture, 10 mL hydrosol, and 5 mL hydrosol, had significantly higher darkling beetle repellency (P<0.0001) compared to the rest of the treatments. Study II yielded similar results as study one, with significantly higher repellency in cedar shavings with 30% moisture, 10 mL, and 5 mL hydrosol compared to the control (P<0.0001). These data suggest that Eastern Redcedar could become a potential plant-based insect repellent against darkling beetles and their larva.

Key Words: Repellent, Cedar, Darkling beetle, Pest, Management

M136 Effect of thermal variation during late-stage incubation on broiler chicken growth performance, carcass characteristics, and meat quality defects Jorge Banegas*[1GS], Brittany Wall[1], Martha Rueda[2], Jeremiah Davis[2], Joseph Purswell[3], Charles Starkey[1], Jessica Starkey[1] *[1]Department of Poultry Science, Auburn University, [2]National Poultry Technology Center, Auburn University, [3]USDA ARS Poultry Research Unit*

The objective of this experiment was to evaluate effects of air temperature during late-stage incubation and sex on broiler growth performance, carcass characteristics, and the incidence and severity of the breast meat quality defects, Wooden Breast (WB) and White Striping (WS). Ross 708 ´ Yield Plus broiler breeder eggs (n = 2,160) were incubated at 37.5 °C from embryonic day (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 pulled from hatchers simultaneously, vent sexed, placed in floor pens blocked by incubation treatment and sex (n = 6 replicate pens of 30 birds per treatment), fed a common diet in 3 phases (starter d 0 to 9, grower d 10 to 23, and finisher d 23 to 32), and processed at d 33 (n = 15 per pen). Data were analyzed as a 2-way (TV × sex) ANOVA with the GLIMMIX procedure of SAS ver. 9.4. Means were separated at $P \leq 0.05$ with the PDIFF option and tendencies were declared when $0.0501 \leq P \leq 0.10$. No treatment × sex interactions were observed. While chicks from COLD incubators were heaviest on d 0 ($P < 0.0001$), they were the lightest on d 9, but were similar to those from the HOT treatment on d 23 and 32 ($P = 0.0121$). Broilers hatched from CTL incubators had the greatest overall BWG, while those from COLD incubators had the poorest overall FCR ($P = 0.0086$) but had similar BWG as those from HOT incubators ($P = 0.0056$). Broilers from CTL incubators had the heaviest wing weights ($P = 0.0428$) and heavier carcasses than those from HOT incubators ($P = 0.0261$). Thigh and fat pad weights as well as WB severity were lowest in broilers incubated in HOT incubators ($P \leq 0.0438$). Broilers from the COLD incubators had higher incidence of WB score 3 breast fillets than those from the HOT treatment but were similar to those from the CTL incubators ($P = 0.0259$). No differences in WS were observed ($P \geq 0.1650$). Overall, as little as a 1.1°C temperature differential from CTL during late-stage incubation (ED 11 to 18) altered broiler growth performance, carcass and parts weights, and WB severity and highlight the importance of careful incubator management.

Key Words: broiler chicken, incubation temperature, growth performance, carcass characteristics, Wooden Breast

M137 Effect of novel litter amendments on d 0-59 litter quality and broiler performance Jorge Urrutia*[1GS], Dalton Dennehy[1], Hudson Thames[1], Kelley Wamsley[1], Jessica Wells[1], Anuraj Sukumaran[1], Tom Tabler[2], John Linhoss[3] *[1]Department of Poultry Science, Mississippi State University, [2]Department of Animal Science, The University of Tennessee, [3]Biosystem Engineering, Auburn University,*

Poultry litter amendments (LA) are commonly used to improve litter quality (LQ); however, there are concerns over their efficacy beyond 21 d, which leads to the need for an alternative. Previous research from our lab tested different inclusion rates (low, medium, high, and very high) and application methods (top-dress – TD or mixed – MIX), as well as CON (no treatment added) and a commercial product (COMP; sodium bisulfate), were applied to containers of reused litter to evaluate LQ variables. These data led to the selection of high inclusions for AM, BC and SAPs applied as either TD (AM only) or MIX (BC and SAP) based on feasibility and improved LQ to be tested in the current study. The current objective was to evaluate treatments identified in the previous study (compared to CON and COMP) and determine their impact on LQ and broiler performance over a 59 d growout. For LQ, sampling for pH, moisture, water activity, and bacterial load occurred on d 0, 1, 7, 21, 42, and 58. For broiler performance, BW, FI, and mortality were obtained at d 14, 28, 42, and 59. Treatments were arranged as a randomized complete block design (RCBD) to 40 pens (8 replications/treatment; 1.219 x 1.524 m; 25 birds/pen; 0.074m^2 /bird). In general, throughout the study, COMP had the lowest pH, followed by AM; BC and CON had the highest pH and SAPs were intermediate (P<0.05). However, even though significant, it is important to note that at sampling d 42 and 58, the pH values were high for all treatments (ranging from 9.2 to 9.6); thus, unlikely to control bacterial growth – as verified through the lack of statistical differences for APC and coliform counts (P>0.05). Overall, SAPs consistently had the highest moisture content throughout sampling d (P<0.05); AM numerically had the lowest moisture content, though similar to CON. For water activity, results were inconsistent; however, overall SAP tended to have the highest measured, while COMP and AM had the lowest. Litter amendments had no significant impact on live performance variables (P>0.05). Overall, all litter amendments except BC and SAPs in terms of moisture content, improved LQ, mostly within 21 d, and without affecting live performance; however, more research is needed using different sources of reused litter.

Key Words: water activity, poultry litter, litter quality, novel, litter amendments

M138 Effects of yeast cell wall on performance, immune response, and cecal colonization of Campylobacter jejuni inoculated broilers Luis Munoz*[1GS], Marquisha Paul[2], James Krehlng[1], Matthew Bailey[1], William King[2], Cesar Escobar[1], Leticia Orellana[1], Yagya Adhikari[1], Ken Macklin[1] *[1]Auburn University, [2]Alltech, Inc.*

Campylobacter jejuni (CJ) is one of the most prevalent causative agents of gastrointestinal disease in humans, and chickens are recognized as a common reservoir. Reducing colonization levels of CJ in broilers could reduce risk of human exposure and have a significant impact on food safety and public health. Yeast cell wall products (YCW) have been considered as alternatives to antibiotics to improve performance and reduce intestinal colonization of CJ in broilers. The objective of this study was to evaluate the effects of YCW on broiler growth performance, innate immune response, and cecal colonization in a CJ challenge model. A total of 2,240-day-old Ross 708 males were randomly distributed in 64 pens with 8 replicate pens/treatment and 35 birds/pen. Each pen was assigned to 1 of 4 dietary treatments: negative control, positive control (bacitracin, 50 g/ton), YCW

constant dose (400 g/ton), and YCW step-down dose (800, 400, and 200 g/ton in the starter, grower, and finisher periods, respectively) and to 1 of 2 inoculations: phosphate buffered saline or CJ at 10^3 CFU/mL administered via oral gavage on day 16. Three broilers per pen were collected 1-day post inoculation for relative gene expression analysis and three broilers per pen were collected on day 42 for CJ enumeration and prevalence calculation. Data were subjected to ANOVA using GLIMMIX (SAS v 9.4) and means were separated by Tukey's honestly significant difference. The experimental design consisted of a 4x2 factorial using a randomized complete block with pen location being the blocking factor. There were no statistical differences between the interaction of the main factors of diet and inoculation on BW, FI, and FCR ($P>0.05$). However, the YCW step-down diet lowered FCR during the starter period compared to birds fed the negative control diet ($P=0.02$). The type of inoculation did not affect the relative expression of avian beta defensin 10, interleukin 1ß, or interleukin 10. In addition, under the conditions of this study, YCW did not influence the cecal colonization of CJ ($P>0.05$). The results of this experiment indicate that YCW reduced FCR during the starter period, however, it did not affect innate immune response or reduce CJ colonization in the ceca when administered at 10^3 CFU/mL inoculation.

Key Words: prebiotic, yeast cell wall, performance, cecal colonization, innate immune response

M139 Application of Carvacrol nanoemulsion as a natural sanitizer for controlling Salmonella Enteritidis biofilm in poultry farm environment. TRUSHENKUMAR SHAH*[GS], Chen Zhu, Chetna Shah, Abhinav Upadhyay *University of Connecticut*

Introduction: *Salmonella* Enteritidis (SE) is a major poultry-associated foodborne pathogen in the United States that can form sanitizer tolerant biofilms on various surfaces. The biofilm forming capability of SE facilitates its survival on farm equipment such as feeders and waterers. Conventional sanitization methods such as 200 ppm chlorine is not completely effective in killing SE biofilms. This study investigated the efficacy of a Generally Recognized as Safe-status phytochemical *Carvacrol, in its nanoemulsion form,* in inactivating SE biofilms developed on plastic surface. In addition, the effect of carvacrol nanoemulsion on plastic degradation was studied by thermogravimetric analysis (TGA). Moreover, the effect of carvacrol on SE genes critical for biofilm formation was studied using real-time quantitative PCR.

Methods: Carvacrol nanoemulsion (CRNE) was prepared using a high energy sonication method with Gum arabic and lecithin as emulsifier. Mature SE biofilms were developed on polystyrene surface at 25°C followed by CRNE wash treatments (0.125, 0.25, 0.5, 1%) for 1, 5 or 15 min. Surviving SE in the biofilms were enumerated on XLD agar. TGA was performed after exposing plastic surface to 0.5 & 1 % of CR and CRNE for a period of 14 days. All treatments had duplicate samples and the study was replicated at least 3 times.

Results: All CRNE and CR treatments were effective in significantly reducing SE in biofilm, as early as 1 min of treatment time ($P<0.05$). The lowest dose of CRNE (0.125 %) reduced pathogen population by ~3 log CFU/ml as early as 1 min of treatment ($P<0.05$). After 15 min of exposure period, 0.125 % CRNE reduced pathogen population by ~4.5 log CFU/ml ($P<0.05$). Thermogravimetric analysis showed that CRNE did not induce plastic degradation as compared to control ($P>0.05$). Carvacrol down-regulated the expression of SE genes (*hilA, hilC, flhD, csgA, csgD, sdiA)* responsible for biofilm formation ($P<0.05$).

Significance: Results indicate that CRNE could potentially be used as a natural sanitizer to inactivate *S.* Enteritidis biofilm without causing degradation of farm equipment.

Key Words: Salmonella, Poultry, Biofilm, Carvacrol, nanoemulsion

Metabolism & Nutrition VII General Nutrition

T142 Variability in energy value, nutrient and trypsin inhibitor content of extruded full-fat soybeans due to genetics and agronomic conditions Nicolas Mejia-Abaunza*[1], Paula Lozano-Cruz[1], Joaquín Cabanas-Ojeda[1], Valmiro Lima Aragão Neto[1], Michael Joseph[1], Danny Patino[1], Rachel Vann[2], Edgar Oviedo-Rondon[1] *[1]Prestage Department of Poultry Science, North Carolina State University, [2]Department of Crop and Soil Sciences, North Carolina State University*

Dry extruded full-fat soybean meal (FF) is used as a feed ingredient for poultry. Meal quality can vary due to processing, soybean variety, and production conditions. This study evaluated the proximate and predicted energy values, and trypsin inhibitor activity (TIA) of FF affected by soy genetics, location, and seasons in a factorial experiment. Two soybean varieties (SV), AG56X8 (X) and S62XT09 (Y) were planted in three North Carolina counties (A, B, C location) at two planting dates: double crop and full season. 12 treatments resulted from the 2x3x2 factorial arrangement. Soybeans were harvested as they reached maturity, dried to 8-8.5%, and dry-extruded between 148-154.5°C in a single screw extruder, Insta-Pro-2000R. Five samples were collected from raw soybeans and FF, ground in Retsch mill to 0.5 mm, and scanned in triplicate in NIRS. The spectra were analyzed using AminoNIR (EVONIK) calibration curves. The data was standardized to DM basis and analyzed using a three-way ANOVA with mean separation using Tukey's test. Three-way interaction effects (P<0.001) were observed on nutrient composition. Soybean grown in full season with variety X in locations A and B produced FF with the highest crude protein (CP), compared with the double crop with the same SV in location C, which contained the lowest CP and highest ether extract (EE). However, there was a negative correlation between CP and EE. Higher crude fiber (CF) was observed in both SV in location C regardless of planting date and in SV X in the double crop at location C had the highest value. Both SVs had the highest AMEn in location B for the double crop. In this location, SV X had higher AMEn values in the full season. The AMEn was positively correlated to EE content. The SV X in locations A and B planted in full season contained the lowest TIA. Both SV in location C planted in double crop had the highest TIA values. The correlation (P<0.001) between raw soybean and FF in CP, EE, CF, and TIA were 0.86, 0.66, 0.65, and 0.58, respectively. The TIA in FF can be predicted (P<0.001) from its content in the raw soybeans (Y= -20.39+1.15X; R^2 =0.34). In conclusion, agronomic factors may influence nutrient content, TIA values, and controlling these factors may improve FF quality and reduce variability.

Key Words: Full-fat soybean, Variety, Location, Season, Trypsin inhibitor

T143 Effect of combined doses of fumonisin, deoxynivalenol and zearalenone on production performance and amino acid digestibility in broiler chickens Revathi Shanmugasundaram*[1], Mary Davis[1], Walid Hakeem[2], Shahna Fathima[2], Bikas Shah[2], Anthony Pokoo-Aikins[1], Oluyinka Olukosi[2], Todd Applegate[2], Anthony Glenn[1] *[1]Toxicology and Mycotoxin Research Unit, US National Poultry Research Center, Agricultural Research Service, [2]Department of Poultry Science, University of Georgia*

The individual tolerance levels of fumonisin (FUM) and deoxynivalenol (DON) in chicken diets are 50mg/kg and 5mg/kg, respectively. FUM, DON, and zearalenone (ZEN) mycotoxins typically co-occur in poultry feed ingredients and may exacerbate the effects of each other. The objective of this study is to identify the effects of subclinical doses of combined FUM, DON, and ZEN doses, on production performances, digestibility, and immune parameters. A total of 960 one-day-old chicks were assigned to eight treatments. (1) Control diet (0.8mg FUM and 0.4mg DON) (2) 33mg FUM + 3mg DON +1.0mg ZEN; (3) 26mg FUM + 1.0mg DON + 0.2mg ZEN; (4) 14mg FUM + 3.5mg DON + 0.7mg ZEN; (5) 7.7mg FUM + 0.4mg DON + 0.1mg ZEN; (6) 3.6mg FUM + 2.5mg DON + 0.9mg ZEN; (7) 0.8mg FUM + 1.0mg DON + 0.3mg ZEN; and (8) 1mg FUM + 0.5mg DON + 0.1mg ZEN per kg diet. Each treatment had 6 replicates with 20 birds per pen. Birds in all the treatment groups received coccidial vaccine at d0. At d35, birds in the 33mg FUM + 3mg DON + 1.0mg ZEN group had a significantly lower BWG ($P = 0.05$) and increased FCR by 12 points compared to those in the control group. Birds in the 3.6mg FUM + 2.5mg DON + 0.9mg ZEN group had no significant ($P > 0.05$) loss in BWG and FCR compared to the control group. At d21, splenic macrophages from birds in the 33mg FUM + 3mg DON + 1.0mg ZEN groups had significantly decreased nitric oxide production ($P < 0.05$) compared to that in the control group. At d21, birds in the 21mg FUM + 3mg DON + 1.0mg ZEN groups had significantly decreased digestibility for aspartic acid and serine ($P < 0.05$); tended to have decreased ($P < 0.10$) digestibility for threonine, glutamine, and tyrosine compared to the control. Further, at d21, birds in the 21mg FUM + 3mg DON + 1.0mg ZEN group had an increased ($P < 0.05$) serum FITC-D concentration (a measure of gut integrity). In summary, broilers fed diets contaminated with 33mg FUM along with 3mg DON and 1.0mg ZEN/kg diet had decreased growth performance, decreased amino acid digestibility, and a loss in gut integrity. It can be concluded that broiler birds are sensitive to the presence of multiple mycotoxins, and tolerance levels of individual mycotoxins decrease when multiple mycotoxins are present in the feed.

Key Words: Broilers, Mycotoxins, Fumonisins, Deoxynivalenol, Production performance

T144 Body composition and fasting heat production of modern broilers and their relationship with dietary productive energy (Arkansas Net Energy) Diego Martinez*, Nawin Suesuttajit, Katie Hilton, Jordan Weil, Cole Umberson, Abdullah Scott, Craig Coon *Department of Poultry Science, University of Arkansas*

The heat production (HP) of broilers has been shown to increase through the years associated with higher breast meat yield, supporting the use of an energy system that better accounts for HP differences caused by changes in genetics, body composition (BC), nutrition, and environmental temperature. This study aimed to determine the influence of BC on fasting HP (FHP) and the sensitivity of models based on metabolic body weight (MBW) alone or including BC to determine the maintenance net energy of broilers. Four experiments (E1 to E4) were conducted to determine the influence of BC on FHP and develop prediction models, and two experiments (E5, E6) to validate them. In E1 to E4, a total of 2400 Cobb 500 broilers distributed into 96 floor pens, were fed three dietary treatments to induce BC differences. The FHP (indirect calorimetry) and body protein-to-fat ratio (PFR; Dual-Energy X-Ray Absorptiometry) were determined from 1 to 56 d. The metabolic allometric coefficient was estimated using non-linear modeling and multiple linear models (response variable: FHP) were adjusted to the data using JMP Pro 16.0.0. In E5, broilers fed one of six dietary treatments were tested for FHP and BC three times within 1-42 d. In E6, broilers of two genetic lines distributed into 54 pens (45 birds

each) and fed three dietary treatments were also tested twice within 1-42 d. The allometric coefficient was determined to be not different from 0.75 (P >0.05). Four models developed (R^2 >0.98), considering body weight, PFR, and age, were validated (R^2 >0.92; error <3%). Results showed that the leaner the bird, the higher the FHP (P <0.0001). Models based on MBW alone underestimate (+10% dif.) the FHP of lean birds being the estimations unreliable (regression assumptions not met), but including BC allows detecting +18% FHP differences. FHP and body energy retention (ER), expressed per unit of feed intake, account for productive energy (Arkansas Net Energy; Ark NE). In conclusion, BC influences the FHP and energy partitioning in modern broilers. This study (i) indicates that energy systems assuming the ER, total HP, or FHP as a function of the BW or MBW alone do not detect the actual energy value of the diet and ingredients, and (ii) supports the use of a productive energy (Ark NE) system.

Key Words: heat production, productive energy, energy partitioning, Arkansas Net Energy, meat production

T145 Energy and nutrient composition of corn particle fractions generated by three hammer mill settings Paula Lozano-Cruz, Nicolas Mejia-Abaunza, Joaquín Cabanas-Ojeda, Edgar Oviedo-Rondon* *Prestage Department of Poultry Science, North Carolina State University*

Corn particle size (PS) is an important factor related to poultry gut health and nutrient utilization efficiency. However, corn particle fractions differ not only in physical properties, but also in nutrient composition. Previous studies have shown that corn kernel hardness and post-harvest drying temperatures (DT) affected nutrient composition and PS. Therefore, this study aimed at evaluating energy and nutrient composition of corn particle fractions generated at three hammermill settings. Two corn hybrids with hard (H) and average (A) endosperm hardness from contiguous plot areas were harvested, dried at 120 and 35ºC, and ground at 3 hammermill settings to obtain d_{gw} of nearly 450, 700, and 1250 microns (µm), for fine, mid, and coarse PS. The PS d_{gw} and S_{gw} were obtained by the sieving method ASAE S319.3. The contents of each sieve plate were retained, accumulated from 5 replicate samples per treatment combination, and grouped in 7 categories (0-52, 53-149, 150-296, 297-840, 841-1,190, 1,191-1,679 and 1,680-more µm). These 7 groups were ground in a Retsch mill to 0.5 mm, and analyzed by NIRS to obtain moisture, protein solubility index (PSI), vitreousness, AME, starch, protein, and amino acid (AA) content using ABVista calibration curves. Data was analyzed in a 2x2x7 factorial arrangement in a randomized complete block design with kernel hardness, DT, and PS fractions as main effects and grinding settings as blocks. Three-way interaction effects were observed (P<0.001) for all corn traits at each grinding setting. Moisture increased as PS decreased. In contrast, lower PSI was observed as PS decreased. The AME decreased linearly as PS reduced for every corn and DT combination in all grinding settings. But, the AME dispersion as PS decreased was greater for corn dried at 35ºC (176 to 88 kcal/kg) than for corn dried at 120ºC (145 to 77 kcal/kg), and mostly bigger in corn ground coarse and mid than in fine. Protein and AA were higher in the middle PS groups (297 to 1,679 µm), and the very fine PS had the lowest content for all corn treatments evaluated. In conclusion, the energy and nutrient content varies among the different corn PS generated during grinding, independently of grinding settings. The middle particle size had the best balance among all nutrients.

Key Words: Corn, Nutrient content, particle size, AME

T146 Effects of feeding varying proportions of pellets and fines on growth performance and carcass yield of broilers during a 63-day production period Klint McCafferty*, Joseph Purswell *USDA-ARS Poultry Research Unit*

An experiment was conducted to evaluate the effects of feeding various proportions of pellets and fines on performance and carcass yield of broilers during a 63-d production period. Five hundred-four YPM × Ross 708 straight-run broilers chicks were feather-sexed, weighed, and equally distributed into 42 floor pens (6 males and 6 females per pen; 0.10 m^2 per bird). Broilers were fed 7 dietary treatments (6 replicates per treatment) with varying percentages of pellets and fines from 14 to 63 d of age: 1) 100:0, 2) 75:25, 3) 56:44, 4) 42:58, 5) 31:69, 6) 0:100%, and 7) 0:100% mill fines (feed particles that passed through a 2,000 µm sieve), respectively. Cumulatively, no differences (P > 0.05) in BW, carcass yield, and abdominal fat yield were observed between those fed treatments 1 to 6, but broilers fed treatment 7 had a lower (P < 0.05) BW, and carcass yield than those fed treatments 2, 4, and 5. Birds fed treatment 7 had the highest (P < 0.05) FCR and abdominal fat yield, and lowest (P < 0.05) total breast meat yield of all treatments. Broilers fed treatments 5 and 6 had a lower (P < 0.05) FCR than those fed treatment 1. These data indicated that increasing the proportion of pellets to fines in diets did not incrementally improve the performance and yield of large broilers. However, feeding broilers mill fines negatively affected growth performance and carcass yield of large broilers.

Key Words: feed form, pellet, mill fine, broiler, fine

T147 The detection and co-occurrence of mycotoxins in feed corn using HPLC/MS analysis Anthony Pokoo-Aikins*, Callie McDonough, Jaci Hawkins, Trevor Mitchell, Revathi Shanmugasundaram, Scott Gold, Anthony Glenn *United States National Poultry Research Center, Toxicology and Mycotoxin Research Unit, USDA-ARS*

Feed safety is critical for poultry diets and feed ingredients must be evaluated prior to feed formulation. The main feed ingredient, corn, accounts for about 60% of poultry diets. Corn is often contaminated with multiple mycotoxins in the field and during storage. Due to the detrimental effects that mycotoxins can have on poultry health and performance, particularly in multi-toxin combinations, it is critical assess the mixed mycotoxin content in corn. The objective of this study was to gain an overview of mycotoxin co-contamination and develop effective procedures to consistently quantify the mixed mycotoxin content of corn samples. To obtain an unbiased overview of mixed mycotoxin load in corn intended for poultry feed, as an initial experimental design we received 95 random pre-formulation corn samples from collaborators in three states, GA, NY and MD. Corn samples were analyzed for fumonisins (FUM), deoxynivalenol (DON), aflatoxins (AFLA), zearalenone (ZEA), and ochratoxin A (OTA) content using HPLC/MS. Only one of the samples had no quantifiable mycotoxin contamination. All other samples (99%) had quantifiable levels of FUM, however, all analyzed samples were below the 100 ppm guidance level for poultry. Statistically, analysis indicated that 87% of the samples had less than 5 ppm DON, while 6% of the samples had above 5 ppm DON. Of the total samples, approximately 43% had one, 52% two, and 3% three quantifiable mycotoxins, with FUM and DON by far the most prevalent combination. Additionally, approximately 4% of the samples had detectable AFLA below and 2% above the 20 ppb actionable level. 34% and 15% of tested samples had less and more than the guidance level of 1 ppm ZEA, respectively. Overall, 99%, 55%, 43%, and 4% of the samples were contaminated with quantifiable levels of FUM, DON, ZEA, and AFLA, respectively. There was no statistical difference between mycotoxin level by state according to Tukey HSD. We conclude that multiple mycotoxin contamination, particularly FUM and DON, is the rule in feed corn. Because combinations of mycotoxins may be synergistic well below their individual guidance, as we have shown with combinations of FUM and DON, knowledge of their co-occurrence and combined action is imperative for maximal poultry productivity.

Key Words: poultry, nutrition, mycotoxin, corn, feed safety

T148 Dose dependent responses of young broilers to soy β-conglycinin in mash and pelleted diets Kyle Teague*[1], Guillermo Tellez-Isaias[1], Alfred Blanch[2], Simone Rasmussen[2], Samuel Rochell[3] *[1]University of Arkansas System Division of Agriculture, [2]Hamlet Protein, [3]Auburn University*

This experiment investigated the effects of soy β-conglycinin (BCON), a well-documented allergenic protein in other species, on performance and health of young broilers. Dose responses were evaluated in mash and pelleted feeds due to potential BCON denaturation with pelleting. Purified BCON was added to soy-protein isolate-based diets to achieve 0 (null), 25,000 (moderate), or 50,000 (high) mg/kg BCON and fed in mash or pelleted (conditioning time of 10 s at 38°C; 3 mm die) form. Treatments were fed to 9 replicate battery cages of 6 chicks from 0 to 14 d, with a common pelleted diet fed from 14 to 21 d. Body weight and feed consumption were recorded weekly. At d 14, whole blood was collected for complete blood cell counts, and prior to collection, 2 birds/cage birds were gavaged with fluorescein isothiocyanate-dextran (FITCd) for subsequent measurement in the serum. Blood was also analyzed for completed blood counts, serum vitamin E, and superoxide dismutase (SOD) activity. Intestinal tissue was collected to measure mucosal SOD activity. Respective analyzed BCON levels for the null, moderate, and high BCON diets were 10, 11,989, and 46,937 mg/kg in mash feed and 17, 5,412, and 15,783 mg/kg in pelleted feed. From 0 to 14 d, interactive effects ($P < 0.05$) of BCON and feed form on FI and BWG were observed. Feed intake was reduced by high BCON, but not by moderate BCON in mash feeds, and BCON did not influence the FI of pelleted diets. Relative to birds fed no added BCON, BWG was reduced by high BCON in mash diets only, but by both moderate and high BCON in pelleted diets. Feed conversion of chicks (0 to 14 d) was independently reduced ($P < 0.05$) in pelleted (1.099) compared with mash (1.216) diets and increased ($P < 0.05$) with high BCON (1.218) compared with moderate (1.142) and null BCON (1.114). There were no treatment effects ($P > 0.05$) on differential blood cell counts, serum vitamin E levels, or serum or mucosal SOD activity. Serum FITCd was increased ($P < 0.05$) by pelleting but was not influenced ($P > 0.05$) by BCON addition. In conclusion, these data demonstrated that feed pelleting reduced soy-derived purified BCON concentrations in the diet, and that BCON dose-dependently impaired the growth performance of young broilers.

Key Words: soybean, anti-nutritional factor, β-conglycinin, broiler, gut health

T149 Effect of emulsifier supplementation on growth, performance nutrient digestibility, fecal microbial, fecal gas emission, and blood profiles in growing pigs. In Ho Kim[1], Srijit Tripathi*[2], Dilraj Bhatia[2] *[1]DANKOOK UNIVERSITY, [2]VETLINE, A DIVISION OF SIMFA LABS PVT. LTD.*

Purpose: Objective of the present study was to evaluate the effect of dietary supplementation of emulsifiers on growth performance, nutrient digestibility, fecal microbial count, and blood profiles in growing pigs.

Experimental Design: A total of 180 growing-finishing pigs [(Landrace × Yorkshire) × Duroc] with an average initial BW of 25.07 ± 2.38 kg were used in this 6-week feeding trial to evaluate the effect of dietary emulsifier supplementation on various parameters in growing pigs. Pigs were randomly allotted to 4 dietary treatments with 9 replicates per treatment having 5 pigs per replicate. Throughout all the experimental period, each pen was equipped with a 1-sided self-feeder and a nipple drinker to allow the pigs ad libitum access to feed and water. Dietary treatments include: 1) CON, PC (Basal Diet), T1, PC - 50Kcal (Fat), T2, T1 + 0.05% (Emulsifier product, X, M/S Simfa Labs Pvt. Ltd., India) and T3, T1 + 0.05% (competitor biological surfactant, Y).

Results and Statistics: Initial BW gain, ADG and FCR were found to have improvement in the emulsifier X treated group (T2). The blood parameters were much improved in group T2 as compared to other groups. The beneficial bacterial counts were increased while the harmful one was reduced in group T2. Excellent improvement in the nutrient digestibility of DM, lipids and Amino acids were there in group T2. All data were analyzed using General Linear Model procedure of SAS. The pen was used as the experimental unit. For microbial counts, data were log-transformed prior to statistical analysis. Duncan's multiple range test was adopted to compare means of the treatments. Variability in the data was expressed as the pooled SEM. $P < 0.05$ was considered statistically significant.

Conclusion: The emulsifier product X has been found to have positive results even when there was a reduction of 50 Kcal in the feed. There was better nutrient digestibility that accounts to better growth, Production and FCR. Improvement in the gut microbial composition is an additional advantage seen in the group T2. Improved blood profile leads to a healthy production of pigs in T2 group. Overall emulsifier X treated group has a much better productivity and other parameters in this experimental trial in comparison to other groups.

Key Words: emulsifier, Feed additive, FCR

T150 Productive energy (Ark NE), apparent metabolizable energy (AMEn) and net energy (NE) values for diets and ingredients for 5- 56 d broiler grow-out studies Nawin Suesuttajit*, Jordan Weil, Cole Umberson, Diego Martinez, Abdullah Scott, Criag Coon *Center of Excellence for Poultry Science, University of Arkansas*

Arkansas net energy (Ark NE) is a productive energy system based on actual dietary energy utilized for gain and maintenance. A total of 48 diets with varying levels of total digestible amino acids (TDAA), digestible starch (DSTAR), digestible fat (DFAT), and NSP were utilized in 5-56d broiler studies to determine the performance and energy value of diets and ingredients using DEXA and indirect calorimetry system. Data were subjected to a one-way ANOVA and Tukey's HSD to compare separate treatment means with significance set at 0.05 using JMP pro16. Multiple linear regression models were computed to generate prediction equations for the energy values of ingredients. Treatment diets significantly impacted broiler performance, ADG, and FCR. FCR provided a much stronger negative relationship to Ark NE ($R^2 = 0.52$) compared to AMEn ($R^2 = 0.16$) and classic NE (R^2=0.17). The TDAA, DFAT, and DSTAR were positively related to Ark NE ($P<0.05$), while total NSPs negatively affected Ark NE for 56d broiler performance. Each percentage increase of TDAA, DFAT and DSTAR increased the predicted Ark NE value by 74, 58, and 32 kcal/kg, respectively, while increase of NSP decreased the predicted Ark NE value by 18 kcal/kg in the multiple linear regression model. Conversely, AMEn and classic NE systems were strongly influenced by DFAT compared to TDAA and DSTAR in the same diets. The average energy efficiency (EE) of Ark NE compared to AMEn (Ark NE/AMEn) was 85.48% and the EE of classic NE compared to AMEn was 75.49% for the same diet. The predicted Ark NE values of SBM, corn, and corn oil were 3340, 2511, and 5,728 kcal/kg for 56 d broiler performance, respectively. The studies indicated digestible amino acid calories are the most important calories in 56d broiler grow-out because digestible amino acids enhance performance and contribute more calories for productive energy, whereas digestible dietary protein and amino acids decrease AME and classic NE calories. The AME and classic NE systems undervalue protein ingredients such as soybean meal and overvalue energy sources such as corn oil and corn. The AMEn and classic NE energy systems are less sensitive to broiler performance and type of gain compared to Ark NE.

Key Words: Ark NE, Digestible AA Calories, AME, NE, Energy System Comparison

T151 Dietary hemp seed cake enhances performance and egg quality parameters in white caged laying Hens. Fausto Solis*[1], Rajasekhar Kasula[1], Byron Shaffer[2], Frank Connett[2], Chris Barrett[2], Rodney Cocker[2], Eric Willinghan[3] *[1]Wenger Animal Nutrient & Technology Innovation Center, The Wenger Group, [2]Kreider Farms, 1461 Lancaster Rd, Manheim, PA 17545, [3]Winfield Veterinary Consulting, Inc.,*

Background and Objective: Hemp seed and hemp seed products such as Hemp Seed Cake (HSC) have shown to increase unsaturated fatty acid (FA) profile in eggs, including linoleic acid, known to increase egg weight and α-linolenic fatty acids. However, the use of hemp products in animal feed is still a concern due to the potential residues of the of Δ-9 tetrahydrocannabinol, a psychoactive substance present in the hemp plant. No significant published research is available on the effect of dietary HSC on egg quality parameters in commercial laying hens. The objectives of this study was to determine the effect of dietary HSC on egg quality, external (egg weight, egg mass, eggshell strength, eggshell thickness) and internal (Haugh units, egg yolk pigmentation, egg lutein, egg fatty acids, egg heavy metals and egg cannabinoid residues). Materials and methods: Eight hundred (800) Bovan caged hens in lay at 30 weeks of age were distributed into 4 treatments of 200 hens per treatment based on inclusion levels (0%, 10%, 20% and 30%) of hemp seed cake (HSC). Each treatment comprised of 8 cages of 25 hens each that served as replicates. The observations per protocol were made over a period of 16 weeks following a 3-week acclimation. Results: HSC feeding to commercial laying hens did not adversely affect egg weigh, egg mass; however, positive effects of HSC supplementation was observed on eggshell strength, and the polyunsaturated fatty acids including linoleic and linolenic fatty acids. HSC also improved egg lutein, yolk pigmentation and Haugh units. The cannabinoids residues in eggs was below the detectable level. Conclusion: The results of this study confirm that HSC fed to laying hens enhanced the overall value of the eggs with increased deposition of beneficial unsaturated fatty acids, yolk pigmentation, Haugh units and lutein content and the trial also demonstrated that feeding HSC to laying hens did not contribute to tetrahydrocannabinol (THC) or cannabinoid residues in eggs.

Key Words: Hemp seed cake, eggs, tetrahydrocannabinol,, cannabinoids, Laying hens

SCAD II

T152 Characterization of fowl typhoid outbreaks occurring in Latin America Martha Pulido-Landinez* *Mississippi State University*

Fowl typhoid caused by *Salmonella* Gallinarum continues to be a significant problem in the Latin American (L.A.) poultry industry. For many years this disease was observed in brown layers and later in broiler chickens. In the last two years, an increase in cases of broiler breeders has been reported.

This study aims to characterize the fowl typhoid outbreaks in L.A., paying particular attention to cases related to broiler breeders and broilers. The main characteristics of its presentation were evaluated by compiling information from field cases. The ages of identification of the disease were established as follows: In broilers, as early as three days of age, up to 21 to 35 days. In breeders, it is reported from 35- 40 to 58-60 weeks. In commercial layers, the presentation ages are variable, with a special presentation in hens older than 60 weeks.

Depression, egg drop production, bright greenish feces, impaired fertility, decreased hatchability, and increased mortality has been observed in affected breeders. Progeny from positive breeders shows depression, bright greenish feces, low feed intake and weight gain, and poor uniformity. Reports of mortality fluctuate from 3 to 30%. These findings highlight the importance of the vertical transmission of this bacterium.

A second part of the work was performed at the Poultry Research and Diagnostic Laboratory of Mississippi State University. *Salmonella spp* DNA samples from L.A. were received for serotyping using intergenic sequencing ribotyping. From 2018 to 2022, 33 samples have been identified as S. Gallinarum, gallinarum, genovar 2978. These samples include S. Gallinarum from commercial brown layers, broilers, and broiler breeders. To analyze the most relevant characteristics of S. Gallinarum isolated from L.A., 20 samples (10 commercial layers, five broiler breeders, and five broiler chickens) were selected.

The characterization of S. Gallinarum will allow identifying if the predominant bacteria correspond to a field strain or if it is related to a vaccine strain. This information will help establish control mechanisms that will reduce the impact of this bacterium in the L.A. poultry industry.

Key Words: Fowl Typhoid, Salmonella Gallinarum, Risk analysis

T153 Behavior of naturally occurring recombinant infectious bronchitis viruses Camila Cuadrado*[GS], Cassandra Breedlove, Haroldo Toro *Auburn University*

Novel variants of infectious bronchitis virus (IBV) continue to emerge from selection of vaccine subpopulations and/or naturally occurring recombination events. Little is known about the biological properties of such novel variants. Previously, we genetically characterized virulent ArkDPI vaccine subpopulations as well as ArkDPI vaccine viruses showing recombination obtained from broilers flocks in Alabama. We now evaluated pathogenicity and protection conferred by a commercial ArkDPI vaccine against strains homologous to the major parent, and against both virulent ArkDPI subpopulations and naturally occurring ArkDPI recombinants. SPF layer-type chickens were vaccinated at day of hatch and challenged 21 days post-vaccination with either homologous virulent virus, an ArkDPI virulent subpopulation, or an ArkDPI/Mass recombinant virus. Spike gene sequencing was performed to confirm the stability of the inoculated viruses. Five days post-challenge significant differences were detected between groups in tracheal viral load, antibody responses and histological damage.

Key Words: Infectious bronchitis virus, vaccine, recombinant IBV, poultry

T154 Impact of embryonic exposure to Enterococcus cecorum strains isolated from field outbreaks on early performance and septicemia in broiler chickens Marcela Arango*[GS], Latasha Gray, Randy Moore, Abdiel Atencio, Carolina Trujillo, Aaron Forga, Guillermo Tellez-Isaias, Billy Hargis, Juan Latorre, Danielle Graham *Department of Poultry Science, University of Arkansas Division of Agriculture*

Recently, *Enterococcus cecorum* (EC) has been associated with septicemia and mortality in young broiler chickens. There is limited research investigating the pathogenicity of EC strains isolates obtained from affected birds in the field. The purpose of the present study was to evaluate the impact of *in-ovo* administration into the amnion of different EC isolates at day 18 of embryogenesis (DOE18). In a preliminary study, EC field isolates (n=7; EC1-EC7) were selected based on phenotypic characteristics and evaluated at different concentrations ($1x10^2$, $1x10^4$, and $1x10^6$ CFU/200uL/embryo) to assess the impact on early performance and macroscopic lesions in broiler chickens. Based on the results, isolates (n=3; EC2, EC5, EC7) were selected for further evaluation based on the significant ($P<0.05$) reduction in d0-21 BWG and presence of lesions during

necropsy at d14 and d21 compared to the non-challenged control (NC). An additional isolate previously associated with enterococcal spondylitis was also evaluated. Treatment groups included: 1) NC, 2) EC2, 3) EC5, 4) EC7, and 5) EC11B (n=90-120/embryos/group). Groups 2-5 received $1x10^2$ CFU/200uL/embryo by *in-ovo* injection into the amnion at DOE18. Chicks were placed in battery cages and pen BW was recorded at d0, d7, d14, and d21 to determine average BW and BWG. At d14 and d21 post-hatch, liver, spleen, free thoracic vertebrae (FTV), and femoral head (FH) were collected to enumerate *Enterococcus* spp. using Chromagar Orientation as the selective media. There was a significant ($P<0.05$) reduction in BW at d21 and BWG from d14-21 and d0-21, for EC7 and EC11B isolates. Additionally, EC was recovered from the FTV of all challenged groups. The most representative lesions were pericarditis, heart blood vessels congestion, focal heart necrosis, FH osteomyelitis, and hydropericardium. However, lesions were not uniform across challenged groups or ages (d14 and d21). These results highlight the differences in pathogenicity and performance impacts of EC strains isolated from field cases. Further research investigating the effects of horizontal transmission of virulent EC during the hatching phase is underway.

Key Words: Enterococcus cecorum, Broiler, Performance, Lesions, Chicken

T155 Effects of monoglycerides of butyric, caprylic, and capric fatty acids on the performance of pullets vaccinated with a commercial coccidiosis vaccine Gerald Self, Jr.*[1GS], Adebayo Sokale[2], Michael Elliot[3], Daniel Chesser[4], Kelley Wamsley[1], Pratima Adhikari[1] *[1]Poultry Science, Mississippi State University,, [2]BASF Corporation-Animal Nutrition, North America,, [3]A&E Nutrition Services, LLC, [4]Agricultural and Biological Engineering, Mississippi State University*

As the industry continues to move toward cage-free egg production, the need for additional control methods of intestinal diseases such as coccidiosis become more imperative. One method being the inclusion of diets containing increased amounts of small and medium chain fatty acids. The objective of the experiment was to evaluate the effects of monoglycerides of short and medium chain fatty acids (SMCG) on the performance of pullets vaccinated with COCCIVAC-D2. 800-day-old Hy-Line W-36 pullets were randomly assigned into 4 treatments with 10 replications (20 birds/rep). The 4 treatments consisted of an unvaccinated negative control (NC; T1), vaccinated positive control (PC; T2), SMCG at 0.05% (T3) and SMCG at 0.1% (T4). Birds were fed a diet composed of corn, soybean meal, dried distillers' grain (DDGS), and meat and bone meal (MBM) in 2 phases (0-3 weeks and 3-6 weeks). Diets were formulated to breeder recommendations. Pullets in T2-T4 were vaccinated in-feed on days 0, 7, 14 and 21. Feed consumption (FC) and body weights (BW) were recorded weekly. Five birds were wing-banded at placement and were weighed weekly to determine BW uniformity. Fecal samples were collected on days 7, 14, 21, and 28 for oocyst per gram of feces (OPG) counts. Performance data were analyzed using Proc GLM procedure of SAS version 9.4, where uniformity was subjected to Tukey's range test. There was significance in the OPG at days 7 and 14, where counts were lower for the NC compared to the PC, T3, and T4. There were two instances of significance in FC and one instance for BW during weeks 0-3. For FC, there was significance in T3 and T4 for weeks 1 and 3 (P=0.008 and 0.016), respectively, where FC for T3 and T4 surpassed the NC and PC. Similarly, there was a significant increase in BW for T4 during week 3, where pullets had higher BW than the pullets in NC (P=0.041). Additionally, throughout weeks 0-6, there were two instances of significant BW uniformity at week 1 and 5. In both instances, SMCG treated groups showed higher uniformity than the control groups. In conclusion, the study showed that SMCG supplementation at 0.1% can improve the performance of pullets over an extended time when vaccinated with COCCIVAC-D2.

Key Words: Body Weight, Coccidiosis, Fatty Acid Chains, Oocysts per Gram, Pullets

T156 Transcriptomic analysis of chicken harderian gland, trachea, cecal tonsil and spleen after vaccination with the LaSota Newcastle Disease Virus Jannis Nankemann*[GS], Andrea Pietruska, Raimundo Espejo, Cassandra Kitchens, Haroldo Toro, Rüdiger Hauck *Auburn University*

Newcastle Disease (ND) causes substantial economic losses in the global poultry industry, and vaccination, especially with the LaSota strain, is an important tool for its prevention. The objective of the present experiment was to analyze the expression of immune genes in chicken trachea, harderian gland, cecal tonsils and spleen upon vaccination with the LaSota strain as a foundation for further experiments evaluating alternative vaccines. 13-day old specific pathogen free chickens were vaccinated by the naso-lacrimal route. Tissues were sampled at 12h, 24h and 48h post vaccination, and total RNA was extracted. RNA-seq and Gene Expression Quantification and Differential Expression Analysis were carried out to identify differentially expressed genes (DEGs) in vaccinated versus unvaccinated chickens. To determine the immunologic functions of the DEG products, GO Enrichment Analysis (GOEA) was performed.

Transcriptome analysis revealed 1 significant DEG at 12h, 404 significant DEGs at 24h and 2001 significant DEGs at 48h post vaccination. In cecal tonsils, at 24h, MHC class I and II complex including antigen processing and presenting, T cell mediated immunity and T cell cytokine production were significantly downregulated. In harderian glands, at 24h, virus response, type I interferon production, regulation and signaling, as well as MyD88-independent and TRIF-dependent toll-like receptor signaling were significantly upregulated. In harderian glands, at 48h, T cell proliferation was significantly downregulated, and pathways related to virus response, MHC protein complex assembly, toll-like receptor signaling and type I interferon signaling and response were significantly upregulated. In spleens, at 24h, lymphoid progenitor cell differentiation and defense response were significantly upregulated. In trachea, at 48h, T cell mediated immunity, T cell differentiation and activation were significantly down regulated, while type I interferon production and regulation, MyD88-independent and TRIF-dependent toll-like receptor signaling and response to dsRNA were significantly upregulated. Overall, this study helps to build a better understanding of the chicken immune reaction after vaccination against ND.

Key Words: Newcastle Disease, transcriptome, immune response, chicken, vaccination

T157 Bacteriology findings and microbiological composition analysis of Focal Duodenal Necrosis (FDN) Yu-Yang Tsai*[GS], Sonsiray Narvaez, Nicolle Barbieri, Catherine Logue *Poultry Diagnostic and Research Center, Department of Population Health, The University of Georgia*

Focal Duodenal Necrosis (FDN), an intestinal disease is among the top five concerns in table egg layers. The economic impact of FDN is associated with a decrease in egg case weight and a drop in egg production. In this research, we used bacteriological analysis to analyze, the microbiological composition of FDN lesions.

Five duodenal samples with characteristic FDN lesions were examined bacteriologically and a total of 114 colonies grew on TSA, MacConkey, blood agar, and PEA agar plates incubated aerobically and anaerobically. Through 16S rRNA gene PCR and Sanger sequencing, 50/114 colonies were identified as *Escherichia coli*. PCR for avian pathogenic *E. coli* (APEC) virulence genes revealed 14% of isolates are considered APEC-like. PCR panels examining for virulence genes associated with intestinal pathogenic *E. coli* showed 84% of isolates possessed multiple virulence genes.

To understand the detailed genomic information of *E. coli,* isolates that possessed multiple virulence genes associated with intestinal pathogenic strains were sequenced. Therefore, five *E. coli* isolates were chosen for whole genome sequencing. Phylogenetic analysis revealed that one isolate had a closer relationship to IBD associated *E. coli* while the others were closer to extraintestinal pathogenic *E. coli* (ExPEC) and APEC strains.

Examination of the microbial composition of duodenal FDN lesions can provide more insight about the roles of which organisms contribute to the disease. Ten frozen duodenal lesion samples were used for microbiome analysis. The Powersoil kit was used for DNA extraction and the DNA then subjected to 16S rRNA sequencing using PacBio sequencing. *Clostridium perfringens, Enterococcus cecorum, E. coli, E. fergusonii, Lactobacillus aviaries* and *Tyzzerella clostridium colinum* were found. These bacterial species can be considered as contributing to the pathogenesis of FDN.

FDN appears to be a multifactorial inflammatory intestinal disease associated with multiple bacterial species and *E. coli* isolated from the duodenal lesions is linked with other intestinal disease strains.

Key Words: Focal duodenal necrosis, intestinal disease, layers, Escherichia coli

SCAD III

T158 Using the Waterfowl Alert Network to Model Waterfowl Density Near Commercial Poultry in the Atlantic Flyway and Beyond Maurice Pitesky*[1,2], Joseph Gendreau[1,2], Tristan Bond[1], Shane Feirer[1,3], Jaclyn Smolinsky[1,4], Jeff Buler[1,5] *[1]AgriNerds Inc, [2]University of California, Davis, [3]University of California-Agriculture Natural Resources, [4]Cherokee Nation System Solutions, [5]University of Delaware*

The current outbreak of Highly Pathogenic Avian Influenza (HPAI) in North America has led to the depopulation of over 50 million poultry in 46 states. Current prevention efforts by commercial producers are primarily focused on structural and operational biosecurity. Because waterfowl are the primary reservoir of HPAI, understanding where waterfowl are in close proximity to commercial poultry would provide a novel and useful stream of information for various poultry stakeholders (e.g. commercial producers, state and federal stakeholders and backyard poultry owners alike). New and emerging remote sensing technologies have the capability to offer a suite of science based information on waterfowl roosting, flight and feeding movements. The Waterfowl Alert Network is the world's first commercial subscription based service which offers daily predictions of waterfowl density at 250m granularity between November 1st and March 31st, which is when waterfowl are at their greatest abundance in the U.S. The technology builds an ensemble of Boosted Regression Tree (BRT) models trained using various historical remote sensing data from the national weather surveillance radar network (NEXRAD), MODIS satellites, PRISM climate dataset, and CroplandCROS agricultural crop data layer. Near-real time environmental data are used to make daily waterfowl density maps from the models. The Waterfowl Alert Network ranks farms based on predicted bird biomass density within various spatial (1,2,3, and 4 km radius of a selected farm) and temporal (daily, weekly, monthly, and seasonal) windows. Current models use data from 25 of the 160 NEXRAD radars, which cover parts or all of 15 states in three flyways. Here we present redacted BRT model predictions for over 450 farms in the Mississippi and Atlantic Flyways during 2022. Initial results for November of 2022 show the greatest reflectivity (i.e. waterfowl density) in the upper Mississippi flyway with the lowest reflectivities in the North Eastern Plains and North East of the Appalachian Mountains. These types of data offer novel insights for various poultry stakeholders on husbandry, biosecurity and food security.

Key Words: avian influenza, commercial poultry, remote sensing, waterfowl, daily prediction

T159 Molecular characterization of Mycoplasma gallisepticum on poultry farms in Nigeria Eniope Oluwayinka*[1], Oni Oluwole[2], Kadiri Amina[2], Steven Fini[1], Marianne Dos Santos[1], Jin Qian[1], Naola Ferguson-Noel[1] *[1]Poultry Diagnostic and Research Center, University of Georgia, [2]College of Veterinary Medicine, Federal University of Agriculture Abeokuta*

Chronic respiratory disease, caused by *Mycoplasma gallisepticum* (MG), is one of the important respiratory disease of poultry in Nigeria causing reduced egg production, decreased feed efficiency and poor carcass quality. In this study, we characterized MG from chicken carcasses with a history of respiratory disease submitted to some diagnostic laboratories in three south-western states of Nigeria. A total of forty-eight flocks submitted samples during the study period, July - December 2021, including twenty samples from layer farms and twenty-six from broiler farms. Pooled tracheal scrapings from at most four carcasses per flock were spotted on FTA cards, DNA was extracted, conventional PCR was done to identify MG positive samples followed by the sequencing of short MG cytadhesin 2 (mgc2S) and MG 16S-23 rRNA intergenic spacer region (IGSR). Samples from eighteen flocks (37.5%) were MG positive; about 33% (6/18) of these positive samples were from flocks that were less than 4 weeks old. Phylogenetic analysis of the mgc2sIGSR targets demonstrated the presence of 6/85 MG vaccine strain in three unvaccinated flocks; and a wild-type MG strain in a vaccinated flock. The Nigeria MG wild-type strain shared approximately 98.71- 99.84% identity with strains from Egypt, Israel, Jordan, Thailand and Colorado. This study sheds light on the prevalence of MG in Nigeria, and provides important information that can be used to better understand the epidemiology of MG and help develop control programs in Nigeria.

Key Words: Molecular, characterization, Mycoplasma gallisepticum, Nigeria

T160 Chicken lymphoid cellular immune responses against necrotic enteritis producing Clostridium perfringens strains Ravi Kulkarni*[1], Carissa Gaghan[1], Khaled Abdelaziz[2] *[1]North Carolina State University, [2]Clemson University*

Necrotic enteritis (NE), caused by virulent strains of *Clostridium perfringens*, is an economically important disease of chickens. Although much work has been invested in understanding the NE pathogenesis, the avian host cellular responses against C. perfringens strains have been poorly characterized. In the present study, using immunophenotyping, cellular responses in cecal tonsil (CT), bursa of Fabricius and spleen were evaluated in chickens infected with strains representing three levels of virulence, namely, avirulent (strain CP5), virulent (strain CP18) and very virulent (strain CP26). Results showed that the macrophage frequency in CT and spleen were significantly higher in CP18 and CP26-infected chickens compared to uninfected control, while the gdT cells in the CT of CP26-infected chickens were significantly higher than rest of the groups. Similarly, B cell frequency in the CT, spleen and bursa of CP26-infected chickens was higher than rest of the groups. Evaluation of T cellular activation showed that chickens infected with CP18 or CP26 had significantly higher frequencies of splenic CD4+ and CD8+ T cells expressing CD44 and CD28 molecules compared to uninfected or CP5-infected groups. Additionally, CP26-infected chickens also had significantly increased CT frequency of these activated CD4+ and CD8+ T cells compared to uninfected or CP5-infected groups. Collectively, the findings suggested that host cellular responses, including activation of T cells, are selectively induced against virulent, specifically the very virulent C. perfringens strains and that the virulence trait of this pathogen may influence the outcome of immunity to NE.

Key Words: Necrotic enteritis, Clostridium perfringens, immune response, virulence, pathogenesis

T161 Effects of quorum sensing inhibition product on broilers in necrotic enteritis challenge. Charles Hofacre*[1], Alain Labbe[2], Matthew Jones[1], Thomas Coulson[2], Ana Jaramillo[2] *[1]Southern Poultry Research Group, Inc, [2]MicroSintesis Inc*

MicroSintesis has developed a technology based on quorum sensing inhibition peptides produced by a proprietary fermentation process. The product is formulated using cell free spent media and is shown to reduce the expression of virulence genes in pathogenic bacteria *in vitro*. Necrotic enteritis is the result of intestinal damage in broiler chickens caused by the protozoan *Eimeria maxima* and the subsequent infection by toxigenic *Clostridium perfringens.* Two battery cage studies were performed to evaluate the effectiveness of quorum sensing inhibition product Nuvio Poultry to prevent the negative effects of Necrotic enteritis caused by *C. perfringens* on broiler health (clinical necrotic enteritis) and performance (subclinical NE). DESIGN: Broilers were housed in battery cages (10 birds/cage) and fed a non-medicated broiler starter diet. The treatments (15 cages/treatment) were administered in drinking water at 12mg/kg body weight prior to *Eimeria maxima* oocyst challenge (on day 14) and continued to day 28 (study termination). *C. perfringens* was administered on days 19 and 20 to reach 15% to 30% mortality. RESULTS: In the first study, there was 16.36% NE mortality observed in the challenge control while Nuvio Poultry had a significant reduction in NE mortality at 6.67% ($p<0.05$). There were no statistically significant differences in performance between treatments although Nuvio Poultry had the largest numerical reduction in non-adjusted FCR from 1.775 in control group to 1.670. In the second study, the control had 30.91% NE mortality indicating a strong challenge. Both Nuvio Poultry and an alternate formulation (IVP3) had significantly lower necrotic enteritis mortality (16.97% and 18.18% respectively, $p<0.05$). At study termination, feed intake was statistically greater in Nuvio poultry (15.65kg/cage) and IVP 3 (15.65kg/cage) relative to the challenge control (14.14kg/cage). Non-adjusted FCR was not significantly different. However, Nuvio and IVP 3 had numerically lower non-adjusted FCR (1.81 and 1.78 respectively) compared to control group (2.09). CONCLUSION: Nuvio demonstrated a reduction in NE mortality and numerical improvements in non-adjusted FCR. This study supports that Nuvio may reduce the negative impact of *C.perfringens* in broilers.

Key Words: Necrotic enteritis, Clostridium perfringens, Quorum sensing inhibition, clinical study

T162 Applications of online data for understanding backyard poultry distributions. Joseph Gendreau*, Maurice Pitesky *University of California, Davis School of Veterinary Medicine*

Understanding the spatial distribution of backyard poultry (BYP) is important to epidemiological analysis of and response to outbreaks as well as assessing disease risk for commercial poultry facilities. The efficacy of traditional survey methods to understand BYP distributions is limited, especially in regions with a history of BYP depopulation for disease control. Online platforms such as classified advertisement websites and forums provide a large amount of publicly available self-reported data on BYP locations. In this study, we monitored a classified advertisement site covering counties in southern California historically impacted by Newcastle Disease outbreaks over the course of one year for bird sales to better understand the spatio-temporal trends in private parties selling BYP. Data was collected once per week in Los Angeles, San Bernardino, Riverside, San Diego, and Imperial counties. "Title", "description", "url", "postdate", and "city or census designated place (CDP) of posting" were cataloged. Since sales posts can be active over multiple collection periods, we defined "unique posts" as a unique url and only counted unique posts once. A total of 13,318 unique posts were collected with ranges of 982 to 1,229 unique posts per month. Geographically, the highest number of posts were in the cities of Vista (n = 760) and La Mesa (n = 754) in San Diego County. These San Diego County locations are associated with posts advertising non-poultry species and laying hens, while the cities with the next highest post counts, Perris (n = 632) and Riverside (n = 516) in Riverside County, have significantly higher numbers of posts advertising gamefowl. Initial analysis suggests a high correlation between cities and CDPs with high volumes of bird sales and areas with high case counts during the 2018-2020 Newcastle Disease outbreak. Temporal trends differed by county, which may be due to differences in the species and type of poultry (i.e., regular backyard poultry vs. gamefowl) sold in the area. This and other web data have the potential to better inform disease models as well as improve allocation of resources during outbreaks.

Key Words: Newcastle Disease, Biosurveillance, Backyard Poultry, Gamefowl

T163 Protection studies of recombinant HVT-IBD and/or live IBD vaccine against 14-day or 25-day AL2 challenge in commercial broilers Kalen Cookson*, Manuel Da Costa, John Dickson, Jon Schaeffer *Zoetis*

AL2 is the most prevalent IBD virus in U.S. broilers, accounting for as much as half of today's field isolations. The newest recombinant rHVT-IBD vaccine was introduced in 2021. Previous studies in broilers with no IBD maternal antibodies showed this vaccine gave high levels of protection (70-78%) against AL2 challenge by 18-19 days. This paper will present 2 studies conducted to measure AL2 protection in commercial broilers. Study Design: Ross 708 broilers from a single breeder source were given a full dose of rHVT-IBD and/or a live IBD vaccine *in ovo* and then raised in separate rooms on litter. At 14 days all vaccine treatments including controls were commingled. One room of birds was challenged at 14 days and the other at 25 days. Challenged birds received 3.5 EID50 AL2 by eye/nose drop. At 7 days post challenge, bursameter scores were recorded and bursa to body weight ratios (B:BWs) were determined to calculate protection. Challenged birds with no bursal edema and a mean B:BW within 2 standard deviations of the non-challenged controls were considered protected. Histopathology scoring of protection will be presented once completed. Results: The AL2 challenge "took" well even at 14 days. All vaccine treatments had bigger bursas (less atrophy) than challenge controls after both 14-day and 25-day challenges. The live IBD treatment showed a modest but not significant improvement in bursa sizes and protection in both challenge intervals while the recombinant vaccine showed significant protection (67-69%) vs. challenge controls (0-3%). The use of both vaccines together resulted in further improvements in bursa size and protection (81%) against the 25-day challenge. Discussion: This pair of studies demonstrated that the newest rHVT-IBD vaccine can offer significant AL2 protection in broilers with marginal maternal immunity that would otherwise be susceptible to challenge by 14 to 25 days of age. While the live IBD vaccine only afforded modest levels of protection by itself, administering it along with the recombinant vaccine resulted in the highest bursa sizes and 25-day IBDV challenge protection—demonstrating the compatibility and potential utility in using these two vaccination strategies together in commercial broilers.

Key Words: IBDV, recombinant, live, vaccine, AL2

T164 Evaluation of NutriQuest® NUQuest AC-e™ with a coccidiosis vaccine during an Eimeria challenge in broilers Charles Hofacre*[1], Matthew Jones[1], Gregory Mathis[2], Ran Song[3], Kim Friesen[3], Chet Wiernusz[3], Lorraine Fuller[4], Myrna Olvera[5], Julio Baltazar[5], Gonzalo Villar[5] *[1]Southern Poultry Research Group, Inc., [2]Southern Poultry Research, Inc., [3]Nutriquest, [4]The University of Georgia, [5]NUTEC*

Plant and herbal extracts can enhance anticoccidial effects in broilers when used in combination with chemical, ionophore and cocci vaccine programs. Vaccine programs typically increase the immune response and impede optimal performance. Three floor pen studies evaluated the use of NutriQuest® NUQuest AC-e™ (NQ-ACe), a proprietary blend of plant and herbal extracts, fed to coccidia vaccinated broilers. Flocks in the trials were placed on new litter and in challenge treatments challenged with approximately 100,000 oocysts per bird of *E. acervulina*; 50,000 oocysts per bird of*E. maxima*; and 75,000 oocysts per bird of *E. tenella* on Day

21 (Trial 2) or Day 22 (Trial 1 and Trial 3) post-hatch. Birds were fed a commercial-type broiler starter DOT 0-14 (crumble), grower 14-28 (pellet), finisher 28-42 (pellet) and all feed was heated to 80 ºC. All trials used a completely randomized block design. Trial 1 was conducted utilizing 1,200 chicks in a completely randomized block experiment and allocated to 5 treatments: 1) no feed additive, non-challenged; 2) no feed additive, challenged; 3) no feed additive, coccidia vaccinated, challenged; 4) Salinomycin at 60 ppm, challenged; 5) NQ-ACe fed at 0.25 kg/metric ton, challenged. Trial 2 and 3 were designed similarly to Trial 1. Trial 1 study results indicated that NQ-ACe when fed with a vaccine improved ($P < 0.05$) FCR and body weight gain compared to the coccidia vaccinated alone and no feed additive challenged treatments and similar to the salinomycin treatment group. In addition, NQ-ACe when fed with a vaccine reduced ($P < 0.05$) cocci related mortality compared to the no feed additive, challenged treatment group. The results of Trial 2 and Trial 3 indicated that NQ-ACe when fed with a vaccine improved FCR and body weight gain compared to the coccidia vaccinated alone and no feed additive challenged treatment groups. In conclusion, NutriQuest® NUQuest AC-e™ can be added to a coccidia vaccine program to help support a normal immune system in broilers.

Key Words: broiler, coccidiosis, growth performance, anticoccidial, plant/herbal extracts

Metabolism and Nutrition VIII Feed Additives

T166 Comparative evaluation of scent leaf meal (Occimum grattissimum) and antibiotic growth promoter on broiler performance and blood components AKINTUNDE ADEDOYIN* *UNIVERSITY OF IBADAN NIGERIA*

A study was conducted to evaluate the effects of scent leaf meal (SLM) as alternative to antibiotic feed additive on performance and some selected blood components of broiler chickens. A Completely Randomized Design (CRD) was adopted by using 150 one-week old abhor acre broiler chicks, allotted to six treatments with five replicates of five birds each in a 49-day feeding trial, including a week acclimatization period. Commercial broiler diets used were prepared such that diet 1 (negative control contained no leaf meal or antibiotics), diet 2 (positive control contained 0.10% oxytetracycline), while diets 3, 4, 5 and 6, contained 0.5%, 1.0%, 1.5% and 2.0% scent leaf meal respectively.

Results showed that both the oxy-tetracycline and SLM supplementation improved feed intake only at finisher phase, but significantly improved average body weight gain (ABWG), FCR and cost/kg weight gain at both the starter and finisher phases. Percentage mortality (2.0%) was however, significantly ($P>0.05$) higher in diet 1 (negative control) compared to other diets. White blood cell levels only varied significantly ($P>0.05$) in birds fed SLM supplemented diets 3, 4, 5 and 6, 17.09, 17.84, 17.92 and 17.12, closely followed by the diet 2 (positive control supplemented 0.10% oxytetracycline) (17.01) respectively compared with diet 1.

It can be concluded that scent leaf meal holds high potential as a natural feed additive to replace synthetic antibiotic in broiler diets in the developing countries.

Key Words: broilers, scent leaf meal, synthetic antibiotic, white blood cell

T167 Comparative evaluation of various hepato-stimulants on growth, immunity, carcass traits, histology and cost economics in Broiler birds. Srijit Tripathi*[1], Mukund Kadam[2], A.B. Mandal[3], Dilraj Bhatia[1] *[1]VETLINE, A DIVISION OF SIMFA LABS PVT. LTD., [2]Maharashtra Animal & Fishery Sciences University, [3]CENTRAL AVIAN RESEARCH INSTITUTE*

Purpose: Liver related disorders are a worldwide health concern in Poultry even today. Objective of the present experimental trial was to study the effect of a hepato-stimulant product Bytox Premium feed premix (M/S SIMFA LABS Pvt. Ltd., India) in broiler birds.

Experimental Design: A study was conducted for 42 days to evaluate the efficacy of a a hepato-stimulant product Bytox Premium feed premix other groups. Gross observations suggest that incorporation of Bytox Premium (@ 1.0 kg per ton of feed) on growth and performance of broiler birds in comparison to other liver tonics. A total of 560 healthy day old VenCobb430Y broiler chicks of nearly similar live body weight were divided into 4 groups of 140 birds each with four replicates of 35 Birds in each group. The key parameters under study were Feed Intake, body weight gain, feed conversion ratio (FCR), Immunological parameters, carcass traits, Histological studies of hepatic tissues, feed-cost of rearing the broiler chicks for the entire experimental period.

Results and Statistics: Significant improvement in terms of growth performance, histology, carcass characteristics and cost economics were obtained in the group supplemented with Bytox Premium feed premix as compared to other groups. Gross observations suggest that incorporation of Bytox Premium @ 1.0 kg per ton of feed gave the better results compared to other dietary treatments in terms of improved live weight gain, FCR, carcass traits, immunity, histology, and feed-cost of production. The data were subjected to statistical analysis following one way analysis (CRD) using SPSS 2021. Each replicate was used as one experimental unit for statistical analysis of feed intake, live weight, feed conversion ratio and feed cost.

Conclusion: On the basis of the results, it can be concluded that Bytox Premium is hepatoprotective and a performance enhancer product. Bytox Premium proved beneficial in terms of growth performance, structural liver health, carcass traits and cost-effective production of broiler chickens in comparison to other comparative products tested in this feeding trial and hence, addition of liver tonic is must in diets since pre-starter growth phase.

Key Words: Bytox, Liver, Immunity

T168 Effect of herbal liver tonic and growth promoter (Livoliv DS) on meat quality attributes of chicken and lean meat production A Devangre[1], Shivi Maini*[2], Mohd Raziuddin[1], G Gadegoankar[1], R Kulkarni[1] *[1]Department of livestock products technology, College of Veterinary and Animal Sciences, MAFSU, [2]General Manager Technical, Indian Herbs Specialities*

The aim of this study was to determine effect of herbal hepato-protectant, growth promoter and liver tonic Livoliv DS on growth, performance, carcass characteristics, chemical composition of breast muscles and selected meat quality attributes (MQA) in broiler chicken. The experiment was performed on 80 cobb 500 broiler birdsf four replicates per group, day old chicks were randomly divided into two groups, control and treatment . Birds were fed with standard basal ration. Treatment group was supplemented Livoliv DS@250g/ton of feed, 0-6 weeks. The data collected were subjected to one-way ANOVA according to the general linear procedure of SPSS. A statistical analysis revealed that Livoliv DS had a beneficial influence on the final body weights of birds, average daily gain (ADG), feed conversion ratio (FCR), physico-chemical meat quality, proximate composition of meat, meat texture and instrumental colour profile. FCR was 1.56 in treatment as compared to 1.58 in control. Supplementing herbal liver tonic significantly improved water holding capacity (WHC) (0.4 sq.cm) as compared to control (0.2 sq.cm). Cooking loss was significantly reduced in treatment (30%) vs. control (32%). Proximate analysis

of meat revealed significantly higher protein and ash content (23.22g & 2.13 %) in treatment than control (21.15g and 1.32%). Meat obtained from broilers fed with LivoLiv DS had higher moisture and protein & lower fat than meat obtained from control birds. Texture profile analysis chicken exhibited advantage of supplementing herbal liver tonic to improve hardness, cohesiveness, gumminess, chewiness and springiness of meat. The findings of this study indicate efficacy of Livoliv DS in improving meat quality attributes of chicken. Addition of 250g/ton of Livoliv DS had enhanced physico-chemical attributes, meat textural, sensory and lean meat quality. Significantly better MQA can be correlated to the influence of active principles present in herbal liver tonic Livoliv DS. It was concluded on basis of trial findings that supplementation of herbal liver tonic and growth promoter LivoLiv DS had additional advantages to improve MQA in addition to its hepatoprotectant, liver tonic, performance enhancer an growth promoter properties.

Key Words: meat quality, texture, lean meat, herbal, liver tonic

T169 Microbiome Modulation, Microbiome Protein Metabolism Index, and growth performance of broilers supplemented with a precision biotic Cristiano Bortoluzzi*[1], Jose Sorbara[1], Ian Tamburini[2], Jack Geremia[1] *[1]DSM Nutritional Products, [2]University of California*

The objective of the present studies was to evaluate: (1) the *in vivo* impact of supplementation with a Precision Biotic (PB) on the growth performance and microbiome modulation of broiler chickens; (2) the modulation of functional pathways of the microbiome collected from animals with low and high body weight gain, and (3) to develop a Microbiome Protein Metabolism Index (MPMI) derived from gut metagenomic data to link microbial protein metabolism with performance. The *in vivo* work consisted of two experiments with two treatments: Control vs PB at 1.1 kg/MT of PB with 21 or 14 replicates of 40 birds per replicate, in experiments 1 and 2, respectively. Growth performance was evaluated in both experiments, and from experiment 1, cecal samples from one bird/replicate was collected on d 21 and 42 (n = 21/treatment) to evaluate the microbiome through whole genome sequencing. In the *ex vivo* assay, 6 cecal samples were collected from low body weight (BW) birds (at 10% below average), and 6 samples from high BW birds (at least 10% above average). The samples were incubated in the presence or absence of PB. After incubation, DNA was isolated to develop a functional genomic assay and the supernatant was separated to measure short-chain fatty acid (SCFA) production. The MPMI is the sum of beneficial genes in the pathways related to protein metabolism. In the *in vivo* grow out experiments, it was observed that the supplementation improved the BW gain by 3% in both studies, and the corrected feed conversion ratio (cFCR) by 3.7 and 3.4% in studies 1 and 2, respectively ($P < 0.05$). The functional microbiome analysis revealed that the PB shift the microbiome pathways towards a beneficial increase in protein utilization, as reflected by higher MPMI. In the *ex vivo* experiment, an increased abundance of genes related to the beneficial metabolism of protein (Quantitative MPMI) were observed in the PB treated microbiome, and the concentration of SCFA, regardless of the underline BW of the chickens. Taken together, the microbiome metabolic shift observed in the *in vivo* study that reflected higher MPMI, plus the observations from the *ex vivo* assay with increased SFCA production, may explain the improvement in growth performance obtained with the supplementation of PB.

Key Words: Broilers, microbiome metabolism, precision biotic, metagenome

T170 Efficiency trial of 4 different technological toxin binders against mycotoxins multiple contamination in broiler diets. Jose Fierro*, Patrica Gómez, Juan Medina, Elizabeth Rodríguez *Sanfer Animal Health*

The objective of the following study, was to evaluate the efficiency of 4 different commercial toxin binders used to prevent from the potential toxicity of multiple mycotoxin contamination within broilers diet during 28 days trial period. The selected products for this trail, were based upon the different offered technologies and potential benefits claimed in literature for each. Classified as (MA). Product (A) Organoaluminosilicate. Product (B) blend of Bentonite, Diatomite, Eubacterium spp. Product (C) based on Seaweed extract inserted in Sodium Calcium Aluminosilicate. Product (D) based on seaweed extract, glucomannan, yeast walls extracts and Sodium Calcium Aluminosilicate.

The experiment was conducted at Nutek experimental Toxicology unit. Toxin binders A, B, C & D were evaluated at inclusion rate of 1.5 kg/Ton each. A commercial broiler diet was used and feed contaminated with five different natural mycotoxins identified as (MMC) composed by: Aflatoxin B1 (AFB1) at 100ppb, Fumonisin B1 (FB1) at 2,500ppb, Deoxynivalenol (DON) at 1,200ppb , Ochratoxin A (OTA) at 50 ppb and T-2 Toxin (T-2) at 100ppb. A total 180 chickens of 1 day of age were used, distributed in 6 treatments within 3 repetitions with 10 chickens each. Treatments were identified as: Treatment 1 Negative control without mycotoxin additive (MA) and without the MMC. Treatment 2 positive control that included food contaminated with MMC, without MA. Treatment 3 included A product; treatment 4 included B, treatment 5 was with C and finally treatment 6 was with D product, these all treatments all contained the MMC. We use ANOVA Tukey $p < 0.05$ *SYSTAT* for all results.

There was a significant difference between treatments groups A,B,C D Toxin Binders against MMC positive group ($p < 0.05$). We have concluded the selected technologies against MMC to be effective as no difference exist between negative control group. Also, weight gain and feed conversion versus Positive control, Product A & C had an effectiveness in weight gain of 76 & 77%, respectively, Product B 58% & D 47%. However, to demonstrate their true effectiveness. All toxin binders must be evaluated "*in vivo*" and result must be analyzed to understand better their true effectivness.

Key Words: Multiple Mycotoxin contamination, Toxin binder, toxicity, mycotoxin, broiler

T171 Effect of different probiotics and EnzaPro® on growth performance, and gut health parameters of broiler raised under mild coccidia challenge Sudhir Yadav*, Yun-mei Lin, Sandra Rodrigues, Rasha Qudsieh *BioResource International, Inc*

A study was designed to evaluate the effect of different *Bacillus spp.*-based commercial probiotics, EnzaPro, a unique combination of direct-fed microbial and xylanase, and an antibiotic growth promoter (AGP) on growth performance, and gut health of broilers raised under mild coccidia challenge in 42 d-study. The AGP, Bacitracin Methylene Disalicylate (BMD), was supplemented at 0.055 g/kg diet, whereas other products were supplemented at their manufacturer's recommended dose. A total of 864, day-old Ross 308 male chicks were raised on used litter in 48-floor pens with 6 treatments and 8 replicates per treatment. Mash, corn-SBM-based diets were fed in three phases (starter, 0-14 d; grower, 15-28 d; and finisher, 29-42 d). The 6 dietary treatments were: Control (no additives supplementation), EnzaPro, AGP, and the remaining 3 different *Bacillus spp.* probiotics-based treatments. All pens were challenged with a 5X dose of Huvepharma Advent coccidia vaccine on 7 and 14 d via oral gavage. Body weight (BW) and feed intake (FI) were recorded on a weekly basis while feed conversion ratio (FCR) was calculated per feed phase. On 21 and 42 d, intestinal lesion score and oocyst shedding in the litter were measured. Data were analyzed using one-way ANOVA, and means were separated using least significant difference at $P<0.05$. EnzaPro improved BW on 35 d ($P=0.05$) and 42 d ($P=0.04$) by 6% compared to non-supplemented challenged control. Furthermore, EnzaPro improved overall body weight gain (BWG; $P=0.04$) by 6% compared to non-supplemented

control and one of the probiotic treatments, with no differences in overall FI or FCR. EnzaPro tended to improve FCR (P=0.06) by 8% compared to the control for the periods of 21-27 d and 28-35 d. There was no difference in intestinal lesion scores (P>0.05) while litter oocysts counts were below detection limits since the challenge was mild. In summary, EnzaPro improved BWG beyond one of the probiotics-fed treatments while being comparable to AGP during mild disease challenge induced by the coccidia vaccine. Thus, under mild coccidia challenge, EnzaPro can be used in broiler production to mitigate the reduction in growth parameters while maintaining gut health.

Key Words: broiler, coccidiosis, direct-fed microbial, gut health, xylanase

T173 Effect of inactivated Pichia guilliermondii postbiotic on performance parameters in broilers: systematic review and multiple trial analysis Clementine Oguey*[1], Mohamad Mortada[2] [1]*ADM International,* [2]*ADM Animal Nutrition*

Yeast-derived postbiotics, like *Pichia guilliermondii* (Pg), are rich in β-glucans and mannan oligosaccharides that can prime the innate immune system in broilers resulting in increased resistance to various pathogens. This multiple-trial analysis aimed to systematically synthesize results from 28 research trials focusing on the effect of Pg (Citristim, ADM) on performance in unchallenged and challenged conditions. Evaluated performance parameters included final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F) in broilers. The analysis included 60 pairwise comparisons of Pg vs. Control (CTL). Twenty-two comparisons were from controlled challenge trials, including 18 coccidiosis, 2 LPS, and 2 *Clostridium perfringens*, and 38 comparisons were from unchallenged conditions. The average dose of Pg was 1,031 g/mt of feed (range: 100 - 2,000 g/mt), and the average trial duration was 35 days of age (range: 21 – 43). Data were analyzed using a mixed model procedure with the trial as a random effect and treatment (Pg or CTL), challenge (with or without), and their interaction as fixed effects. Mean values were calculated using the LSMEANS procedure of Minitab 18.1, weighing the data for SEM among trials. Pre-planned Pg vs. CTL comparisons were conducted, and significance was reported at $P<0.05$. During unchallenged conditions, compared to the control, Pg supplementation did not affect final BW (2,383 vs. 2,397 g; $P=0.273$) and ADFI (mean 101.2 g/d; $P=0.604$), significantly decreased ADG (66.72 vs. 67.14 g/day; $P=0.046$) as well as G:F ratio (663.7 vs. 666.4 kg/g; $P=0.038$). However, during challenge conditions, Pg supplementation significantly increased final BW by 43g (2,622 vs. 2,579 g; $P=0.02$), ADG by 1.06 g/day (64.84 vs. 63.74 g/day; $P=0.003$) and ADFI by 1.08 g/day compared to the control (99.96 vs. 98.88 g/day; $P=0.02$). As a result, Pg tended to increase G:F ratio during challenge conditions (649.11 vs. 645.03 g/day; $P=0.065$). Commercial broilers are exposed to various microbial stressors, and *Pichia guilliermondii* supplementation might alleviate the loss in performance. Future studies will focus on understanding the mechanism of action of *Pichia guilliermondii* postbiotic during challenging conditions in broilers.

Key Words: Broiler performance, Pichia guilliermondii, postbiotics, microbial stressors, systematic review

T174 Effect of supplementing a proprietary yeast-based product on the efficacy of a live salmonella vaccine in broilers Sharon Miller*[1], Charles Hofacre[2], Muhammed Shameer Abdul Rasheed[1], Chris Rude[1], Mark LaVorgna[1], Mahmoud Masadeh[1], Kristy Dorton[1] [1]*Devenish Nutrition,* [2]*Southern Poultry Research Group*

A study was conducted at Southern Poultry Research to determine if supplementation of a proprietary yeast-based product (HT; HY-Tech, Devenish Nutrition, Fairmont, MN) had a negative effect on the live *Salmonella* vaccine, Megan Vac 1 (MV1), in broilers. One day old Ross male broiler chicks (n = 400) were randomly assigned to 8 pens (2 pens/treatment; 50 birds/pen). The 4 treatment groups were: Challenge Control (no test product, no vaccine), MV1 alone (no test product, vaccinated), MV1 + HT (HT at 2 lb/ton of feed, vaccinated), HT alone (HT at 2 lb/ton of feed, no vaccine). Broilers received a standard corn/soybean based pelleted diet that contained a coccidiostat (113.5 g/ton amprolium). Prior to placement, birds in MV1 alone and MV1 + HT treatment groups were vaccinated by coarse spray with a live *Salmonella* vaccine (MV1 at 0.25 ml/chick; Elanco Animal Health, Greenfield, Indiana). Three days post vaccination, ceca, liver, and spleen were collected from 4 birds per pen and cultured for the presence of the MV1 *Salmonella* Typhimurium. On day 7, all birds were challenged with *Salmonella* Infantis at 5.0 X 10^7 CFU/chick. At 21 days post challenge, cloacal swabs were collected from 15 birds per pen for *S.* Infantis culture. Growth performance was assessed at d 21, 35, and 42. *Salmonella* vaccine recovery in various samples were compared using

Fisher's exact test. *Salmonella* prevalence in ceca were compared using logistic regression. ANOVA was performed for the growth performance parameters using generalized linear model. Differences were considered statistically significant at $P \leq 0.05$. Analyses were performed using Stata (version 17.0, StataCorp LLC, College Station, TX). There were no significant differences in body weight, FCR, feed intake, mortality, or *S*. Infantis colonization in the cloacal swabs between treatment groups. A higher percentage of *salmonella* vaccine recovery in the cecal samples collected from both MV 1 alone (38% recovery) and MV1 + HT (68% recovery) treatments were observed. Similarly, the liver and spleen samples in the 2 treatment groups had 100% recovery of vaccine *Salmonella* strain. This suggests that HT does not interfere with the colonization of *Salmonella* vaccine in the intestine (ceca) or internal organs (spleen/liver).

Key Words: Salmonella Vaccine, Yeast, Broiler, non-interference

T175 Effect of a proprietary oregano product on weight gain, feed conversion, and intestinal lesions of Cobb 500 broilers during a mixed Eimeria challenge infection Muhammed Shameer Abdul Rasheed*[1], Greg Mathis[2], Brett Lumpkins[2], Kristjan Bregendahl[1], Mark LaVorgna[1], Mahmoud Masadeh[1], Kristy Dorton[1] *[1]Devenish Nutrition, [2]Southern Poultry Feed and Research*

Two studies were conducted at Southern Poultry Feed and Research (Athens, GA) to evaluate an oregano product (OP, OregoPro; Devenish Nutrition, Fairmont, MN) on growth performance and intestinal lesions after a challenge infection with *Eimeria Spp*. One day old Cobb 500 broiler chicks (as hatched) were randomly allocated to battery cages (Study 1: n = 144, Study 2: n = 192) with 6 reps/treatment (8 chicks/cage). Treatments in both studies included: uninfected-unsupplemented control (UUC), infected-unsupplemented control (IUC), and infected plus OP, fed at 0.25 kg/ton from d 1. Additionally in Study 2, a competitive oregano product was tested at 0.45 kg/ton. On d 14, all birds except for UUC were orally gavaged with a coccidia inoculum (100,000; 50,000; and 75,000 sporulated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella*). Birds were fed a corn-soy mash diet. Body weight and feed intake were measured on d 0, 14, and 20. Feed conversion ratio (FCR) was calculated. On d 20, intestinal lesions (0 = none to 4 = severe) were evaluated (5 birds/cage). Both studies used a randomized complete block design with cage as the experimental unit. Data were analyzed by ANOVA and means separated with Fisher's least significant difference at $P \leq 0.05$. Overall weight gain (Study 1: $P \leq 0.0002$; 0.52 vs. 0.38 kg; Study 2: 0.59 vs. 0.46 kg) and feed conversion (Study 1: $P \leq 0.001$; 1.37 vs. 1.76, Study 2: $P < 0.0001$ 1.40 vs. 1.78) were negatively affected and lesion scores were higher (Study 1: $P < 0.0001$; 0 vs. 2.43, Study 2: 0 vs. 2.50) in IUC compared with UUC, which confirmed a successful *Eimeria* challenge. After the challenge (d 14 to 20), broilers supplemented with OP had improved FCR (Study 1: $P = 0.001$; 4.08 vs. 7.12, Study 2: $P < 0.0001$; 1.89 vs. 2.25) and reduced *Eimeria* lesion scores (Study 1: $P < 0.0001$; 2.04 vs. 2.43, Study 2: $P < 0.0001$; 1.70 vs. 2.50) compared to IUC. When compared to broilers supplemented with the competitive oregano product (Study 2), broilers receiving OP had better FCR ($P < 0.0001$; 1.89 vs. 2.08) and lower lesion scores ($P < 0.0001$; 1.70 vs. 2.12). The results show that supplementation of OP improves FCR and minimizes lesions during an *Eimeria* infection. Additionally, OP exhibited superior results compared to the competitive oregano product.

Key Words: Oregano, Broiler, Eimeria, Growth Performance, Lesion score

T176 Determining the effect of a proprietary all-natural feed additive on the performance of Ross 708 broilers to 35 days of age under a coccidiosis challenge Michael Blair*, Mark LaVorgna, Mahmoud Masadeh, Muhammed Shameer Abdul Rasheed, Kristy Dorton *Devenish Nutrition*

A study was conducted to evaluate a proprietary all-natural feed additive product (UPtimum, Devenish Nutrition, Fairmont, MN) on growth performance of broilers to 35 days of age. One day old Ross 708 (YPM x Ross 708) broiler chicks (as hatched) were randomly allocated to 18 floor pens with 9 reps/treatment (50 chicks/pen). Treatments included: Control (C) diet (corn-soybean meal) and UPtimum (UP) diet with UPtimum added to the C diet at 0.40 lbs/ton. On the day before placement of the chicks and weekly through d 35, the litter (1 cycle old) was sprayed with water to ensure adequate oocyst sporulation for a successful coccidiosis challenge. On d 3, all birds were challenged with live oocysts of *E. acervulina*, *E. maxima*, *E. mivati* and *E. tenella via* CocciVac B-52 (Merck Animal Health, Madison, NJ) sprayed on the feed at 20 times the recommended dose. Pen body weight (BW) and feed intake (FI) were measured on d 16 and 35. Body weight gain (BWG), feed conversion ratio adjusted for mortality (FCR) and FCR adjusted to a common body weight (4 lb; Adjusted FCR) were calculated. The study used a randomized complete block design with individual pens as the experimental unit. A one-way-ANOVA was used to evaluate the effects of treatments. Means were separated using Fisher's protected LSD and differences were considered significant at $P \leq 0.05$. Body weight ($P = 0.02$; 1.05 vs. 1.01 lb) and BWG ($P = 0.01$; 0.065 vs 0.063 lb/d) at d 16 were significantly greater for broilers fed the UP diet *versus* those fed the C diet. By d 35, broilers fed the UP diet had significantly greater BW ($P < 0.02$; 4.09 vs. 3.80 lb) and BWG ($P = 0.02$; 0.117 vs. 0.109 lb/d) than those fed the C diet. From d 0 to 35, broilers fed the UP diet had better ($P = 0.03$) FCR than those fed the C treatment (1.357 vs. 1.426). Adjusted FCR was lower ($P = 0.02$) for those birds fed the UP diet as compared to those fed the C diet (1.346 vs. 1.445). The results show that supplementation of UPtimum to a corn-soybean meal diet significantly improves the body weight, body weight gain and adjusted FCR of Ross 708 straight-run broilers to 35 d of age when undergoing a 20x normal dose of coccidiosis vaccine challenge.

Key Words: All-natural, Broiler, Eimeria, Growth performance

T177 Effects of a commercial triple-strain Bacillus-based probiotic on broiler performance and welfare Eric Sobotik*[1], Steve Lerner[1], Rogerio Frozza[1], Alberto Inoue[1], Antoine Meuter[1,2], Ibiara Almeida Paz[3] *[1]Animal and Plant Health & Nutrition, Chr. Hansen, Inc., [2]Animal and Plant Health & Nutrition, A/S, [3]Department of Animal Production and Preventative Veterinary Medicine, São Paulo State University*

Previous investigations have shown bi-directional interactions within the gut-brain-microbiome axis. Gut microbes communicate to the central nervous system through at least three parallel and interacting channels involving nervous, endocrine, and immune signaling mechanisms. A commercial triple-strain *Bacillus*-based probiotic was tested to determine its effect on the performance and welfare of broilers. Four treatments were tested with eight replicate groups, each containing 400 day-of-hatch AP95 male broilers. On top of a standard diet, the treatments were: 1) no supplement (Negative Control, NC), 2) Halquinol (Positive Control, PC), 3) Probiotic (PRO), and 4) Organic Acid and Essential Oil (OA+EO). Body weight (BW) and feed intake of birds 1 to 42 d were measured to calculate feed conversion ratio (FCR). Behavioral responses were measured using latency-to-lie and approximation tests. Plasma concentrations of corticosterone (CORT) and serotonin (5HT) were measured as indicators of stress. Intestinal morphology was measured using villus height (VH), villus width (VW), and villus to crypt ratio (V/C). Data were analyzed using JMP and Tukey's HSD was used for means separation. Compared with NC (1.51), PC (1.52), and OA+EO (1.51), FCR was lower ($P<0.05$) in PRO (1.48). BW at 42 d was greater ($P<0.05$) in PRO (3,206 g) compared with NC (3,084 g), PC (3,120 g), and OA+EO (3,166 g). Birds fed

an effective probiotic were more comfortable and less skittish compared with other treatment groups, as evidenced by longer standing times and allowed proximity (P<0.05). PRO had lower CORT (80 µg/mL) and higher 5HT (402 µg/mL) compared to NC (166 and 100 µg/mL), PC (183 and 91 µg/mL), and OA+EO (133 and 316 µg/mL), respectively (P<0.05). Furthermore, the mechanisms that enable the beneficial synaptic actions of 5HT (5-HTT, SLC6A4, and TPH1) were expressed to a greater degree in PRO. For all measures of intestinal morphology, response values were numerically highest in PRO. Based on the dimensions of the duodenal microvilli, birds fed PRO had 23% greater surface area for absorption of nutrients compared to the NC. In conclusion, the commercial triple-strain *Bacillus*-based probiotic improved the performance and well-being of broilers via the gut-brain-microbiome axis.

Key Words: bacillus, probiotic, broiler, performance, welfare

Metabolism and Nutrition IX Feed Additives

T179 Broiler gut barrier function enhancement by a synergistic in-feed technology under dietary-induced chronic inflammation in real farming conditions Alireza Khadem*[1,2], Eirini Griela[3], Milena Sevastiyanova[2], Konstantinos C. Mountzouris[3], DON Ritter[2], Christos Gougoulias[2] *[1]Ghent University, [2]Innovad SA, [3]Department of Animal Science, Agricultural University of Athens*

Dysfunction of the intestinal barrier has been associated not only with impaired nutrient absorption and reduced growth performance in broilers but also with increased microbial translocation and disease risk. Here, we evaluated the protective gut barrier effects of 1 or 2 kg/ton feed of Lumance® (Innovad, Belgium; esterified butyrate, combined with plant extracts, essential oils, and other fatty acids), in a dietary-induced chronic inflammation model (high NSP diet: 60% Wheat + 5% rye, without NSPase and coccidiostats), via the distal-jejunal mRNA gene expression of mucins and tight-junction proteins in broilers. Importantly, the pens (n=8 pens/treatment; n=30 birds/pen) were housed inside a commercial production unit of 55,000 broilers so that the experimental birds could get exposed to the same (real) farming conditions. One broiler per pen was randomly selected on D28 and D35 for the determination of the intestinal expression of tight junctions and the plasma level of FITC-dextran (the latter only at D28). ANOVA with Tukey Post-hoc analysis revealed that Lumance® 1 and 2 kg/ton when compared to the control treatment, increased significantly the BW of broilers at D35 by 3% and 4%, respectively (P = 0.040) and reduced the FCR (1.64, 1.62 and 1.57, respectively; P = 0.01). FI was not affected by the treatments. Importantly, Lumance® 1 kg/ton and 2 kg/ton resulted in a profound increase of intestinal expression of Claudin-1, Occludin, and Mucin-2 (approximately, a 4- and 5-fold increase for the former and a 2- and 3-fold increase, respectively, for the latter two genes) compared to the control, both at D28 and D35 (P < 0.005 in all cases). Additionally, a strong trend in the reduction of FITC-d levels in plasma (P = 0.098) was seen at D28 in birds receiving Lumance® compared to the control. In this study, broilers under chronic inflammation in real conditions, when fed with an in-feed synergistic technology conferred a superior protective effect on gut barrier function, accompanied by enhanced growth performance. The positive results warrant further work for the elucidation of related mechanisms.

Key Words: Intestinal barrier function, growth performance, gene expression, tight junctions, FITC-dextran

T180 Effect of activated diatoms on the regulation of intestinal passage rate, digestibility, and performance in broiler chicken Felipe Mendy*[1], David Díez[2], Anna Tesouro[2], Maria Soriano[2], Connie Gallardo[3] *[1]IFTA USA Inc, [2]Biovet S.A., [3]Universidad Científica del Sur*

Statement of the purpose

Rapid transit is common in commercial farming. Feed does not remain in the gut long enough for the proper absorption of nutrients. Undigested feed in feces and watery dejections may be observed, as well as decreased performance, worsening of gut health, and poor litter quality. Activated diatoms are intended to regulate the intestinal passage rate and prevent rapid transit.

Experimental design

A trial was conducted to evaluate the effect of specific species of activated diatoms (DTM) to regulate the intestinal passage rate of broiler chicken. For 42 days, 440 birds were distributed into 4 treatment groups with 5 replicates per treatment and 22 birds per replicate. Control groups had no additional additives and DTM groups received activated diatoms at 0.5 kg/t. Results were analyzed separately for males and females. Intestinal retention time, digestibility and litter moisture were evaluated at days 21 and 42. Performance was assessed weekly. Differences with P<0.05 were set as statistically significant.

Results

Intestinal retention time was higher in DTM groups (P<0.001), of 16 minutes on average compared to control. DTM groups showed a significant increase in the digestibility of protein (4.26%) (P<0.001), ashes (3.14%) and calcium (2.46%) (P<0.05) and numerical increase in the digestibility of crude fiber, energy and phosphorus. Litter moisture (%) was significatively reduced (P<0.05) with DTM. Weight and feed conversion ratio (FCR) of DTM were superior to control throughout the trial, and significatively better in days 28, 35 and 42 (P<0.05). Average final weight difference was of 92 g/bird and average final FCR difference of 0.07 pts. DTM had higher uniformity during all the trial, with 3.26 pts. average difference at day 42 (P<0.001). Breast yield was enhanced in the DTM groups, both in males (P<0.001) and females (P<0.05). Mortality was significantly reduced in the DTM groups (P<0.05) at weeks 2, 3 and 4 of the cycle.

Conclusions

Activated diatoms are inert natural additives which are demonstrated to be effective to control rapid transit in birds, allowing to improve the feed utilization and performance of the flock by increasing final weight (+3%), uniformity (+4%), and breast yield (+7%), improving FCR (-4%) and reducing mortality.

Key Words: broiler, diatoms, rapid transit, performance, feed additives

T181 IMW50® improves immune modulation response in aflatoxin challenge broilers Melina Bonato*[1], Max Ingbergman[2], Breno Beirão[2], Fernando Souza[1] *[1]ICC Industrial Comércio Exportação e Importação SA, [2]Imunova Análises Biológicas Ltda ME.*

The objective of this study was to evaluate the effect of IMW50® (source of β-glucans and MOS) to modulate the immune responses in aflatoxin-challenged broilers. It was used 152 one-day-old chicks randomly distributed in 4 treatments: NC (negative control, basal diet without contamination), AFB (positive control, basal diet with 2.5 ppm of aflatoxin in diet administration), IMW50 (basal diet with 0.5 kg/MT from *Saccharomyces cerevisiae's* cell wall – IMW50®) and IMW50+AFB (IMW50 diet with 2.5 ppm contamination of aflatoxin). The experimental period was 35 days with the diets and on the 14th day was administrated the Newcastle vaccine. On days 1 and 14, blood samples were collected with anticoagulants for ELISA antibodies against Newcastle Disease (NDV) to determine maternal titer (10 birds/treat). ELISA for antibodies against NDV was assessed in blood samples at days 28 and 35 (8 birds/treat). The blood collection samples with anticoagulant for determination of cell profile by flow cytometry, the phagocytic capacity of peripheral blood lymphocytes, and the assessment of intestinal permeability (using Dextran-FITC, 3-5 kD) were performed at days 7, 28, and 35 (8 birds/treat). The data from each analysis were submitted to the D'Agostino-Pearson omnibus K2 normality test. However, the data that failed this test were submitted to non-parametric analyses, Kruskal-Wallis test (GraphPad Software). The challenge with aflatoxin was verified and resulted in 2.24±0.28 ppm in the challenged groups. The Aflatoxin challenge promoted the depletion of cytotoxic T lymphocytes on day 14. The IMW50+AFB promoted an increase (P<0.05) in diverse populations of immune cells, including total lymphocytes, T and B lymphocytes, in addition to phagocytic macrophages and heterophils (at day 28). Even in the absence of an aflatoxin challenge, animals treated with IMW50 showed higher production of NDV antibodies (P<0.05) at day 35. No differences between the treatments were found in intestinal permeability. The IMW50 supplementation in broilers challenged with 2.5 ppm of aflatoxin showed an immune modulation effect; in non-challenged birds, it was observed an impact in higher production of antibodies probably through the trained immunity mechanism (cytotoxic T lymphocytes).

Key Words: Yeast cell wall, Saccharomyces cerevisiae, vaccine titers

T182 Effects of a phytogenic blend on broiler growth performance with used litter challenge Meghan Schwartz*, Stacie Crowder *PMI*

Two studies were conducted to evaluate the effects of a phytogenic feed additive on broiler performance with used litter challenges. Both trials were complete randomized block designs where block was based on pen location within the research facility. Treatments were 1) Control (C), 2) Supplant™ D (PHYTO) at 150 mg/kg (PMI, Arden Hills, MN). Body weight gain (BWG), feed intake (FI), and feed conversion ratio adjusted for mortality (mFCR) were evaluated at 14, 28, and 42 days of age. Statistical analysis was performed using Proc Mixed procedure in SAS and significant means were compared by Least Significant Difference (P<0.05). In trial 1, 720 day-old Ross 708 males were assigned to 12 replicate pens per treatment (30 birds/pen). Throughout trial 1, no significant effect on FI, mFCR, and mortality was observed. PHYTO treatment improved BWG at 28 (1.342 vs 1.258 kg, P = 0.002) and 42 days of age (2.823 vs 2.911 kg, P = 0.003). In trial 2, 1,300 day-old Cobb 500 males were assigned to 13 replicate pens per treatment (50 birds/pen). Throughout trial 2, no significant effect on mortality was observed. At 28 days, PHYTO improved mFCR (1.388 vs 1.452, P<0.001) with reduced FI (85.87 vs 95.54 kg, P = 0.002). At 42 days, PHYTO treatment had no significant effect on BWG but improved mFCR (1.650 vs 1.739, P<0.001). Inclusion of this phytogenic blend improved BWG in trial 1 and mFCR in trial 2. Based on these results, including this phytogenic blend in broiler diets improved growth performance.

Key Words: feed additive, broilers, phytogenic

T183 Macroalgae extracts limits Marek's disease virus replication and cell-to-cell spread in vitro. Maria RODRIGUEZ*[1], Frédérick Bussy[1], Pi Nyvall-Collen[1], Laëticia Trapp-Fragnet[2] *[1]Olmix S.A., [2]INRAE*

Marek's disease (MD) is a highly contagious lymphoproliferative disease of chickens caused by an alphaherpesvirus, Marek's disease virus (MDV). MD is presently controlled by systematic vaccination of animals, which protects efficiently against clinical disease. Although vaccines are available, they only confer protection against tumor development but do not prevent virus replication and shedding in poultry flocks. Therefore, MDV persists to be a major problem for the poultry industry generating economic losses estimated at 1-2 billion dollars per year. The development of new sustainable alternative strategies to control MDV is needed. Macroalgae extracts have previously shown to exert antiviral and immunomodulatory activities that could support the animals to better resist to this challenge. The objective of the assay was to study the effect of sulphated green macroalgae (*Ulva. sp*) extract (MSP®$_{IMMUNITY}$) on MDV lytic replication and cell-to-cell spread *in-vitro*. In the experimental design, chicken embryo fibroblasts (CEFs) were infected with MDV and incubated with increasing doses of the algae extract added in the cell incubation medium (0; 0.5; 1; 1.5 and 2ml/l). Samples were removed for analysis at 24, 48, 72 and 96 hours post treatment (hpt). Viral replication in CEFs was determined by genome copy numbers and quantified by q-PCR and cell-to-cell spread was analyzed on CEFs by plaque size assay. The Kruskal-Wallis test was used to compare differences in multiple groups and the Mann-Whitney (two-tailed) test was used to compare non-parametric variables between two groups; p values < 0.05 were considered statistically significant. The results showed that MSP®$_{IMMUNITY}$ significantly decreased MDV lytic replication in CEFs in a dose-dependent manner (p<0.05) with the strongest effect observed with concentrations of 1ml/l. From 24 hpt, MDV lytic replication was reduced by 80% with the algae extract at a concentration of 2ml/l and this is maintained over time and reached 94% at 96 hpt. A substantial decrease in MDV plaque size (from 2 to 3-fold; p<0.0001) was demonstrated suggesting that macroalgae extract impede MDV cell-to-cell spread. This study provides the first evidence that the use of the macroalgae extracts could be a good alternative to limit MDV infection

in poultry. It could be used in in-feed or in drinking water formulation to support the animals to fight viral challenges.

Key Words: Marek disease virus, macroalgae, ulva sp., viral replication, viral dissemination

T184 Feeding a protected complex of biofactors and antioxidants to breeder hens provides transgenerational protection against Salmonella enterica serovar Enteritidis to progeny chicks Christi Swaggerty*[1], Ramon Malheiros[2], Casey Johnson[1], Ludovic Lahaye[3], Hector Salgado[3], J Byrd[1], Kenneth Genovese[1], Haiqi He[1], Elizabeth Santin[3], Michael Kogut[1] *[1]U.S. Department of Agriculture, Agricultural Research Service, [2]North Carolina State University, [3]Jefo Nutrition, Inc.*

Addition of vitamins and antioxidants have been long associated with increased immunity and are commonly used in the poultry industry; however, less is known regarding their use in broiler breeder hens. The objective of this study was to determine if feeding a complex of protected biofactors and antioxidants composed of vitamins and fermentation extracts to broiler breeder hens conferred resistance against *Salmonella enterica* serovar Enteritidis (*S*. Enteritidis) in the progeny chicks. Three-day-old chicks from control- and supplement-fed hens were challenged with *S*. Enteritidis and necropsied 4- and 11-days post challenge (dpc) to determine if there were differences in invasion and colonization. Serum and jejunum were evaluated for various cytokine and chemokine production. Fewer (P=0.002) chicks from supplement-fed hens had detectable *S*. Enteritidis in the ceca (32.6%) compared to chicks from control-fed hens (64%). By 11dpc, significantly (P<0.001) fewer chicks from supplement-fed hens were positive for *S*. Enteritidis (liver [36%]; ceca [16%]) compared to chicks from the control hens (liver [76%]; ceca [76%]). The recoverable *S*. Enteritidis in the cecal content was also lower (P=0.01) at 11dpc. In additional to the differences in invasion and colonization, cytokine and chemokine production were distinct between the two groups of chicks. Chicks from supplement-fed hens had increased production of IL-16, IL-6, MIP-3a, and RANTES in the jejunum while IL-16 and MIP-1b were higher in the serum of chicks from the control-fed hens. By 11dpc, production of IFN-g was decreased in the jejunum of chicks from supplement-fed hens. Collectively, these data demonstrate adding a protected complex of biofactors and antioxidants to the diet of broiler breeder hens offers a measure of transgenerational protection to the progeny against *S*. Enteritidis infection and reduces colonization that is mediated, in part, by a robust and distinct cytokine and chemokine response locally at the intestine and systemically in the blood.

Key Words: Cytokine and chemokine, Feed additive, Protected vitamins, Salmonella, Transgenerational protection

T185 Effect of dietary supplementation of Solergy as a replacement of soybean oil on productive performance, egg quality traits and liver function parameters in laying hens from 32 to 47 weeks of age. Juan Miguel Ruiz Rodríguez*, Octavi Colom, Ignacio Josa Prado, Carlos Domènech *Igusol Advance S.A.*

The study was conducted to evaluate the efficacy, of an energetic metabolism enhancer called SOLERGY added to replace soybean oil, on productive performance, egg quality traits and liver function parameters of laying hens from 32 up to 47 weeks old. Solergy is marketed by the company Igusol Advance S.A., Spain. The product is composed of gluconeogenic and phytogenic compounds. The study was conducted at the facilities of Agrivet Research & Advisory P Ltd., India. A total of 150 Lohmann LSL Lite of 32 weeks age were distributed randomly to cages containing 3 birds per cage. Cages were randomly assigned to control or treatment diet. Diets were AME 2,825 kcal/kg; CP 18 %; Ca 3.8 %; avP 0,42 %. Control (CONT) contained 2.8 % soybean oil, which was substituted by 0.8 % soybean oil, 0.2 % SOLERGY, and 1.8 % rice hull in treatment (SLGY) group. 2 weeks previous to the start of the study the birds were acclimated to control diet and housing. At the end of the trial blood was collected from the right brachial vein. And analysed for aspartate amino transferase (AST) and alanine transaminase (ALT). Cage was the experimental unit, and all data were pooled cage-wise and analysed by multivariate analysis of variance in the general linear model of SPSS (v 26.0). Probability of $P < 0.05$ was expressed as significant. Laying rate was comparatively lower in the control (96.7 %) group than in SOLERGY group (97.1 %). Although no statistically significant different. Weekly measurements of egg mass (57.32 and 56.38 g), body weight (1650.5 and 1616.3 g), rejected eggs (5.03 and 4.55 %), and broken eggs (0.81 and 0.97 %) did not show statistical differences between CONT and SLGY groups. Egg parameters were measured on weeks 33, 37, 41, and 45. Albumen height, yolk fan score, haugh unit, egg shell strength, egg shell thickness yolk height, yolk diameter and yolk index did not show statistically significant differences in any measurement. Liver function parameters showed statistical differences in AST (38.62 and 31.75 IU/L), and no differences for ALT (157.78 and 148.4 IU/L). It is concluded that Solergy is capable of maintaining performance and egg traits in 32 to 47 weeks old laying hens. Liver function parameters are improved as AST is lower in a diet supplemented with SOLERGY.

Key Words: SOLERGY, lipids, fat, soybean oil, energy

T186 EVALUATION OF CELLUTEIN® ON TURKEY GROWTH PERFORMANCE AND FEED EFFICIENCY Mathew Vaughn*[1], Michael Barnas[2], James McNaughton[2], John Gonzalez[3] *[1]Puretein Bioscience, [2]AHPharma, [3]University of Georgia*

Yeast and yeast byproducts have been used as feed supplements for many years. Though many benefits are universal, many strain-specific benefits exist. An *in-vitro* study indicated celluTEIN® (CT) yeast contained promoted immune and skeletal muscle cell activity through mTOR pathway signaling. Poults (N=1,152) were assigned to 48 pens (24 poults/pen) and pens were randomly assigned to 1 of 4 treatments: 0 ppm CT throughout (CON), 300 ppm CT from day 0-28 and 50 ppm CT from day 29-84 (TRT2), 600 ppm CT from day 0-28 and 50 ppm CT from day 29-84 (TRT3), and 600 ppm CT from day 0-28 and 100 ppm CT from day 29-84 (TRT4). There was no treatment effect (P>0.05) for day one average body weight (BW), but there were treatment effects for feed conversion ratio (FCR), coefficient of variation (CV), and European production efficiency factor (EPEF) on all days (P<0.05). Turkeys from all 3 CT treatments had greater BW and less CV of BW compared to CON turkeys (P<0.05), but did not differ from each other (P>0.05). Over the course of the 84-day trial, CT supplemented turkeys had smaller FCR compared to CON turkeys (P<0.05), TRT4 FCR was smaller (P<0.05) than TRT3, and TRT 2 FCR did not differ from TRT3 or 4 (P>0.05). The European production efficiency factor (EPEF) takes into account gain, survival rate, and FCR. The EPEF did not differ between all CT treatments (P>0.05), TRT2 and TRT4 had greater EPEF than CON turkeys (P<0.05), and CON and TRT3 did not differ in EPEF (P>0.05). These data illustrate CT supplementation improved turkey performance during the first 84 days of feeding. Additionally, the smaller CT dose was as effective as the greater dose, which would result in a greater return on investment.

Key Words: Turkey, Feed effiency, celluTEIN

T187 Evaluation of the effect of Balancius on growth performance of commercial turkeys Shelby Corray*[1], April Levy[1], Jeffre Firman[2], Randy Mitchell[3] *[1]DSM Nutritional Products, [2]Missouri Contract Poultry Research, [3]Perdue Research Farm*

The objective of present two studies was to evaluate the effect of Balancius (muramidase) on commercial turkey growth performance. In trial 1, 780 male turkeys were randomly allocated to 1 of 3 treatment groups. T1: Non-supplemented control diet (C); T2: C + Balancius 25,000LSU/kg; T3: C + 45,000 LSU/kg. Each group consisted of 13 replicates with 20 birds per pen. Birds were fed for 63 days in three feeding phases. Growth performance parameters and livability were determined on d 21, 42, and

63. On d63, litter quality was assessed and digesta was collected from the jejunum to evaluate soluble peptidoglycans. In trial 2, 2,624 male turkeys were randomly allocated to 1 of 4 treatment groups. T1: Positive Control, T2: Negative control, T3: NC + Balancius 35,000 LSU/kg, T4: NC + Balancius 55,000 LSU/kg. Each group consisted of 8 replicates with 82 birds per pen. Turkeys were fed for 19 weeks. Growth performance parameters and livability were determined on d 56, 84, and 133. In trial 1 and 2 the data were submitted to two-way ANOVA ($P \leq 0.05$) and LSMeans Tukey was used to separate the means. The results from trial 1 showed no significant differences in performance or mortality at d21;however, there were numerical differences in BW and FCR with T3 having the highest BW and the lowest FCR. By d42, it was observed that T2 and T3 had higher FCR ($P=0.052$) as well as significantly higher feed intake ($P=0.018$). By the end of the trial on d63, T3 had significantly improved FCR compared to T1 ($P=0.04$). Although there were no significant differences, litter quality, litter pH and moisture decreased as Balancius increased in the diet. Paw scores were numerically lower in T2 and T3 with T2 having the lowest score. A linear increase in soluble peptidoglycans was observed as the dose of Balancius increased. In trial 2, results at d56 presented a significant increase in BW for T3 and T4 compared to T1 and no significant differences in FCR or mortality. By d84, results continue to show an increase in BW for T3 and T4 ($P=0.007$). On d133, although not significant, turkeys fed T3 were 489g heavier than T2 and FCR was improved by 4 points. (2.241 vs 2.198). Overall, Balancius was effective in improving the performance of turkeys by increasing body weight and decreasing feed conversion.

Key Words: turkeys, Balancius, Muramidase, peptidoglycan

T189 Growth performance response in tom turkeys fed conventional diet supplemented with monoglycerides of short and medium chain fatty acids A. O. Sokale*[1], A. Barri[2], J. Firman[3], N. Tillman[4] *[1]BASF Corporation, [2]BASF SE, [3]Missouri Contract Poultry Research, [4]Nutritional Statistics LLC*

This study aimed to investigate the effects of butyric, caprylic, and capric monoglycerides (MG) on the growth performance of male turkeys when supplemented at 0, 300, 600, 900, 1200, 1500 and 1800 g/MT feed. A total of 840 male Hybrid Converters turkeys (15 birds × 8 pens/treatment) were randomly allotted to the 7 dietary treatments (Trt) in a randomized complete block design. A corn-soybean meal-based diet with fixed minimal amount of meat meal and DDGS per phase was formulated and fed in 6 phases (d0 - 21, d21 - 42, d42 - 63, d63 - 84, d84 - 105, and d105 - 119). All diets were formulated to meet the breeder's recommendations and to simulate a standard US turkey diet. Feed intake (FI) and body weight (BW) data were collected for each phase during the 17-weeks study. Body weight gain (BWG) and feed conversion rate adjust for chick days (FCRa) were calculated. The experimental unit was the pen and data were analyzed using mixed model of JMP®. Quadratic polynomial (QP) model was used to fit the titrated levels for both Trt LSMeans and pen means. The level which maximized BW or minimized FCRa from the QP model are reported. Only the responses with significant Goodness of fit ($P<0.05$) were reported, which were the supplementation of MG at 0 - 1800 g/MT during 0-3 weeks period. The results suggested that to maximize BWG from 0-3 weeks, it would require 624 g/MT using Trt LSMeans and 644 g/MT using pen means while FCRa was minimized at 482 g/MT using Trt LSMeans and 478 g/MT using pen means. Although, One-Way ANOVA showed no significant differences among 7 treatments for BWG and FCRa for any phase, consistent numerical improvement in BWG and coefficient of Variation (CV) were observed for all phases in comparison to the diets with no MG. The improvement in BWG at 300 g/MT ranged from 1.42 - 4.93 %, while the CV improvement at the same level ranged from 18 - 70 %. This trial showed that feeding MG from 478 - 644 g/MT to male turkeys during 0 - 3 weeks improved BWG and FCRa and, while feeding at least 300 g/MT across phases up to 119 days showed consistent improvement in BWG and weight uniformity.

Key Words: fatty acids monoglycerides, performance, turkeys

Metabolism and Nutrition X Enzymes

T191 Evaluating the benefits of a novel multi-component protease supplementation on broiler chicken in a commercial organic production system Anhao Wang[1], Aizwarya Thanabalan[2], Rob Patterson*[1], Paul Garvey[1], Mike Edwards[3], Elijah Kiarie[2] *[1]CBS Bio-Platforms, [2]University of Guelph, Animal Biosciences, [3]Jones Feed Mill*

The aim of this study was to evaluate the beneficial effects of dietary supplementation of a novel multi-component protease (Pro) in diets with reduced crude protein. A total of 16,000 unsexed day old-Ross x Ross 708 broiler chicks were placed at a certified organic commercial farm in Ontario, Canada. At placement, a subsample of 378 birds were randomly selected from the main flock and placed in eighteen identical floor pens with 21 birds/pen for a 35-d feeding study. The experimental pens provided 920 cm^2/bird floor space, located within the barn and fitted with individual feeders and waterers. Three dietary treatments: corn-wheat-soy based control diets without protease supplementation (Control); reduced protein diets (RP) with 125 mg/kg Pro (125Pro); RP with 250 mg/kg Pro (250Pro) were allocated to pens to give 6 replicates per diet. The control diet was formulated to meet nutrient requirements and had 0.5% higher

crude protein than their RP counterparts. All diets contained a commercially available multi-carbohydrase and enzymatically hydrolyzed yeast carbohydrates product. Body weight, average daily gain, average daily feed intake and feed conversion ratio (FCR) were determined at 21 and 35-d. The fresh excreta samples were collected from each pen on 21-d, for determination of apparent retention (AR) of dry matter, crude protein, ash and gross energy using indigestible ash as the digestibility marker. At 21-d, one bird/pen was randomly selected, weighed, bled for plasma metabolites, sacrificed for liver, spleen and bursa weight and ceca digesta for the short-chain fatty acid profile. Supplementing Pro in RP diets quadratically improved FCR during the 22-35 d and 0-35 d, respectively ($p < 0.05$). At 35-d, the birds fed RP diets with Pro had similar body weights to the control birds ($p = 0.449$). At 21-d, Pro quadratically improved the AR of ash and plasma uric acid concentration ($p < 0.05$). Pro-supplementations quadratically reduced relative bursa weight ($p = 0.009$) and tended to reduce iso-butyric concentration ($p = 0.090$). Taken together, multi-component protease supplementation improved organic broiler chicken performance and nutrient metabolism which may result in greater economic returns.

Key Words: multi-component protease, broiler chickens performance, crude protein density, nutrient digestibility, organic production system

T192 Use of a novel mannanase isolated or in combination on broeilers peformance Rodrigo Messias*[1], Rita Vieira[2], Anna Fickler[3], Bruno Wernick[1], Michele Lima[4], Edney Silva[2] *[1]BASF SA, [2]UNESP, [3]BASF SE, [4]Universidade Brasil*

Non-starch polysaccharide (NSP)-degrading enzymes can improve energy digestibility in poultry by breaking down dietary NSP such as ß-mannans, arabinoxylans and ß-glucans. ß-mannanase degrades ß-mannans which are commonly present in soybean meal and other mannan-rich feed ingredients. Xylanase and ß-glucanase degrade dietary arabinoxylans and ß-glucans, respectively, which are the main NSP in cereal grains. Although these enzymes have been used to improve energy release, it is crucial to understand their interaction with regards to energy release. Thus, the objective of this study was to estimate the impact on the energy efficiency of a novel ß-mannanase alone or in combination with xylanase and ß-glucanase.

A trial was conducted at UNESP-Jaboticabal in Brazil, using 3096 one-day old male broilers fed corn-soybean meal-based diets fed in two phases (1-21 and 22-42 days). Birds were allocated in

floor pens with 6 treatments (T1-T6), 12 replicates and 43 birds per replicate. All diets were formulated according to nutritional recommendations of Rostagno et al. (2017). Phytase was added to all diets at 500 FTU/kg feed with matrix values according to the manufacturer (BASF SE). The energy level of the diets was stepwise reduced from T1 to T4 to estimate the regression describing the effect of energy level on feed conversion ratio (FCR). T5 and T6 were designed to estimate the energy efficiency of enzymes used alone or in combination: T1: 3050 and 3200 kcal/kg in phase 1 and 2 respectively; T2: T1-50 kcal/kg; T3: T1-100 kcal/kg; T4: T1-150 kcal/kg; T5: T4 + mannanase; T6: T4 + mannanase + xylanase + ß-glucanase. Performance parameters were measured and analyzed via ANOVA and regression analysis considering a significance level of 5%.

FCR was significantly ($P<0.05$) affected by the stepwise reduction of dietary energy and thus, used to estimate a linear equation. Based on this, the dietary inclusion of ß-mannanase delivered +30 kcal/kg when used alone on top of phytase. When ß-mannanase was used in combination with xylanase and ß-glucanase on top of phytase, +73 kcal/kg of energy was released.

In conclusion, dietary ß-mannanase can release energy when used alone (on top of phytase) and in combination with xylanase and ß-glucanase (and phytase), respectively.

Key Words: Enzymes, Mannanase, Energy, Xylanase, Carbohydrase

T193 Measuring the in vitro anti-clostridial activity of a phage endolysin expressed in Saccharomyces cerevisiae. Gregory Siragusa*[1], Mike Barnas[2], Wendy Attuquayefio[2], Jennifer Timmons[3], Chris Skory[4], Rose Hammond[4], David Donovan[5], James McNaughton[2] *[1]Scout Microbiology, [2]AHPharma, Inc, [3]University of Maryland Eastern Shore, [4]USDA Agricultural Research Service, [5]Morgan State University*

Prior work has demonstrated that a proprietary bacteriophage endolysin (X) exhibits lytic activity against *Clostridium perfringens* (*CP*) when expressed from *Escherichia coli*. Five replicate experiments were conducted to measure endolysin X's *in vitro* anti-*CP* activity when expressed in transgenic *Saccharomyces cerevisiae.* Experimental *CP* was produced by inoculating a *CP* (CP509) colony into 50 mL Fluid Thioglycollate (FTG) Broth and incubating anaerobically for 24h at 37°C. The culture was then pelleted at 4000 × g for 10min, the supernatant discarded, and the pellet resuspended to 1.0 OD_{600} using phosphate-buffered saline (PBS). The *CP* was divided into three aliquots for the lytic (killing) assays: (T1) *CP* alone control, containing 1mL CP509 cells only; (T2) non-transgenic yeast control, including 1mL of *CP* cells + 50µL of unmodified *S. cerevisiae* cells containing no endolysin X; and (T3) yeast + endolysin X, including 1mL of CP cells + 50µL of *S. cerevisiae* cells expressing the transgenic enzyme X at 450µg/ml concentration as estimated by western analysis. Lytic assays were incubated at 23°C for 20min, then serial diluted in PBS (range of 10^{-1}-10^{-4}), and 100µL of each dilution was pour-plated in triplicate using TSC *CP* indicator agar; incubated anaerobically for 24h at 37°C and presumptive *CP* colonies counted to calculate the average $\log^{10}$ CFU/mL of *CP* for each treatment within a single experiment. Individual treatment averages (N=5) were analyzed for a one-way ANOVA using the GLM procedure to determine the anti-CP activity of endolysin X. The T1 control assay containing only CP509 cells had an average colony count of 5.07±0.63 $\log^{10}$ CFU/mL. The T2 yeast control assay containing *S. cerevisiae* cells with no endolysin averaged 5.08±0.64 $\log^{10}$ CFU/mL, [not significantly different from the T1 control ($P>0.05$)]. However, the T3 assays containing yeast + endolysin averaged 0.6±1.34 log10 CFU/mL *CP*, equating to a 99.997% (4.47 log10 CFU/mL) reduction ($P<0.0001$). These results indicate that the proprietary bacteriophage endolysin X expressed in *S. cerevisiae* cells is highly effective at reducing the recovery of *CP in vitro.* Future studies will measure the inhibitory effect *in vivo* by administering yeast cells containing the endolysin to broilers via feed additives.

Key Words: Endolysin, Phage, Clostridium, Lysis, Yeast

T194 Effect of supplementing a multi-carbohydrase enzyme in conjunction with phytase to broiler chickens on growth performance and carcass traits Hector Leyva-Jimenez, Yemi Burden*, Carlos Soto, Katherine McCormick, Adrienne Woodward *United Animal Health Inc.*

This study evaluated the effects of supplementing a multi-carbohydrase enzyme (MCE) or an MCE+phytase combo to corn-SBM and DDGS-based diets on broiler performance and carcass traits. The study consisted of a complete randomized block of 6 treatments including positive control (PC), negative control (NC; -125 kcal/kg of ME compared with the PC), and the NC supplemented with either the MCE (Enspira+; United Animal Health, US) at 75 (MCE75), 100 (MCE100), and 125 (MCE125) ppm, or the MCE+phytase combo (MCEP; Enspira+Phytase; United Animal Health, US) at 100 ppm. All experimental diets were supplemented with a commercially available phytase at 1,000 FTU/kg except for the MCEP treatment which was supplemented with the proprietary MCE+phytase combo at 1,000 FTU/kg. Both phytase products were expected to deliver 0.18% non-phytate P and 0.20% Ca. Diets were pelleted and offered ad libitum. Each treatment included 10 replicate floor pens with 40 male broilers per pen. The feeding program consisted of 3 dietary phases (starter 0-14 d; grower 14-28 d; finisher 28-42 d). Feed intake, body weight (BW), and FCR (corrected for mortality) were determined. Five birds per pen were processed for the evaluation of carcass traits. Data were subjected to ANOVA and means were separated using Duncan's multiple range test ($P<0.05$). Cumulatively after 42 d, the NC diet numerically reduced

(P>0.05) BW and increased (P<0.05) FCR. All MCE and MCE+phytase-supplemented treatments improved FCR to levels comparable to the PC (P>0.05). A linear response in FCR (P<0.05) was observed with increasing inclusion levels of MCE to the NC diet. When the FCR was adjusted to a common weight (2.94 kg) an 8.4 point difference (P<0.05) was detected between the PC (1.553) and NC (1.637) groups. When compared to the NC group MEC125 and MCEP treatments recovered 8.3 (1.554) and 7.3 (1.564) FCR points respectively and were not different (P>0.05) from the PC. Whole carcass yield was numerically increased with MCE and MCE+phytase combo supplementation compared to the NC. Breast yield linearly increased (P<0.05) with increasing inclusion level of MCE. In conclusion, the supplementation of MCE or MCE+phytase combo recovered broiler performance in energy-reduced diets with positive effects on carcass traits.

Key Words: Broiler, energy, phosphorus, non-starch polysaccharide, performance

T195 Application of full matrix for a novel consensus bacterial 6-phytase variant alone and in combination with a xylanase-amylase-protease mixture in broilers improved economic benefit and sustainability A. Bello*[1,2], R. D. Gimenez-Rico[2], S. Gilani[2], B. C. Hillen[2], K. M. Venter[3], P. Plumstead[3], Y. Dersjant-Li[2] *[1]Danisco Animal Nutrition & Health, IFF - USA, [2]Danisco Animal Nutrition & Health, IFF - Netherlands, [3]Neuro Livestock Research, South Africa*

This study evaluated the ability of a novel consensus bacterial 6-phytase variant (PhyG) alone or with a xylanase-amylase-protease mixture (XAP) to alleviate the effects of nutrient and energy downspec based on respective full matrix (dig P, Ca, Na, dig AA and ME). Growth performance, bone quality and carcass characteristics were measured and economic benefit and carbon footprint level were calculated. A total of 1,760 as-hatch broilers (55 birds/pen) were randomly assigned to 8 floor pens × 4 dietary treatments. The diets were based on corn, wheat, SBM, sunflower meal and canola meal and were formulated for starter (1-10 d), grower (10-21 d) and finisher (21-32 d) phases. The diets were 1) a nutrient and energy adequate positive control (PC); 2) a diet reduced in Ca by 0.24 – 0.21% point (p), digestible P by 0.20 – 0.18% p, Na by 0.05% p, dig AA by up to 0.06 – 0.04% p and ME by 79 – 44 kcal/kg vs PC across the 3 phases (NC1); 3) NC1 supplemented with PhyG at 2,000, 1,500, and 1,000 FTU/kg (NC1+PhyG) in starter, grower and finisher phase, respectively; and 4) the NC1+PhyG with additional reduction in dig AA by up to 0.02% p and ME by 75 kcal/kg and supplemented with XAP (xylanase at 2,000 XU/kg, amylase at 200 U/kg and protease at 4,000 U/kg) in all dietary phases (NC2+PhyG+XAP). Data were analyzed using JMP 16.1 and means separated by Tukey test ($P \leq 0.05$ as significant). The 32 d BW and 1-32 d FCR were worsened ($P<0.05$) by the NC1 (1,555 g and 1.448) vs PC (2032 g and 1.413), and were maintained by both NC1+PhyG (2045 g and 1.417) and NC2+PhyG+XAP (2005 g and 1.425) vs PC ($P>0.05$). And similar diet effects were observed for bone ash contents at d 10 and 32 and each of carcass, breast, thigh, and leg meat weights ($P<0.05$). The overall feed cost, USD/kg BWG and total carbon footprint, g CO2 eq/kg BWG were decreased by NC1+PhyG (0.491 and 1943) vs PC (0.529 and 2208), and further decreased by NC2+PhyG+XAP (0.475 and 1,838) vs NC1+PhyG ($P<0.05$). In conclusion, supplementation of the novel consensus bacterial 6-phytase variant alone or with a xylanase-amylase-protease mixture to diets with full matrix application cost-effectively maintain broiler growth performance, bone ash, carcass characteristics and contribute to sustainable broiler production.

Key Words: phytase, broiler growth performance, xylanase-amylase-protease mixture, economic benefits, carbon footprint

T196 Effect of new generation bacterial 6-phytase on recovery in feed and performance, bone mineralization and calcium and phosphorus apparent ileal digestibility in 21-day-old broilers. Mohamad Mortada*, Amanda Hesse, Cole Schralla, Grant Scheuermann, Nathan Martin, Laura Griffith, Ayoub Mousstaaid *ADM Animal Nutrition*

Two studies were conducted to evaluate a new generation bacterial 6-phytase recovery in feed and effect on broilers' performance. The first study assessed the recovery of 10 FTU/g of Empirical® Phytase in a corn-SBM basal diet pelleted at pilot-scale at 103, 145, and 175°F. Analyzed by ISO assay, phytase activity in mash was 9.3 FTU/g for the phytase-supplemented treatment compared to 0.2 FTU/g for the control ($P<0.01$). Relative to the phytase-supplemented mash treatment, bacterial 6-phytase was recovered at 88%, 91%, and 74% at 103, 145, and 175°F, respectively. The second study evaluated the effect of phytase on performance, bone mineralization, and apparent ileal digestibility (AID) of phosphorus and calcium. 576 Ross 308 male chicks were randomly assigned to five dietary treatments. The positive control (PC) treatment received a corn-SBM diet with 0.43% available phosphorus(aP) and 0.86% Ca. The negative control (NC) treatment received a corn-SBM meal diet with 0.20% and 0.22% reductions in aP and Ca at monocalcium phosphate (MCP) expense. In addition, three treatments received NC diet with Empirical® Phytase at 500, 1,000, or 1,500 FTU/kg. Treatments were replicated in 9 battery cages with 8 birds/cage until 21 Days-of-age. Data were analyzed using 1-way ANOVA. Fisher's LSD test was used to separate the means. The NC treatment had a reduction in feed intake (FI; 1,017 Vs. 1,069g; $P<0.01$) and a decrease in body weight gain (BWG; 796 Vs. 847g; $P<0.01$) compared to the PC. Phytase supplementation at 500, 1,000, or 1,500 FTU/kg significantly restored the drop in FI and BWG back to equivalent PC levels ($P<0.05$). The NC treatment significantly reduced tibia ash percentage (46 Vs. 50%) and tibia phosphorus content (16.87 Vs. 17.27%; $P<0.0001$), and decreased AID of P (56 Vs. 60%; $P=0.07$) compared to the PC. Phytase supplementation at 500, 1,000, or 1,500 FTU/kg linearly increased bone ash, P tibia content and P AID ($P<0.01$). A 500 FTU/kg dose of bacterial 6-phytase recovered the decline in performance (FI and BWG) and tibia P content. While, 1,500 FTU/kg recovered the decrease in bone ash to PC levels. Doses as low as 500 FTU/g of bacterial 6-phytase reduce 9.5 kg/mt of MCP in feed resulting in reduced feed cost and the P excretion into the environment.

Key Words: Bacterial 6-phytase, Phytase activity recovery, Broilers, Phosphorus, Bone mineralization

Metabolism and Nutrition XI Vitamins and Minerals

T197 Maternal supplementation of different levels bis-chelated trace minerals on reproductive performance of broiler breeder and growth performance of progeny subjected to coccidia challenge Song Bin[1], Shiping Bai[1], Keying Zhang[1], Xuemei Ding[1], Jianping Wang[1], Qiufeng Zeng[1], Quan Tan[2], Yuyun Mu[2], Juxing Chen*[2] *[1]Animal Nutrition Institute, Sichuan Agricultural University, [2]Novus International Inc*

Trace minerals Zn, Cu and Mn play important roles in poultry production. The objective of this study was to determine maternal supplementation of different levels bis-chelated trace mineral on breeder production and progeny growth and gut health. A total of 640 50-wk old Cobb female broiler breeders were randomly assigned to 5 dietary treatments with 8 cages of 8 hens/cage for 12 wks: No supplemental trace minerals (CON); inorganic trace minerals Zn:Cu:Mn at 110:10:120 ppm in sulfate (ITM100); and 3 levels of Zn:Cu:Mn from mineral methionine hydroxy analog chelate (MMHAC): 27.5: 2.5:30 ppm (MMHAC25), 55:5:60 ppm (MMHAC50) and 110:10:120 ppm (MMHAC100). The progeny hatched from eggs laid at wk 12 in each maternal treatment were allotted randomly into 10 cages of 10 chicks each, fed the same diet for 28 d. All birds were orally gavaged

30× recommended dose of coccidia vaccine on d14. Data were subject to one-way ANOVA and contrasts were used to compare CON or ITM100 with other treatments. In the breeders, MMHAC50 tended to increase (P<0.1) egg production per pen housed compared to ITM100 and settable egg production per hen-day compared to CON during wk 9-12. Compared to CON and ITM100, MMHAC 50 and MMHAC100 tended to (P<0.1) increase fertility of settable eggs, MMHAC25 and MMHAC50 (P < 0.05) increased first-grade chick percentage. On wk 12, compared to CON, all MMHAC treatments increased (P<0.05) eggshell breaking strength; compared to CON and ITM, MMHAC25 and MMHAC50 (P<0.05) increased eggshell weight, MMHAC25 (P<0.05) increased eggshell thickness. In progeny, there was no difference in d1 body weight, body weight gain and feed intake. MMHAC50 (P < 0.05) improved FCR during d1-21 and tended to (P<0.1) improve FCR during d1-28 compared to CON and ITM100. MMHAC50 and MMHAC100 (P < 0.05) decreased serum IFN-γ level compared to CON, and all maternal MMHAC treatments (P < 0.05) decreased serum IL-10 level compared to ITM100 on d 21. In conclusion, feeding broiler breeders MMHAC Zn, Cu, and Mn at 50% ITM levels (MMHAC50) demonstrated the most consistent benefits in both breeder and progeny, it not only improved the reproductive performance of broiler breeders, but also carried over the effect to progeny by improving growth performance and reducing inflammation.

Key Words: breeder production, bis-chelated trace minerals, gut health, maternal supplementation, progeny

T198 Maternal supplementation of trace mineral minerals on breeder production and progeny growth and health Juxing Chen*[1], Raquel Araujo[2], Fabricia Roque[2], Guilherme Silva[2], Cristiane Araujo[2], Brunna Leite[2], Lúcio Araujo[2], David Torres[1], Mercedes Vazquez-Anon[1] *[1]Novus International Inc., [2]Universidade de São Paulo*

Trace mineral minerals Zn, Cu and Mn play important roles in breeder production and progeny performance. The objective of this study was to determine maternal supplementation of trace mineral minerals on breeder production and progeny growth and development. Five hundred forty breeders were randomly assigned to 3 dietary treatments with 15 pens of 12 birds/pen at d1 of age and 4 birds/pen during production phase (24-66 wks): (1) inorganic trace minerals in sulfate (ITM), (2) mineral methionine hydroxy analog chelate (MMHAC) and (3) mineral amino acid complex (OTM) with 100:16:100, 50:8:50, and 50:8:50 ppm Zn:Cu:Mn, respectively. When females were 20 wks of age, 40 males at the same age were placed to the same treatments. Breeder production, gut heath and egg quality were measured. At 28 wks of age, eggs from breeder treatments were hatched for progeny trial, 10 pens with 6 males and 6 female birds per pen were fed a common diet with ITM for 45d. Growth performance and gut health parameters were measured in progeny. Data were analyzed by one-way ANOVA, means were separated by Fisher's protected LSD test. A P-Value ≤ 0.05 was considered statistically different. In the hens, MMHAC improved (P < 0.01) egg yolk color vs ITM and OTM at 40, 50, 58 and 65 wks, increased (P < 0.01) eggshell thickness and resistance vs OTM at 58 wks, and reduced (P<0.05) jejunal NF-kB gene expression vs OTM at 24 wks. MMHAC numerically (0.05<P<0.1) improved egg production % vs ITM at 24-66 weeks and OTM was not different from MMHAC and ITM. In progeny, MMHAC increased (P < 0.0001) BW vs ITM and OTM at hatch, reduced (P<0.05) FI vs ITM at d14 and 28, and improved (P<0.01) FCR and performance index vs OTM at d28. MMHAC reduced (P<0.01) NF-kB gene expression vs OTM on d0 and reduced (P<0.01) NF-kB and IL1b gene expression vs ITM on d14 in progeny jejunum, which is probably caused by the upregulation (P<0.05) of A20 gene expression. These results suggest that supplementation of chelated trace minerals MMHAC in the breeder diet not only increased breeder production and egg quality and decreased jejunal inflammation, but also carry over to the progeny by improving growth performance and reducing jejunal inflammation in comparison to ITM and mineral amino acid complex.

Key Words: breeder production, chelated trace minerals, gut health, maternal supplementation, progeny

T199 Comparative efficacy evaluation of natural choline (BioCholine) and synthetic choline chloride on broiler growth, performance and meat quality attributes A Devangare[1], Shivi Maini*[2], Mohd Raziuddin[1], G Gadegaonkar[1], R Kulkarni[1] *[1]Department of livestock products technology, College of veterinary and animal sciences, MAFSU, [2]General Manager technical, Indian Herbs Specialities*

The aim of this study was to determine comparatively on growth, performance and selected meat quality attributes (MQA) in chicken. The experiment was performed on 80 cobb 500 broilers, day old chicks were randomly divided into two treatments (T1 &T2) of four replicates per group. Birds of both groups were fed with standard basal ration from 0-6 weeks. T1 birds were supplemented synthetic choline chloride 60%@1kg/ton of feed and T2 was supplemented natural choline supplement BioCholine@750g/ton of feed by completely replacing synthetic choline from basal ration. A statistical analysis revealed that natural choline supplement (BioCholine) had beneficial influence on zootechnical performance, liveability, physico-chemical meat quality, proximate composition of meat, meat texture and instrumental colour profile. The data collected were subjected to one-way ANOVA according to general linear procedure of SPSS. Supplementing herbal choline lead to significant (P>0.05) reduction in mortality in T2 (2.85%) than T1 (5.71%). In previous studies, BioCholine supplementation is well proven for its efficacy for regulating key allosteric effectors of hepatic fat and energy pathways. In this trial, supplementing natural choline supplementation BioCholine lead to higher protein deposition in meat (22.12%) as compared to 21.15 % in synthetic choline supplemented group. Lightness and redness of chicken fed BioCholine (53.16, 6.29) was significantly (P>0.05) desirable than synthetic choline group (51.18, 5.32). Physico-chemical meat quality indicated cooking loss to be significantly (P>0.05) lower (30%) and higher pH of meat, 6.04 (within normal range) in T2 than T1 (cooking loss 32% and pH 5.02). Texture profile analysis exhibited advantage of supplementing herbal choline to improve hardness, cohesiveness, gumminess, chewiness and springiness of meat of T2 than T1. The findings of this study indicate efficacy of herbal choline over synthetic choline chloride in improving meat quality attributes of chicken. BioCholine@750g/ton enhanced zootechnical performance and MQA. BioCholine can completely replace synthetic choline chloride in broiler feed for prevention of fatty liver, improving zootechnical performance and for improving overall meat quality attributes in chicken.

Key Words: natural choline, fat metabolism, lean meat, choline chloride, meat texture

T200 Effect of calcium pidolate dietary supplementation in a context of phosphate withdrawal in broiler feed Clémence Marecaille[1], Julián Melo*[2], Samuel Roulleau[3], Xavier Roulleau[1] *[1]Dietaxion SAS, [2]Universidad Nacional de Luján, [3]MG2MIX*

In animal nutrition, phosphorus is an important mineral mostly supplied through phosphates, meat & bone meal (MBM) and phytates. But the global phosphates reserves are limited, and the prices keep increasing. One strategy known to reduce the need of phosphate is to increase the dose of phytase in broiler diets to release as much phosphorus of phytic acid from grains as possible. Besides, calcium pidolate is known as an ingredient directly promoting the absorption of calcium and indirectly phosphorus. The objective of this trial was to study the effect of calcium pidolate on performance and bones of broilers fed diets free of phosphate and containing a high dose of phytase. A total of 2048 Day-old male chicks (Ross 308) were allocated in floor pens in a completely randomized design (8 replications/treatment). Starter (1-10 d) and grower (11-35 d) diets were used for dietary treatments. Control diets (CTRL) were formulated

without phosphate and MBM but with triple and double phytase doses for starter and grower diets, respectively (Ca_{tot} 0.51 and 0.5 ; P_{dig} 0.255 and 0.25). PCa diets were supplemented with 300 ppm of calcium pidolate (PIDOLin PCa®, DIETAXION) in the starter diet and 150 ppm in the grower diet. The parameters studied were feed intake (FI), average daily gain (ADG), feed conversion ratio (FCR), mortality rate (MO) and production index (EPI) from 1 to 31 days old. At 35 days of age the breaking strength and elasticity of the tibia were determined in one bird per pen. Parametric data were analyzed using ANOVA and non-parametric data through the Kruskal-Wallis test. For MO a chi-square test was done. Feed intake increased 2.4% ($P<0.05$) and ADG 3.7% ($P<0.05$) supplementing PCa in feed. FCR tended to be improved in the PCa group ($P<0.1$). There was no difference in MO ($P>0.05$), but the EPI also was improved by 4.6% in broilers supplemented with calcium pidolate ($P<0.05$). There was no difference in bone breaking strength at day 35 ($P<0.05$), but the bone elasticity was improved 32% ($P<0.05$) including PCa in the diets. In a context of high prices of phosphates, adding calcium pidolate to broiler diets containing high doses of phytase enables to make savings by formulating by far with lower levels of phosphorus than what is applied today in broiler feed.

Key Words: calcium pidolate, broiler, phosphate, phytase

T201 Effect of plant-based 1,25(OH)2D3-glycosides cross-supplementation to breeders and broiler offspring on zootechnical performance, bone ash and intestinal integrity Ricardo Nunes[1], Jonivan Paloschi[2], Javier Gonzalez*[2], Marine Dewez[2], Valentin Bartholomey[2], Jan Dirk Van der Klis[3], Kathrin Buehler[3], Katia Pedrosa[3], Thiago dos Santos Andrade[1], Nilton Rohloff Junior[1], Edevaldo Iachinski[1] *[1]Universidade Estadual do Oeste do Paraná, [2]Nuproxa Switzerland, [3]Herbonis*

This study evaluated plant based 1,25 dihydroxycholecalciferol glycoside (1,25DHC-G; Panbonis®) cross fed to broiler breeders (BB) and their progeny, on performance, bone quality and intestine histology. Previous work demonstrated better performance in broilers when their parent stock (Ross 308, 27 wk) was fed 1,25DHC-G (100 g/ton). In this study, a total of 1,536 one-day-old, male broilers (Ross 308 AP) were distributed into 8 treatments (T) and 8 replicate pens in a 2 x 4 factorial design. Chicks were fed a corn-soybean meal basal diet supplemented with 0, 50 and 100 g/ton of 1,25DHC-G from 1 to 21 d (T1 to T3, respectively) or 100 g/ton of 1,25DHC-G from 1 to 42 d (T4). The same regime was applied in T5 to T8. Broilers in T1 to T4 and T5 to T8 were hatched from BB (Ross 308, 45 wk) fed with and without 1,25DHC-G (100 g/ton), respectively. The average feed intake (FI), weight gain (WG), feed conversion ratio (FCR), viability and productive efficiency index (IEP) were calculated at 1, 10, 21 and 42 d of age; intestinal histology and bone parameters at 21 and 42 d; and processing yield at 42 d. Data were submitted to the normality test and then to a two-way ANOVA at 5% significance. The main effects were analyzed using the F test for the BB and the SNK test for the progeny, all at 5% significance. The inclusion of 100 g/ton of 1,25DHC-G in BB did not ($p<.05$) influence broiler performance, however, the inclusion of 100 g of 1,25DHC-G /ton in broilers for either 21 or 42 d improved body weight and WG ($p=.003$) at 42 d. Carcass and breast yield were improved ($p<.03$) in birds hatched from BB fed with 1,25DHC-G. Bone breaking strength was not influenced ($p>.05$) by treatments. Tibia ash at 21 d was higher in broilers fed 1,25DHC-G and hatch from BB supplemented with the additive, but not in the birds hatch from the non-supplemented BB. In contrast, at 21 d, 1,25DHC-G increased tibia ash in broilers hatch from non-supplemented BB, but not in broilers hatch from supplemented BB. Histology analysis revealed that 1,25DHC-G increased duodenum crypt depth at 42 d. In conclusion, 1,25DHC-G at 100 g/ton demonstrated to positively influence broiler performance and processing yield. The mode of action of 1,25DHC-G requires further research.

Key Words: broilers, 1,25 dihydroxycholecalciferol glycoside, broiler breeders, performance, tibia ash

T202 In vivo amino acid digestibility is inversely correlated to the trypsin and chymotrypsin inhibitor contents of commercial solvent extracted soybean meal Nelson Ruiz*[1], Carl Parsons[2], Benjamin Parsons[3], Keshun Liu[4] *[1]Nelson Ruiz Nutrition, LLC, [2]Department of Animal Sciences, University of Illinois, [3]Department of Poultry Science, University of Arkansas, [4]Grain Chemistry and Utilization Laboratory, National Small Grains and Potato Germplasm Research Unit, USDA*

The objective of this study was to determine the relationship between *in vivo* amino acid digestibility and protease inhibitors in commercial soybean meal (CSBM). A total of 12 CSBM samples were analyzed for: (1) trypsin inhibitor activity (TIA) utilizing the new official AOCS method (Ba 12a-2020). (2) chymotrypsin inhibitor activity (CIA) by the latest optimized method of Liu (2022. J. Food Sci. https://doi.org//10.1111/1750-3841.16141), and (3) *in vivo* amino acid (AA) digestibility determined utilizing the precision-fed cecectomized rooster bioassay as described by Corray et al. (2018. Poult. Sci. 97:3987-3991). Linear and quadratic regressions of digestible AA coefficients on TIA and the summation of TIA and CIA (TIA+CIA) were conducted using PROC GLM in SAS (2013). The TIA ranged from 3.53 to 12.82 units inhibited (UI)/mg of CSBM. The TIA+CIA ranged from 8.74 to 23.40 UI/mg of CSBM. All 17 determined digestible AA coefficients for each of the 12 CSBM samples were negatively correlated with TIA and TIA+CIA values. For TIA, linear regression R-square values ranged from 0.48 (r= -0.69) for digestible lysine to 0.72 (r= -0.85) for digestible serine ($P<0.05$). There was no significant quadratic effect of TIA vs. digestible AA for all AA. For TIA+CIA, linear regression R-square values ranged from 0.41 (r= -0.64) for digestible cysteine to 0.77 (r= -0.88) for digestible serine ($P<0.05$); there was no significant quadratic effect of TIA+CIA vs. digestible AA for all AA. Given the fact that TIA values above 5.37 UI/mg of CSBM which is approximately equivalent to 3.58 mg trypsin inhibited (TId)/g of CSBM) were associated with rapid feed passage syndrome outbreaks in the field as previously reported in broilers and broiler breeders [2005 Poult. Sci. 84(Suppl. 1):70; 2008 Poult. Sci. 87(Suppl. 1):30], these data suggest that TIA and TIA+CIA contents in CSBM lots are partially responsible for the indigestible AA fraction in CSBM, and that TIA and CIA are relevant in the formulation of CSBM in broiler and broiler breeder feeds.

Key Words: Soybean meal, Trypsin inhibitor activity, Chymotrypsin inhibitor activity, Amino acid digestibility, Rapid feed passage

POSTER ABSTRACTS
Physiology, Endocrinology and Reproduction: Broilers, Turkeys

P203 Effects of antibiotic growth promoters on growth and metabolism in broilers of high or low feed efficiency Charles Meeks*[GS], Laura Ellestad *University of Georgia*

The ban on the practice of using antibiotic growth promoters (AGPs), or subtherapeutic levels of antibiotics to enhance broiler production efficiency, has generated a need to establish AGP alternatives. However, the mechanisms by which AGPs influence broiler growth performance remains unknown, and there is a lack of information as to how AGPs affect physiological systems of broilers that differ in feed efficiency. The objective of this experiment was to determine how AGPs influence growth and metabolism in broilers with high (HE) or low feed efficiency (LE). Individually caged male broilers (n=216) received either an antibiotic-free control diet (C) or a diet containing 55 mg/kg bacitracin methylene dis-

alicylate (A). Plasma was collected on day (D) 21, D28, and D36 to assess circulating insulin-like growth factors 1 and 2 (IGF1 and IGF2), thyroxine (T_4), and triiodothyronine (T_3). On D36, broilers were sampled based on their cumulative feed conversion ratio [FCR; g feed intake/g body weight gain]. Birds at the tails of the FCR distribution were sampled, resulting in four groups: LE-C, LE-A, HE-C, and HE-A (n=8). *Pectoralis major* was collected to measure expression of nutrient transporters and genes related to hormonal control of metabolism by RT-qPCR. Data were analyzed with ANOVA followed by Fisher's LSD test when ANOVA indicated significance ($P \leq 0.05$). LE-A broilers were found to have a lower FCR than LE-C broilers but higher FCR than HE broilers (P=0.0158). Circulating IGF2 was observed to be highest in D21 LE-A broilers (P=0.0022). Circulating T_4 levels were lower in A-fed birds than C-fed birds (P=0.0359). Circulating levels of both T_4 and T_3 were lower in HE birds than LE birds (P=0.0144 and P=0.0020, respectively). *Pectoralis major* expression of *IGF1* (P<0.0001), *IGF2* (P=0.0100), cationic amino acid transporter 2 Y+L system (P=0.0254), and the fructose transporter glucose transport protein 5 (P=0.0025) was higher in HE birds than LE birds. These data suggest AGPs primarily enhance growth performance of underperforming broilers, and differences in feed efficiency between broilers may be attributed to upregulation of somatotropic signaling, downregulation of thyrotropic signaling, and upregulation of nutrient transporter expression in breast muscle.

Key Words: Antibiotic growth promoters, feed efficiency, metabolism, nutrient partitioning, broilers

P204 Understanding the impact of selection for water conversion ratio in broilers John Helt*[1,2UG], Travis Tabler[2], Sara Orlowski[2], Kirsten Shafer[2], Maricela Maqueda[2] [1]*Department of Animal Science, University of Tennessee,* [2]*Department of Poultry Science, Division of Agriculture, University of Arkansas*

With the global population growing and continuing to grow in the years to come, finding methods to improve sustainability is of utmost importance. One resource that is often overlooked is water usage. With nearly 1/5th of the global population living in a water scarce region and that number growing year after year, it is imperative to focus on methods of decreasing water usage in poultry production to create a sustainable, food secure and water secure future. Selection for water use efficiency has not yet been implemented into a primary breeder's selection program as it remains unclear how inclusion of a water efficiency related trait may impact other traits of economic importance. Therefore, two lines were developed from a modern random population (MRB) at the University of Arkansas. These lines were selected for water conversion ratio (WCR). The divergent populations include the low WCR (LWCR) line selected for improved water efficiency and the high WCR (HWCR) selected for poor water efficiency. The purpose of this study was to evaluate the LWCR and HWCR lines during the fourth generation of selection. Line comparisons were assessed using a one way-ANOVA with means separated by Tukey's HSD at $p<0.05$. Daily water and daily feed intake were evaluated on sire families from the three lines (MRB, LWCR and HWCR) from hatch to 4 weeks. The LWCR had the lowest water intake (3773.6±158.6) during the 4-week selection period, while the HWCR line had the highest (4541.84±391.1). Feed intake was lower in both the LWCR and HWCR line compared to the MRB control. Overall, the LWCR line had the lowest WCR from 0-4 weeks (2.83±0.08), the HWCR the highest (3.42±0.31) while the MRB line was an intermediate (3.01±0.03). Body weights did not differ between the three populations. After the 4th generation of selection, it appears that selection for WCR does not have an impact on economically important traits such as FCR and body weight and could be a useful selection parameter for primary breeder companies. This research was part of an undergraduate internship program awarded to J. Heldt funded by USDA NIFA SAS #2019-69012-29905.

Key Words: Divergent selection, water conversion ratio, broiler, sustainability

P205 The impact of pre-lay inseminations on the sperm storage tubule transcriptome in turkey hens Kristen Brady*, Julie Long *Animal Biosciences and Biotechnology Laboratory, BARC, ARS, USDA*

Pre-lay inseminations are used in the poultry industry to boost fertility rates. Pre-lay inseminations are hypothesized to fill sperm storage tubules (SSTs) to allow for proper sperm concentrations at the initiation of egg lay. However, the molecular mechanisms surrounding pre-lay insemination in SST dynamics are not understood. The transcriptomes of SSTs isolated from hens receiving two pre-lay or no pre-lay inseminations were compared on days 7 (D7), 30 (D30), and 60 (D60) (n=4 per group/timepoint). All hens received weekly post-lay inseminations using pooled semen per industry standard. Bioinformatic analysis was performed using CLC Genomics and DAVID. A total of 295, 26, and 19 differentially expressed genes (DEGs) were obtained through the comparison of pre-lay and no pre-lay SST samples at D7, D30, and D60, respectively ($q<0.05$, |fold change|>1.5, FPKM>1). At D7, enriched gene ontology (GO) terms were superoxide metabolism, fibroblast proliferation, endothelial cell migration, and proteoglycan, methylated histone, and hyaluronic acid binding, with localization at the cell surface, extracellular matrix (ECM), and cytoplasm. At D30, enriched GO terms were ECM organization, cellular oxidant detoxification, cell-substrate adhesion, and integrin, oxygen, and ECM binding, with localization to the extracellular space, collagen type I trimer, and hemoglobin complex. At D60, enriched GO terms were neuron projection development, kinase activity, and cell migration biological processes, and transmembrane receptor protein tyrosine kinase activity, with localization to the integral component of the plasma membrane. In addition, KEGG pathway analysis revealed enrichment of FoxO signaling, ECM-receptor interaction, and tyrosine kinase signaling pathways in DEGs obtained at D7, D30, and D90 respectively. These results demonstrate that the inclusion of pre-lay inseminations impacts SST gene expression for up to 60 days after the first post-lay insemination, with the largest impact to the SST transcriptome at the earliest timepoint. At a molecular level, pre-lay insemination appears to increase SST developmental processes and sperm maintenance capabilities when compared to no pre-lay insemination samples at D7, ultimately increasing fertility rates.

Key Words: Turkey, Sperm Storage Tubules, Pre-lay insemination, Transcriptome, Gene Expression

Physiology, Endocrinology and Reproduction: Layers, Breeders

P206 Effects of sampling methodologies on Mycoplasma gallisepticum tissue populations in commercial egg-laying pullets Sethulakshmi Sasidharan*[1GS], Jeffrey Evans[2], Katie Elliott[2], Quentin Read[3], E. David Peebles[1] [1]*Department of Poultry Science, Mississippi State University,* [2]*USDA-ARS, Poultry Research Unit,* [3]*USDA-ARS, Southeast Area, North Carolina State University*

Mycoplasma gallisepticum (MG) is an economically important pathogen in layers, as it is responsible for chronic respiratory disease. Routine detection of MG is performed via swabbing of the trachea or choanal cleft. However, the possible impact of repeated sampling events on *in vivo* MG population dynamics within individual subjects have not been investigated. Therefore, 11 wk-of-age Hy-Line W-36 layer pullets (n = 80) were infected with MG and housed in one of 4 identical rooms. Two wk post-infection, birds in each room were assigned to sampling schedules of 2, 4, 8, or 16 d and were maintained for an additional 32 d. At each sampling event, the birds were swabbed in both the choanal cleft and trachea, and

sample-associated MG populations were quantified via detection of MG DNA using real-time PCR. Five birds were allocated to each treatment, with the treatment groups being completely randomized within each of 4 rooms. Room was considered as an experimental block, so that a randomized complete block design was employed. Data analysis involved repeated measures within birds. Undetected bacterial populations were considered as a negative sample. A Bayesian generalized linear mixed model was best suited to analyze the data and determined the trend of variation in bacterial abundance differing by sampling schedule, sites, and the variation in positivity rates of infection due to variation in sites. The median estimates of the difference in trend between sampling schedules were close to zero with wide uncertainty on either side of zero, which indicated little or no evidence of any difference in trend over time of microbial abundance due to different sampling schedules. However, the choanal cleft had 5.58 times more MG populations than the trachea with a 95% credible interval between 2.17 and 2.79 averaged across all sampling frequencies. It was concluded that the site of sampling influenced MG population observations with the sampling interval exerting no additional influence.

Key Words: DNA, Layer pullets, Mycoplasma gallisepticum, Real time PCR, Trachea

P207 Effect of preincubation heating, turning and physiological zero on hatchability rate and post hatch growth performance of broiler from a 37-week old flock Jordan Smith*[UG], Oscar Tejeda *Southern Arkansas University*

Storage of fertile eggs is a common practice that allows the broiler industry to synchronize hatchability of eggs coming from different flocks. However, storage time is inversely related with hatchability and chick survival. Therefore, the objective of the experiment was to evaluate the effect of preincubation heating and turning on eggs stored for 10 days. A total of 400 fertile eggs were collected from a 37-week old breeder flock. All eggs were divided in four treatments: PC eggs that were stored for 4 days; NC eggs were stored for 10 days; T1 eggs were stored for 10 days and turned every 8 hours; T2 eggs were stored 10 days, turned every 8 hours and were exposed to preincubation heating at 85 °F for 5 hours at storage day 5. Chicks were raised for 21 days. Body weight, feed disappearance and FCR were measured on days 7, 14, and 21. Statistical analyses were conducted using the general linear model of SPSS statistical software version 21. The highest hatchability was observed the control group (97% hatchability) followed by T2 (70%) the negative control had 61% hatchability. Statistical tendencies were observed where birds from T2 tended to have heavier body weight gain compared to NC birds that had the lowest weight gain. Furthermore, a tendency for better feed conversion ratio in birds from T2 was observed whereas the highest FCR was observed in the NC group (P = 0.09). In conclusion, preincubation heating may have profound implications in the hatchability and post-hatch performance of broiler chickens and can be used as a method to improve performance.

Key Words: preincubation heating, fertile egg, broiler chicken, storage, hatchability

P208 Changes of nutritional absorption-related genes and intestinal histology in laying Japanese quail (Coturnix japonica) at different ages Hamid reza Rafieian-Naeini*[1GS], Mahdi Zhandi[2], Maryam Taghipoor-Shahbandi[2], Amir Reza Sabzian[3], Ali Reza Yousefi[4], Woo Kim[1]
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Quail is an efficient poultry species known for many years, and its productivity is highly affected by gastrointestinal function. The decline in egg production with aging after the peak of the laying stage, is a great concern of poultry farmers. Therefore, the most potent issues that may be affected by aging are the gut health, structure and function of the digestion capacity. This study aimed to evaluate the mRNA abundance of nutritional absorption-related genes and intestinal histology changes of laying Japanese quail at different production ages. A total of 135 quails from a commercial flock at 11 (Young), 26 (Adult), and 45 (old) weeks of age were randomly selected (n=45/age group) and reared for two weeks of experimental period. Production performance was evaluated during the experimental period, and tissue samples were taken from duodenum, jejunum, and ileum for histological analysis and mRNA abundance purposes at the end of experiment. The mRNA abundance of *CALB1, SGLT1, SGLT5, EAAT3,* and *NaPi-IIb* genes were compared between groups. The continuous data were then subjected to analysis of variance using GLM procedure, and egg production was analyzed by GENMOD procedure using a logit odds ratio link function. Tukey's multiple comparison tests were used to determine significant differences among experimental groups and $P \leq 0.05$ was considered statistically significant level. Age did not affect the eggshell thickness, goblet cell number, mRNA abundance of *CALB1, EAAT3*, and *NaPi-IIb* ($P<0.05$). The old quails had the highest egg weight and FCR compared to younger quails ($P<0.01$). Egg production and egg mass of the young and adult quails were higher than that of old quails ($P<0.01$). Moreover, young quails had the highest villus height /crypt depth (VH:CD) ratio in the duodenum and jejunum and the lowest VH:CD in the ileum compared with the elder quails ($P<0.01$). The lowest *SGLT1* mRNA abundance was noted in the adult quails, while young quails had the highest *SGLT5* mRNA abundance ($P<0.05$).

In conclusion, although histology parameter changes were in line with the lowest performance observed in the old quails, the current results failed to show a relationship between mRNA abundance changes and production performance attributes at different ages.

Key Words: Age, gene expression, Production, Histology, quail

P209 Effects of herd and sex on telomere length among three chicken herds Sang-Hyon OH*, Sea-Hwan Sohn *Gyeonsang National University*

This study is to figure out the difference of telomeric amount among three chicken herds (PL, PS, and CC). In this study, we investigated the amount of telomeric DNA using Quantitative Fluorescence *in situ* Hybridization technique on interphase nuclei in lymphocytes collected from chickens at the tenth week of age. The chicken flock of the present study was raised at the University farm of Gyeongsang National University, Jinju, Republic of Korea. The experimental facility was solid-sided and light and temperature controlled. Ventilation consisted of a single fan producing positive pressure in the house. Birds were kept in wire cages at the university experimental farm in a room equipped with control of temperature (23-33°C) and relative humidity (around 50%). Peripheral blood (3 ml) was collected from the brachial wing vein into heparin tubes using standard blood collection procedures. After collection, the lymphocytes were separated using the method described by Lovoie and Grassman (2005). The data were analyzed using the General Linear Model procedure of Statistical Analysis System Institute (SAS Institute Inc., Cary, NC, USA) with fixed effects of herd and sex. The interaction between herd and sex was excluded because it wasn't significant in the preliminary analyses. The fixed effects were significantly different ($p<0.05$). The least squares means of telomeric amount were 2.587±0.008, 2.856±0.007, and 2.540±0.007 in PL, PS, and CC, respectively. The least squares means in female and male were 2.674±0.004 and 2.648±0.009, respectively. As a result, the telomere length of offspring at the tenth week of age was different significantly in herds and sexes.

Key Words: chicken, telomere, herd, sex, length

P210 Effects of four Gallus gallus domesticus strains on body weight gain and vital internal organs allometry Lizia Carvalho*[1], Dimitri Malheiros[2], Emmillie Boot[2], Benjamin Alig[2], Kari Karding[2], Kenneth Anderson[2], Ramon Malheiros[2] *[1]UNESP, [2]Prestage Department of Poultry Science - NCSU*

Domestic chicken was intensively selected for meat production on an industrial scale at the beginning of the 20th century. Nowadays, modern lines are constantly being improved to produce birds that are highly efficient in converting feed into body weight or egg. Comparative studies have been developed between lines of *Gallus gallus domesticus*, and are useful for morphological considerations that these birds may present over the years of selection. Therefore, this study aims to describe a comparison of body weight (BW) and internal organs allometry between four strains: Hy-Line 2016 (HL), Leghorn 1940 strain (S1), Athens Canadian Random Breed (ACR), and Jungle Fowl (JF) (their common ancestor), from 1 to 28 days of age. A total of 135 day-old chicks were used for the test, 48 ACR, 13 S1, 38 HL, and 36 JF. The birds were housed in alternative-design battery cages, with heat, light, and feed + water *ad libitum* during the experimental period (28days). Feed formulation requirements were provided by the Hy-Line recommendation. Birds were weighed weekly from day 1 post-hatch. On day 28 post-hatch, 10 birds of each strain were weighed and euthanized. Individual organs were weighed, including the heart, liver, proventriculus, gizzard, and whole intestine. Data were analyzed using the SAS GLM procedure. Tukey's test was used to determine differences between strains. Strain differences had a significant effect on body weight. At hatch two strains showed significantly increased body weight (HL 32.96 g and ACR 32.02 g) when compared to S1 (28.98 g) and JF (20.77 g). The ACR strain significantly outperformed the others until the end of 28 days and the HL and S1 strains did not differ significantly. The results obtained in the allometric measurements of the internal organs compared with the BW, the heart (0.93%) and the liver (2.96%) did not show significant differences between the strains studied. The ACR strain had a lower allometric value for the proventriculus and whole intestine than the other strains, 4.36% and 5,14%, 6.37% and 7.39% for ACR and average, respectively. In conclusion, the genetic selection over almost 80 years, did not change the allometric correlation between organ size and BW, during the first 28 days of the chicken's life in strains evaluated.

Key Words: Organs allometry, Red Jungle Fowls, Body weight, Chickens, Genetic

Processing and Products

P211 - Rapid Detection of Septicemia-Toxemia in Eviscerated Carcasses Using a Handheld Fluorescence Imaging System Micah Black*[1GS], Bet Wu Alvarado[1], Luis Guzman[1], Aftab Siddique[1], Laura Garner[1], Amit Morey[1], Jianwei Qin[2], Diane Chan[2], Insuck Baek[2], Moon Kim[2], Nicholas MacKinnon[3], Stanislov Sokolov[3], Alireza Akhbardeh[3], Fartash Vasefi[3] *[1]Auburn University, [2]USDA-ARS, [3]SafetySpect Inc.*

Introduction:

Each broiler carcass that is processed is mandated to be visually inspected by a USDA inspector or a trained processing plant personnel for carcass condemnations which is a cumbersome process and may lead to errors. The objective of the research was to identify and differentiate normal broilers and broilers with septicemia-toxemia (septox) using a handheld, rapid fluorescence imaging system.

Materials and Methods:

Freshly processed eviscerated broiler carcasses (8-10 lb live weight), both normal (n=200) and septox (n=195), were collected from a local commercial processor. Broiler carcasses were photographed using a handheld fluorescence spectral imaging system called the CSI-D+, a RGB camera with Ultraviolet (UV) camera for fluorescence. The spectral camera and carcasses were placed in a box with a shackle attachment and images were taken. Normal broiler and septox carcasses had two images captured for different fluorescence exposure times (175 and 350nm; 230 and 270 nm). Collected images for both experiments were randomly selected and saved in three different folders for the model development with a random split of 70::30 (training and testing) with two subfolders folders in each training and testing with normal and septox images. Images in validation folder were not repeated in any previously mentioned folder. Image analysis was performed using the Convolution Neural Network (CNN) algorithm. Data augmentation transformers (Resize, RandomHorizontal flip, RandomVertical flip, and RandomRotation) were used to transform the collected images for analysis. CNN model was developed on Google collaboratory platform using CUDA 11.2 and Pytorch machine learning framework with 3 ConvNet layers. Adam Optimizer (learning rate =0.01, weight decay = 0.0001) with cross entropy loss function was used for training of model with 20 epoch.

Results:

Septox and normal images were differentiated with a 98% accuracy with the testing model and 100% accuracy with the validation model. Results indicate that the CSI-D+ imaging system can provide image data to eliminate error in sorting septox affected broiler carcasses through fluorescent imaging.

Key Words: Septox, Spectral imaging, Fluorescence

P212 Phenotypic variations of woody and normal condition in broiler breast meat Sang-Hyon OH*[1], Euyeon Noh[2], Byungrok Min[2] *[1]Gyeongsang National University, [2]University of Maryland Eastern Shore*

This study was to determine the potential for a genetic selection of woody breast (WB) in live broilers. Phenotypic variations of muscle stiffness in WB were examined using a non-invasive digital palpation device less than 24 hours after harvest. The device can measure oscillation frequency, stiffness, elasticity, mechanical stress relaxation time, and the ratio of deformation and relaxation time of muscle *in vivo*. If the variations are detected, the phenotypes can be utilized a marker for the genetic selection. Thirty-five chicken breast fillets (Pectoralis major) with woody and normal (NB) conditions, respectively, were obtained from a local processing plant one day after slaughtering at three different times. The breast fillets were sorted into WB and NB at the processing plant based on the plant processing procedure. Subesquently, the samples were subjected to physical and physicochemical analyses: pH, color (L*, a*, b*), drip loss, texture (firmness, compression energe), and muscle stiffness for raw meat and cooking yield, color, and texture (shear force and energy) for cooked meat. After raw meat parameters were measured, the meat was vacuum-packed and cooked in boiling water until the internal temperature reached 74°C. Two-way ANOVA was used to analyze the data as a statistical model with fixed effects of trial and group. The least squares means of the following variables were significantly different between WB and NB ($p<0.01$): pH (6.00 vs 5.85), cooking yield (62.15 vs 71.88; %), drip loss (11.79 vs 9.58), color in raw meat (L*, 65.56 vs 62.19; a*, 1.53 vs 0.72; b*, 9.93 vs 8.30), firmness (39.09 vs 14.64 N), compression energy (60.52 vs 26.41 N·s), stiffness (596.5 vs 533.6 N/m), elasticity (1.40 vs 1.55), color in cooked meat (L*, 76.76 vs 82.97; a*, 1.96 vs 0.85; b*, 16.01 vs 15.36), shear force (13.56 vs 10.86 N) and shear energy (19.85 vs 14.64 N·s),. These results

showed that the physical characteristics, especially muscle stiffness measured by the digital palpation device, of WB significantly differed from NB. Further study investigating the measurability of muscle stiffness in live birds using the palpation device is needed to verify its applicability for the genetic selection program to prevent WB.

Key Words: Broiler, Woody, Breast, Phenotypic, Variation

P213 Buffered peptone water formulation does not influence growth of pESI-positive Salmonella serovar Infantis Elizabeth McMillan*[1], Mark Berrang[1], Quentin Read[2], Surendra Rasamsetti[3], Amber Richards[3], Nikki Shariat[3], Jonathan Frye[1] *[1]United States Department of Agriculture, Agricultural Research Service, U.S. National Poultry Research Service, Poultry Microbiological Safety and Processing Research Unit, [2]United States Department of Agriculture, Agricultural Research Service, Southeast Area, [3]Department of Population Health, University of Georgia*

Purpose: Since 2016, a marked increase in the detection of *Salmonella* serovar Infantis has been noted from raw poultry samples. Many isolates of this serovar also carry the pESI plasmid. Coincidentally, also in 2016, the Food Safety and Inspection Service (USDA-FSIS) modified the Hazard Analysis and Critical Control Point (HACCP) method to use neutralizing buffered peptone water (nBPW) instead of BPW as the sampling and overnight storage broth for raw poultry samples. It has been hypothesized that the increase in detection of pESI-positive ser. Infantis could be due to isolates carrying the plasmid recovering better in nBPW compared to the original formulation of BPW. In the current study we tested whether nBPW provides a growth or survival advantage to pESI-positive ser. Infantis isolates.

Methods: Two experiments were performed: a six-hour growth experiment, and a simulation of USDA-FSIS methods. Briefly, ten ser. Infantis isolates, nine which carried pESI, were grown in both BPW and nBPW. Log CFU/mL was measured hourly for six hours. For the FSIS method simulation, six isolates, three carrying pESI and three without, were inoculated at low concentrations and held in nBPW and BPW (4°, overnight) and prior to incubation with additional BPW. All samples were plated to BGS agar (35°, 24 hours) to enumerate log CFU/mL.

Results: In the growth experiment, bacterial concentration increased by a mean of 2.4 log CFU/mL for nBPW cultures and 2.8 log CFU/mL for BPW cultures. When analyzed using a Bayesian model, there was weak evidence for a negative effect of nBPW on the growth rate but no difference in the carrying capacity. In the FSIS methods simulation, all isolates increased over 8 log CFU/mL regardless of storage media or plasmid carriage. There was no significant difference in final concentration of isolates stored in nBPW versus those stored in BPW (t-test, $p>0.05$).

Conclusions: Given these results, we were able to conclude that nBPW does not provide a growth advantage for pESI positive *Salmonella* serovar Infantis and that the increased isolation rate of serovar Infantis is due to other factors.

Key Words: Salmonella, Infantis, nBPW, pESI

P214 Chicken packed in chicken: Developing a fully biodegradable packaging film for poultry meat. Katherine Sierra*[GS], Amit Morey *Auburn University*

Sustainable packaging is seen as the next step in the food industry. Packaging made using biodegradable materials has been studied but the incorporation of new bioactive molecules recovered from existing food is scarce. Poultry processing products such as chicken skins are sold at a very low price to the rendering industry. Chicken skin is rich in collagen which has a wide range of applications including cosmetics and pharmaceuticals, but the research using chicken collagen in packaging is limited. The collagen was converted into gelatin to be used in the developing of the biodegradable packaging films. A total of eight film formulations with three repetitions (n=24) were tested. Treatment 1 has 2% of chicken gelatin (CG) and 0% of nanocellulose (NC), treatment 2 has 2% CG; 1% NC, treatment 3 has 2% CG; 3% NC, treatment 4 has 2% CG; 3% NC, treatment 5 has 3% CG; 0% NC, treatment 6 has 3% CG; 1% NC, treatment 7 has 3% CG; 3% NC, treatment 8 has 3% CG; 4% NC. Thus, all the treatment contains the same percentage of starch (1%), glycerol (6%), and water (85-91%). In addition, film with 0% CG was formulated as control, however, this formulation did not form a film and could not be tested. To create the film, starch and CG in different beakers were dissolved in water (50% water for CG and 50% for starch) (45°C for CG and 85°C for starch) for 30 min, then the mixture was combined and mixed at 45°C (30 min). NC was added to the solution and mixed at 45°C for 15 min. Finally, glycerol was added and mixed at 45°C for 15 min. Tensile force, young´s modulus, elongation, punching force, solubility, and water vapor permeability (WVP) were analyzed. Data were analyzed using ANOVA with significant differences in the means at $p<0.05$ (Tukey's HSD). The highest value for young´s modulus and punching forces were 0.88 Mpa and 12.08 N/mm respectively (3% CG and 4% NC). The lowest value for WVP was 0.00077 and 0.00084 g/m.h.Kpa (2% CG, 4% NC, and 3% CG, 1% NC). The highest tensile force was 0.295 and 0.269 Mpa (3% CG, 3% NC and 3% GC, 4% NC). Thus, the highest elongation was 41.57% (3% CG and 0% NC). In conclusion, the chicken gelatin combined with nanocellulose can form films and could be considered used for packaged chicken.

Key Words: Chicken collagen, Nanocellulose, Packaging, Sustainable

P215 Rapid assessment of flavor profiles of raw and cooked chicken meat by electronic tongue and electronic nose Linda Barahona*[1,2UG], Michelle Hayden[1], Md. Moazzem[1], Sungeun Cho[1] *[1]Auburn University, [2]Zamorano University*

An investigation was carried out to evaluate the taste and aroma profiles of different parts of chicken (breast, leg, and thigh) using electronic senses. The raw and cooked (boiled for 5 min) chicken samples (20 g) in 200 mL distilled water were homogenized, filtered, and centrifuged to get supernatant for the electronic tongue (*a*-Astree II E-tongue, Alpha MOS, Toulouse, France) analysis. To perform electronic nose analysis (Heracles Neo E-nose, Alpha MOS), 2 g of minced raw and cooked chicken samples were sealed in 20 mL vials and incubated for 20 min at 60°C for headspace analysis. The results revealed that the e-tongue could distinguish different parts of chicken meat with high discrimination index (DI) both for cooked (DI=98) and uncooked (DI=96) samples. The Principal Component Analysis (PCA) of taste profiles could explain 96.9% and 93.8% variations of the e-tongue data for raw and cooked samples, respectively. Moderate DI values (70 and 64 for raw and cooked samples) were observed for their discrimination capability with the different parts being well separated in the PCA biplot. The PCA of the odor profiles could explain 65.2% and 58.1% of the e-nose data variability by the first two principal components of raw and cooked samples, respectively. The results suggest that a combination of e-tongue and e-nose can be an effective and powerful tool for the rapid assessment of sensory profiles of chicken meat. These instruments also have potential uses in quality detection or shelf-life evaluation when correlated with human sensory data.

Key Words: Electronic tongue, Electronic nose, Flavor profiles, Chicken meat

P216 Identifying Signature Micro-Wave Frequencies for the Non-invasive, Rapid Detection of Woody Breast Myopathy in Live and Processed Broilers Using Machine Learning Algorithms Aftab Siddique*[1GS], Laura Garner[1], Wilmer Pacheco[1], Ryan Freeman[2], Amit Morey[1] *[1]Auburn University, [2]Compass Technology Group, LLC*

Development of time and cost effective, rapid, non-contact technologies to detect muscle myopathies in broilers is an area of great interest in the poultry industry. We investigated radio-frequency waves from S-band to KU band (2GHz to 18 GHz) from the electromagnetic spectrum and identified specific signature frequencies that can detect muscle myopathies.

Ross 708 broilers (48 days old; n = 107) were analyzed using radio-wave with frequency ranging from 2 GHz to 18 GHz. Data was collected in amplitude and phase from during the process. Deboned fillets were hand-palpated and scored for the severity (Normal = 0; Moderate = 1; and Severe = 2) and were used to train the BPNN (Back Propagation Neural Network) algorithm after pre-processing of data. A subset of 100 measured frequencies were chosen to eliminate false positive results and simplify the analysis. Variable clustering was used to identify the signature frequencies form top 100 predictor frequencies based on the 1-R^2 values [1-R^2 = (1-R^2) $_{(own)}$) / (1 — R^2) $_{(nearest)}$]. For the development of BPNN model, variable clustered identified signature frequencies data set was normalized between the value of 0 and 1 using normalize function (logistic function 0 to 1 sigmoid) in R and spilt into training and testing set (55::45). Model was trained on different hidden layer (5~15) with 2~10 random repetition using different random weights with threshold value of 0.015, and learning rate of 0.01~0.001 with 200,000~500,000 maximum number of iterations. For live birds, we have identified 6 frequencies for normal (2.14 GHz, 2.33 GHz, 8.73 GHz, 10.21 GHz, 12.60 GHz, and 16.95 GHz); 6 for moderate (6.06 GHz, 8.41GHz, 9.27GHz, 10.36GHz, 12.61GHz, and 16.95 GHz) and 4 for severe condition (8.77 GHz, 10.21 GHz, 12.61 GHz, and 16.95 GHz). In Pre-chill WOG's data, for normal 6 frequencies (3.21 GHz, 4.60 GHz, 7.50 GHz, 10.06 GHz, 11.16 GHz, 16.09 GHz), for moderate 7 frequencies (3.15 GHz, 3.92 GHz, 4.85 GHz, 6.93 GHz, 7.69 GHz, 9.97 GHz, 16.07 GHz) and for severe 6 frequencies (3.33 GHz, 4.75 GHz, 5.90 GHz, 8.52 GHz, 10.00 GHz, and 15.99 GHz) were identified respectively. The generated BPNN model was able to classify normal, moderate and severe breast meat in live birds at 90-96.1%, and pre-chilled WOG's with accuracy of 78.9 % to 97.1%. Neural network-based classification algorithms along with feature extraction techniques can be used to develop predictive models and perform classification during in-line processing.

Key Words: BackPropagationNeuralNetwork, Variable clustering, Myopathic conditions, Radio-wave frequencies

P217 Changes in the quality of eggs during storage depending on the laying hen rearing system at 30 weeks of age Serkan BÜYÜKÜNAL[1], Karlo MURATOĞLU[1], Ali ÇALIK[2], Abdurrahman KIZIL[3], Mustafa ALATAŞ[4], Oğuzhan KAHRAMAN[4], Ahmet PEKEL*[3] *[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, 34320, [2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ankara University, 06110, [3]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, 34320, [4]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Selçuk University, 42003*

The effects of storage time (0, 7, 14, 21, and 28 day) at room temperature and the rearing system (enriched cage and cage-free) on the quality of eggs from layers (Lohmann Brown-Classic) at 30-wk of age were studied. A total of 250 eggs (125 eggs per rearing system) were collected on the first day of 30-wk of age and placed on plastic egg trays. Eggs were numbered, weighed, and assigned to each experimental storage time for each rearing system (25 eggs per storage time for each rearing system). The design of the experiment was completely randomized with a 2 x 5 factorial arrangement. All data were analyzed using the GLM procedure in SPSS software. Egg weight, yolk height, yolk index, haugh unit, albumen height, and eggshell strength decreased linearly as egg storage time increased (P<0.05). On the contrary, albumen pH, yolk diameter, albumen length, albumen width, and air cell diameter increased linearly as egg storage time increased (P<0.001). Albumen height, yolk index, yolk height, and haugh unit of eggs from the enriched cage system were higher than that of the cage-free system (P<0.05). Eggs from the cage-free system had higher albumen length than that of the enriched cage system (P<0.05). An interaction (P<0.01) was observed between storage time and the rearing system led to a higher decrease in albumen height of eggs from the enriched cage system (64%) when compared to those from the cage-free system (53%) at the end of 28-day storage time. An interaction (P<0.01) was observed between storage time and rearing system led to a higher increase in albumen width of eggs from the enriched cage system (45%) when compared to those from the cage-free system (25%) at the end of the 28-day storage time. In conclusion, fresh eggs (0-day) from the enriched cage system had better interior quality (higher albumen height and haugh unit, higher yolk height and index, and lower albumen length). On the other hand, the interior quality of eggs from the cage-free system remained superior to those from the enriched cages during 28-day room temperature storage.

Key Words: cage-free, egg quality, enriched cage, laying hen, storage time

P218 Interactions between high pressure processing and natural antimicrobials in ground white-meat chicken: An analysis of microbial and physicochemical changes Amalia Diaz, Belinda Cochran, Julie Northcutt, Paul Dawson, George Cavender* *Clemson University*

Comminuted poultry products pose multiple challenges regarding shelf stability and safety, many of which stem from the peculiarities of the processes involved in making them. Grinding increases surface area, while mixing not only exposes more of that surface area to air, potentially aiding in the formation of oxidative byproducts, it also can distribute any localized microbial contaminants, potentially leading to shelf life and safety issues for entire batches. Processors have, quite expectedly, attempted to mitigate these concerns using a variety of techniques. One of the most common is the use of natural antimicrobials like essential oils, buffered vinegar and citrus juices. Another option that has seen more limited use is High Pressure Processing (HPP). Although it can eliminate spoilage and illness-causing microbes, HPP can also have undesirable effects on the texture, color and oxidation rate, making some processors hesitant to adopt it. In order to address these issues, we examined the effects of two different HPP treatments on ground white-meat chicken with or without one of two common antimicrobials (Buffered concentrated vinegar with rosemary essential oil (V+REO)and a lemon juice-vinegar blend) Samples were inoculated with a non-pathogenic E. coli strain and individually packaged in polyethylene bags. Refrigerated samples with or without antimicrobial additive were then processed at pressures of either 300 MPa or 600 MPa each with a three-minute hold time. Measurements of color were taken prior to processing, within 4 hours of processing, and after a 24 hour refrigerated storage. E.coli, Pseudomonas spp, and Aerobic plate counts were also performed at the 24 hour mark, along with pH, TBARS assays to measure levels of oxidation, texture profile analysis (TPA) of raw samples and TPA and yield of cooked samples. All samples at 600 MPa showed greater than 5 log reductions in E. coli, while non HPP samples saw more modest reductions in samples which had been treated with antimicrobials. The interaction of V+REO and HPP proved complex, particularly as it related to bacterial inactivation, and the antioxidant properties of the V+REO having a minor positive effect on the TBARS value. While some of the results showed promise, further validation is needed.

Key Words: High Pressure Processing, Essential Oil, Microbiology

P219 Efficacy of atmospheric-pressure plasma jet to eliminate Salmonella Typhimurium, Heidelberg and Enteritidis inoculated on chicken skin Bet Wu*[1GS], Amit Morey[1], Aftab Siddique[1], Charles Herron[1], Garret Royster[1], Katherine Sierra[1], Laura Garner[1], Luis Guzman[1], Micah Black[1], Ryan Sheinberg[1], Saikat Chakraborty Thakur[2] *[1]Poultry Science Department, Auburn University, [2]Physics Department, Auburn University*

Consumer trends indicate a higher demand for low thermally processed foods without synthetic antimicrobials. Atmospheric-pressure plasma jet (APPJ) consists of reactive oxygen and nitrogen species which affect the cell membrane and intracellular components of the bacterial cell thus eventually kill it. The objective of the study was to determine the efficacy of APPJ to eliminate three *Salmonella* serovars on chicken skin.

Fresh chicken skin coupons (1 sq. cm) (n = 189) were cut were cut from the breast skin and stored at 4ºC. The coupons were inoculated individually with *Salmonella* Enteritidis (SE), *Salmonella* Heidelberg (SH) and, *Salmonella*Typhimurium (ST) and to obtain a target inoculum of 10^4 CFU/cm^2. After a 30 min attachment period at 4˚C, the inoculated coupons were treated with APPJ for 0, 10 and 20 min with different combinations of helium (He), hydrogen peroxide (H_2O_2) and APPJ using 5.20 and 5.75kV potential. Post-treated samples were vortex 30 seconds in cryovials with buffered peptone water (2 mL), serially diluted and then spread plated on XLT4 agar plates which were incubated for 24 h at 37˚C. Typical *Salmonella* colonies were counted and reported as log CFU/sq. cm. The experiment was repeated as three separate trials with three samples per treatment for each trial. Data was analyzed using ANOVA ($P < 0.05$) with Tukey's HSD to determine significant differences between treatment means.

Overall, APPJ exposure at 10 and 20 min significantly reduced (*P<0.05*) the three *Salmonella* serovars inoculated on chicken skin. Hydrogen peroxide and helium alone or in combination without the application of electric potential did not significantly reduce (*P>0.*05) pathogen levels on chicken skin coupons. The APPJ treatment, 5.75 kV + H_2O_2 at 10- and 20-min exposure was the most effective treatment irrespective of serovars. However, for SE AAPJ at 5.75 kV without H_2O_2 for 20 min eliminated 2.46 log CFU/g. The study demonstrates the potential to use APPJ as an additional hurdle to eliminate *Salmonella* on raw poultry and improve food safety.

Key Words: Exposure time, Food quality, Foodborne reduction, Food Safety, Reactive oxygen species

P220 Impacts of acidified peracetic acid on the survival of Salmonella and Campylobacter on poultry thighs Jessica Brown*[1GS], Dana Dittoe[1], C.B. Austin[1], Kara Mikkelson[2], Billy Hughes[2], Steven Ricke[1] *[1]University of Wisconsin-Madison, [2]Hydrite*

Recently, the acidification of peracetic acid (PAA) with an organic acid has been of interest to the industry as a processing aid to reduce foodborne pathogens on poultry carcasses. The objective of this study was to determine if altering the pH of PAA would result in improved reductions of *Salmonella* and *Campylobacter* on inoculated poultry thighs. Chicken thighs (N=65, n=5, k=13) were inoculated with a cocktail of *Salmonella*Typhimurium (S-9), Heidelberg (S-13), Enteritidis (E40), Infantis (6424), Kentucky (M-09-0001A-1) and *Campylobacter jejuni* (NCTC 11168) at 10^8 CFU/mL and incubated at 37°C for 30 min for a total attachment of 10^7 and 10^5 CFU/g of *Salmonella* and *Campylobacter,* respectively. Inoculated thighs were either not treated (NT) or sprayed in a modified spray cabinet for 60 sec with one of the following treatments: tap water (TW), TW pH 2.0 (2.0 pH), TW pH 1.5 (1.5 pH), 200, 400, and 800 ppm PAA, 200, 400, and 800 ppm PAA pH 2.0, and 200, 400, and 800 ppm PAA pH 1.5. Treatments were prepared at the onset of the study and pH was adjusted using an acid blend of citric and muriatic acid. After treatment, samples were rested for 2 min and rinsed with 150 mL of NBPW for 1 min. Rinsates were then serially diluted and spread plated on XLD and mCCDA agar for the enumeration of *Salmonella* and *Campylobacter*. Data were log transformed and analyzed in a one-way ANOVA in JMP15 with means separated using Tukey's protected HSD ($P \leq 0.05$). There was a significant main effect of treatment on the load of *Salmonella* ($P<0.05$) and *Campylobacter* ($P<0.05$). Treatment with 800 ppm PAA (4.96 log CFU/g) and 200 ppm PAA pH 1.5 (5.15 log CFU/g) significantly reduced *Salmonella* compared to TW (5.93 log CFU/g) and NT (6.94 log CFU/g), while a pH of 2.0 with or without the addition of PAA was most effective at mitigating *Campylobacter*. Treatment with TW 2.0 pH resulted in lower levels of *Campylobacter* (3.37 log CFU/g) compared to TW (4.54 log CFU/g) and NT (5.58 log CFU/g). Overall, acidification of PAA with an acid blend of citric and muriatic acid is more effective at reducing *Campylobacter* but does not improve efficacy against *Salmonella.*

Key Words: poultry, peracetic acid, acidification, Salmonella, Campylobacter

Pathology

P221 Respiratory Cryptosporidiosis in commercial broiler chickens in northern Georgia. Silvia Carnaccini*, Maurice Raccoursier Frost, Jenny Nicholds, John French, Karen Grogan *University of Georgia*

Cryptosporidiosis is a sporadic disease of chicken and turkeys accompanied by respiratory and/or gastrointestinal disease, resulting in considerable morbidity and mortality. In October 2022, a total of 12 (9 live and 3 dead) 7-week-old Ross708 broiler chickens were received for macroscopic examination. Five out of 9 live birds had a respiratory noise and swelling of the eyelids. Macroscopically, all birds had muco-catarrhal exudate in the trachea, and white-yellow fibrinous exudate deposition in lungs (1/12), air sacs (5/12), pericardium (4/12), liver (1/12) and subcutis (1/12). Escherichia coli was isolated in pure culture from (2/3) pericardial sacs. Bursas were diffusely small (<0.8 cm in diameter - 5/5), and some (6/12) birds had lesions compatible with femoral head degeneration/necrosis. Histology revealed moderate to severe lymphoplasmacytic conjunctivitis (6/6 sections) and tracheitis (17/17 sections) compatible with infectious bronchitis virus, as confirmed by polymerase chain reaction. In (2/6 sections) conjunctiva and (2/17 sections) tracheas had concurrent moderate heterophilic and histiocytic inflammation associated with deciliation and marked protozoal colonization of the epithelial brush border. Protozoa were also observed colonizing the surface epithelium of (3/5 sections) cloacal bursas in course of severe diffuse lymphoid depletion. These were morphologically compatible with *Cryptosporidium spp*. *Cryptosporidium baileyi* has been previously associated with recurrent outbreaks of respiratory disease in broiler chickens in northern Georgia. This is a case report of respiratory cryptosporidiosis in broiler chickens in course of multiple co-morbidities.

Key Words: Cryptosporidium, chicken, respiratory

P222 Botanical solution in feed additive for intestinal release to control blackhead disease (Histomoniasis) in turkeys Sebastian Decap*[1], Hans Konsens[1], Victoria Tapia[1], Rosa Navarro[1], Miguel Guzman[2] *[1]Plantae Labs SpA, [2]Avian Pathology Lab, Universidad de Chile*

Introduction

Blackhead disease, also known as Histomoniasis, is an important poultry disease caused by Histomona meleagridis, which is a protozoa that spreads in the bird by the roundworm *Heterakis gallinarum*, and infects the cecum and liver. Turkeys are highly susceptible to this disease. Although mortalities in turkey flocks reach 80-100% and economic losses exceed 2 million USD/year, there are lack of approved drugs against blackhead disease. Therefore, phytogenic compounds are new and natural alternatives.

Methodology

Samples were obtained by mixing Quillaja extract and different polymer combinations, commonly used in encapsulation technology, to achieve a

control release in the intestinal tract. The mixture was obtained by homogenization, pH variation and spray dry. Sample (QSAP): Quillaja extract + fiber+ 1 polymer, Sample (QSBP):Quillaja extract + 2 fiber+ 1 soluble polymer, Sample (BQP): Quillaja extract + 2 fiber+ 2 complex polymer. All samples were characterized by measurement of moisture, saponin content by HPLC, solubility, saponin release kinetic and in vivo evaluation. The in vivo study was conducted at the Avian Pathology Lab (Universidad de Chile) using a total of 200 hen turkey poults. Birds were reared from 0-28 days of age in cages and randomly assigned to treatments. There were 10 cages with 20 birds/cage, and 2 cages/treatment except for Control (CON) and MED. All additives were administered via feed from day 21. On day 28, all birds except CON were individually inoculated per cloaca with ~100,000 cells/ml with *H. meleagridis* 14810. Body weight (BW), Feed intake (FI), and FCR was recorded. Dead birds were registered and removed for necropsy along with the assay to determine the liver and cecal lesion score using a scale of 0 to 4, with 0 being normal and 4 being the most severe. The lesion score was analyzed using Kruskal-Wallis test. One-way ANOVA and subsequent Tukey test (α=0.05) were carried out using Statgraphic software, except for body weights.

Results

To use the same drying conditions, it was necessary to adjust the content of saponins to standardize the active compound. The moisture content was similar in all samples. There were clear differences between treatments in the saponin kinetic release and solubility, where the samples QPP and QQP had a significant lower solubility ($p<0.05$).

For the period 0-21 days, BW and FCR in POS, was higher than CON; 28 % increase in FCR and 2 % increase in BW. Compared with untreated/challenged birds (POS), QSAP had a reduction (15%) in FCR and 4% increase in BWG. BWG and FCR did not differ significantly between MED and BQP. For the period 21-35 days, compared with untreated/challenged birds (POS), QSAP and BQP group had a significant increase ($p<0.05$), with 9 and 13% in BW, respectively, and similar to MED. Also, compared with birds (POS), the highest reduction in FCR were observed in samples QSAP and BQP.

There was a clear treatment effect on mortality. Compared with untreated/challenged turkeys (POS), BQP had a significant reduction (69%) in mortality (p-value<0.05), followed by MED. The average cecum lesion score in BQP and QSAP was 4 and 2 times lower than in the POS group, respectively. In the liver, MED-treated birds had the lowest lesion scores followed by the BQP group, being 8 and 3.5 lower than POS.

Conclusion

There was a clear impact of the *Histomona* challenge on the performance of the bids. BQP treated groups showed promising results regarding decreased mortality and low lesion scores compared with POS and it was comparable with a natural and synthetic solution available in the market.

Key Words: Histomoniasis, turkeys, Blackhead disease, feed additives, botanical solutions

P223 Analysis of serum biochemistry profile in turkeys challenged with Histomonas meleagridis Vijay Durairaj*, Ryan Vander Veen *Huvepharma, Inc.,*

Histomoniasis is a deadly disease in turkeys caused by a protozoal parasite, *Histomonas meleagridis*. *H. meleagridis* causes systemic disease in turkeys and induces characteristic lesions in ceca and liver. Since histomoniasis is a systemic disease, evaluating the serum biochemistry may provide more insights in understanding the pathogenesis of this disease. With this objective, three experimental studies were conducted and serum samples were analyzed. In study 1, two of three groups were challenged with $1x10^3$ *H. meleagridis* (HMA) by the cloacal-drop and intra-cloacal routes, respectively. Birds were bled at 9 days post-challenge and serum samples were analyzed. In study 2, two of three groups were challenged with $1x10^4$ and $1x10^5$ *H. meleagridis* (HMA)/dose by the intra-cloacal route. Blood samples were collected at 9 days post-challenge and serum samples were analyzed. In study 3, two of three groups were challenged with two *H. meleagridis* field isolates, HMA and HMB, at doses of $1x10^5$ and $2x10^5$ by the intra-cloacal route, respectively. The birds were bled at 5 and 8 days post-challenge and serum samples were analyzed. In all these studies, significantly lower levels of serum cholesterol and alkaline phosphatase were noticed in the *H. meleagridis* challenged birds compared to the controls. Significantly lower cholesterol and alkaline phosphatase was noticed in the serum as early as 5 days post-challenge.

Key Words: Histomoniasis, Histomonas meleagridis, Blackhead disease, Serum biochemistry, Cholesterol

P224 Assessment of mRNA abundance of key cytokines during histomoniasis in turkey poults in a lateral transmission model Abhisek Niraula*[GS], Candice Blue, Davis Fenster, Nima Emami, Rami Dalloul *University of Georgia*

Histomoniasis is a protozoal disease of poultry that primarily affects the ceca and liver resulting in high mortality and causing major economic losses. This study aimed to evaluate the mRNA abundance of key cytokines in the liver of turkey poults during histomoniasis. Day-old female poults (n=400) were individually wing tagged and assigned to two groups: a non-inoculated control group (NC) and a challenge group, reared in separate rooms with 8 floor pens and 25 birds/pen. On day 9, 30% of the challenge group poults were directly inoculated (DI) with 1 mL inoculum/bird (~80,000 histomonads) intracloacally, whereas the remaining birds were designated as a non-directly inoculated (NDI) group via lateral transmission of the parasite. On days 13,16,19 and 22, liver samples from 2 birds/pen were collected to assess the relative abundance of mRNA of two key cytokines, IL-10 and IFN-γ. The data were analyzed by one-way ANOVA using JMP and significance ($P \leq 0.05$) between treatments was determined by using LSD test. On day 13, the mRNA levels of IL-10 were significantly greater in both DI and NDI groups compared to NC. On day 16, the mRNA abundance of both cytokines in DI and NDI birds was significantly greater than in NC birds. On d 19, DI group displayed significantly higher mRNA abundance of IFN-γ and IL-10 compared to birds of NC and NDI groups. The sustained elevated levels of IL-10 mRNA in DI birds could be to limit an inflammatory response while comparable IFN-γ levels on d 13 among all groups could be due to absence of parasites in the liver at that particular timepoint. For more comprehensive understanding of host immunity to this disease, abundance of additional immune response markers in the liver as well in the ceca of turkey poults need to be evaluated.

Key Words: histomoniasis, immune response, cytokines, turkey, lateral transmission

P225 In-vitro and In-vivo evaluations of plant bio-actives to directly inhibit the growth of Histomonas Meleagridis Bertrand Medina*[1], Ivan GIRARD[1], Chongxiao (Sean) CHEN[2,3] *[1]Probiotech International Inc., [2]Dept. of Poultry Science, University of Georgia, [3]North Carolina State University*

Histomonas meleagridis (*HM*) is the protozoan responsible for histomoniasis in turkey poults and adults. Since the ban of molecules such as dimetridazole and nifursol, the capacity of botanical extracts (BE) to limit this flagellated protozoa development has been investigated. Both *in-vitro* (Study#1) and *in-vivo* (Study#2) assays were carried out to determine the abilities of 9 single BE bio-actives and 1 new-designed blend to directly inhibit the growth of *Histomonas.* Three BEs were selected for their high content in saponins (#1), polyphenols (#2, 3) and 5 volatile BEs for their high content in phenylpro-

panoids (#4, 7, 8), phenols (#5), thiol-esters (#6), and terpenes (#9). Study#1: 100,000 *HM* cells grown in log phase were added to Dwyer's media with the plant bio-actives to be tested (with two concentrations: 5000, 500 ppm) and incubated at 40°C for 48 hours. Metronidazole (MDZ@12.5 ppm) was used as a positive control and Dwyer's media considered as a negative control. *HM* counts were performed using a Neubauer hemocytometer (3 replications per treatment and each replicate was counted 4 times). One ml of culture was spun down, media removed, and the pellet resuspended in 1 ml of water. Bacterial concentrations were determined at OD600 to ensure that inhibition of growth was not caused by the loss of bacteria in the culture. Study#2: At day 18 of age, turkey poults were cloacally challenged with ~100,000 *HM* cells per bird. Poults were monitored daily for mortality. The experiment achieved 10 days after infection, and all poults were weighed, necropsied and scored for the presence of blackhead disease signs in the liver and cecal on a 0-4 scale (with 0 as no lesions and 4 as severe lesions). Study#1: At 5000 ppm, all BEs and the novel blend had anti-histomonas activity *in-vitro* ($P<0.01$) as efficient as MDZ@12.5 ppm. At 500 ppm, BEs #3, 5 and the blend inhibited the growth of *HM* ($P<0.05$) and BEs #2, 6, 9 had an intermediate ability against *HM*'s growth. Study#2: Cumulative mortality curves showed differences between the NC and the two liquid and powder forms of the tested novel blend of BEs. These last ones tended to decrease the cecal lesions *vs* NC ($P<0.11$). Only the powder form decreased ($P<0.05$) the liver lesion score *vs* NC. Further studies should be completed.

Key Words: histomonas meleagridis, growth inhibition, Turkeys, plant bio-actives, Essential oils

P226 Productive monitoring of a complete cycle of broilers subjected to an infection process with field strains of Eimeria spp. under experimental farm conditions Jenny Chaparro Gutiérrez*[1], Carolina Mesa Pineda[2], Oscar Múnera Bedoya[2], Jorge Duque Noreña[2], Sara López Osorio[1] [1]*Grupo de Investigación CIBAV, Facultad de Ciencias Agrarias, Universidad de Antioquia,* [2]*Grupo de Investigación Nutri-Solla, SOLLA S.A*

Background: the accelerated growth of the population, the global economic crisis post-Covid 19 and the problems between countries-war: Ukraine-Russia, increase the concern for the food security. Considering that the poultry industry is the main supplier of protein for human consumption in the world, with an estimated production of chicken meat for the year 2023 of 2% (102.7 million tons), it is important to continuously monitor the pathogens that affect said production, such as *Eimeria* spp. main responsible for reducing the productive parameters in broiler. Objective: to evaluate the performance of chickens infected with *Eimeria* spp. field during a production cycle under experimental farm conditions in Rionegro-Colombia. Methods: 56 Ross 308-AP males, 12 days old, were place in seven wire-floor cages, with *ad libitum access* to water and feed without anticoccidials; on day 14 an oral inoculation with 2mL of distilled water with 800 million oocysts of *Eimeria* spp. previously obtained from commercial farms and prepared in the laboratory. On days 18 to 21, 28, 35 and 42 of life, group feces were collect to evaluate OPG production and feed consumption and group weight gains were check, comparing with the Ross 308-AP 2022 guide. On days 21, 28, and 42, 2, 2, and 4 animals were randomly select per cage, to evaluate OPG and Intestinal Lesion Score. Results: OPG-groups, a gradual increase in oocyst production is evidenced between days 18 and 21 of life, reaching a maximum of 83.808 OPG on day 19, decreasing on days 28(9.216 OPG), 35(35.712) and 42(12.096); coinciding with the decrease in feed consumption(66.3g vs 90g) and weight gain during the first stage of evaluation (274g vs 304g). Productive parameters are improve on days 28, 35 and 42 of life, reaching an average final weight of 2.861g, but lower than that suggested by the Ross 308-Ap guide(3.316g). In the individual weekly evaluation, the maximum scores for intestinal lesions were on day 21 mainly *E. acervulina* (1.92±1.32). On day 28 a score of *E. maxima* and *E. tenella* were observe with a score of one. The latter remained until day 42. Conclusions: the results agree with the literature reports, evidencing how birds that are expose to challenges by coccidia can considerably compromise their productive response.

Key Words: gut health, anticoccidials, coccidia,, food safety

P227 Eimeria infection with different doses in broilers raised in floor pens for 35 days Janghan Choi*, Doyun Goo, Milan Sharma, Hanseo Ko, Thiago Belem, Jihwan Lee, Woo Kyun Kim *Department of Poultry Science, University of Georgia*

The objective of the study was to investigate the effects of Eimeria infection with different doses on growth performance, apparent ileal digestibility (AID) of nutrients, intestinal morphology, gut microbiota, Eimeria gene expression, and body composition in broilers raised in floor pens for 35 days. A total of 750 fifteen-day-old Cobb 500 male broiler chickens were randomly allocated to 5 treatments with 6 replicates of 25 birds per replicate. The five treatments included treatment 1 (T1): PBS gavage as a control; treatment 2 (T2): *E. acervulina*: 31,250/*E. maxima*: 6,250/*E. tenella*: 6,250; treatment 3 (T3): double dosages of T2; treatment 4 (T4): double dosages of T3; and treatment 5 (T5): double dosages of T4. Eimeria infection was performed on D 15 via oral gavage. Orthogonal polynomial contrasts were performed to analyze the significance of linear or quadratic effects of different *Eimeria* dosages. On D 21, growth performance parameters including body weight (BW), average daily gain, average daily feed intake, and feed conversion ratio were linearly impaired by increased dosages of Eimeria inoculation ($P < 0.01$). On D 35, the Eimeria infected groups had lower BW compared to the T1 group ($P < 0.01$). Increased Eimeria inoculation dosages resulted in a linear increase in gut permeability ($P < 0.01$) on D 20. Increased Eimeria inoculation dosages linearly decreased AID of crude protein (CP) and ether extract (EE) ($P < 0.01$) on D 21. Duodenal and jejunal villus height:crypt depth ratios (VH:CD) were linearly reduced by increased Eimeria inoculation dosages ($P < 0.01$), whereas cecal CD was linearly deepened by increased Eimeria inoculation dosages on D 21 ($P < 0.01$). Higher Eimeria dosages linearly upregulated *E. maxima* genes related to viability and sexual reproduction such as APN, EF2, GAM56 and GAM82 ($P < 0.05$). Increased Eimeria inoculation dosages tended to increase the relative abundance of the phylum Proteobacteria ($P = 0.098$) on D 21. On D 35, lean:fat ratio was linearly reduced by increased Eimeria inoculation dosages ($P < 0.05$). Eimeria infection negatively influenced growth performance and gut health in broilers in the acute phase, and the negative effects were prolonged to D 35 in floor pens.

Key Words: Eimeria, gut health, body composition, floor pen, broilers

P228 Establishment of chicken apical-out three-dimensional enteroids Bingqi Dong*[GS], Rami Dalloul *University of Georgia*

Chicken enteroids are 3D cells derived from chicken intestinal stem cells. Enteroids, the so-called 'mini-guts' contain a variety of differentiated intestinal cells, providing valuable ex vivo models that recapitulate intestinal functions and mechanisms of host-pathogen interactions. However, enteroids that grow in an extracellular matrix have a closed structure and present difficulties for gut functional studies. Our objective is to establish apical-out intestinal villi-derived enteroids from the chicken embryonic intestine and subsequent enteroid culture from cryogenic preservation. The intestinal embryonic villi from the duodenum, jejunum, ileum, and ceca were collected from 10 chicks at embryonic day 18, pooled by intestinal segments, and cryopreserved in liquid nitrogen. Cryopreserved embryonic intestinal villi were cultured by floating in growth factor supplemented media. These embryonic small intestinal villi were successfully recovered from the cryopreserved stock and grew into extensively budding enteroids and maintained for 6 days of culture. Simply floating the chicken villi from 4 intestinal segments in growth factor supplemented culture media significantly improved enteroid morphology by rapidly forming spheroid

structures on the first day of culture, and multiple budding domains were developed from the second day of culture. Numerous elongated buds were formed by days 2-3 of culture and continued to grow over the 6-day culture period in all four segment-based cultures. Apical-out chicken enteroids afford an ideal model to investigate how chicken specific intestinal tissues respond to microorganisms, parasites, and nutrients.

Key Words: enteroid, gut development, intestinal stem cells, embryonic, chicken

P229 Understanding the adhesion and invasion characteristics of avian E. coli isolated from clinical and non-clinical samples using avian macrophages cell line HD11 Priyanka Devkota*[1GS], Linan Jia[1], Xue Zhang[2], Anuraj Sukumaran[1], Aaron Kiess[3], Jeffrey Evans[4], Reshma Ramachandran[1], Pratima Adhikari[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, [3]Prestage Department of Poultry Science, North Carolina State University, [4]USDA, Agriculture Research Service, Poultry Research Unit*

Avian pathogenic *Escherichia coli* (APEC) causes respiratory and systemic disease in chickens, commonly known as colibacillosis. The bacteria enter the bloodstream via the respiratory tract making them highly susceptible to bacterial colonization and invasion. For the study of APEC-host cell interactions, the avian macrophage cell line HD11 was used which helps to mimic bacterial host interaction. The main objective of this study was to evaluate the adhesion and invasion of avian *E. coli* isolated from clinical and non-clinical samples using macrophage cell lines HD11. A total of 66 *E. coli* isolates were collected, out of which 44 were obtained from lesions of diseased broilers (considered clinical isolates), and 22 were collected from asymptomatic broilers and environmental samples (considered non-clinical isolates). Cell adhesion and invasion assays were performed on the HD11 cell line. The adhesion and invasion assay data analysis was conducted using the Mann-Whitney U test in SAS 9.4 software. The mean adhesion level of clinical strains was 10.1% whereas that of non-clinical strains was 4.6%. The *E. coli* attachment rate on chicken macrophage HD11 cell lines was significantly higher in clinical strains as compared to non-clinical strains ($P = 0.0337$). The mean invasion percentage for clinical strains was 0.125% and that for nonclinical was 0.04% ($P = 0.9648$). In conclusion, the data on the adhesion assay indicated that avian *E. coli* isolated from clinical samples were able to attach to the macrophage HD11 cell line. However, no difference was observed between clinical and non-clinical samples in terms of invasion. Further investigation and examination are necessary to study the mechanisms of macrophage adhesion and invasion among avian *E. coli* to understand its pathogenic mechanism.

Key Words: Avian Escherichia coli, Adhesion, Invasion, Macrophages cell line, Broiler

P230 Avian polyclonal B-cell lymphocytosis-a stress indication or a consequence of infection? P.F. Cotter* *Cotter Laboratory*

Polyclonal B-cell lymphocytosis has been known for nearly 40 yrs. as a benign dyscrasia. It was identified in human females who smoked but were otherwise healthy. Circulating B-cells with atypical morphological features and IgM hyperglobulinemia are characteristics. Binucleate cells of the plasmacyte series are distinguishing cytological features. In some cases, an unusual chromosome rearrangement i(3q) has been described. During a study of chicken blood films, a high frequency of binucleate plasmacytoid cells was detected. Some of these atypical cells were large enough to be classified as giant cells. Moreover, some of the individual nuclei of such cells were large enough to be polyploid [A_N ~50 μm^2]. Method: They were identified during standard differential counts of Wright-Giemsa stained peripheral blood and photographed with a light microscope. The purpose is to describe these unusual cells. Results: Binucleate cells of varying sizes ranging from ~ 80 - > ~200 μm^2 were in the blood of controls, Rispens vaccinated, MD challenged, and vaccinated/challenged SPF Leghorn chickens through 5 woa. Many of the nuclei were of unequal size; others appeared to be in the process of division by amitosis. Some displayed cytoplasmic Hofs features of cells with the potential to secrete. The patchy chromatin arrangement of most resembled cobblestones. Bacteria were often found in fields with binucleate cells. These cells were found in samples whose total white cell count (TWBC) ranged from low TWBC (leukopenia, 5K/μL) to high TWBC (leukemoid reaction, 200K/μL) levels (Ave 50K/μL). Binucleate cells were found in samples with Ave H/L ~ 0.4, falsely suggesting homeostasis. Conclusions: Binucleate cells share some characteristics with classic plasmacytes and may be indicators of infectious processes; possibly of viral origin. Their presence in a hemogram, as should other members of the plasma cell series, acts to nullify the simple H/L ratio as a stress indicator. This description of polyclonal B-cell lymphocytosis is the first report of an avian species. As Marek's disease remains important in the poultry sector these observations should have a wide interest.

Key Words: binucleate, poly B-cell lymphocytosis, plasmacyte, stress, H/L ratio

P231 Comparison of the efficacy of a live bivalent Salmonella vaccine with a monovalent vaccine against a challenge with a Salmonella Typhimurium field strain Priscilla Koerich*[1], Doris Doblies[2] *[1]Elanco, [2]Elanco Austria GmbH*

Salmonella Enteritidis (SE) is one of the most important causes of human salmonellosis worldwide, and chickens can be asymptomatic carriers of infection, therefore posing a public health risk mainly through the consumption of contaminated eggs and egg products.

In the early 1980s, an epidemic due to SE infection of breeding and laying flocks started to unfold, and vaccination of breeding and laying hens was one of the major factors to reduce the prevalence of SE in poultry, followed by a reduction in human cases.

Today, laying hens in many countries are vaccinated during rear using live vaccines, and it is desired that shedding of the vaccine has stopped by the time egg production starts. It is therefore important to use a live vaccine with a short shedding phase following the third dose during rear.

A trial was performed, comparing the shedding period of two live SE vaccines (AviPro™ Salmonella VacE and a competitor product) after the third vaccination. Both products were applied three times during rear according to manufacturer's instructions.

In group A, 25 birds were vaccinated with AviPro™ Salmonella VacE and in group B, 25 birds were vaccinated with the competitor product. 25 birds were used as unvaccinated controls. Birds were monitored until 37 weeks of age, with regular samples collected and analyzed for the presence of the respective vaccine strain. Samples included individual cloacal swabs, boot covers and eggshells.

In group A, not a single sample was positive for the vaccine strain in weeks 26, 31 and 36.

In group B on the contrary, 10% of eggshells, 10% of cloacal swabs and 11.1% of boot covers still tested positive for the vaccine strain in week 36, indicating prolonged shedding of the competitor product well into production period.

The results show that AviPro™ Salmonella VacE was not detected during production period in any sample, indicating that the product is safe to use, also from the consumer's point of view. The competitor product, on the other hand, demonstrated prolonged shedding until at least week 36 of age, with positive samples found on eggshells, cloacal swab and boot covers.

Key Words: Salmonella, public health risk, SE vaccines

P232 Understanding the adhesion and invasion characteristics of avian E. coli isolated from clinical and non-clinical samples using avian macrophages cell line HD11 Priyanka Devkota*[1GS], Linan Jia[1], Xue Zhang[2], Anuraj Sukumaran[1], Aaron Kiess[3], Jeffrey Evans[4], Reshma Ramachandran[1], Pratima Adhikari[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, [3]Prestage Department of Poultry Science, North Carolina State University, [4]USDA, Agriculture Research Service, Poultry Research Unit*

Avian pathogenic *Escherichia coli* (APEC) causes respiratory and systemic disease in chickens, commonly known as colibacillosis. The bacteria enter the bloodstream via the respiratory tract making them highly susceptible to bacterial colonization and invasion. For the study of APEC-host cell interactions, the avian macrophage cell line HD11 was used which helps to mimic bacterial host interaction. The main objective of this study was to evaluate the adhesion and invasion of avian *E. coli* isolated from clinical and non-clinical samples using macrophage cell lines HD11. A total of 66 *E. coli* isolates were collected, out of which 44 were obtained from lesions of diseased broilers (considered clinical isolates), and 22 were collected from asymptomatic broilers and environmental samples (considered non-clinical isolates). Cell adhesion and invasion assays were performed on the HD11 cell line. The adhesion and invasion assay data analysis was conducted using the Mann-Whitney U test in SAS 9.4 software. The mean adhesion level of clinical strains was 10.1% whereas that of non-clinical strains was 4.6%. The *E. coli* attachment rate on chicken macrophage HD11 cell lines was significantly higher in clinical strains as compared to non-clinical strains ($P = 0.0337$). The mean invasion percentage for clinical strains was 0.125% and that for nonclinical was 0.04% ($P = 0.9648$). In conclusion, the data on the adhesion assay indicated that avian *E. coli* isolated from clinical samples were able to attach to the macrophage HD11 cell line. However, no difference was observed between clinical and non-clinical samples in terms of invasion. Further investigation and examination are necessary to study the mechanisms of macrophage adhesion and invasion among avian *E. coli* to understand its pathogenic mechanism.

Key Words: Avian Escherichia coli, Adhesion, Invasion, Macrophages cell line, Broiler

P233 High-throughput avian pathogenic Escherichia coli (APEC) multilocus sequence typing (MLST) using Oxford Nanopore Technologies Linan Jia*[1], Mark Arick II[2], Chuan-Yu Hsu[2], Daniel Peterson[2], Jeffrey Evans[3], Anuraj Sukumaran[1], Reshma Ramachandran[1], Pratima Adhikari[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, [3]USDA, Agriculture Research Service, Poultry Research Unit*

Avian pathogenic *Escherichia coli* (APEC) causes avian colibacillosis and identifying and accurately distinguishing infectious isolates is critical to controlling APEC transmission. Multilocus sequence typing (MLST) is an accurate and efficient strain identification method for epidemiological surveillance. The traditional MLST process using Sanger sequencing is time-consuming and costly. This research aimed to develop a workflow that simultaneously sequences seven alleles of multiple *E. coli* isolates on a single MinION flow cell using the Oxford Nanopore system. 66 *E. coli* strains were isolated from broiler farms and their genome sequences were obtained using the Illumina sequencing platform. The genomic DNA of 66 *E. coli* isolates was isolated using a commercial DNA isolation kit. Seven housekeeping genes used were adenosine kinase (*adk*), fumarate hydratase (*fumC*), DNA gyrase subunit B (*gyrB*), isocitrate dehydrogenase (*icd*), malate dehydrogenase (*mdh*), purine-rich element binding protein A (*purA*), and recA bacterial DNA recombination protein (*recA*). Seven housekeeping genes were multiplex amplified using Phusion High-Fidelity PCR Master Mix. The reaction was started with 2 min at 98°C followed by thirty-five cycles of 98°C for10s, 65°C for 15s, and 72°C for 30s. The final extension lasted 5 min at 72°C. PCR amplicon yield and quality were then assessed using agarose gel electrophoresis. The amplicons of seven housekeeping genes from the same strain were pooled together and purified with AMPure XP beads. The pooled amplicons were barcoded and ligated with Nanopore sequencing adaptor using the Native Barcoding Kit (EXP-NBD196) and Ligation Sequencing Kit (SQK-LSK109) respectively, then followed by sequencing on an R9.4 MinION flow cell using the Nanopore GridION sequencer (Oxford Nanopore Technologies, Oxford, UK). After basecalling, we used Medaka workflows to determine the consensus sequences of each allele. The MLST profile of each isolate was determined using the PubMLST database. The results showed that the allele assignments obtained by Nanopore workflow were identical to those obtained by Illumina sequencing. With the advantages of high-throughput and low cost of Nanopore sequencing, this study provides a rapid and cost-effective workflow for *E. coli* typing.

Key Words: Escherichia coli, multilocus sequence typing, Oxford Nanopore, Illumina, high-throughput

P234 Relation between ISI Sys macroscopic lesion results and performance in commercial broilers flocks of South America Igor Soares*, Bruna Belote, Otto Figueiró, Elizabeth Santin *ISI Institute*

The quick identification of sanitary issues is essential to ensure a sustainable poultry production. To reach this level of efficiency, a systematic, simple, and reliable sanitary monitoring tool is necessaire. The ISI Sys® is a flock monitoring software based on the I See Inside® (ISI) methodology, which converts the birds' overall health into numbers through the scoring of 31 parameters evaluated during necropsy. Briefly, each parameter is scored (S) from zero to 3, according to the extension and intensity of the alteration, and then multiplied by the impact factor (IF), which varies from 1 to 3 according to the negative effect of the lesion on the tissue functionality. Finally, the ISI Score is assessed by the formula $ISI = \sum (IF*S)$. The present study aimed to evaluate the relation between the ISI Sys results with FCR and mortality in broilers flocks raised in South America. For each flock, five birds from 20 to 30 days of age are euthanized and submitted to necropsy. The macroscopic alterations are scored by the evaluator in the ISI Sys mobile application, and the flock ISI Score is automatically calculated. A total of 1,895 broiler flocks monitored in the period of January 5th of 2020 to October 4th of 2022 were gathered according to their ISI Score in 7 groups varying in 5 points (G1: ISI Score of 0-4; G2: 5-9; G3: 10-14; G4: 15-19; G5: 20-24; G6: 25-29; G7: ≥30). The mortality (%) and FCR were obtained at the slaughter of the flocks (mean age of 45 days) and the mean values per group were calculated. The range for mortality and FCR were, respectively, 3.6% (G1) to 6,4% (G7) and 1.622 (G1) to 1.684 (G7), demonstrating that the higher the ISI Score, the higher the mortality and worse the FCR. The FCR was worsened in 1.3% from G3 to G4 and the mortality increased 19.1% from G5 to G6, demonstrating the sensitivity of the ISI Sys to detect and predict losses in days before the end of flock. In the period, the five main alterations compromising the birds' health were (top to bottom) airsacculitis, pododermatitis, mucus or epithelial shedding in duodenum, hydropericardium, and mucus or epithelial shedding in jejunum. The results demonstrate that ISI Sys is an efficient sanitary management tool to predict zootechnical results in broiler production.

Key Words: I See Inside, Gut health, Scoring method, Benchmark, Monitoring

P235 Effect of phytogenic water supplement on Campylobacter colonization in broiler challenged with Campylobacter jejuni Hanseo Ko*[1], Emily Kimminau[2], Doyun Goo[1], Janghan Choi[1], Venkata Sesha Reddy Choppa[1], Jihwan Lee[1], Woo Kyun Kim[1] *[1]University of Georgia, [2]Purina Animal Nutrition*

The objective of this study was to evaluate the effect of phytogenic water supplement levels on growth performance, water consumption, and *Cam-*

pylobacter colonization in broiler challenged with *Campylobacter jejuni* (*C. jejuni*). Total of 360 broilers (as hatched, male Cobb 500) were allocated into five treatments with six replicate cages (12 birds per cage) for 21 d. Five treatments were comprised of non-challenged control and *C. jejuni* challenged groups with different phytogenic water supplementation levels (0, 0.100, 0.125, and 0.150 g/L drinking water). On d 0, all birds in the *C. jejuni* challenge groups and NC group were orally challenged with *C. jejuni* (0.5 ml of $7.0x10^8$ CFU/ml per bird) and the sterilized PBS (0.5 ml per bird), respectively. Growth performance, mortality, and water consumption were measured weekly. The *C. jejuni* loads in small intestinal digesta, total ceca, and liver were evaluated at 4, 7, 14, and 21 d. One-way ANOVA with Tukey's posthoc test was used for all data analyses, and the orthogonal polynomial contrast was applied to compare the *C. jejuni* challenge groups for evaluating the phytogenic water supplementation levels. During 0-7 and 7-14 d, the phytogenic water supplementation levels quadratically reduced ($P = 0.028$, and $P = 0.012$, respectively) FI. During 0–7, 0.125 g/L phytogenic water supplement improved ($P = 0.009$) FCR compared to the *C. jejuni* challenge control group (CC). The pyrogenic supplement groups (0.100 and 0.125 g/L) improved FCR during 7–21 d ($P = 0.004$) and 0–21 d ($P = 0.003$) as compared with the CC. The phytogenic water supplement reduced ($P < 0.001$) the water consumption compared to the NC and CC, during 0–21 d. The phytogenic water supplement linearly decreased ($P < 0.010$) the cecal *Campylobacter* loads at 4, 7, 14, and 21 d. At 4 and 21 d, the phytogenic water supplement linearly decreased ($P = 0.003$, and $P = 0.015$) the *Campylobacter* loads in digesta. At 7 and 21 d, the phytogenic water supplement linearly decreased ($P = 0.011$, and $P = 0.038$) the *Campylobacter* loads in the liver. In conclusion, the phytogenic water supplement inhibited *Campylobacter* colonization of broilers challenged with *Campylobacter jejuni* in a dose-dependent manner and reduced water consumption without adverse effects on growth performance.

Key Words: Campylobacter jejuni, Phytogenics, Water supplement, Broiler, Foodborne pathogen

P236 Novel natural feed additive efficacy during a clinical necrotic enteritis challenge in broilers Candice BLUE*, Bertrand Medina, Ashley WAGNER, Rami DALLOUL

This experiment evaluated the effects of a proprietary blend of phytogenics (AP, Probiotech International Inc.) on broiler chickens' performance during a necrotic enteritis challenge. Day (D) -old male chicks (n=450) were weighed, and randomly assigned to floor pens (25 birds/pen, 6 replicate pens). There were three dietary treatments: Treatment #1 (NC) consisted of chicks fed a non-medicated corn soybean meal basal diet; Treatment #2 (PC) consisted of chicks fed the basal diet with Avilamycin 20g/ton and Treatment #3 (AP) consisted of chicks fed the basal diet with AP at 400 g/ton for starter/grower and 300 g/ton finisher (AP). Growth performance, including body weight gain, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), were monitored throughout the 35-day experimental period. On D 14, all birds were orally gavaged with 5000 oocysts of Eimeria maxima. Five- and six days post *Eimeria* infection (D 19 and 20), birds were orally gavaged with approximately 1.0×10^8 CFU/bird of *Clostridium perfringens*. On D21, four birds per pen were randomly selected and sampled to determine necrotic enteritis pathology via lesion scoring. Data were analyzed using ANOVA procedure (JMP) and significance ($P \leq 0.05$) between treatments were detected by LSD test. High cumulative mortality among all treatments (16.7, 24.0 and 26.7% for NC, PC and AP, respectively) demonstrated a clinical necrotic enteritis instead of the planned subclinical form, likely due to the 1-day heat supply interruption early on. FCR for the NC (1.42) group was higher ($P \leq 0.05$) than PC (1.30) and AP (1.33), during D 0-14. AP had higher ADG (NS) than PC and NC (76.0, 71.8 and 60.4 g/d), during D 14-21. On D21, PC birds had lower (NS) jejunum and ileum lesion scores than all other ones. Over the 35-day trial, NC (2.57 kg) had lower live-weight (LW) than PC (2.87 kg) and AP (2.69 kg) groups. Only, Avilamycin reduced (NS) lesions while both Avilamycin and AP did assist to improve (NS) slaughter LW. Finally, the trial needs to be repeated to better understand how this phytogenic blend could enhance performance and reduce the impact of subclinical necrotic enteritis.

Key Words: phytogenics, broilers, necrotic enteritis, clinical challenge, avilamycin

P237 Analyzing host transcript data to improve host depletion in next generation sequencing for poultry pathogens Abhijeet Bakre*, Henry Karithii, Dawn Williams-Coplin, Edna Espinoza, David Suarez
Exotic and Emerging Avian Viral Disease Research Unit, Southeast Poultry Research Laboratory, United States National Poultry Research Center

Non-targeted next generation sequencing (NGS) of poultry samples yields unbiased information on the diversity and abundance of pathogens in clinical samples and can be useful to characterize disease outbreaks and help guide control measures like autologous vaccine development. However, host and environmental transcripts can significantly reduce the sensitivity for detecting pathogens. Previous efforts have focused on using DNA probes to selectively degrade host and bacterial rRNA using RNAse H to improve viral pathogen sensitivity. In this study we show how analysis of host transcripts can help identify the top contaminating host reads in our NGS runs to design and validate novel probes for additional host depletion. We established a transcript quantitation assay following RNAse H mediated host depletion to validate efficacy of the probes we designed and added to our pool. We showed that current probe concentrations are optimal for the RNAseH assay and demonstrated that an alternative commercial duplex buffer supports RNAseH digestion just as well as the current buffer. The open platform of DNA probes to deplete targeted RNAs with RNAse H shows that further optimization can increase sensitivity for viral pathogen detection which moves this procedure closer to routine diagnostic testing.

Key Words: Next generation sequencing, RNAse H, host depletion, DNA probes, alternative buffers

P240 Enriched culture and PCR protocols improve recovery and detection of pathogenic Enterococcus cecorum in broiler hatchery samples Grayson Walker*, M Suyemoto, Undine Taldo, Luke Borst *North Carolina State University College of Veterinary Medicine*

Enterococcus cecorum (EC) is the dominant enteric commensal in adult broilers, however; pathogenic strains of EC cause increased morbid-ity and mortality in broiler production worldwide. Gut colonization by pathogenic EC occurs as early as week 1, followed by septicemia and the development of enterococcal spondylitis. EC infection can present as pericarditis and paralytic spinal lesions which readily grow pathogenic EC when cultured on Columbia agar with colistin, nalidixic acid and 5% sheep blood (CNA) incubated at 37°C with 5% CO_2. However, the in-ability to distinguish between commensal and pathogenic EC strains has confounded the search for the source of pathogenic EC in environment or hatchery samples and is exacerbated by poor sensitivity of standard sampling and culture methods. Our previously reported comparative ge-nomic analysis of EC isolates identified a core capsule region with a large downstream variable region which is conserved in pathogenic strains but absent in commensal strains. Based on a capsular synthesis gene in the variable region, *cpsO*, and the EC species-specific *sodA* primers reported by Jackson et al., we designed a standard multiplex PCR to distinguish pathogenic EC from commensal EC strains. To allow for increased sample throughput, a real-time PCR protocol was also developed based on detec-tion of these genes. To increase the culture sensitivity, we have created selective enrichment protocols using Todd-Hewitt broth with 1% yeast extract and several antibiotics which have enabled the isolation of patho-genic EC from transfer residue and culled eggs at hatcheries. Pulsed-field gel electrophoresis and band-based cluster analysis was used to genotype recovered hatchery isolates, which identified clonal pathogenic EC strains isolated from transfer residue and a spinal lesion of a broiler on a recipi-ent farm. Although further investigation is warranted to confirm vertical transmission, the ability to distinguish pathogenic EC from the common commensal EC coupled with modified culture methods will facilitate im-proved surveillance of pathogenic EC throughout the broiler industry ide-ally leading to decreased incidence or eradication of this disease.

Key Words: Enterococcus cecorum, PCR, Hatchery, Culture methods

SCAD

P241 Description of insect populations in and around broiler breeder pullet farms with regard to potential vectors of Histomonas meleagridis Ruediger Hauck[1], Ken Macklin[1], John Beckmannb[1], Mark Burleson[2], Alan Jeon[1], Maria Tereza Terra-Long*[1] [1]*Auburn University,* [2]*Wayne-Sanderson Farms*

Histomonas meleagridis is commonly found in broiler breeder pullet farms, where it can cause disease characterized by typical lesions in ceca and liver. *Histomonas* can use the eggs of the cecal worm *Heterakis gallinarum* as vectors and reservoir. Moreover, cecal worm eggs are not only very resistant in the environment but can also be carried by arthropods. However, little is known about which arthropod species are the most robust vectors. The aim of this study was to define relevant arthropod vectors of *H. meleagridis* and *H. gallinarum* in broiler breeder pullet flocks. Over a period of one year, four broiler breeder pullet farms were sampled every four months. On each farm, three types of traps were set inside and outside two houses and remained for a period of one week. Trapped insect specimens were morphologically identified at order level. Fifty-one hundred ninety-nine individual insects from 11 orders were counted and attributed to 319 different types. Traps kept outside had a higher abundance of arthropods with 3459 individuals from 10 orders than inside traps with 1740 individuals from 8 orders. Alpha diversity of the insect populations was significantly higher in traps kept outside and beta diversity analysis showed that individuals clustered together based on their location and seasons. Diptera was the most common order and darkling species the most common type with 1806 specimens. 99% of the darkling beetles were found inside the houses and only one was found on a hanging glue trap, which suggests that darkling beetles are unlikely to carry diseases from house to house or even farm to farm. There was an outbreak of Histomoniasis at one farm during the second collection. PCR analysis showed that to 50% of beetles were positive for Histomonas DNA four months after the outbreak. *H. gallinarum* DNA was sporadically detected in beetles on all farms. In addition, 170 other insects were selected for DNA extraction based on their detection at several time points and inside as well as outside the houses. Out of those, 4 samples were positive for Histomonas and one for *Heterakis.* Three of these insects were also collected 4 mounts after

the blackhead outbreak. The role of these insects as potential vectors for *Histomonas* should be further investigated.

Key Words: Histomonas, Heterakis, broiler breeders, insect vectors

P242 Evaluation of two different day-of-age IBV spray vaccination programs on early protection against DMV/1639 in Leghorn pullets Fernando Ruiz-Jimenez*, Kalen Cookson, Manuel Da Costa, John Dickson, Jon Schaeffer *Zoetis - US Poultry*

Early protection against the DMV/1639 strain of the Infectious Bronchitis Virus (IBV) is critical to prevent the development of false layer syndrome in layers. The administration of monovalent IBV vaccines via cabinet sprayer at day-of-age in the hatchery has significantly reduced the incidence of this condition in the field. While day-of-age use of serotype combinations has been shown to improve cross-protection by 3-4 weeks of age, it is unclear if presenting more than one IBV serotype during this primer vaccination would further improve the protection against early DMV/1639 infections. In this study, we compared a monovalent (Mass only) vs. a bivalent (Mass+Ark) post-hatch IBV program to evaluate their protection of internal organs to a DMV/1639 challenge at 10 and 21 days of age. The bivalent program showed significantly better protection against colonization based on DMV/1639 PCR recovery from all organs, demonstrating that it is beneficial to present the Mass+Ark serotypes instead of Mass alone at day-of-age vaccination.

Key Words: Bronchitis, Vaccine, DMV/1639, Hatchery, Layers

P243 An update of wild type IBDV from diagnostic bursal surveys of broiler flocks in the United States since 2009 Therese Anderson*, Andrew Barker, Jennifer Strickland, Kalen Cookson, Manuel Da Costa, Jonathan Shaeffer *Zoetis*

Infectious Bursal Disease Virus (IBDV) is a highly infectious viral disease that infects young chickens and causes notable immune suppression in birds 3 weeks of age or younger. IBDV frequently mutates due to being an RNA virus, and this can lead to a wide range of antigenic variation in the field. The data herein summarize the wild type IBDV isolations from 2009 to 2022 with the relative strain shifts over time. Survey Design: Typically five to six bursas per flock were collected for each survey submission. Samples were tested by qRT-PCR for the presence of IBDV. Positive samples were confirmed by amplifying the VP2 region via RT-PCR and electrophoresis, followed by genetic sequencing of the VP2 hypervariable region. Since 2009, over 500 broiler flock samples have been sequenced and divided into the following categories: AL2, Del-E, Group-6, T1, "Hybrids", and Other. Results: Strain frequencies were grouped into three time blocks; 2009-2014, 2015-2020, and 2021-2022. During each date range, AL2 was the most prevalent strain (48.9%, 50.8%, 30.9%, respectively). However, in recent years there was a shift where Group-6 viruses started occurring much more frequently (2009-2014=14.8%; 2015-2020=16.8%; 2021-2022=24.8%). Discussion: Despite AL2 continuously being the most prevalent strain found in surveys (44.7% overall), in the past 5 years there has been an increased detection of other strains. Group 6 viruses are the most prevalent on the East Coast and Deep South, whereas AL2 is still predominant in the rest of the country. Observing regional differences will be important as we continue to survey IBDV prevalence across the country.

Key Words: IBDV, Survey, Diagnostic, AL2, Group-6

P244 Molecular serology and virulence characterization of avian pathogenic Escherichia coli (APEC) recovered from Georgia poultry Muhammad Haque*[GS], Klao Runcharoon, Bellanirys Garcia, Meaghan M. Young, Catherine M. Logue *Department of Population Health, College of Veterinary Medicine, University of Georgia*

Avian Pathogenic *Escherichia coli* (APEC) is the causative agent of avian colibacillosis a disease of production birds that causes significant losses annually. However, there are diverse strains and isolates of *E. coli* which remain a problem for poultry producers and consumers including public health professionals. In this study, we performed PCR analysis to assess the O-types (serogroups) for all *E. coli* from clinical diagnostic cases of colibacillosis and we also assessed these isolates for genes associated with virulence that typify the APEC pathotype. A total of 145 isolates were tested from poultry submitted for analysis of virulence associated genes and molecular serology. These isolates originated from birds identified as broilers, broiler breeders, pet/hobby birds and game birds. Approximately 54% of APEC isolates could not be O-typed; of the remaining 46% (typeable) the common O-types identified included O25 (43%), O78 (28%), O86 (17%), O1 (11%), O8 (9%) and O91 (6%). The serogroups O154, O104, O155, and O65 were detected in less than 2% of isolates. When examined by organ/tissue source, for broiler, pet hobby birds and broiler breeder the most common sources were liver, body cavity, yolk sac and heart. A lower prevalence (2%) was associated with lungs, air sac, joints and peritoneum. A multiplex PCR panel targeting nine virulence genes was used to screen the 145 avian *E. coli* isolates. From this analysis 6 genes are plasmid associated and typify the APEC pathotype, while two other genes are associated with the chromosome. Our data found APEC virulence genes ≥4 in 68% of all isolates and were most commonly found in broiler breeder pullet/cockerel birds and broilers, while the pet hobby birds (5%) were less associated with pathogenic strains. This data demonstrates the diversity of APEC causing significant losses to the poultry industry in Georgia and also remains an important concern for public health professionals. This analysis may be helpful to clarify the potential role of APEC in poultry outbreaks, and human disease and may be useful to identify effective targets for vaccine development and therapeutics to effectively control avian colibacillosis.

Key Words: APEC

P245 Temporal Salmonella prevalence and quantity in broiler production Emily Cason*[1GS], Tomi Obe[2], Nikki Shariat[1] *[1]Department of Population Health, University of Georgia, [2]Department of Poultry Science, University of Arkansas*

Despite reduced *Salmonella* incidence in broilers at processing, an estimated 16.8% salmonellosis cases are linked to consumption of contaminated broilers. Reducing pre-harvest *Salmonella* contamination of poultry is critical to lowering the burden of *Salmonella* on broilers arriving for processing. To effectively implement pre-harvest *Salmonella* screening and interventions, there is a need to determine *Salmonella* prevalence and to identify any temporal changes in *Salmonella* shedding. This study was designed to evaluate *Salmonella* prevalence and quantity in broilers during production. We utilized bootsocks to assess environmental *Salmonella* at days 0, 6, 12, 18, 30, 42, 54, and 66 via selective enrichment and plating, with additional quantification data collected using the BAX® qPCR assay. Chick baskets (one composite swab of ten baskets per house) were also analyzed to determine whether incoming chicks were colonized with *Salmonella* pre-placement. Samples were collected from four farms across two integrators (A and B), with three houses sampled on each farm. For all houses, 83.3% (10/12) were *Salmonella* positive before chick placement, showing that residual *Salmonella* from previous flocks was present. A total of 41.7% (5/12) chick baskets were positive, suggesting that upstream contamination may contribute to *Salmonella* colonization within a flock. For all houses, *Salmonella* prevalence peaked at 100% between 12-30 days of production and decreased during the final two weeks of production. By the last day of production, 41.7% of houses remained *Salmonella* positive. A generalized linear model showed significant correlation between day of production and *Salmonella* prevalence ($p = 0.00159$). The *Salmonella* level was below the level of detection of the assay for the majority of *Salmonella* positive samples. For Integrator A, levels peaked at days 6-18 and day 66; for Integrator 2, *Salmonella* levels were only detected at day 42, despite 100% prevalence at earlier time points. Collectively, this data shows that *Salmonella* shedding during broiler growth

is dynamic. Importantly, *Salmonella* detected mid-way through growth may not best reflect *Salmonella* present on broilers at harvest, which might impact the timing of pre-harvest *Salmonella* monitoring and interventions.

Key Words: Broilers, Salmonella, Pre-harvest, Food safety, Litter

P246 Effect of a Saccharomyces cerevisiae postbiotic on Salmonella Enteritidis colonization of cecal and ovarian tissues in commercial layer pullets William Chaney*[1], Devin Hanson[1], Hannah McBride[2], George Girgis[2] *[1]Diamond V, Cargill, Inc., [2]Nevysta Laboratory*

Feed additive technologies are often evaluated as pre-harvest food safety interventions against *Salmonella enterica* colonization in poultry. Efficacy may be influenced by factors including, but not limited to, bird age, *Salmonella* serovar, exposure dose or route, exposure duration, feed composition, mechanism of action, sample type or collection day among others in both controlled and real-world research. The purpose of this study was to evaluate the effects of postbiotic (Diamond V Original XPC) inclusion on the colonization of cecal and ovarian tissues of commercial pullets directly and horizontally exposed to *Salmonella* Enteritidis. Four hundred-eighty commercial, day-of-age W36 chicks were randomly allotted to 60 cages per treatment in two identical BSL-2 isolation rooms (Iowa State University) with four birds per cage and fed control (CON) or treatment (TRT) diets for the duration of study. At 16 weeks, two birds per cage were directly challenged via oral gavage with 1.1 x 10^9 CFU of a nalidixic acid resistant *Salmonella* Enteritidis (SE) isolate. The remaining two birds in each cage were indirectly exposed to the SE challenge. At 3-, 7- and 14- days post-challenge (DPC), 20 cages per group were harvested and sampled for SE colonization and load. No significant differences were observed between groups for SE prevalence in the ceca or ovary tissues of directly challenged birds. For the contact exposed cohort, SE cecal prevalence was significantly lower for TRT at 7 DPC (50.0%) vs. CON (72.5%) (P=0.037) and, likewise, demonstrated significantly lower mean SE cecal load (1.69 Log_{10}) vs. CON (2.83 Log_{10}) (P=0.0053). At 14 DPC, no significant differences were detected but ~10% fewer individuals remained positive in the TRT group vs. CON. Postbiotic supplemented diets may be a useful tool for mitigating SE colonization and promoting clearance in horizontally exposed pullets and may further support pre-harvest food safety strategies.

Key Words: Layers, Salmonella, Postbiotic, Preharvest Food Safety, Feed Additive

P247 Assessing incidence of house-to-house Salmonella transmission among breeder flocks by deep serotyping Amy Siceloff*[1GS], Doug Waltman[2], Nikki Shariat[1] *[1]University of Georgia, [2]Georgia Poultry Laboratory Network*

There is an increasing need to control *Salmonella* during poultry production as mitigation at processing is not sufficient. Successful control relies on a robust surveillance platform. Typical *Salmonella* surveillance identifies the most abundant serovars in a sample and lacks the resolution to characterize multiserovar populations, demonstrating a need for deep serotyping. CRISPR-SeroSeq is an amplicon-based next-generation sequencing approach that uses the native CRISPR spacer sequences in *Salmonella* to detect and determine the relative frequency of multiple serovars in a sample.

Each month, ~1,900 routine surveillance samples from breeder flocks in the southeast are screened for *Salmonella*. Over seven months, an unbiased subset of *Salmonella*-positive samples were analyzed each week by CRISPR-SeroSeq from the overnight tetrathionate enrichment cultures. CRISPR-SeroSeq libraries were prepared and sequenced from 128 samples, representing 64 pairs of breeder houses across 13 different companies. On average, traditional culture methods found a single serovar (1.1) per sample and found 20 different serovars across the dataset. CRISPR-SeroSeq was able to detect an average of two serovars (1.5) per sample and found 24 serovars. In 23% (29/128), we detected two or more serovars, and as many as five serovars were identified in one sample. Bray-Curtis statistical analysis showed that 72% (46/64) of paired samples had similar serovar composition, while 22% (14/64) contained different serovars; the remaining samples had serovar content that was a moderate match (6.3%; 4/64). In comparison, conventional serotyping showed that 66% (42/64) of paired samples had the same serotype.

These results demonstrate that multiserovar populations frequently occur in poultry, and the increased resolution of deep serotyping reveals a greater level of complexity within house-to-house transmission than is observed by traditional *Salmonella* isolation. This suggests that pathogen transmission occurs at the farm level rather than the house level, and highlights the need to maintain on-farm biosecurity to limit *Salmonella* introduction and transmission between houses.

Key Words: Breeders, Salmonella, Surveillance, Preharvest, CRISPR-SeroSeq

P248 Effects of dietary supplementation of Provia Prime™ on intestinal permeability and immune response in broiler chickens during a coccidia challenge Saheed Osho*, Kevin Bolek, Kari Saddoris-Clemons, Brooke Humphrey, Miriam Garcia-Orellana *Phibro Animal Health Corporation*

Maintaining intestinal health supports optimal gut function and influences overall performance of broilers. Provia Prime™ (PP) contains a unique combination of four strains of *Bacillus spp* selected to support a healthy gut which may help improve performance. The aim was to determine the effects of PP supplementation on intestinal health and immunity of broilers challenged with a mixed coccidia infection during peak (d0 to d6 post-challenge (PC)) and recovery phases (d6 to d13 PC). A total of 120 male, 4d-old Ross 708, broiler chicks were allotted to 3 treatment groups (8 replicate cages; 5 birds/cage) in a randomized complete block design. Treatments included a non-challenge (NC), a cocci challenge (CC), and CC fed PP (5×10^5 CFUs/g of diet; PP). Diets were corn-soybean meal-based and fed in mash form. At 11d post-hatch, all birds except for NC were orally gavaged with 3× the recommended coccidia vaccine (Coccivac B52®) using 25 doses/kg BW. On d6 and d13 PC, birds were orally gavaged with fluorescein isothiocyanate conjugate dextran (FD4). Plasma and mid-jejunum tissues were collected 2h later. On d6 PC, duodenal lesions from 2 birds/cage were scored and excreta was collected for oocyst enumeration. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated over the experimental period. Data were analyzed with GLIMMIX procedure of SAS. During the peak phase, CC birds had reduced BWG (23%) and FCR (15%) compared to NC birds ($P<0.05$), while birds fed PP had similar BWG and FCR compared to NC ($P>0.05$). On d6 PC, CC birds had higher lesion scores and oocyst shedding, 2× increase in serum FD4, and higher jejunum IL-10, and IFN-γ mRNA compared to NC ($P<0.05$). Birds fed PP had decreased lesion scores and oocyst shedding, and reduced plasma FD4 compared to CC birds ($P<0.05$) and similar IL-10 and IFN-γ mRNA to CC (P>0.05). On d13 PC, CC birds had increased plasma FD4 and higher jejunum IL-10 and IFN-γ mRNA compared to NC birds ($P<0.05$). Birds fed PP had lower plasma FD4, jejunum IL-10 and IFN-γ mRNA compared to CC birds ($P<0.05$), but similar IL-10 and IFN-γ mRNA to NC ($P>0.05$), except for IFN-γ. This study confirms PP helps improve intestinal health and alters mucosal immune responses during peak and recovery phases following a mixed coccidiosis challenge.

Key Words: broiler chickens, coccidiosis, cytokines, intestinal permeability, Provia Prime

P249 Effect of a formaldehyde-based feed sanitizer on the control of necrotic enteritis in broiler chickens Callie Selby*[1], Nicole Holcombe[1], Cheryl Shaffer[1], Enrique Montiel[1], Dan Moore[2] *[1]Anitox Corporation, [2]Colorado Quality Research, Inc*

Necrotic Enteritis (NE) is one of the most economically harmful diseases to the poultry industry. This disease, caused by the bacterium, *Clostridium*

perfringens, is ubiquitous with the environments in which commercial poultry are reared. Because the pathogen is a natural habitant of the GI tract of poultry, predisposing factors that favor *C. perfringens* growth are often associated with the pathogen's ability to produce NE in broilers. Antibiotic-growth promoters were previously utilized to control NE in chickens. However, with recent bans on AGP usage, other prevention strategies are being investigated. The use of a feed sanitizer to control or prevent NE in broiler chickens has not previously been studied. Feed sanitizers have been utilized for decades to reduce the colonization of pathogenic bacteria stemming from feed ingredient contamination. In the present study, broiler chickens were vaccinated against coccidiosis on day-of-hatch and challenged with *C. perfringens* on d17. Birds were fed a starter, grower, or finisher diet sanitized with Termin-8, a formaldehyde + propionic acid + terpene -based feed sanitizer. Under challenge conditions, broiler body weight gain was significantly (P<0.0001) increased in birds fed a sanitizer diet versus those fed a control diet. Significant reductions in feed conversion (P<0.0001), mortality (P<0.0001), oocyst shedding (P=0.0001), and NE lesion scores (P=0.0014) were also observed in broilers fed a sanitized diet under challenge conditions. These data suggest that feeding broiler chickens a diet sanitized with Termin-8 feed sanitizer can reduce the impact of NE, further increasing performance and livability in broiler chickens

Key Words: Broiler, Necrotic Enteritis, Feed Sanitizer, Formaldehyde

P250 Evaluating mRNA abundance of host defense peptide genes in heritage and modern broiler breeds during subclinical necrotic enteritis Candice Blue*[GS], Laney Froebel, Rami Dalloul *University of Georgia*

In broilers, necrotic enteritis (NE) is caused by *Clostridium perfringens* toxins following damage caused to the gut by a predisposing factor such as *Eimeria*. Variation in susceptibility to NE can be contributed to genetic differences among different breeds. In this comparative study, we evaluated mRNA abundance of host defense peptides in the spleen and cecal tonsils of Athens Canadian Random Bred (ACRB) and Cobb broilers in response to subclinical NE. The design was a 2×2 factorial with breed (ACRB and Cobb) and challenge (no challenge and NE) as main factors. On day of hatch, 96 male chicks (48 ACRB and 48 Cobb) were allocated to the four experimental treatments with 8 replicate cages and 3 birds/cage. On d 14, birds in the NE-challenged groups were orally gavaged with ~3,000 *Eimeria maxima* sporulated oocysts followed by two doses of approximately 1×10^8 CFU of *C. perfringens* on d 19 and d 20. On d 21, spleen and cecal tonsil samples were collected to measure mRNA abundance of avian beta defensins (AvBD)-8, AvBD-10, AvBD-13, and liver-expressed antimicrobial peptide (LEAP)-2. Data were analyzed by 2-way ANOVA (JMP Pro 16) and significance ($P\leq0.05$) between treatments was evaluated by LSD test. In the spleen, mRNA abundance of AvBD-8, AvBD-13, and LEAP-2 in the NE-Challenged Cobb birds was significantly greater compared to all other treatments ($P\leq0.05$). While mRNA abundance of AvBD-10 was significantly greater in the NE-challenged ACRB birds. When comparing ACRB and Cobb birds, mRNA abundance of AvBD-8 was significantly greater in cecal tonsils of the ACRB compared to Cobb birds. mRNA abundance of AvBD-10, AvBD-13, and LEAP-2 was not significantly different (P>0.05) in the cecal tonsils between treatment groups. Previous analysis from this study showed that proinflammatory cytokines (IL-1β, IL-18, and TNF-α) and chemokine ligands (CCL5, and CCL20) were greater in Cobb broilers. These results indicate that systemic expression of these antimicrobial peptides could be important in the regulation of inflammation and host innate immunity to this infection.

Key Words: Necrotic enteritis, Avian beta defensins, ACRB, LEAP-2, Broilers

P251 Impact of quorum sensing inhibition peptides on Clostridium perfringens quorum-sensing regulated virulence factors. Thomas Coulson*[1], Ana Jaramillo[1], Laura O'Neill[1], Alain Labbe[1], Charles Hofacre[2] *[1]MicroSintesis Inc, [2]Southern Poultry Research Group, Inc*

Clostridium perfringens is the causative agent of necrotic enteritis (NE) in broiler chickens. It can synthesize multiple toxins that contribute to pathogenesis. Bacteria use quorum-sensing (QS) to coordinate their behavior through regulation of gene expression, including the virulence genes responsible for NE. MicroSintesis has a technology based on quorum sensing inhibition peptides, Nuvio Poultry, which reduces virulence genes expression *in vitro*. In this study, we investigated the effects of Nuvio on virulence in *C. perfringens in vitro*. Expression of key QS-regulated toxin genes (*pfoA, plc, cloSI*) is reduced 59.6-91.0% in Nuvio treated cultures.

Furthermore, the NE toxin gene *netB* is decreased 61.9% in a clinical strain of *C. perfringens.* Nuvio reduced hemolysis of red blood cells by *C. perfringens* by 95.7% *in vitro*.

A clinical study was performed to determine the effects of the products on NE. Broilers were treated with Nuvio Poultry in drinking water prior to *E. maxima* oocyst challenge and *C. perfringens* administration. The NE mortality was reduced from 16.4% in control to 6.7% ($p<0.05$) with Nuvio. Animals were sacrificed for lesion scoring 3 days after challenge and samples from the intestinal lumen were analyzed for microbiome composition. Microbiome diversity indices like Shannon, Simpson and Chao1 were calculated. Shannon showed no significant differences ($p> 0.05$) for the untreated group (2.22) and the Nuvio treatment group (2.27). At phylum level, both groups shared the most prevalent phyla (Firmicutes and Proteobacteria), with a relative abundance of 90.6% and 9%, respectively, for the untreated group and 88% and 9.7%, respectively for the treated group. No statistical differences were observed ($p > 0.05$). At the species level, *Lactobacillus sp*. and *C. perfringens* were the most prevalent in both groups, with a relative abundance of 20% and 19%, respectively, for the untreated group and 23% and 11%, respectively, for the treated group. No statistical differences were observed ($p>0.05$). Conclusion: Nuvio poultry demonstrates potent anti-virulence activity against *C. perfringens in vitro*. Furthermore, Nuvio does not disrupt the composition of the intestinal microbiome while simultaneously reducing disease symptoms such as NE induced mortality.

Key Words: virulence genes, clostridium perfringens, hemolysis, microbiome, quorum sensing

P252 Higher stocking density as a model for applied performance assessment of products Matthew Jones*, Virginia Baxter, Charles Hofacre *Southern Poultry Research Group*

Many feed additives being evaluated for efficacy on poultry performance and health are hypothesized to improve the host's ability to cope with stress. While this may be observed in the field where the stressors are more moderated, controlled challenges are often too severe and overwhelm efficacy of these products. Alternative challenge models which create more moderated stress while not masking a product's efficacy are needed to evaluate these interventions. In the current model, bird density was used in combination with reused litter to assess performance under these conditions. Prior to placement all birds were sprayed with a commercial coccidiosis vaccine at manufacturer recommendations. A low density (LD) group was placed at 23 birds (1.09 ft^2/bird) per pen and 32 birds (0.78 ft^2/bird) per pen were placed in the high density (HD) treatment. Each treatment consisted of eleven replicate pens of male Ross broiler chicks. Body weight and feed intake were calculated on day 0, 14, 28, and 42 to evaluate performance metrics. Data was subjected to an ANOVA and means were separated using Tukey HSD tests. On DOT 14, the birds would have experienced the peak cycling of the *Eimeria* vaccine. The HD group had greater FCR and lower body weight than the LD group in this interval ($P < 0.05$). At 28 days there was no difference in FCR between groups. The body weight was lower in the HD group (1.620kg)

than the LD group (1.670kg). On day 42, non-adjusted FCR was lower in the LD group than the HD group (P=0.039); however, adjusted FCR was not different (P=0.101). Body weight gain was greater in the LD group (3.164 kg) compared to HD (3.036kg) ($P < 0.05$). Total mortality was not different between the two groups (P=0.158). In experiments with greater mortality in challenged groups, at 42 days there is often no difference in performance between the challenged and unchallenged groups. This observation has been attributed to the increase in space within groups that experience greater mortality early. The current study supports that finding in that the lower density exhibited greater growth metrics than the higher density group. The increased density under controlled conditions creates more pressure which may be ideal for assessing product efficacy during applied stressors.

Key Words: Broiler, Stocking Density, Intestinal Challenge, Product Efficacy

P253 In vitro testing of an alpha-monoglyceride blend's effectiveness against inhibition of poultry pathogens Jennie Baxter*[1], Charles Hofacre[1], Matthew Jones[1], Stacie Appleton[2], Kevin Watkins[3] *[1]Southern Poultry Research Group, Inc., [2]Alura Animal Health and Nutrition, Inc., [3]FoodFirst, LLC*

A recent interagency report from FSIS and CDC attributed 23% of foodborne *Salmonella* illnesses to poultry. In addition, most poultry companies are now following raised-without-antibiotic guidelines which can result in greater intestinal bacteria challenge. This has increased focus on food safety and intestinal health and resulted in many new interventions. FractalÔ is a plant-sourced blend of primarily short- and medium-chain alpha monoglycerides and glycerol. Organic acids and their derivatives are reported to enhance intestinal integrity, optimize production performance, and reduce pathogen load in poultry. *In vitro* testing is a useful way to determine a product's effectiveness to inhibit poultry pathogens. This method is helpful in determining a products potential *in vitro* effectiveness before moving to larger scale *in vivo* testing. In this *in vitro* assay, FractalÔ, was tested against multiple isolates of S*almonella*, *Escherichia coli*, *Enterococcus cecorum*, *Campylobacter jejuni*, and *Clostridium perfringens*. The bacterial isolates were grown then added into a 96 well microtiter plate with multiple dilutions of the test product. Each plate was incubated and read kinetically (630nm) with a 96 well optical density plate reader at different time periods over 24-48 hours while maintaining temperature at 37° C. All bacterial isolates were grown under different conditions to emulate the growth requirement for each organism. Negative and positive controls were included in each assay and results were analyzed and graphed to evaluate whether FractalÔ was effective at slowing or inhibiting the growth of the test bacteria. In this study, FractalÔ greatly reduced the growth of *S. typhimurium, S. kentucky, S. infantis, S. heidelberg, S. enteritidis, E. coli* (APEC), *C. perfringens* (2 Net B isolates), *C. jejuni* and *E. cecorum* (4 isolates). The effectiveness of the inhibition after 6 hours tended to be inclusion-level dependent. While lower inclusions did not inhibit the growth of the bacterial isolates as effectively as the higher inclusions of FractalÔ, all inclusion levels showed inhibition against all isolates. The results of *in vitro* screening suggests this monoglyceride blend is a good candidate for applied food safety and intestinal health applications.

Key Words: In Vitro, Monoglyceride Blend, Salmonella, Clostridium, Other bacteria

P254 Effect of an antibiotic and bioactive clay on live performance in broilers challenged with Clostridium perfringens Charles Hofacre*[1], Matthew Jones[1], Gregory Mathis[2], Ran Song[3], Kim Friesen[3], Chet Wiernusz[3] *[1]Southern Poultry Research Group, Inc., [2]Southern Poultry Research, Inc., [3]Nutriquest*

Certain naturally occurring clay deposits contain antimicrobial properties against bacteria as demonstrated in in-vitro studies. NutriQuest has identified a unique clay with bioactive minerals (NQ-A) which establishes a geochemical cycle between iron, pyrite and smectite. The cycle causes a release of radicals that inhibits bacterial pathogens. In addition to the clay the product contains a combination of *Bacillus*-DFMs. In recent years, the U.S. broiler industry has shifted to predominantly antibiotic free production which has created a need for additional solutions to help mitigate necrotic enteritis. The objective of two floor pen studies was to evaluate the use of NQ-A in broilers challenged with *Clostridium perfringens*. Birds were fed a commercial-type broiler starter DOT 0-14 (crumble), grower 14-28 (pellet) and finisher 28-41/42 (pellet). Both trials were conducted in a completely randomized block experiment and allocated to 3 NE challenged treatments: 1) no feed additive; 2) bacitracin methylene disalicylate (BMD 50 gm/ton); 3) NQ-A. The flock in Trial 1 was placed on new litter and challenged with coccidiosis and *Clostridium perfringens* in a necrotic enteritis (NE) challenge model. Broilers fed NQ-A at 1 kg/MT had decreased adjusted FCR, non-adjusted FCR and increased body weight gain at 41 days of age compared to the no feed additive treatment group ($P < 0.05$). Live performance results were similar in the NQ-A and BMD fed groups. Birds in the second trial were placed on used litter from a previous NE challenge trial. Trial 2 results were similar to Trial 1. Broilers fed NQ-A at 0.75 kg/MT had decreased adjusted FCR ($P < 0.05$), non-adjusted FCR and increased ($P < 0.05$) body weight gain at 42 days of age compared to the no feed additive treatment group. In conclusion, the clay and DFM product aided broilers in a NE challenge by decreasing the negative effects of *Clostridium perfringens*

Key Words: broiler, necrotic enteritis, growth performance, antimicrobial, clay

P255 Prevention and control of necrotic enteritis with an essential oil (OregoStim™) and an organic acid (pHorce™) Charles Hofacre*[1], Laura Corbett[2], Matthew Jones[1], Wendy Wakeman[2], Elizabeth Krushinskie[3] *[1]Southern Poultry Research Group, Inc., [2]Anparío, [3]FSRM Consulting LLC*

Necrotic enteritis (N.E.) caused by both coccidia and *Clostridium perfringens* (C.P.) results in broiler mortality and reduces body weight and feed efficiency (FCR). Oregano essential oil, in a highly purified product, OregoStimä, and a blend of formic and propionic acid in a micropearl carrier, pHorceä, were evaluated in an N.E. challenge model. The 1800 Ross male broilers were coccidia vaccinated by coarse spray at day of age. The birds were placed in 36 floor pens. Nine replicate blocks of 4 treatments were evaluated: Untreated-challenged; Essential oil (1 kg/MT); Essential oil (0.5 kg/MT); Essential oil (0.3 kg/MT) with organic acid blend (0.5 kg/MT). At peak of coccidia vaccine cycling, C.P. #6 (1.0×10^8 CFU/ml) was administered to all birds by applying C.P. culture on feeders. The statistical analysis used ANOVA with comparison of means using LSD at both 0.05 and 0.10. The N.E. lesions compared by Kruskal-Wallis procedure and means were separated using Dunn's Post Hoc test at 0.05. The C.P. challenge resulted in $9.33\%^{A}$N.E. mortality for challenge control and all treatments were numerically lower at $P = 0.05$ and all were significantly lower at $P = 0.10$. N.E. mortality in these groups were: OregoStim (1 kg) 5.78%; OregoStim (0.5 kg) 6.00%; and OregoStim and pHorce 5.56%. There was a numerical reduction in N.E. lesion scores in the OregoStim (1 kg) 0.44^{B}versus challenge control 0.78^{AB}. The OregoStim (1 kg) had the lowest FCR (1.615^{A}) versus challenge control (1.639^{A}). At 42 days, all treatments had body weights that were numerically heavier than the challenge control and all had numerically lower FCR. Overall, the oregano product had a significant ($P = 0.10$) impact on preventing clinical necrotic enteritis and consistent numerical prevention of the negative effects of *Clostridium perfringens* effect on the performance parameters of body weight and FCR.

Key Words: oregano, organic acid, necrotic enteritis, performance

Environment, Management: Animal Well-Being (stress, welfare, behavior)

P256 Monitoring mislaying behaviors of cage-free hens with deep learning Ramesh Bist*[GS], Xiao Yang, Sachin Subedi, Lilong Chai *Department of Poultry Science, University of Georgia*

While cage-free housing allows hens to perform more natural behaviors such as foraging and dustbathing on litter floors, a particular challenge is floor egg-laying behavior (FELB). Floor eggs mislaid eggs on the litter floor, have a higher chance of being contaminated and damaged, and thus result in economic loss and egg safety concerns. Egg farm management strategies such as light intensity, nests, perches, and robots have been tested to control floor eggs. The primary objectives of this research were to develop and test a new deep-learning model to detect floor-laying behaviors and evaluate the model's performance in four research cage-free facilities. Video data were converted into images with the help of Free Video to JPG Converter App, then labeled using the image labeler website (Makesense.AI). About 80% of images were used in training, and 20% of images were used for validation. Images were analyzed using Google Colab (Python 3 google compute engine backend 10 GB GPU, 12.68 GB RAM, & 78.19 GB disk). For imaging processing and analysis, training images were labelled as FELB or not based on manual observation. Statistical analyses were done using one-way ANOVA and compared using the Tukey HSD method using R for predicting floor-laying and non-floor-laying behaviors at a significance level of $p < 0.05$. Results show that the newly developed deep learning had reached 90% or higher accuracy. In addition, the precision, and recall of some tracking reached 99% or 100%. The newly developed and trained model has acceptable accuracy in detecting FELB in research houses with 800 Hy-Line W-36 hens ($p < 0.01$). In the future, we will test the model in commercial cage-free houses with thousands of hens.

Key Words: Laying hen, Cage-free system, Precision farming, Animal behavior, Floor egg

P257 Detecting Floor Eggs with Machine Vision Technologies Sachin Subedi*[GS], Ramesh Bist, Xiao Yang, Lilong Chai *Department of Poultry Science, University of Georgia*

The European Commission set a goal to eliminate cages for farmed animals by the year 2027. In the USA, the primary restaurants or grocers have pledged to buy cage-free (CF) eggs only by 2025 or 2030. A particular challenge for CF production is floor eggs management. Floor eggs have a high chance of contamination as they are in direct contact with litter and are often get pecked by birds resulting in egg-eating behavior. The manual collection of eggs is laborious and time-consuming. The implementation of precision poultry farming technology to detect floor eggs is necessary. The objectives of this study were to develop a machine vision method to track eggs on litter floor and test the performance under research house conditions. A new deep learning model "YOLOv5-egg" network was developed to detect floor eggs of laying in four research cage-free facilities at the University of Georgia. A dataset of 1200 images (i.e., 750 for training, 250 for validations, and 200 for testing) from 30-35 weeks of age was created. The one-way ANOVA and Tukey HSD analysis were conducted using R to determine whether there are significant differences between predicted number of floor eggs. The difference was considered significant at $p<0.05$. After training, the YOLOv5-egg model can automatically and effectively detect floor eggs with a predicted bounding box, including an objectness score ranging from 0 to 1 in test images. The precision is 0.89, the ratio of correctly predicted positive observations of egg to the total positive observations predicted. The recall is 0.87, the ratio of correctly predicted positive observations to all observations in the actual class of egg. The mean average precision (mAP) is 0.9, which is a change in the precision of egg detection with a change in the recall. Our datasets were trained with a batch size of 16 for 200 epochs using Virtual Machine GPU 3.1 provided by Oracle Cloud Infrastructure (OCI) that contains 6 Oracle CPUs and 90 GB of memory. The detection of single image time by YOLOv5-egg was 0.021 seconds, and the model size was 14.4 MB. Future studies are guaranteed to test the system in commercial houses.

Key Words: Cage-free system, problematic behavior, mislaid eggs, artificial intelligence

P258 Impact of platforms on broiler density and behavior in a commercial house Yudith Hernandez-Valencia*[UG], Monica Franco, Katy Tarrant *California State University, Fresno*

Platform enrichments serve as a method to encourage natural behaviors to promote animal well-being in conventional broiler houses. Yet, much remains unknown about the impact platforms can have on production traits, behavior, preference, and best practices in using such enrichments. In this study, we aim to understand how the presence of platforms impact bird density and select behaviors in a conventional broiler house. Twelve 2.32 m^2 plots were mapped out down the middle of a 1,484 m^2 broiler house. Feed pans and water lines were included in the square plot boundaries. Plots were designated as control or platform based on the absence/presence of a rectangular platform placed equal distance from the water and feed lines. Bird density and behavior counts (eating, drinking, active) were recorded at five daily time points over a 14-day period during grow out of a 20,000 head flock with unrestricted access to plots using a cc-TV system. Means were compared using a t-test in JMP Pro v.16.0.0. When comparing all time points, bird count by plot was significantly different averaging 31.70 ± 0.97 and 50.50 ± 0.97 in control and platform plots, respectively ($P < 0.0001$). Birds engaged in active movements were calculated at 11.4 % of the birds counted within the platform plots, versus 8.7 % of the control plots ($P = 0.003$). Drinking and eating behaviors were negatively impacted by the presence of the platform, calculated at 18.8 % versus 38.7 % displaying feeding behaviors in platform versus control plots ($P < 0.0001$), and 7.8% versus 12.0% displaying drinking behaviors ($P < 0.0001$). Interestingly, the presence of platforms was associated with increased bird density and activity within plots. Additional study is needed to further understand how litter quality and the impact of exercise on leg health may be impacted by platform enrichments. Further, more information is needed to understand the impact of feeding and drinking behavior on production traits when platforms are present.

Key Words: broiler, enrichment, platform

P259 Effect of photometric sensor orientation on the measurement of light intensity (illuminance) in commercial broiler houses during tunnel ventilation William Westerlund*[1UG], Jeremiah Davis[1], John Linhoss[1], Joseph Purswell[2], Brooke Morgan[1], Cody Smith[1], Martha Rueda[1], Josh Etherton[1], Olamide Durodola[1], Olumide Falana[1] *[1]National Poultry Technology Center at Auburn University, [2]USDA ARS Poultry Research Unit*

Light intensity in commercial broiler houses has typically been performed with photometric meters placed on the floor with the sensor facing the ceiling. This orientation is useful in adjusting light intensity from bulbs but does not account for light intrusion through the tunnel fans. The objective of this project was to evaluate the effect of sensor orientation on the measurement of light intensities in commercial broiler houses. A commercial broiler farm with four 18.3 x 182.9 m modern broiler houses in south-eastern Alabama was used in this study. The houses were fitted with 17 tunnel fans; four mounted on the end wall and 13 on the sidewalls. Four 45-cm stands were constructed to measure light intensity in five directions [evaporative pad end-wall, fan end-wall, both sidewalls, upward (ceiling)] at each measurement location at bird height. Target light intensity was set at 0.2 lux with the meter directly under a bulb. Directional light intensity measurements were simultaneously taken down the center of the long-axis of each house near solar noon (± 1 hr) with all tunnel fans running. Stands were moved to record the light intensity levels alternating under

each bulb and between bulbs every 2.44 m down the length beginning 2.44-m from the fan end-wall. The five sensor orientations were simultaneously measured in each house (n=3) at each location (n=74) down the house. A one-way (orientation by location) ANOVA was performed using the mixed model procedure in SAS ver. 9.4, means were separated using Fisher's LSD. Solar light intensity (mean 55.6±1.5 klux) was included in the model as a covariate to account for variations due to cloud cover across the sampling period. Statistical significance was established at $P \leq 0.05$ level. Sidewall facing sensors measured highest intensities (between 10 and 269 lux) in the tunnel fan section of the house. Beyond the fan section, fan end-wall facing sensors consistently measured the highest light intensity up to 78.0 m (42.6% of house length) from the fan end-wall due to light ingress from running fans. This research shows that during tunnel ventilation, light control is being achieved in roughly 57.4% of the house length. In the remaining 42.6% of the house, light levels are above target due to fan light ingress.

Key Words: light uniformity, light meter, tunnel ventilation

P260 Spatial evaluation of temperature using simulated eggs in Natureform incubators Olamide Durodola*[1GS], Jeremiah Davis[1], John Linhoss[1], Jessica Starkey[2], Brittany Wall[2], Katie Elliott[3] *[1]National Poultry Technology Center at Auburn University, [2]Auburn University, Poultry Science Department, [3]USDA ARS Poultry Research Unit*

Understanding temperature variations during incubation have been a critical concern for the optimal development of egg embryos. Temperature variation can alter embryonic mortality, egg moisture loss, hatchability, and post-hatch growth. The objective of this study was to evaluate the spatial temperature profile of a Natureform NPMC 2000 incubator using simulated eggs. The incubator housed 12 egg trays, each holding 90 eggs for total of 1080 eggs. The air temperature was measured using self-contained iButton data loggers (DS1923-F5) fitted to the top of 3D-printed eggs that allowed the measurement of air temperature at the surface of the simulated egg during the first stage of incubation. Each egg tray was fitted with eight iButton-eggs and 82 simulated eggs. The incubator was set to 37.5 °C with 60 % RH for three days. Comparisons were made horizontally (left trays vs. right trays) and vertically (levels 1 – 6). Data were analyzed with one-way ANOVA with the MIXED procedure of SAS ver. 9.4. There was a 0.2 °C difference ($P \leq 0.0001$) between the left and right egg trays, the right being hotter (37.3 °C vs. 37.1 °C); a 0.2 °C difference ($P \leq 0.0001$) between egg tray levels, with L1 (top) being the coldest (37.1°C) and L5 being the hottest (37.3°C). Results from this study show that temperature within the incubator varied significantly with location and that 3D-printed eggs fitted with iButtons can be used to capture air temperature near the egg surface.

Key Words: Incubation, temperature, simulated eggs, spatial mapping, iButtons

P261 Mitigation of heat stress in broiler chickens using dietary supplementation of microalgae (Spirulina platensis) Ajay Chaudhary*[GS], Pravin Mishra, Razib Das, Sadid Amaz, Prem Mahato, Rajesh Jha, Birendra Mishra *Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa*

Microalgae (*Spirulina platensis*) are rich in antioxidants and have several health benefits. However, its role in health and production, along with the underlying mechanism in heat-stressed broilers, is obscure. This study aimed to determine the effect of microalgae supplementation on the health markers associated with production in heat-stressed broilers. Cobb500 day-old chicks (N=144) were raised in floor pens (6 pens/treatment and 8 birds/pen). The treatment groups were a) no heat stress (NHS), b) heat stress (HS), and c) heat stress+3% microalgae (HS+MAG). The birds in the NHS and HS were fed with a standard broiler diet, whereas the HS+MAG group was supplemented with 3% microalgae. The birds in the NHS were raised under standard broiler management, whereas the birds in the HS and HS+MAG were subjected to cyclic heat stress from day 22-35 (32-33ºC for 8 hours). All data were presented as mean ± SEM after performing one-way ANOVA Tukey's post hoc test using GraphPad and microbial bioinformatics was performed in QIIME2 and RStudio at $P<0.05$. Heat stress significantly decreased the body weight, whereas the supplementation of microalgae increased the body weight of broilers ($P<0.05$). Heat stress showed no significant effect on the expression of ileal antioxidant (*GPX3*), heat shock (*HSF3*), immune-related (*IL4*, *TLR4*), and tight-junction (*CLDN1*, *CLDN2*, *ZO2*) genes compared to NHS group, whereas supplementation of microalgae significantly increased these genes compared to NHS and HS birds ($P<0.05$). In the HS+MAG, villus height, crypt depth, and villus height: crypt depth ratio of ileum were also improved ($P<0.05$). However, there was no change in feed conversion ratio, cecal volatile fatty acids production, and plasma IgA and IgG ($P>0.05$). Gut microbial metagenomics showed a higher alpha diversity ($P<0.05$) in the HS+MAG group, but no change in the beta diversity ($P>0.05$). The abundance of Enterobacteriaceae, Ruminococcaceae, and Oscillospiraceae family was higher in HS+MAG compared to HS. The study revealed that heat stress negatively impacted production by disturbing the physiogenomics, which was improved by dietary supplementation of microalgae. Thus, the inclusion of microalgae in the broilers' diet can potentially be used to mitigate heat stress in broilers.

Key Words: broiler, heat stress, microalgae, antioxidant, health

P262 Influence of perch provision timing on anxiety and fearfulness in laying hens Mallory Anderson*[GS], Alexa Johnson, Ahmed Ali *Clemson University*

Many studies indicate possible positive effects of perch provision on the behavior and welfare of laying hens. However, the age during which birds are experiencing perch provision may significantly influence such effects. Therefore, we aimed to investigate the short- and long-term effects of early or later access to perches on anxiety and fear in laying hens, measured through attention bias (AB) and tonic immobility (TI) tests. Pullets (n=800) were housed in floor pens either with or without multi-level perches (n=10 pens/treatment) until 17 weeks of age (woa); perch provision was then switched until 37 woa, resulting in 4 treatment groups (n=5 pens/treatment): CP (continuous perch access: 0 to 37 woa), EP (early perch access: 0-17 woa), LP (later perch access: 17-37 woa), and NP (no perch access: 0-37 woa). AB was conducted on weeks 21 and 37 (n=90/week), and TI was performed on weeks 20, 25, and 37 (n=90/week). Differences across weeks and treatments were assessed using GLMM (α set at 0.05) in R 3.3.1. In the AB test during weeks 21 and 37, CP hens began feeding faster (202.6, 189.7s) than EP (288.6, 228.9s), LP (293.2, 259.6s), and NP (296.9, 289.8s) hens (all $P \leq 0.05$). However, there was no difference in feeding latencies between weeks 21 and 37 for CP and NP groups, while both EP and LP hens began feeding faster at week 37 compared to week 21 (all $P \leq 0.05$). In the TI test during week 20, CP hens had the shortest TI duration (52.2s), followed by LP (69.9s), NP (83.6s), and EP (90.6s) hens ($p<0.05$). Similarly, CP and LP hens had the shortest TI durations, followed by NP and EP hens, at weeks 25 and 37 (all $P \leq 0.05$). Across weeks, CP and EP hens had longer TI durations at weeks 20 and 25 than at week 37, while LP hens had longer TI durations at week 20 compared to weeks 25 and 37 (all $P \leq 0.05$), with no differences in TI duration between weeks for NP hens. Thus, early and long-term perch provision reduces anxiety and fearfulness, while restricting perch access upon transitioning into the laying phase has a short-term negative impact on anxiety and fear level that dissipates in the long-term. Although there is no evidence of a positive short-term impact of providing access to perches later in life, long-term levels of anxiety and fear are reduced.

Key Words: laying hen, animal welfare, behavior, attention bias, tonic immobility

P263 Continuous glucose supplementation improved feed conversion ratio, carcass yield and composition in chronic heat stressed broiler chickens Oluwatomide Ariyo*[1GS], Ahmed Ghareeb[1], Marie Milfort[1], Bikash Aryal[1], Evan Hartono[1], Josephine Kwakye[1], Selorm Sovi[1], Sommer Hipple[1], Carrienton Stevenson[2], Alberta Fuller[1], Romdhane Rekaya[1], Samuel Aggrey[1] *[1]University of Georgia, [2]Fort Valley State University*

Heat stress (HS) is a prevalent environmental stressor impacting broiler chicken performance by reducing feed intake, growth, feed conversion, and carcass quality resulting in severe economic losses. Chronic HS affects carcass composition by reducing the proportion of breast muscle due to restricting protein synthesis. Energy status is one of the basic muscular protein synthesis regulators. We hypothesized that glucose (Glu) supplementation would rectify the muscular growth reduction induced by HS. A total of 456 Cobb500 broiler chickens were raised on floor pens at ad libitum feed and water, and randomly allocated to 4 treatment groups (6 rep of 19 birds each): thermoneutral groups (TN0 and TN6) were raised at 25°C, HS groups (HS0 and HS6) were exposed to cyclic temperature 35°C from d28 to d35 for 12 hrs daily. TN6 and HS6 received 6% Glu in water. TN0 and HS0 received fresh water. On d35, 3 chickens/rep (n=18/TRT) were randomly selected for processing, and 6 other chickens were bled/TRT to measure blood Glu level. Growth and feed intake were recorded at d28 and d35. The weight of eviscerated carcass, abdominal fat, *pectoralis* (*P.*) *major*, *P. minor*, thighs, drumsticks, and wings were recorded; the proportion values were calculated relative to the eviscerated weight. Either HS or Glu elevated blood Glu levels. ANOVA results showed a significant effect of Glu treatment on FCR ($p=0.03$), and the Glu groups showed lower FCR, compared with their corresponding control groups ($p<0.05$). LS Means/Tukey-Kramer test was used for multiple comparisons. Interestingly, the eviscerated weight and *P. major* proportion of the HS6 chickens were significantly higher than those of the HS0 chickens ($p<0.05$), but not significantly different from those of thermoneutral groups (TN0 and TN6). There was no significant difference between the drumstick proportions of the HS6 group and the TN groups. Herein, continuous supplementation of 6% Glu in water reverted some negative effects of chronic HS on chicken performance by improving carcass yield, and *P. major* and drumstick proportion. A further molecular study is recommended to investigate the role of energy increase by Glu supplementation in preventing the protein synthesis transcriptional factors' suppression induced by HS in broilers.

Key Words: heat stress, glucose supplementation, carcass yield, eviscerated weight, Pectoralis major yield

P264 The effect of different lighting sources on Pekin duck growth and welfare Abbigail LeBlanc*[GS], Gregory Archer *Department of Poultry Science, Texas A&M University*

This study evaluated the effects of various lighting on Pekin duck performance and welfare. Four treatments were tested with 9 replicate groups, each containing 126, day-of-hatch Pekin ducks. The treatments were: 1) Full Spectrum LED lighting (FS) 2) White LED lighting (White), 3) LED Lantern lighting (Lantern), 4) Monochromatic Green LED lighting (Green). Body weight (BW) and feed consumption (FC) of birds from 1 to 35 days of age were measured for each pen and mortality corrected feed conversion ratio (FCR) was calculated. On d35, tonic immobility and physical asymmetry scores were determined. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation. At d35, no difference in FCR were observed between treatments ($P > 0.05$, avg. 1.526). Differences in BW ($P = 0.002$) and FI ($P = 0.002$) were observed at d35 between treatments. The Green and White treatments both had greater ($P < 0.05$) BW (3.28 and 3.27kg) and had higher ($P < 0.05$) FC (145 and 143 g/d) than the Lantern and FS treatments (3.16 and 3.11kg; 138 and 136 g/d). The Green and White treatments had lower ($P < 0.05$) asymmetry scores (1.71 and 1.70) and shorter ($P < 0.05$) latency to right during tonic immobility (161 and 157s) than the Lantern and FS treatments (2.12 and 1.99; 242 and 229s). In conclusion, the Green and White improved performance and welfare over the Lantern and FS treatments. These results continue to illustrate the importance of lighting on poultry performance and welfare.

Key Words: Lighting, Duck, Performance, Welfare

P265 Influence of construction material on platform usage in a commercial broiler house Cynthia Lopez*[GS], Monica Franco, Katy Tarrant *California State University, Fresno*

Introducing platforms to broiler chickens is one method to promote enriched environments and natural behaviors. We aim to understand how construction material impacts broiler usage of an ideal platform design previously identified. All platforms measured 0.91 m x 0.28 m x 0.14 m in height. Three platforms were covered in each of the material types (wood, PVC plastic, or rubber), for a total of nine platforms placed in a commercial broiler house. Platform placement was randomized through the center of the broiler house, but maintained equal distances between water and feed lines. Platform usage was recorded with a ccTV system, where usage was defined as bird counts on top of the platform at five different time points per day from day 5 to day 42 over two flocks. Adjusted usage was also taken into account due to the amount of space a broiler takes on the platform at different weeks of the grow-out period (adjusted frequency = bird count / possible bird count). Means were compared using JMP v.16 and means were separated using Tukey's HSD. When comparing usage of platforms by week, week 2 had the highest average usage at a mean of 10.054 ± 0.219 ($P < 0.0001$). Usage decreased by week throughout the flock, where week six usage was lowered at an average count of 2.381 ± 0.179 ($P < 0.0001$). Taking into account the change in bird size, the calculated adjusted frequency indicated that week 6 usage was significantly larger than the rest of the flock with an average of 0.627 ± 0.016 ($P < 0.0001$). During the entire grow-out period, the rubber material platform adjusted frequency was significantly larger in weeks 2-6, compared to the other materials ($P \leq 0.0119$). Over two flocks in this study, data show that broiler chickens have a preference on platform material throughout the grow-out period. In order to ensure best practices in promoting bird well-being and minimizing the economic impact of utilizing platform enrichments in commercial broiler facilities, it becomes imperative that we understand all parameters related to platforms prior to deployment.

Key Words: broiler, enrichment, platform

P266 The effect of continuous glucose supplementation on the mRNA expression of ileum nutrient transporter in chronic heat stressed broiler chickens Ahmed Ghareeb*[1GS], Oluwatomide Ariyo[1], Marie Milfort[1], Bikash Aryal[1], Evan Hartono[1], Josephine Kwakye[1], Selorm Sovi[1], Sommer Hipple[1], Carrienton Stevenson[2], Alberta Fuller[1], Romdhane Rekaya[1], Samuel Aggrey[1] *[1]University of Georgia, [2]Fort Valley State University*

Heat stress (HS) in broiler houses is a persistent challenge affecting chicken performance by decreasing growth, feed intake, and feed conversion leading to huge economic losses. Chronic HS impacts gut function by altering the intestinal transporters' expression and inducing enterocytic oxidative damage. Chickens dramatically increase water consumption to compensate for the water evaporated by panting. We herein investigated the effect of oral glucose (Glu) supplementation on mRNA expression of ileum monosaccharide, fatty acid, and amino acids (AAs) transporters; and kidney water channels (AQPs) in broiler chickens. Birds (456 males Cobb500) were raised on floor pens with ad libitum feed and water, randomly assigned to 4 treatment groups (6 rep./19 birds each): thermoneutral groups (TN0 and TN6) were raised at 25°C, HS groups (HS0 and HS6) were exposed to cyclic temperature 35°C from d28 to d35 (12hrs/d). TN6 and HS6 continuously received 6% Glu in water, while TN0 and HS0 received fresh water. On d35, ileum and kidney tissues were collected from 6 randomly selected chickens/treatment for RNA extraction and gene expression analysis. LS Means/Tukey-Kramer test was used for statistical multiple comparisons and only significant changes were

reported ($\alpha \leq 0.05$). AQP1, 2, and 3 were upregulated in the HS0 group compared with the other treatment groups. The HS6 had the highest expression level of ileum GLUT1, FATP1, FABP6, rBAT, $b^{0,+}$AT, SNAT2, and SNAT7 transporters compared with the other treatment groups. The TN6 group showed significant upregulation of GLUT5 compared with the other treatment groups. The HS groups showed upregulation of GLUT12 and FABP2 compared with the TN groups. Supplying heat-stressed chickens with water containing 6% Glu significantly altered the expression of ileum nutrient transporters, and induced upregulation of AAs transporters responsible for the absorption of glutamine and neutral AAs, particularly cysteine. Enterocytes utilize glutamine for energy production. Cystine is essential for the production of glutathione antioxidant protecting cells from HS-induced oxidative damage. Studying the effect of Glu supplementation on digestibility, intestinal morphology and permeability, and blood parameters of heat stressed chickens is recommended.

Key Words: heat stress, glucose supplementation, monosaccharide transporters, fatty acid transporters, amino acid transporters

P267 Assessing the effect of commercially available brooder type on body weight, fearfulness, and behavior of chicks. Ally Jackson*[GS], Bethany Baker-Cook, Brigid McCrea *Auburn University*

Brooding period is critical for chick development and the brooding environment can impact bird health, productivity, and welfare. To assess the difference in a traditional galvanized brooder (GB) and a multi-level Hatching Time brooder (HTB) on chick development, body weight (BW), average daily gain (ADG), fear response, and behavior were compared. Ross 308 chicks (n = 48) were placed in brooder cages at one day of age with a stocking density of ~ten chicks per cage/level. BW and ADG were taken over a three-week period. To assess fear a novel object test was conducted on day 15. Chick behaviors of sitting, standing, sleeping, active (walking or running), preening, and stretching were assessed via scan sampling at 30-minute intervals on day (d) 4, 7, and 11. One-way ANOVA with brooder as main effect was performed (Proc Mixed, SAS 9.4). BW and ADG were higher in the GB (W1 | BW=147.6, ADG =21.1; W2 | BW=338.3, ADG=48.3) then HTB (BW=113.6, ADG=16.2; BW=264.3, ADG=37.8). No difference was found in BW and ADG when comparing within levels of HTB at any age. Five interactions with novel object occurred within 1 minute and 15 seconds in the GB, whilst no physical interactions occurred in the HTB within 15 minutes. However, the object was approached six times in level two, once in level three, and never in level one and four of HTB. On d4 and d7, birds spent more time sitting (d4 =19.6, d7=27.6) in the GB than in HTB (d4=19.0, d7=13.4). Birds also spent more time standing in GB on d7 (24.1) than HTB (28.6). On d4 and d7, a preening (2.6;2.8) and active behavior (8.1;10.2) occurred more in the HTB than GB (1.1;1.7) (5.9;6.1). Sleeping was observed most often in the HTB (47.4) versus the GB (40.9) on d11. Within the levels of the HTB, sitting was highest in level two (28.1) (L1=14.8, L4=14.0) on d4, standing was highest in level 4 (42.4) (L1=21.2, L2=25.7) on d7. On d11, active and preening were higher in level one (5.9;9.5) than level two (3.6;1.8). No difference was seen for stretching at any age. Overall, the galvanized brooder was found to have higher bodyweight and average daily gain, presented lower fearfulness with more time spent sitting and standing and less time performing comfort (preening) and active behaviors compared to the Hatching Time brooder.

Key Words: brooding, chick development, novel object test, active behavior, comfort behavior

P268 Dietary oregano essential oil improves performance, blood lipid profile, and antioxidant capacity in laying pullets. Alexa Johnson*[GS], Gracie Anderson, Mireille Arguelles-Ramos, Ahmed Ali *Clemson University*

National bans on the use of antibiotics as growth promoters in livestock have given rise to natural alternatives, one being essential oils. Oregano essential oil has been used in many poultry species with positive effects, but there is little to no research on its effects during the pullet phase. This study examined the effects of dietary oregano oil on production performance, blood lipid profile, and antioxidant capacity of pullets. A total of 290 day-old HyLine Brown chicks were randomly housed in floor pens, provided with a corn-soybean meal-based diet either supplemented with (4 pens/treatment with 29 birds/pen) the oregano oil at 0.275 g/kg basal diet (OEO) or not (CON). Body weight (BW) and average daily feed intake (FI) were measured starting at 1 week of age (woa) and continued every other week until 17 woa. Blood samples were collected (3 birds/pen) at 11 and 17 woa, and blood lipids (High-density lipoproteins: HDL, cholesterol: CHO) and antioxidant measures (total antioxidant capacity: TAC, malondialdehyde: MDA, and superoxide dismutase: SOD) were determined. Differences across weeks and treatments were assessed using GLMM (α=0.05) in R 3.3.1. Weights at 3, 11, 13, and 17 woa was significantly higher in OEO hens than in CON ($p \leq 0.05$). The average daily weight gain was significantly higher in OEO birds at 9 and 13 woa than in CON birds ($p \leq 0.05$), while no differences were observed in FI. Levels of HDL (3.0 vs 1.3 mmol/L, p=0.01) and CHO (4.9 vs 3.9 mmol/L, p=0.03) were both higher in OEO birds compared to CON birds at 11 woa. At 17 woa, HDL levels were higher (1.2 vs 2.6 mmol/L, p=0.01) in OEO birds compared to CON birds, with no difference in CHO levels across groups. At both 11 and 17 woa, TAC (11woa: 9.8 vs 7.0 mmol/L, p=0.01; 17woa: 11.4 vs 7.0, p=0.01) and SOD (11woa: 380 vs 260 U/mg, p=0.01; 17woa: 358 vs 274 p=0.01) levels were significantly higher in OEO birds compared to CON birds. MDA levels were significantly lower (11woa: 10.7 vs 19.8 mmol/mg, p=0.01; 17woa: 12.7 vs 21.3, p=0.01) in OEO group birds than in the CON group. Thus, oregano supplementation improved weight gain without impacting feed intake, influenced blood lipids, and improved antioxidant measures, indicating a reduction in oxidative stress; but further reaseach is needed.

Key Words: Pullets, Oregano Essential Oil, Production Performance, Blood Lipid Profile, Antioxidant Capacity

P269 Conventional and aviary-style housing drive unique feather microbiota diversity and composition in laying hens Ashley Tarcin*[UG], Dana Dittoe, Samar Tolba, Vanessa Leone, Steven Ricke *Meat Science and Animal Biologics Discovery, Animal and Dairy Sciences, University of Wisconsin-Madison*

Chicken feather microbiota has been sparsely examined, with little information regarding differences across rearing systems. The objective was to elucidate the wing, breast, and dorsal feather microbiota diversity and composition of single-comb white leghorn commercial laying hens across aviary and conventional rearing methods via 16S rRNA gene amplicon sequencing. Individually housed hens (n = 120) were continuously maintained under conventional battery cages (CONV) or transitioned to aviary-style housing (AVIARY) (60 hens/trt, 4 blocks of 15 hens) at 58 to 60 weeks of age. One month post transition, the wing, breast, and dorsal feathers were swabbed from 24 hens per housing system. Swabs were hydrated in 10 mL of neutralizing buffered peptone water. Swabbing was repeated 10x with applied pressure for each side of the swab per location. Genomic DNA was extracted from the swab's diluent and the V4 region of the 16S rRNA gene was sequenced via Illumina MiSeq and analyzed with QIIME2-2022.2. Alpha and beta diversity differences were determined using Kruskal-Wallis and ANOSIM and compositional differences were determined using ANCOM. Significance was determined at $P < 0.05$ and $Q < 0.05$. Within alpha diversity, both housing system and feather site impacted microbiota community richness ($P < 0.05$), but not evenness ($P > 0.05$). The breast and dorsal feather microbiota richness was different across housing systems ($Q < 0.05$). Within beta diversity, both presence and abundance of taxa were relatively consistent across all feather locations ($P > 0.05$). However, beta diversity was driven by rearing system, AVIARY vs. CONV ($Q < 0.05$), with significant differences detected among taxa at the genus level ($P < 0.05$). ANCOM revealed 46 of ~800

unique taxa ($P < 0.05$, $W > 698$) were significantly impacted by housing system. Together, these data indicate housing system elicits a stronger impact on the subsequent feather microbiota community membership as compared to feather swab location. Further investigation is required to establish a complete transition of the feather microbiome over the lifespan of the hen within each housing system.

Key Words: laying hens, rearing system, microbiota, feathers

P270 Differences in the relationship between tibia breaking strength and ash in two fast growing commercial strains of broiler. Gregory Archer* *Texas A&M University*

xx. Leg health is a constant welfare concern in broiler production. Typical measures of this include bone breaking strength (BS) and bone ash percentage (BA). Generally, it has been considered that these two measures are highly correlated. Though there is a lack of confirmatory data of this across different strains of modern commercial broilers. Therefore, the objective of this study was to evaluate relationship between these two measures and their overall values in two commercial strains of broiler. To do this data collected from numerous studies was pooled and evaluated. Two time points were compared d21 and d42. Strain A had 1453 birds at d21 and 1879 at d42. Strain B had 1194 birds at d21 and 1206 at d42. Strains differed in both average BS and BA. Strain A had greater BS ($P < 0.001$, 23.1g) and BA ($P < 0.001$, 50.4%) than Strain B (18.7g and 48.6%) at d21. Strain A had lower BS ($P < 0.001$, 45.6g) and greater ($P < 0.001$, 47.4%) BA than Strain B (47.5g and 46.9%) at d42. At d21 Strain A had a correlation (0.19) between BS and BA that was linear ($P < 0.001$) and quadratic ($P < 0.001$). At d42 Strain A had a correlation (0.46) between BS and BA that was linear ($P < 0.001$). At d21 Strain B had a correlation (0.56) between BS and BA that was linear ($P < 0.001$) and quadratic ($P = 0.01$). At d42 Strain B no correlation (-0.002) between BS and BA. These results indicate that strain of broiler can affect the overall magnitude of BS and BA and their correlation. Using these measures without considering the strain and age could result in inaccurate conclusions about leg strength especially if both parameters are not used simultaneously.

Key Words: Broiler, Leg Health, Strain

P271 The effects walking with or without obstacles on broiler and duck growth and leg health. Kyle Trejo, Gregory Archer* *Texas A&M University*

Leg health is a constant welfare concern in poultry production. Typically, poultry have feed and water located so they do not have to locomote far to reach one or the other which may promote poor leg health. The objective of this study was to evaluate increasing walking alone or in combination with an obstacle could on growth and leg health in broiler chickens and ducks. Broiler chickens and Pekin ducks were reared under one of four conditions from day of hatch until market weight: In a short pen (2m long) with feed and water in close proximity (control), in a long pen (6m long) with feed and water at opposite ends (walk), in a long pen with a step over obstacle in the middle of the pen (obst), or a long pen with a ramp in the middle of the pen (ramp). Stocking density was the same in all treatments. Feed consumption, bird weight, feed conversion, mortality, gait score, and tibia ash and breaking strength were all measured. A LSD post hoc test was used for mean separation. Data was analyzed using GLM, and $P < 0.05$ was considered a significant difference. No differences ($P > 0.05$) were observed between treatments in mortality, or tibia ash in either the broilers or ducks. There were, however, differences observed between treatments in feed consumption, bird weight, gait score, and breaking strength in the broilers ($P < 0.05$). Ducks also had similar differences observed between treatments with the exception of gait score where no differences were observed ($P > 0.05$) and differences in FCR were observed ($P < 0.05$). In the broilers, the control birds consumed less feed (92g/d), weighed less at day 42 (2.50kg), had worse gait scores (2.5), and lower tibia breaking strength(37g) ($P < 0.05$) than the other three treatments (105g/d, 2.84kg, 2.0, 43g). In the ducks, the control birds consumed more feed (125g/d), weighed less at day 35 (2.98kg), higher FCR (1.80) and lower tibia breaking strength (27g) ($P < 0.05$) than the other three treatments (107g/d, 3.28kg, 1.39, 34g). No differences were observed between the walk, obst, or ramp treatments in either broilers or ducks ($P > 0.05$). These results indicate that encouraging broilers or ducks to walk between feed and water with or without additional obstacles can improve production and leg health.

Key Words: duck, broiler, leg health, production, excercise

P272 Vocalizations as a flock welfare indicator and enrichment in the poultry industry Jenna Schober, Jeff Lucas, Gregory Fraley* *Purdue University*

Sounds have been shown to affect the behavior and overall welfare of birds in captivity. We wish to build upon this knowledge to develop duck vocalizations that can be used as a type of environmental enrichment in commercial duck barns. However, we first need to develop a full repertoire of duck vocalizations. To accomplish this goal, we utilized 23 (19 hens and 4 drakes) Pekin ducks ranging from 35 to 45 weeks old to develop a vocal repertoire. The ducks were put in a soundproof chamber, and their vocalizations and behaviors were recorded using a Zoom H5 recorder, an Audio Technica AT4022 omnidirectional microphone and a WYZE Bluetooth camera. We placed a range of 1 to 4 ducks of mixed sex ratios into the chamber for several minutes to record initial vocalizations and to acclimate the ducks to the sound chamber. Once acclimated, different enrichment or aversive stimuli were used to encourage new vocalizations. Each stimulus was recorded for 20-30 minutes. Audio recordings were analyzed using Adobe Audition, Adobe Premiere Pro, and Praat, a phonetics software. Vocalizations were characterized based on the following criteria: number of pulses, amplitude, frequency and the shape of any frequency modulation. Our results showed that ducks can produce up to 36 different vocalizations. A cluster analysis using SAS 9.4 indicated that the environmental stimuli had no effect on the repertoire produced by the ducks. However, all outlier clusters included hens, suggesting that hens use a unique set of vocalizations not recorded from males. Shannon entropy (*H*) was used to describe the diversity of the vocal repertoire under different conditions. This analysis indicated that the vocal repertoire increased with an increase in the number of hens in the chamber ($F_{2,20} = 4.05$, $p<0.05$) and that the number of drakes had no effect on repertoire diversity ($F_{2,20} = 0.73$, $p=0.49$). Our results will allow us to next determine how playbacks of these vocalizations can affect ducks' physiology, such as heart and respiratory rate, or pupillary light reflex. We can then evaluate which sounds may produce calming effects on ducks that could be used as environmental enrichment in commercial barns, or they could be used as an indicator of overall barn welfare.

Key Words: duck, amplitude modulation, frequency modulation

P273 The effects of a botanical extract blend (Phytozen®Liquid) on stress response, enrichment use and behaviour of laying hens housed in a furnished cage system Taiwo Makinde*, Deborah Adewole *Dalhousie University*

Phytogenic additives have been used in the treatment of anxiety and physical and emotional imbalances because of their antioxidant, anxiolytic, and antidepressant activities. However, no study has investigated the effects of a blend of plant extracts on the stress response, environmental enrichment use, and behavior of laying hens housed in furnished cages. This study examined the effects of Phytozen® Liquid, a commercially formulated blend of botanical extracts (BE), on the stress response, enrichment use and behavior of laying hens in a furnished cage system.

280 Lohmann Lite hens (38-week-old) were randomly allocated to either BE (40 ml Phytozen® Liquid in 200 L of drinking water) or a control treatment group (without BE in drinking water) for 16 weeks (4 x 4-week periods, each period consisting of 3 wk of measurement + 1 wk washout period) in a multi-tier furnished cage system. Each group had four furnished cages with 35 hens/cage. At 40, 44, 48, and 52 wk of age, blood samples from 5 birds per cage were taken from the brachial vein, scan samples from daily video recordings were obtained to analyze for enrichment use and behavior, and pecking blocks were weighed weekly. All data sets were analyzed using the GLIMMIX procedure of Statistical Analysis Software (S.A.S., version 9.4) for repeated measures.

The BE reduced (P=0.02) serum corticosterone concentration by18.00% compared to the control, irrespective of the periods. A significant interaction (P=0.04) between age and BE was observed for perch use by hens in the afternoon. In period 3, the BE supplemented hens used the perches 2.06% more in the afternoon than in the control groups. Similarly, the BE improved (P<0.01) scratch mat usage in the afternoon by 6.44% compared to the control. In the BE group, nest box use increased (P<0.01) by 11.37% in the morning and 15.10% in the afternoon, compared to the control group. However, BE did not affect hens pecking behavior, pecking block usage, preening, and injurious pecking, irrespective of time and period. The result of the physiological response and enrichment use by Lohmann Lite hens housed in furnished cages showed that in-water supplementation of Phytozen could improve laying hens' positive affective state and overall quality of life.

Key Words: environmental enrichment, furnished cages, phytozen, welfare, botanical extracts

P274 Effect of stocking density and antimicrobial inclusion on growth performance and carcass characteristics of broilers grown to 3 kg at 42 days of age Hammed Olanrewaju*, Christopher Magee, Stephanie Collier, Joseph Purswell *USDA-ARS Poultry Research Unit*

Lower stocking densities may play a significant role in minimizing difficulties such as necrotic enteritis when reducing or eliminating antimicrobials (antibiotic free; ABF) in poultry diets. This study investigated the effects of stocking densities and antimicrobial inclusion on growth performance and carcass characteristics of broilers grown to 3 kg at 42 days of age. A total of 888 1-d- old Ross x Ross 708 chicks were randomly distributed into 24 pens based on stocking density treatments assignment. The treatments consisted of 4 densities (29, 33, 39, and 42 kg/m^2) with six replicates. Treatments were blocked within the room to account for any variations in room conditions. Treatment assignments were randomized within each block. Used bedding was obtained from commercial farms to simulate commercial conditions. Feed and water provided *ad libitum*. Birds were provided a three phase-feeding program (Starter: 0-14 d, Grower: 15-28 d, and Finisher: 29-43 d). Birds and feed were weighed on 1, 14, 28, and 42 d of age for growth performance. On d 43, 10 (5 males and 5 females) birds from each pen were processed to determine weights and yields. A mixed model ANOVA employing PROC MIXED procedure of SAS software was used to analyze the data. The BW and BW gain, live weight and carcass weights were not affected by stocking density. However, FI for the starter period ($P \leq 0.001$) and overall FCR ($P = 0.0064$) were significantly affected by stocking density. In conclusion, stocking densities up to 42 kg/m^2 may affect production efficiency in ABF broilers.

Key Words: Stocking-density, antimicrobial, growth-performance, broilers, welfare

P275 Evaluation of differences between two fast growing and two slow growing commercial strains of broiler in performance, fear and stress Melinda Grimes*, Gregory Archer *Department of Poultry Science, Texas A&M*

This study evaluated the performance, fear and stress of two fast growing and two slow growing commercial broiler strains. Four strains were tested with 10 replicate groups, each containing 200, day-of-hatch chicks. The treatments were: 1) slow growing strain A (SGA) 2) fast growing strain A (FGA), 3) slow growing strain B (SGB), 4) fast growing strain B (FGB). Body weight (BW) and mortality corrected feed conversion ratio (FCR) was calculated. On d41, tonic immobility (TI), physical asymmetry scores (ASc), CBC, heterophil/lymphocyte ratio (HL), organ weights (heart, spleen, liver) were determined. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation. FGA took more attempts to induce tonic immobility than all other strains ($P < 0.05$). SGA had longer latency to right during TI than all other strains ($P < 0.05$). FGA had lower ASc than both B strains. No differences were observed in CBC or HL ($P > 0.05$). The FGB had heavier hearts than both A strains ($P < 0.05$) and no differences were observed in spleen or liver weights ($P > 0.05$). FG strains weighed more than SG strains at d14 and d 28 ($P < 0.05$) and SB weighed more than SA at d14 and d28 ($P < 0.05$). No differences were observed in BW at d42 ($P > 0.05$). FG strains had lower FCR than SG through d28 ($P < 0.05$) but no differences were observed at d42 ($P. > 0.05$). Overall mortality was higher in strain B compared to strain A. In conclusion, these results demonstrate that when strains are reared under the same conditions and fed the same diet, performance mainly varies early in growth between FG and SG but these diminish by d42 and there may be some differences in fear and stress measures.

Key Words: Broiler, Performance, Welfare, Strain

P277 Effect of the probiotic strain Enterococcus faecium 669 on intestinal colonization and influence on the microbiome composition of broiler chickens Antoine Meuter*[1], Dorthe Sandvang[1], Christophe Bostvironnois[1], Eric Sobotik[2] *[1]Animal and Plant Health & Nutrition, Chr. Hansen, A/S, [2]Animal and Plant Health & Nutrition, Chr. Hansen, Inc.*

The intestinal tract of the newly hatched chickens plays a crucial role in immune cell formation and immune challenges. The interaction between pathogenic microorganisms such as *Escherichia coli (major* causative agent of early mortality and the host commensal microbiota. The aim of this study was to evaluate the effect of the probiotic strain *Enterococcus faecium* 669 after early life application on intestinal colonization and mi-

crobiome composition of broiler chickens. Chicks were inoculated by oral gavage to decrease individual variation to mimic the post hatch spray application. Inoculation dose was 4.2 X 10^8 CFU/ bird, while the broiler chickens were fed ad libitum. Infectious bronchitis aerosol vaccine was used and no other treatments were applied during the trial period.

At day 4 and day 7 after hatch, 15 birds from each group (control and probiotic) were euthanatized, DNA from cecal sacks were purified and 16s RNA was sequenced. *Enterococcus faecium* 669 seemed to be present in caeca, as represented by an increase in Enterococcus in the treated birds. Furthermore, *Enterococcus faecium* 669 modulated the cecal microbiome by increasing the diversity (as measured by the CHAO1 index and based on t-test, P: 0.037) of the microbiome and shaping the composition in a beneficial direction with fewer potentially pathogenic species and more potentially probiotic species. The increase in Enterococcus and other potentially beneficial bacteria could have happened on the expense of reduction in Bifidobacteriaceae.

This modulation of the microbiome can translate into improved gut health and performance, as the *Enterococcus faecium*669-treated chicks showed strong potential for counteracting infections due to competitive exclusion and improved immunity.

Key Words: probiotic, microbiome, hatch, colonization, diversity

P278 The link between broiler flock heterogeneity and cecal microbiome composition Dorthe Sandvang*, Antoine Meuter, Steve Lerner *Chr. Hansen, A/S*

Despite low genetic variation of broilers and deployment of considerate management practices, there still exists considerable body weight (BW) heterogeneity within broiler flocks which adversely affects the commercial value. The purpose of this study was to investigate the role of the cecal microbiome in weight differences between animals. Understanding how the gut microbiome may contribute to flock heterogeneity helps to pave the road for identifying methods to improve flock uniformity and performance. Two hundred eighteen male broiler chicks were housed in the same pen, reared for 37 days, and at study end the 25 birds with highest BW (Big) and the 25 birds with lowest BW (Small) were selected for microbiome analysis. Cecal contents were analyzed by a hybrid metagenomic sequencing approach combining long and short read sequencing. For calculation of Shannon index diversity and Bray-Curtis values, significance testing was done by using a non-parametric Wilcoxon rank sum test. Looking at the data from the two groups, the group of Big birds was more uniform than the group of Small birds. For the group of Small birds, the mean body weight was 1,808 grams, with a standard deviation of 122 grams and a coefficient of variation (CV) of 6.75%. In contrast, the Big birds were almost 1,100 grams heavier, with a smaller standard deviation of 100 grams and a CV that was half that of the group of small birds. Regarding gut microbiota analysis, we found that Big birds displayed significantly higher microbial alpha diversity (p:0.017). Higher microbiome uniformity (lower beta diversity) was also observed within the group of Big birds where the bird-to-bird variation (Bray-Curtis distance) was highly significantly lower (P<0,001) than that of the group of Small birds. Finally, higher levels of SCFA-producing and health-associated bacterial taxa such as Lachnospiraceae, Faecalibacterium, Butyricicoccus and Christensenellales, and lower levels of Akkermansia muciniphila and Escherichia coli as compared to Small birds. Cecal microbiome characteristics could be linked to the size of broiler chickens. Differences in alpha diversity, beta diversity and taxa abundances all seem to be directly associated with growth differences observed in an otherwise similar broiler flock.

Key Words: Gut, Microbiome, Diversity, broiler, uniformity

P279 The effect of the duration of dark period on turkey hens' response to a novel object test at 5 weeks of age Allison Pullin*, Callie Lewis, Jesse Grimes *Prestage Department of Poultry Science, North Carolina State University*

In welfare audit standards, turkeys are hypothesized to become reactive during periods of complete darkness and thus create a higher risk for piling. However, longer dark periods have resulted in less fearfulness in broilers and layers, and this relationship has not been formally studied in turkeys. We aimed to evaluate fearfulness with a novel object test in turkey hens reared under four different dark periods. Nicholas Select hens were housed across 16 rooms with independent light control (55 hens/room). All rooms received 24h light for the first 3d post-placement, then dark period treatments were applied: 0h, 2h, 4h, 8h (n=4 rooms/treatment). At 5 wk of age, a novel object test was conducted with a plastic rod wrapped with colored tape (Welfare Quality® assessment protocol for poultry). Rooms were video recorded for 15 min after placing the object on the floor. Latency to approach, latency to touch (i.e. pecking or stepping on the object), and the average number of hens within one body length, two body lengths, and touching the object with their beak or feet were evaluated. Data were analyzed with linear mixed effects models in R software. Hens were quick to approach the novel object, and this initial approach was not influenced by dark period (p=0.11; 0h: 13.3 ± 2.8, 2h: 13.4 ± 2.8, 4h: 10.0 ± 2.8, 8h: 4.2 ± 3.2 sec, mean ± SE). However, hens reared with 8h dark were faster to touch the object than hens reared with no dark period (p=0.049; 0h: 33.6 ± 6.5, 2h: 22.7 ± 6.5, 4h: 15.8 ± 6.5, 8h: 7.1 ± 7.6 sec). Dark period did not affect the average number of hens within one body length (p=0.93; 0h: 7.5 ± 1.4, 2h: 6.5 ± 1.4, 4h: 6.3 ± 1.4, 8h: 6.5 ± 1.6 hens), two body lengths (p=0.82; 0h: 6.7 ± 1.0, 2h: 7.6 ± 1.0, 4h: 7.8 ± 1.0, 8h: 7.9 ± 1.1 hens), or touching (p=0.94; 0h: 3.2 ± 0.7, 2h: 2.6 ± 0.7, 4h: 2.7 ± 0.7, 8h: 3.1 ± 0.9 hens) the object across the 15 min trial. Contrary to the hypothesis that dark period influences reactivity of turkeys, the duration of dark period had minimal effect on fearfulness to novelty. Furthermore, hens from the longest dark period may be slightly less fearful than hens with no dark period based on latency to touch the object. In future work, we will test additional ages and other types of fear responses.

Key Words: photoperiod, novel object test, turkey, welfare, fearfulness

P280 Impacts of the rearing environment on keel bone integrity, spatial awareness abilities of laying hens Maja Makagon*[1], Richard Blatchford[1], Kristina Horback[1], Suzanne Millman[2], Allison Pullin[1,3], John Tarlton[4], Michael Toscano[5] *[1]University of California, Davis, [2]Iowa State University, [3]North Carolina State University, [4]University of Bristol, [5]University of Bern*

This interdisciplinary project aimed to mitigate the occurrence of keel bone fractures in cage-free housing systems. We hypothesized that the opportunity to move through vertical space during rearing would enhance development of spatial awareness and bone health, facilitating hens' movements during lay and reducing keel fracture prevalence. Dekalb White pullets (55-56 pullets/pen) were reared in 3.05x3.05 m^2 floor pens containing either a single- (ST) or two-tiered aviary (TT), or floor perches (FL; 5 pens/treatment). Rearing systems were replaced with multi-tiered aviaries at 17 wks. Ten pullets/pen were removed at 8 and 16 wks for bone assessment (bone mineral density; biomechanics of tibia, humerus and keel). Spatial abilities were evaluated using depth perception tests at 7-8, 15-16 and 29-30 wks (Y-maze and visual cliff; 50 pullets/treatment/time point), and hippocampal development at 16 wks (dendritic morphology and BDNF expression; 2-3 pullets/pen). Behavioral assessment included use of elevated spaces at flock level and ease of movement across vertical tiers by 10 focal birds/pen in pullet (6 and 16 wks) and layer (17, 19, 23 and 27 wks) housing. Presence and severity of keel fractures were scored for the same focal pullets from radiographs at 18, 26, 28 and 30 wks. Number of eggs laid on pen floors was tallied weekly from 18 wks. Analyses were conducted primarily in R, separately for each bone prop-

erty, depth perception test, behavioral assessment, and floor egg and keel fracture prevalence. Model fits were assessed for deviations from assumptions. Stepwise backwards reductions of the full models determined final models, where appropriate. Alpha was set to 0.05. Bone mineral density was higher in FL than TT and ST birds at 16 wks. As compared to TT and ST birds, FL pullets were less likely to cross the visual cliff at 8 and 16 wks, and were slower to cross at 8wks. TT and ST hens used the layer aviary more than FL hens the first day post-transfer, and laid fewer floor eggs at 18-19 wks. Rearing pullets with height helped facilitate their transition into layer housing, however most rearing treatment differences disappeared by 19 wks. We are currently exploring the prevalence of fractures in the U.S. in relation to commercial cage-free housing systems.

Key Words: Laying hen, Pullet rearing, Keel bone, Cage-free housing, Space use

P281 Primary Growth Plate Chondrocyte Isolated from the Modern Broiler – foundation for in vitro studies Alison Ramser*, Elizabeth Greene, Sami Dridi, Narayan Rath *University of Arkansas, Center of Excellence for Poultry Science*

The study of common diseases in poultry needs relevant and appropriate *in vitro* models. Bacterial chondronecrosis with osteomyelitis (BCO) is a leading cause of lameness affecting the growth plates of the long bones. A major limitation when studying BCO is a lack of chicken immortalized chondrocyte cells. In this study, isolation and high-density culturing of primary chondrocytes from 10-day old chicks was followed by confirmation of cell type, identification of optimal phenotypic expression, and evaluation of cells functionality. Cell lysate and media were collected at each time point for analysis (n = 3). Relative expression of target genes was determined using real-time qPCR and protein was visualized and quantified via Western Blot and Alpha View software. Data were analyzed by one-way ANOVA using GraphPad. Live cell imaging was conducted using Cytation3 plate reader. Results showed a shift from COLII dominant expression in early-culture to COLI dominant expression by late-culture. This also coincided with a loss of other chondrocyte markers, such as Sox9, ACAN, and COLXA1 ($P < 0.05$). Additionally, morphological changes occurred with the shift of phenotype in mid- to late-culture periods indicating a loss of desired phenotype. The functionality of the cultured cells was confirmed using Brefeldin-A treatment which significantly reduced secretion of COLII by day 7 (d7) ($P < 0.05$). Taken as a whole, it is clear that avian growth plate chondrocytes under these conditions exhibit optimal phenotype between d3 to d7. These results provide an accurate road map to isolation and utilization of avian primary chondrocyte cells in the study of bone-related infections and diseases as well as the foundation for future work immortalizing or passaging.

Key Words: primary chondrocytes, avian cell characterization, primary cell isolation, broilers

P282 Broiler mobility assessment aia a Semi-Supervised deep learning model and Neo-DeepSort algorithm Mustafa Jaihuni*[1], Yang Zhao[1], Hao Gan[2], Tom Tabler[1], Hairong Qi[3], Maria Prado[1] *[1]Department of Animal Science, University of Tennessee Knoxville, [2]Department of Biosystems Engineering, University of Tennessee Knoxville, [3]Department of Electrical Engineering, University of Tennessee Knoxville*

Broiler mobility is a vital welfare indicator. Compromised mobility may influence their ability to access feed and water, hence physiologically detrimental. Classical broiler mobility assessment methods are laborious, time-consuming, and cannot provide continuous insights into individual bird's conditions. In this paper, we proposed a semi-supervised Deep Learning (DL) model, YOLOv5, combined with our newly developed variant of the conventional Deep Sort tracking algorithm, Neo-Deep Sort, for individual broiler detection and trajectory tracking, respectively. Two pens with 16 and 12 broiler stocking densities would be utilized in this experiment. Their daily movements would be recorded via two top view cameras for 15 minutes per hour during a 54-day rearing period. Initially, 1650 labeled images from five random days' recordings are employed to train the YOLOv5 model. Through the semi-supervised learning (SSL) which significantly reduced the lengthy data labelling process, this narrowly trained model is then used for pseudo-labeling 2160 images, of which 2153 (99%) were successfully labeled. Thereafter, the YOLOv5 model is fine-tuned on the newly labeled images. Lastly, the meticulously trained YOLOv5 and the Neo-Deep Sort algorithm are applied to detect and track 28 broilers in two pens and consequently categorize them in terms of hourly and daily moved distances and respective speeds. As a result of the SSL, the YOLOv5 model's mean Average Precision (mAP), in identifying birds with a 50%-100% confidence, increased from 81% to 98%. The combined model provided individual broiler's hourly moved distances with a validation accuracy of about 80%. Eventually, individual and flock level mobilities were quantified while overcoming the occlusion, false and miss detection issues. The vision-based algorithm developed in this study may serve effectively to track indicators critical for broiler production performance and welfare.

Key Words: broiler welfare, mobility, YOLOv5, semi-supervised learning, Neo-Deep Sort

Environment, Management: Environmental Impacts

P283 Protecting free range forage with the use of a geogrid system on range forage coverage, forage quality and egg production Benjamin Alig*, Kari Harding, Alea Belflowers, Ramon Malheiros, Madison Spangle, Kenneth Anderson *Prestage Department of Poultry Science, NC State University*

Free range egg production systems are becoming a more widely used system for egg producers as consumer demand expands. One major issue for free range egg production is forage quality and coverage. With proper management, many early issues can be mitigated, however as the hens utilize the range during the production cycle, range quality and recovery ability decreases. GrassWorx™ has developed a geogrid which theoretically, will allow hens access to forage while protecting the roots enabling faster forage recovery. The objective of this study was to evaluate the effectiveness of the geogrid on range forage coverage, quality, and recovery as well as its effect on egg production. We hypothesize that using the geogrid would improve range quality and recoverability while not affecting egg production. Four replicates of ranges with geogrid and control were used for this study (n=8). Each replicate contained 2 equal paddocks which were rotated on a 4 week cycle for recovery. The lay cycle started at 17 weeks of age and continued for 52 wks. Egg production was averaged over 4 week periods for a total of 13 periods. Drone images and forage samples for nutrient analysis were taken at the end of periods 1, 2, 3, 4, 12 and 13 and graded based on a 1-5 scale. Statistical analysis was run as a linear model using JMP PRO 15. As ranges were set up in rotation, recovered pens and used pens were analyzed separately. Egg production, forage analysis and range grades were analyzed by treatment, period, and the interaction of the two. Student's T-test was used to compare treatments and p-values of <0.05 were considered significant. Overall, usage of the geogrid did not affect egg production except hens on the geogrid laid more grade B eggs ($P=0.014$) by about 0.8%. Both recovered ranges ($P=0.041$) and used ranges ($P<0.001$) with the geogrid had better coverage scores than controls by about 0.5 points. Furthermore, forage on the geogrid had less phosphorus by 0.03% ($P<0.001$) and calcium by 0.23% ($P<0.001$) than the control. In conclusion, this study supports that utilizing the geogrid can protect range coverage and quality without diminishing

egg production due to protecting root systems. However, more research is needed to evaluate why nutritional quality was different.

Key Words: Laying Hens, Free Range, Geogrid, Forage Coverage, Forage Protection

P284 No effects of red-green or full spectrum white LEDs on incubation or post-hatch production variables in broilers. Muhammad Riaz[1], Jenna Schober[1], Esther Oluwagbenga[1], Victoria Tetel[1], Karen Christensen[2], Gregory Fraley*[1] *[1]Purdue University, [2]Tyson Foods*

Light plays vital role in visual perception, development, and welfare of birds. Since the advent of LED lights, numerous studies have attempted to obtain effects of lighting on developing embryos, with quite mixed results. Several studies have agreed, however, that red and green lights have beneficial effects on broilers. Further, new evidence has suggested the importance of ultraviolet light in birds. Our study was designed to examine whether red-green lights or full spectrum white lights placed in incubators would benefit production or welfare of broilers. In order to accomplish this goal, fertilized eggs were obtained from a commercial hatchery. 150 eggs were placed in each of 3 incubators that contained control (dark), white light (380-715 nm), or a combination of green (510-540 nm) and red (625-640 nm) LEDs. The photonic energy of each light treatment was normalized at the level of the egg (~2.16 x 10^2 uWatts/cm^2). Incubation settings were set to follow industry standards. Lights were provided for 12 hours per day throughout incubation and the same lights were provided in the respective hatchers. During incubation we measured eggshell temperature (60 eggs per incubator) and embryo weights (10 per incubator) on days 7 and 14. Hatching traits such as hatchability and dead in shell percents were calculated by doing breakout analyses. Following hatch, 20 chicks were placed per pen (6 pens per incubator) and body weight, feed intake, and FCR were calculated on weekly basis beginning the day of hatch until 21 days of age. An isolation vocalization fear test performed (n = 12 per incubator) weekly beginning day 7 through day 21. Birds were placed into an isolation chamber and the time to cease vocalizations was recorded. The complete experiment was then repeated while rotating the lighting treatments among the incubators. All data were analyzed by mixed effects model for ANOVA, or a 2-way ANOVA with repeated measures as appropriate. None of the variables measured during the study showed any significant effects nor trends towards significance. Our results suggest that although light plays a critical role in posthatch development and reproductive status, its role on embryonic tissue remains to be elucidated.

Key Words: incubator lights, incubator environment, welfare

P285 Growth performance of broiler chicks obtained from different type of hatching eggs. Kağan Çubukçu, Serdal Dikmen* *Bursa Uludag University Faculty of Veterinary Medicine Dept. of Animal Science*

Eggs were obtained from Ross 308 breed which were at 54 weeks of age (n=3059). All eggs were collected in the same day and classified into different categories according to their physical characteristics. In the trial design each group had three replicates. Data included growth performance, feed conversion ratio (FCR), carcass characteristics, mortality rates and performance efficiency index of different sex within each group were compared. The difference in hatching weight of male (49.6±0.2 g) and females chicks (47.8±0.2 g) were highly significant (P<0.0001). The average hatching weights of the chicks in large (L), porous (P), normal (N) and misshaped (M) egg groups were determined as 56.7±0.3, 44.9±0.3, 45.7±0.3, 47.5±0.3 g, respectively (P<0.0001). The slaughter weight of male chicks (3121±16.9 g) were greater than female chicks (2567.8±15.7 g) at 6 weeks of age (P<0.0001). The average slaughter weights for the chicks in L, T, N and M groups were found 2893.9±22.5, 2832.6±23.8, 2798.7±22.9 and 2854.3±22.9 g, respectively (P=0.02). The effect of group (P<0.05), sex (P<0.0001) and age (P<0.0001) on feed intake were significant. There was no significant difference in FCR results among groups (P>0.05). But the effects of sex and age was highly significant on FCR results during the study (P<0.0001). The effects of group (P<0.05), sex (P<0.0001) and group X sex interaction (P<0.05) was significant for carcass weights of broilers. The performance efficiency index was also significantly different among groups (P<0.01) and sex (P<0.0001) but the effect of group X sex interaction was not significant (P>0.05). The mortality rate of chicks hatched from large eggs were significantly lower than other groups (P=0.01). As conclusion, the broilers hatched from large eggs and male broilers had better growth performance in all groups.

Key Words: Broiler, egg shell quality, FCR, daily weight gain

P286 Assessment of the hatch of progeny from broiler breeders under different ventilation during morning feedings Katie Elliott*[1], Joseph Purswell[1], Klint McCafferty[1], Leonard Fussell[2] *[1]USDA-ARS Poultry Research Unit, [2]Cobb-Vantress Incorporated*

The purpose of the current study was to evaluate if air speed treatments for broiler breeders had any effect on their eggs and the resulting progeny. Treatments for the breeders included still air (S), constant (C), and variable (V) air speed (increased for 3h at feeding time). Each treatment consisted of 4 replicate pens (22 hens and 2 cocks/pen). For each of 2 trials, 2 replicate trays of eggs (90 egg max capacity trays) were filled representing each broiler breeder pen and randomly placed in a single incubator set at 37.5°C and 55% relative humidity (24 trays/trial). Each tray was weighed for egg moisture loss (ML) at d 12 and 18 of incubation. Fertility was assessed by egg candling and breakout on d 12 of incubation. After hatch, the hatch success and the batch weight of the hatched chicks/tray were noted as well as a residue breakout analysis. The egg wt, ML, and average chick wt were analyzed using proc MIXED and the incidence of hatched chicks and residue egg categories were analyzed using the proc GLIMMIX procedure of SAS. Incubation tray was considered a random effect and any effects of trial, treatment, and trial*treatment interaction were analyzed. Trial*treatment interactions were not found to be significant for any variable. The average egg wt did not differ by treatment at set (P=0.29), and percentage ML did not differ based on treatment on d 12 or 18 (P=0.17 and 0.16, respectively). Trial 2 eggs had greater average egg wt (P<0.0001) due to older birds, and trial 1 eggs had greater ML (P<0.0001). The percent hatch of fertile eggs was marginally significant (P=0.0527) with the greatest hatch in the S treatment (86.0%) which was not different from C (84.4%) with C not differing from V (81.1%). The percentage of infertile eggs was lowest in S (2.4%; P=0.03) which did not differ from C (4.1%) with C not differing from V (4.8%). A similar relationship was found for percentage of late dead (last wk of incubation) embryos (P=0.03). Average chick wt was larger in trial 2 following egg size (P<0.0001) but did not differ due to air speed treatment (P=0.23). Based upon the findings of this study, still air or constant air speed as opposed to varying air speed during broiler breeder feeding may offer some benefit for breeder fertility and hatch success.

Key Words: ventilation, hatch, broiler, incubation, chick

P287 Using spatial modeling to estimate floor area in commercial broiler barns within target brooding and growout light intensity levels. Olumide Falana*[1GS], John Linhoss[1], Jeremiah Davis[1], Carson Edge[1], Abigail Lane[1], Martha Rueda[1], Kelly Griggs[1], Cody Smith[1], Jesse Campbell[1], Joseph Purswell[2] *[1]National Poultry Technology Center, Auburn, AL, [2]USDA-ARS Poultry Research Unit*

Light intensity was measured in twenty 60 × 500 ft commercial broiler houses in South Alabama to determine the percentage of floor area within target light intensity values for brooding and tunnel ventilation conditions. Houses ranged in age from new (never used) to old (8 years old), and all had three rows of lights. Target light intensity for brooding was ≥ 43 lux (lx) and 0.2 lx ± 0.02 for tunnel ventilation. Data were collected at 70 locations in a 40 × 60 ft grid in the following three house sections: evaporative pad (pad), center house (center), and tunnel fans (fan). Brooding light intensity data was reported in two target categories: above or below 43 lx

in the pad and center sections only. Tunnel ventilation data was reported in three target categories: at, above, or below target values in all house sections. Spatial mapping via universal kriging was used to map light intensity distributions. The mean percent of house floor area (arcsine transformed) within each target level was analyzed as a two-way ANOVA (age and house section) using PROC MIXED in SAS for brooding and tunnel ventilation conditions. Means were separated at $P \leq 0.05$ using Fishers' LSD. During brooding, percent of floor area below target (< 43 lx) was significantly higher for older houses than new houses in the pad (old = 99.8%; new = 2.8%) and center sections (old = 99.9%; new = 2.8%). During tunnel ventilation in the new houses, 4.5, 6.0, and 0.0% of the floor area at the pad, center, and fan sections were within the 0.18 – 0.22 lx target. However, in the old houses, only 0.6, 0.7, and 0.0% of the floor area at the pad, center, and fan sections, respectively, were at the target level. All floor area in the fan section was above target due to light intrusion from operating fans. Percent of floor area above the tunnel ventilation target was significantly higher in new houses in both the pad (old = 12.5%; new = 91.3%) and center sections (old = 10.9%; new = 84.7%). This research illustrates that the newer houses did a better job at meeting or exceeding target light intensities and that very little of the floor area in new and old houses during tunnel ventilation was within ±10% of the target. Periodic checking of light intensity is recommended, especially in older houses.

Key Words: light intensity, light variation, light measurement, kriging interpolation, broilers

P288 Endectocides: for the simultaneous control of multiple vector-borne diseases in poultry. Koyle Knape*[1GS], Cassandra Durden[2], Yuexun Tian[3], Macie Garza[3], John Carey[1], Sarah Hamer[2], Gabriel Hamer[3] *[1]Department of Poultry Science, Texas A&M University, [2]Department of Veterinary Integrative Biosciences, Texas A&M University, [3]Department of Entomology, Texas A&M University*

Zoonotic pathogens continue to emerge and threaten both human and animal health. Increased urbanization, globalization, and climate change have facilitated increased transmission of infectious agents, especially those vectored by arthropods. The most common form of arthropod vector control are environmental insecticides. In many cases the use of environmental insecticides are limited, ineffective, and harmful to the environment. An emerging approach is the use of host targeted insecticides(endectocides). It has been shown that domestic poultry provide bloodmeals to the *Culex quinquefasciatus*(Sebring) mosquito which are known vectors of *W. bancrofti*, avian malaria, St. Louis encephalitis virus, Western equine encephalitis virus, Zika virus and West Nile Virus. Our hypothesis was that arthropod vectors that feed on endectocide treated poultry will have a higher rate of mortality compared to control groups. This trial evaluated the efficacy of 3 endectocide products: Safe-Guard® Aquasol (Fenbendazole), Ivomec® Pour-On (Ivermectin), and Bravecto® (Fluralaner), dosed orally. Seventy laying hens were housed in an environmentally controlled layer house in individual cages with a nipple waterer serving individual hens. Fenbendazole and Ivermectin treatments were added to a nipple watering system that only delivers medicated water to the respective treatment group. A weighed, dyed (for visualization) piece of Fluralaner was given to hens mixed with their feed. 50 to 100 hundred *C. quinquefasciatus* mosquitoes were allowed to feed for 45 minutes on live, restrained hens at day 3, day 7, day 14, day 28 and day 56 post-treatment while monitoring the fed vectors for survivability. Statistical survival analysis was visualized using Kaplan-Meier Curves (R Core Team, 2017). This analysis showed that mortality of *C. quinquefasciatus* Sebring mosquitoes feeding on Fluralaner treated hens was significantly higher compared to hens treated with Fenbendazole, Ivermectin and control hens on day 3 and day 7. There were no significant differences found for vector mortality between Fenbendazole, Ivermectin and control treated hens at the later time points. In conclusion we have shown that Bravecto® (Fluralaner) administered to layer hens can suppress populations of *C. quinquefasciatus*.

Key Words: Poultry, Insecticide, Endectocide, Culex, Vector-Borne

P289 Effects of stacked cages versus outdoor moveable pens on broiler growth and performance Matthew Hughes*[1UG], Brigid McCrea[2], Dianna Bourassa[1] *[1]Auburn University, [2]Alabama Cooperative Extension System*

In recent years, there has been a movement by homesteading flock owners to produce their own foods, including poultry. With recent increases in inflation, land prices for new homesteaders has increased and this has caused them to settle for small plots of land. Also, the cost of chicken feed has increased. Since chicken feed is roughly seventy percent of production, every gram of feed saved could save the flock owner money. Stacked cages could help utilize land space while still being able to produce an inexpensive product. The objective of this study was to evaluate two small flock management systems, outdoor moveable pens and stacked cages, for body weight gain, feed intake, and feed conversion. At two weeks of age, 88 Ross 708 broiler chicks were placed in four outside pens with ten birds each (n=40) and four, 4-level stacked cage systems with three birds per level (n=48). The moveable pens measured 150 x 100 x 50 cm LWH. Pens were moved daily to fresh grass and clover mix that was not taller than 8 cm. Moveable pens had gravity fed feeder and cup drinker. Each cage system level measured 91 x 49 x 50 cm LWH. Cages were kept in a covered garage with a large door and no access to grass. Cages had a feed trough on the front and nipple drinkers. All birds were grown for four additional weeks and given the same diet *ad libitum*. Statistical analysis was performed using the TTest procedure for body weight gain, feed intake, and feed conversion and Fisher's Exact Test for mortality in SAS with statistical differences at P0.05. Neither outdoor nor caged management systems had an effect on feed conversion ratios, 1.57 and 1.56, respectively (P=0.8837). The type of management system did have an effect on body weight gain and feed intake (P<0.0001). The birds outside consumed 569 more grams of feed and gained 349 more grams body weight than the birds inside. During the course of the study mortality in the pen system was 4/40 and cage mortality was 0/48 birds (P=0.0392). The additional gain observed for birds raised outside could help the farmer bring birds to market in less time. However, the potential for increased mortality must also be considered.

Key Words: broiler, cages, pens, growth, performance

P290 Evaluating the impact of a geogrid astroturf in free-range layer systems on crop content and egg quality. Taylor O'Lear Reid*, Kari Harding, Benjamin Alig, Alea Belflowers, Rebecca Wysocky, Ramon Malheiros, Kenneth Anderson *North Carolina State University*

Range production used in sectors of the poultry industry has been evolving, to replace the conventional caged housing system and enhance cage free systems. Range quality is particularly important near the poultry house due to denuding of the forages which will decrease the nutritive potential in the free range environment. GrassWorx™ developed a geogrid system, called InstaTurf®, which by design will protect the range and improve forage availability. The objective of this study was to evaluate the effect of the geogrid on hen forage consumption and egg quality. A flock of 520 Brown Bovan egg layers were raised 65 birds/ replicate on range in 4 control replicates with access to the soil and 4 geogrid replicates (n=8). At 17 weeks of age pen populations were set at 60 hens/pen. At 17 and 69 weeks of age 5 and 10 birds, respectively were euthanized and crop contents collected from each replicate to determine the percentages of feed, forage, weed seeds, insects and other (rocks, feathers, eggshells, etc) consumed. These contents were excised into Nalgene cups, placed in cooler, then later dried at 50 C for 72hr. Contents were weighed and distributed into groups based on type and calculated on a percentage basis. JMP 15.1, Student's T test was used. P-value was significant at < 0.05 and the statistical model was age, treatment and the interaction of the two. Shell strength, vitelline membrane strength, shell color, albumen height, Haugh unit, and yolk color were recorded. No significant difference between treatments was noted in the consumption of feed, forage, or the "other" category. However, there was a noted significant increase in "other" consumption

between the start and end of the study (p=0.0175) possibly due seasonal changes that would occur over the course of a year. For egg quality characteristics, no significant difference in egg quality was present between the two treatment groups. However, between the two testing intervals, shell elasticity (p=0.0209), shell color (p=0.0001), and egg weight (p=0.0001) improved. On the other hand, albumen height (p=0.0001), Haugh unit (p=0.0001), and yolk color (p=0.0001) decreased.

Key Words: free-range, layer, egg quality

Metabolism and Nutrition: Amino Acids

P291 Effect of arginine supplementation on production performance and inflammatory response in broilers during necrotic enteritis challenge. Shahna Fathima*[1GS], Walid Al Hakeem[1], Bikas Shah[1], Revathi Shanmugasundaram[2], Ramesh Selvaraj[1] *[1]Department of Poultry Science, University of Georgia, [2]Toxicology and Mycotoxin Research Unit, US National Poultry Research Center*

Restrictions on the use of in-feed antibiotic growth promoters in poultry led to the reemergence of necrotic enteritis (NE) in poultry, necessitating the need to develop alternatives to antibiotic growth promoters (AGPs). Arginine is a conditionally essential amino acid which is the substrate for nitric oxide and ornithine biosynthesis. Arginine can modulate the immune response of birds to *Eimeria* challenge by regulating macrophage differentiation and subsequent inflammatory pathways. This study evaluated the effects of 125% and 135% L-arginine on the production parameters and immunological responses in necrotic enteritis (NE) challenged broilers. A total of 480 day-old chicks were randomly allocated into four treatment groups- 1. Non-infected group fed basal diet 2. NE group fed basal diet 3. NE group fed 125% arginine diet and 4. NE group fed 135% arginine diet. The basal diets were formulated to meet or exceed the Cobb-500 nutrient requirements. NE was induced by inoculating 1×10^4 *Eimeria maxima* at day 14 and 1×10^8 CFU *C. perfringens* at days 19, 20, and 21 of age. All data were analyzed by ANOVA and the means were compared by Tukey's HSD and were considered significantly different at $P \leq 0.05$. NE infection significantly increased the feed conversion ratio by 18 points ($p = 0.01$), intestinal permeability, jejunal lesion score, and decreased the body weight gain and the ratio of $CD4^+$:$CD8^+$ cells in the cecal tonsils. 125% arginine diet increased the feed intake by 30g ($p = 0.02$) and reversed the NE-induced loss in BWG by 70g ($p = 0.12$). 125% arginine diet significantly increased the bile anti-*C. perfringens* IgA concentration by 37.5% ($p = 0.03$). Arginine supplementation significantly decreased the ratio of $CD4^+$:$CD8^+$ cells on day 27 (p0.03) and day 34 (p0.01). Arginine supplementation did not reverse the NE-induced loss in intestinal permeability nor did it have a significant effect on the NE lesion score of the birds. It can be concluded that owing to the growth-promoting and immunomodulatory effects, 125% arginine diet can be used in combination with other feed additives to replace antibiotic growth promoters in broilers.

Key Words: Necrotic enteritis, Broilers, L-Arginine, Nutraceuticals

P292 Methionine supplementation and immune function in broilers experiencing an acute inflammatory challenge Kaitlyn Sommer*[1GS], Juliano de Paula Dorigam[2], Rose Whelan[2], Bradley Gorenz[1], Maci Oelschlager[1], Julianna Jespersen[1], Ryan Dilger[1] *[1]University of Illinois - Urbana Champaign, [2]Evonik Nutrition & Care GmbH*

Methionine is the first-limiting amino acid for broilers and plays a critical role in immune function. This study investigated the impact of graded supplemental levels of methionine in broilers undergoing a lipopolysaccharide (LPS) challenge. A total of 336 male broilers (Ross 308) were housed in batteries and allotted to 1 of 6 dietary treatments, with 7 replicates of 8 birds per replicate. Diets were provided in a 2-phase feeding program with a common starter diet and experimental grower diets provided d 0-10 and 10-28, respectively. Grower diets included a basal diet (0.136% digestible Met) supplemented with 0.360% L-Cys (0%DLM) and five other supplemented diets with graded levels of DL-Met (0.072%DLM-0.360%DLM, with 0.072%DLM increments). To ensure the only limiting amino acid would be Met, other amino acids were included at approximately 15% over the AMINOChick 3.0 recommendations. All diets were provided ad libitum throughout the study. On study d 25 [0 hours post-stimulation (HPS)], all birds were challenged intraperitoneally with LPS derived from *E. coli* (O127:B8) at 2.0 mg/kg BW with 1 bird per pen was euthanized for sample collection at 5 HPS. Body weight gain during the grower phase was lowest (170 g, $P < 0.01$) in 0%DLM compared with 0.216%DLM or higher (1,067 g, 1,103 g and 1,112 g, respectively). Birds receiving 0%DLM had the least efficient feed conversion ratio (3.56 g/g, $P < 0.01$) compared with treatments ≥ 0.216%DLM (1.5). The treatments ≥ 0.216%DLM had, on average, 1.07 percentage points lower ($P < 0.01$) relative liver weights (%BW) at 5 HPS compared with 0%DLM (4.35%). Plasma concentrations of the acute phase protein, α-1-acid glycoprotein (AGP), were elevated (180. 3 μg/mL, $P < 0.01$) in 0%DLM compared with 0.144%DLM (88.3 μg/mL) and 0.288%DLM (76.5 μg/mL) treatments; however, there were no differences in interleukin-6 plasma concentrations. In conclusion, broilers receiving diets deficient in Met exhibited a heightened response to LPS stimulation, as evident by the highest AGP production from hepatocytes, which may have contributed to increased relative liver weights. However, DL-Met supplementation ameliorated the effect of LPS as evidenced by benefits to growth performance, relative liver weights, and AGP concentrations.

Key Words: Broiler, Lipopolysaccharide, methionine, immune function

P293 Effects of maternal feed amino acids profile on quail breeders egg quality parameters. Lizia Carvalho*[1GS], Tatyany Mani[1], Dimitri Malheiros[2], Ramon Malheiros[2], Edney Silva[1] *[1]UNESP, [2]Prestage Department of Poultry Science - NCSU*

Maternal amino acid nutrition is fundamental for egg formation, which subsequently supports embryo development. The ideal amino acid ratio (IAAR) in feed should support protein synthesis of the target tissue. In this research, consists of the proteins that compose the egg during the formation of the yolk, albumen, shell membrane, and eggshell. Thus, the challenge in this research was to apply the deletion method to establish the IAAR based on variables that are related to egg quality. Therefore, the study aims to establish the essential IAAR (Lys, Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His and Phe+Tyr) for Japanese quail breeders based on egg quality using the deletion method. An entirely randomized design was used, with 12 treatments and 10 replicates of one bird each, for a total of 120 breeders at peak egg laying. The treatments consisted of a balanced diet (BD) and 11 other diets obtained from the deletion of 40% of the test amino acid, using as base the BD. The amino acids studied were Lys, Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His and Phr+Tyr. The trial lasted 25 days and at the end egg weight, albumen height and diameter, albumen and yolk index, Haugh unit, pH, shell weight and shell percentage were measured. IAAR calculation was applied when statistical difference was detected by Dunnett test. According to the results, only albumen height was not affected by treatments (P>0.05), in the other variables effects could be detected (P<0.05). Only the variables egg weight (EW) and shell weight (ESW) differed from BD, for all amino acids evaluated. Leucine and valine showed greater limitation in relation to BD, EW, and ESW, respectively. Thus, the IAAR considering Lys as 100% for EW and ESW were Met-Cys 82% and 83%; Thr 60% and 68%; Trp 18% and 21%; Arg 109% and 112%; Gly-Ser 99% and 102%; Val 77% and 87%; Ile61% and 67%; Leu 155% and 141%; His 34% and 37; Phe+Tyr 134% and 133%, respectively. In the present study, it was possible to de-

termine the IAAR for Japanese quail breeder with a methodology not yet described in the literature, based on EW and ESW.

Key Words: amino acids, Quail Breeders, Egg quality, deletion method, egg protein

Metabolism and Nutrition: Enzymes

P294 Ileal digestible, metabolizable, and nitrogen corrected metabolizable energy of soybean meal with inclusions of exogenous carbohydrases for growing broiler chickens Tanner Wise*[1GS], Olayiwola Adeola[1], Mike Blair[2] *[1]Purdue University, [2]United Animal Health*

Enspira (ESP) and Enspira$^+$ (ESP$^+$) are precision engineered blends of exogenous enzymes designed to degrade non-starch polysaccharides in common diet formulations. An experiment was conducted to determine the responses of the ileal digestible energy (IDE), apparent metabolizable energy (AME), and N corrected AME (AMEn) of soybean meal (SBM) to ESP or ESP$^+$ inclusion. Cobb-500 male broiler chickens were obtained and fed a corn-SBM-based starter diet. At 20 d of age birds were individually weighed and 288 birds were allotted to 6 dietary treatments (6 birds/cage) in a randomized complete block design. Three corn-canola meal-based reference diets were created. One contained no enzymes and each of the other 2 contained 125 mg/kg of either ESP or ESP$^+$. Then, 3 test diets were created by adding 150 g/kg SBM to each of the reference diets at the proportional expense of all energy contributing ingredients, producing 3 pairs of diets (control, ESP, ESP$^+$). Experimental diets were fed from d 20 to 25 and excreta collected from d 23 to 25. At d 25, birds were euthanized and digesta collected from the terminal two-thirds of the ileum. Feed, digesta, and excreta samples were dried and analyzed for dry matter (DM), N, Ti, and gross energy (GE) content. Energy values were calculated using a difference method. Data were subjected to an ANOVA and contrasts were used to compare the effect of the enzymes with and with SBM. The addition of 150 g/kg SBM reduced the metabolizability of GE ($P < 0.05$) and N ($P < 0.01$) and produced lower IDE, AME, and AMEn of the test diets ($P < 0.05$). Inclusion of ESP or ESP$^+$ had no effects on the on the metabolizability of DM or GE for either the reference or test diets, although both enzyme blends produce numerical increases in the GE and DM metabolizability of the test diets. Inclusion of ESP elevated ($P < 0.05$) the estimated AME of SBM obtained from this study (405 kcal/kg DM) and ESP$^+$ inclusion tended ($P < 0.10$) to elevate the AME of SBM (304 kcal/kg DM). However, there were no significant differences observed for the IDE or AMEn of SBM. Based on the data from this study, it is possible that inclusions of ESP or ESP$^+$ can improve the energy utilization of SBM for broiler chickens when fed at 125 mg/kg.

Key Words: apparent metabolizable energy, broiler, exogenous carbohydrase, soybean meal

P295 Evaluation of the thermostability of a phytase and its effect on broiler performance, bone mineralization, and mineral digestibility kristina bowen*, elizabeth lynch, tim boltz, victoria ayres, joseph moritz *west virginia university*

The assessment of mixer-added, exogenous phytase enzymes is important for the continual improvement of global broiler production. Two experiments were conducted to evaluate an E. coli derived bacterial 6-phytase. The objective of experiment one was to determine the activity retention of the phytase when conditioned for 30 sec at 75, 80, 85, and 90°C. The objective of experiment two was to evaluate d0-42 broiler performance, bone mineralization, and mineral digestibility when birds were fed different concentrations of the phytase. For experiment one, 1,000 FTU/kg of the phytase was added to replicate batches of a corn and soybean meal-based starter diet. Each batch was conditioned at 75, 80, 85, or 90°C with three replications per temperature, and time of manufacture served as the blocking criteria. Mash and pellet samples were analyzed for phytase activity. For experiment two, seven diets were manufactured, and each were fed to 12 replicate pens of 24 Ross 708, male chicks from d0-42. Diets were conditioned at 75°C for 30 sec, and included a nutritionally adequate Positive Control (PC), a Negative Control (NC) deficient in calcium (Ca) and available phosphorus (P) by 0.2%, and graded levels of NC+phytase (250, 500, 1,000, 1,500, and 3,000 FTU/kg). Results of experiment one indicated a stepwise decrease in pellet mill motor load and increase in hot pellet temperature and pellet durability as conditioning temperature increased ($P<0.0001$). The calculated coefficient of variation for the phytase in mash was 4.9, indicating a thorough mix. Enzyme recovery decreased when diets were conditioned at 85°C relative to 75 and 80°C, and further decreased at 90°C ($P<0.05$; 85, 81,76, and 65% recovery). In experiment two, all phytase concentrations increased apparent ileal digestibility of P (AID P), d42 tibia ash percentage, and d0-42 live weight gain (LWG) relative to the NC diet ($P<0.05$). Phytase concentrations above 1,000 FTU/kg increased AID Ca relative to the NC diet ($P<0.05$). The E. coli derived bacterial 6-phytase demonstrated thermostability below 85°C and efficacy in mineral digestibility, tibia ash percentage, and d0-42 broiler performance.

Key Words: broiler, phytase, mixer added, tibia ash, mineral digestibility

P296 Effect of a specific fumonisin esterase on broiler chickens fed diets contaminated with fumonisins Lorran Gabardo*, Ursula Hofstetter, Verena Starkl *DSM*

Mycotoxins are grain contaminants produced by fungi in crops pre-harvesting or during storage. Among them, Fumonisins (FUM) are the contaminants which are most widespread in commodities worldwide. According to DSM World Mycotoxin Survey, in poultry feed approximately 82% of samples are contaminated by FUM over the last 10 years[SV1] . In USA, corn samples analysed in 2022 show a FUM prevalence of 71% with contamination level of 2,5 ppm. Due to structural FUM is not well adsorbed/binded, and there was a need for a different technology which biotransforms the molecule. Recently, in August 2022 FDA approved the first enzyme a fumonisin esterase (FUMzyme®), which is able to deactivate FUM in poultry species. Therefore, the aim of this study was to prove the capacity of a fumonisin esterase (FUMzyme®) in biotransforming FUM into hydrolised fumonisin (HFB1), a non-toxic and environmentally safe metabolite in broilers through biomarkers (FB1 and HFB1 content in the digesta, sphinganine/sphingosine ratio in blood). A total of 108 one day old broiler chicks were allocated into three experimental groups (G1: Control, G2: FUM, G3: FUM + fumonisin esterase) of 36 animals each with six replicates per group of six birds per pen. FUM contamination was 10 ppm and fumonisin esterase was added in a concentration of 75 Units/kg feed from day 7 to 21. G3 significantly lowered the FB1 content in the digesta when compared to the G2 ($P<0.05$). The metabolite HFB1 was significantly elevated in the G3 in comparation with G2. The sphinganine/sphingosine ratio (Sa/So), a biomarker which indicates FUM contamination, was significantly elevated in G2 as opposed to G1 ($P < 0.001$), while the addition of fumonisin esterase in G3 significantly lowered it.

The results indicated that the specific fumonisin esterase is able to effectively biotransform FUM into HFB1 in broilers. By applying an effective technology, the negative impacts of FUM on broiler health and perfor-

mance can be prevented and the impact of this mycotoxin in poultry production is reduced

Key Words: fumonisins, mycotoxin, broiler

P297 Effects of geographic region of origin and endosperm type on corn energy and nutrient utilization by broiler chickens Catarina Stefanello*[1,2], Sergio Vieira[3], Heitor Rios[3], Cristina Simoes[1], Guilherme Godoy[1], Jessica Starkey[2], Charles Starkey[2] *[1]Federal University of Santa Maria, [2]Auburn University, [3]Federal University of Rio Grande do Sul*

Corn (*Zea mays* L.) is the major energy yielding source in poultry feeds and differences in corn composition can be observed due to corn genetics, field conditions, and soil characteristics. Two experiments (Exp.) were conducted to evaluate nutrient and energy utilization by broiler chickens of corn originating from two geographic regions and of three endosperm types. In Exp. 1, a total of 112 Cobb 500 male chicks were fed semi-dent corn from regions geographically located in the North or South of Brazil, with 8 replicate cages of 7 birds each. In Exp. 2, a total of 168 Cobb 500 male chicks were fed 3 semi-purified feeds using waxy, semi-dent, or semi-flint corn endosperm types, with 8 replicate cages of 7 birds each. In both studies, birds were fed a common basal diet from d 0 to 14. From d 15 to 24, broilers were fed semi-purified test diets with 95.9% corn. Excreta samples were collected from d 21 to 23 and on d 24, ileal content was collected from all birds. Feed, excreta, and ileal digesta were analyzed to determine dry matter (DM), gross energy, crude protein (CP), ether extract (EE), calcium, and phosphorus to calculate their digestibility as well as the apparent metabolizable energy (AME), AME corrected for N retention (AME_n), and ileal digestible energy (IDE). In Exp. 2, starch digestibility was determined in the jejunum on d 24. Data were subjected to analysis of variance using the GLM procedure of SAS. Means were compared by the *t* test (Exp. 1) and Tukey test (Exp. 2) using a 95% confidence interval. Corn from the North region of Brazil had greater AME and AME_n as well as digestibility of CP, DM, and EE compared with that from the South ($P < 0.05$). In Exp. 2, corn endosperm type did not affect AME, AME_n, and IDE nor Ca and P total tract metabolizability for broilers ($P > 0.05$). The waxy corn had the lowest metabolizability of DM, CP, and EE and the lowest digestibility of EE and resistant starch ($P < 0.05$). Waxy and semi-dent corn had higher digestibility of available and total starch compared with the semi-flint corn ($P < 0.001$). In conclusion, energy and nutrient utilization of corn by broilers depended on the region where it was grown. Corn genetics, expressed by the endosperm composition, influenced starch, EE, and CP utilization by broilers.

Key Words: corn, nutrient digestibility, endosperm, starch

P299 Live performance evaluation of HiPhorius™ in turkeys fed to 42 days of age Brooke Jasek*[1], Nelson Ward[1], Dan Moore[2] *[1]DSM Nutritional Products, [2]Colorado Quality Research*

HiPhorius (HP) is a new improved version of HiPhos phytase. The objective of the present study was to evaluate the effect of HP on the growth performance, mortality, and tibia ash of turkey poults during the pre-starter (0 – 28 days) and starter period (28 – 42 days). One-day-old hen poults were placed in a randomized block design arrangement, with 10 replicates/treatment and 15 poults/replicate. The treatments consisted of a positive control (PC; Pre-starter: non-phytate phosphorus (nPP) 0.75%, Ca 1.40%; Starter: nPP 0.73%, Ca 1.38%); 2) Negative control 1 (NC1; Pre-starter: nPP 0.65%, Ca 1.30%; Starter: nPP 0.63%, Ca 1.28%); 3) Negative control 2 (NC2; Pre-starter: nPP 0.55%, Ca 1.20%; Starter: nPP 0.53%, Ca 1.18%); 4) Negative Control 3 (NC3; Pre-starter: nPP 0.45%, Ca 1.10%; Starter: nPP 0.43%, Ca 1.08%); 5) NC3 plus HP at 750 FYT/kg; 6) NC3 plus HP at 1,500 FYT/kg (superdose level); and 7) NC3 plus HP at 3,000 FYT/kg. Poults and remaining feed were weighed at 0, 14, 28, and 42 days (d) of age and average body weight (BW), feed intake, and feed conversion ratio (FCR) were calculated. Tibias were collected from three poults per pen for determination of tibia ash on d 28 and d 42. Data were analyzed by a one-way ANOVA using the PROC GLM procedure in SAS 9.4 as a complete randomized design with pen as the experimental unit. Significance was determined at $P<0.05$. Fisher's LSD t-tests were used to determine means separation between all treatments. On d 14, 28, and 42, a similar trend in live performance was observed where decreasing amounts of nPP and Ca content in the diet significantly reduced BW ($P<0.001$), increased FCR ($P<0.001$), and reduced tibia ash percentage ($P<0.001$). The opposite trend was observed in poults fed NC3 with increasing amounts of HP. Across both phases, the addition of HP at 1,500 and 3,000 FYT/kg met or exceeded the positive control diet for BW ($P<0.001$), FCR ($P<0.001$), and tibia ash percentage ($P<0.001$), suggesting an extra-phosphoric effect on these parameters. In conclusion, the addition of HiPhorius at 1,500 and 3,000 FYT/kg during turkey pre-starter and starter diets resulted in heavier poults, improved FCR, and greater tibial mineral content.

Key Words: Phytase, HiPhorius, Turkeys, Tibia Ash, Live Performance

P300 Finding alternative sources of monogastric feed ingredients through determination of phytase activity in microalgae Kylie Roesler*[1UG], Tao Sun[2], Xingen Lei[2] *[1]Dept. of Poultry Science, Division of Agriculture, University of Arkansas, [2]Department of Animal Science, Cornell University*

Phosphorus is an essential nutrient for poultry's skeletal growth and egg production. However, the majority of inorganic phosphorus in a diet is not bioavailable. This fiscal and efficiency issue is due to the high cost of adding phosphorus to feed especially when the price is increasing due to fertilizer demands. The inefficiency of phosphorus digestibility leads to excess phosphorus in manure being spread on fields which causes an imbalance of minerals. This study aimed to measure the phytase activity from transgenic microalgae and explore experimental measuring conditions. Three transgenic strains of microalgae (PBM3-F3, PBM2-F2, PLRI-F3) and one wild-type microalgae strain (CWM) were tested to estimate phytase activity under different pH and buffers. The supernatant of 5 mg of microalgae homogenate was mixed with 0.1% phytic acid, 0.2M citrate buffer (pH

5.5), and incubated at 37°C for 30 minutes. Then, the released phosphorus was reacted with ammonium molybdate at 55°C to develop blue color and quantified by the absorption at 820 nm. Meanwhile, citrate buffers (pH 2.5, 3.5, 5.5) and glycine buffer (pH 2.5) were used to determine the best reaction condition. Data were analyzed by Student's T-test analysis. All transgenic microalgae increased the phytase activities by 14 to 65% ($P > 0.05$, $n = 2\sim3$) compared with CWM, as PBM3-F3 microalgae showed the highest value (0.124 FTU/mg of protein). Meanwhile, comparing phytase activity under different buffers showed increased phytase activity as the reaction pH dropped. The glycine buffer pH of 2.5 showed the highest phytase activity over the citrate buffer pH of 5.5. In conclusion, all microalgae PBM3-F3, PBM2-F2, and PLRI-F3 showed higher phytase activities than the control, which represents the potential to supplement in a monogastric diet for extra phosphorus release. Meanwhile, various buffers and pH are recommended for testing before estimating the phytase activity of microalgae. This research was part of an undergraduate internship program awarded to K. Roesler by Cornell University and USDA NIFA SAS # 2019-69012-29905.

Key Words: phytase activity, microalgae, phosphorous, citrate buffer

Metabolism and Nutrition: Feed Additives

P301 The expression of nutrient transporters, cecal short chain fatty acids profile and microbial composition in Eimeria-challenged broilers receiving low protein diets with exogenous enzymes and prebiotics
Yang Lin*[GS], Jeferson Lourenco, Oluyinka Olukosi *University of Georgia*

A total of 392 Cobb 500 off-sex male broiler chicks were used in a 21-d experiment to study the potential of exogenous enzymes or prebiotics to help ameliorate *Eimeria*-induced dysbiosis. Birds were allocated to 8 treatments in a 4×2 factorial arrangement (4 diets with or without *Eimeria* challenge). Each treatment had 7 replicates with 7 birds per replicate. The 4 diets were: low protein basal diet without enzyme (NE), basal diet supplemented with protease (PRO), xylanase combined with protease (PRO+XYL), or xylo-oligosaccharides (XOS). Birds and feed were weighed on d 0, 15 and 21. The challenged group were inoculated with a solution containing oocysts of *E. maxima*, *E. tenella*, and *E. acervulina* on d 15. At d 21, jejunal mucosa was collected for nutrient transporter expression analysis, and cecal contents were analyzed for short chain fatty acids and microbial profile. *Eimeria* led to downward ($P < 0.05$) expressions of GLUT2, GLUT5, PepT1, ATP2B1, CaSR, Calbidin D28K, NPT2 and ZnT1 but upward expression ($P < 0.01$) of GLUT1. XOS supplementation upwardly ($P < 0.05$) expressed ATP2B1. There was an *Eimeria* × supplementation interaction for cecal concentration of isobutyrate showing that PRO decreased ($P < 0.05$) isobutyrate concentration in unchallenged birds but not in the challenged ones. *Eimeria* decreased ($P < 0.01$) cecal acetate and propionate, but increased ($P < 0.01$) the branched chain fatty acid isovalerate. PRO+XYL increased ($P < 0.05$) the concentration of butyrate whereas XOS decreased ($P < 0.05$) isobutyrate concentration. *Eimeria* significantly ($P < 0.05$) decreased cecal microbial richness and diversity, and shifted the microbial composition by increasing the abundance of some bacteria such as *Clostridium sensu stricto 1* and decreasing some bacteria such as *Butyricicoccus*, whereas dietary additives had no significant effect on microbial composition. In conclusion, PRO or XOS supplementation produced upward expression of Ca transporter ATP2B1 whereas PRO+XYL and XOS supplementation alleviated *Eimeria*-induced cecal SCFA profile, indicative of lower protein fermentation. However, most of the shifts in microbial composition in the current experiment was in response to *Eimeria* challenge, with no significant effects observed for the supplemented additives.

Key Words: xylanase, protease, xylo-oligosaccharides, broiler chickens, Eimeria

P302 Effects of microalgae, with or without xylanase supplementation, on growth performance and gut health parameters of broiler chickens
Pravin Mishra*[GS], Razib Das, Ajay Chaudhary, Birendra Mishra, Rajesh Jha *Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa*

Microalgae are becoming potential sustainable feed ingredients, while terrestrial feedstuffs are becoming scarce and costly. They are rich in nutritional and functional values but have lower digestibility. Therefore, the study evaluated the effect of microalgae with or without xylanase supplementation on growth performance and gut health of broiler chickens. A total of 162 day-old Cobb 500 chicks were raised for 35 days. Birds were fed with 3 dietary treatments: a) corn-soybean meal-based diet (CON), b) CON + 3% microalgae (MAG), and c) MAG + xylanase (MAG+XYN) in two phases (starter: d0-21 and finisher: d22-35). Weekly body weight (BW) and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). On d35, carcass and organ weight were recorded. The ileum sections were collected on d3 and d35 for total RNA isolation and histomorphology. Ileal gene expression related to the barrier, immune, oxidative, and nutrient transporter was determined using qPCR. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test using the GraphPad Prism program. The significance level was set at $P<0.05$, and the results were expressed as the mean ± SEM. The BW, ADG, and ADFI were significantly higher ($P<0.05$) in MAG and MAG+XYN compared to the CON group. No significant difference ($P>0.05$) was found for FCR. Relative carcass and organ weight also showed no significant differences among treatments ($P>0.05$). The expression of *SLC7A7* (L-amino acid transporter 2) was significantly higher ($P<0.05$) in the MAG group. The expressions of most of the genes were not statistically different ($P>0.05$) but numerically higher in the MAG and MAG+XYN groups than in the CON group. Villi height (VH), crypt depth (CD), VH:CD and villi surface area (VSA) were not different ($P>0.05$) among treatments; however, MAG and MAG+XYN groups had higher VH, CD, VH: CD, and VSA on both d3 and d35. In conclusion, using microalgae with or without xylanase in a corn-soybean meal-based diet promotes growth performance and gut health parameters. In addition, using microalgae in broiler feeding programs helps to produce sustainable, healthy, and cost-effective broilers.

Key Words: Broiler, Growth performance, Gut health, Microalgae, Xylanase

P303 Effects of microalgae, with or without xylanase supplementation, on serum immunoglobulins, cecal short-chain fatty acids, and microbiome of broiler chickens
Pravin Mishra*[GS], Razib Das, Ajay Chaudhary, Birendra Mishra, Rajesh Jha *Department of Human Nutrition, Food and Animal Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa*

The study investigated the effect of microalgae with or without xylanase on serum immunoglobins A and G (IgA and IgG), Cecal short-chain fatty acids (SCFA), and the microbiome of broiler chickens. A total of 162-day-old Cobb 500 chicks were raised for 35 days. Birds were fed with three dietary treatments: a) corn-soybean meal-based diet (CON), b) CON + 3% microalgae (MAG), c) MAG + xylanase (MAG+XYN) in two different phases (starter: d0-21 and finisher: d22-35). Blood was collected for serum IgA and IgG analysis on d35. Cecal digesta were collected for microbiome analysis (d3 and d35) and SCFA analysis (d35). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test using the GraphPad Prism program for IgA, IgG, and SCFA. The bioinformatics tool QIIME2 and analytical tool RStudio were used for

the microbiome analysis. The significance level was set at $P<0.05$, and the data were presented as the mean ± SEM. The concentrations of IgA, IgG, and SCFA were higher in the MAG group, but the differences were not statistically significant ($P>0.05$). Alpha diversity based on observed OTUs, and the Shannon index suggested higher microbiome diversity in the MAG group, followed by MAG+XYN and CON groups. Beta diversity, based on Bray Curtis and Binary Jaccard, displayed statistical variation ($P<0.05$) among the groups. At the phylum level, Firmicutes and Proteobacteria were the major phyla in all groups on d3 and d35. Based on the class, Gammaproteobacteria had a higher abundance on d3 and Clostridia on d35. Based on the order, d3 had a higher abundance of Enterobacterales, and d35 had Lachnospirales, Enterobacterales, and Oscilliospirales. At the family level, Enterobacteriaceae had a higher abundance in d3 and d35, higher abundance of Enterobacteriaceae followed by Lachnospiraceae in the MAG and MAG+XYN group, while Lachnospiraceae was highly abundant in the CON group. The majority of the microbiomes found in our study functioned as a probiotic and increased SCFA production, which maintains the gut health of poultry. In conclusion, using microalgae with or without xylanase in a corn-soybean meal-based diet promotes the chickens' immunity and gut health.

Key Words: Broiler, Cecal microbiome, Gut health, Microalgae, Xylanase

P304 Microbiocenosis of the chicken ceca: impact of in ovo delivered bioactive substances, heat stress, and antibiotic growth promoters Samson Oladokun*[GS], Deborah Adewole *Department of Animal Science and Aquaculture, Dalhousie University*

The study evaluated the gut microbiota modulating potential of *in ovo* delivered probiotics, folic acid (FA), and *in ovo* + in-water delivered essential oil (EO) in broiler chickens, as compared to an in-feed antibiotic growth promoter (AGP), under a heat stress (HS) challenge condition. The experiment was a 5 x 2 factorial experiment involving 5 treatments (Negative control-NC, antibiotics-BMD, in ovo FA- 0.15 mg/egg, in ovo probiotics (*B. subtilis*)- 10×10^6 CFU/egg, and in ovo + in-water EO- 0.2 mL saline + EO (2:1) and EO at 250 mL/1000 L of drinking water) and 2 temperature model (Thermoneutral (TN)- 24 °C ± 0.2 and HS- (8 h/d, 31 °C). Results showed that alpha diversity was not affected by treatments, HS, or their interaction. Compared to other treatments, the AGP treatment caused a significant change in beta diversity. While HS reduced the proportion of members of the *Actinobacteria* phylum, AGP treatment increased the cumulative proportions of bacteria in the family *Lachnospiraceae.* Metagenomic prediction showed that the AGP treatment enriched carbohydrate degradation, carbohydrate synthesis, and vitamin and amino acid biosynthesis-related pathways. The study suggests that microbiota-mediated role of AGP in growth promotion is related to improved biosynthesis of essential nutrients and utilization of carbon sources derived from host diet and microbiome.

Key Words: in ovo, ceca, microbiota, heat stress, antibiotics

P305 Evaluation of different sodium butyrate products on growth performance of 42-day broilers Austin Silva*[1GS], Daniel De Leon[1], Rosana Hirai[1], Macey Randig[1], Audrey McElroy[1], Alexis Thomas[2], Erik Von Hellens[2] *[1]Texas A&M University, [2]SmartFeeds USA*

The objective of this study was to evaluate the effect of Gut Check (S.B.F), an uncoated sodium butyrate powder, against other commercially available sodium butyrate sources (products A, B, C, and D) on the growth performance of 42d male Cobb 500 broilers. A total of 960 broiler chicks were assigned to 6 dietary treatments (Trts) and allocated to 48 pens with used litter top dressed with fresh shavings in a randomized complete block design. A common corn and soybean meal-based basal diet was formulated to meet nutrient requirements and provided ad libitum throughout the trial. Inclusion rate of products B and D were different among each growth phase, but all other products remained constant (starter from 0-14d, grower from 15-28d, and finisher from 29-42d). Dietary Trts included: Trt 1=Basal diet without sodium butyrate (Negative Control, NC); Trt 2=NC + product A at 0.45 kg/ton; Trt 3=NC + product B at 0.45 kg/ton (starter and grower) and 0.34 kg/ton (finisher); Trt 4=NC + S.B.F. at 0.5 kg/ton; Trt 5=NC + product C at 0.5 kg/ton; Trt 6=NC + product D at 1.0 kg/ton (starter) and 0.5 kg/ton (grower and finisher). Data was analyzed in SAS and subjected to an ANOVA test using the GLM procedure. Means were deemed significant at P<0.05 and separated using Tukey's HSD and Fisher's LSD range test. BW, BW gain (BWG), feed intake (FI) and mortality corrected FCR were measured on d14, 28, and 42. During d0-14, S.B.F reduced FCR compared to NC and Trt 2 but performed similarly to Trts 3, 5, and 6. BWG was improved for Trt 6 compared to NC, Trt 3, and S.B.F., but results for Trts 2 and 5 were similar in the starter phase. Trt 2 had a higher FCR and BWG compared to all other treatments for d0-28. No significant differences were observed during the finisher phase of this study. For d0-42, Trt 2 had a higher FCR compared to S.B.F and Trt 5, but results for Trts 1, 3, and 6 were similar to one another. The cumulative data suggests that S.B.F can benefit the growth performance of broilers and could be advantageous when implemented in diets for broilers in antibiotic free production systems.

Key Words: sodium butyrate, growth performance, antibiotic alternatives, broiler

P306 Evaluating the response of Ross x Ross 708 male broilers to varying inclusions of a natural alkaloid sanguinarine (SAN) when challenged with a live coccidiosis vaccine (LCV) Dalton Dennehy*[1GS], Alexandre Zocche[2], Gui Alexandrino[2], Kelley Wamsley[1] *[1]Department of Poultry Science, Mississippi State University, [2]Phytobiotics North America LLC*

Phytogenic feed additive products, such as the natural alkaloid sanguinarine (SAN) derived from *Macleaya cordata*, have been researched as potential alternatives to antibiotics, though their efficacy as an anticoccidial are limited. Two experiments (EXP) were conducted to evaluate the impact of feeding two inclusion levels of SAN challenged with two different live coccidiosis vaccines (LCV; one LCV per EXP) on broiler performance and intestinal lesion scores. In both EXP, 3 dietary treatments were evaluated, including an unchallenged, untreated control (CON), [AZ1] and two treatments containing 60 or 120ppm SAN. Broilers fed SAN were challenged via oral gavage of LCV at d14 (10x dose) and again at d42 (15x dose); late dose to mimic late cycling from LCV field use. In EXP 1, the LCV contained *E. acervulina, E. tenella,* and *E. maxima*; in EXP 2, the LCV used had *E. acervulina, E.tenella, E. mivati,* and 2 strains of *E. maxima.* Results from EXP 1 demonstrated a reduction in mortality for SAN birds vs. CON throughout the EXP (P<0.05). Broilers fed CON had reduced FCR throughout the EXP vs. SAN (P<0.05). This FCR response was likely driven by FI, wherein, broilers fed SAN had increased cumulative FI (P<0.05). No differences (P>0.05) were observed for d21 lesion scores; however, at d49 a numerical benefit for 120ppm SAN was found (P=0.06). Interestingly, broiler responses differed between EXP 1 and 2. Results from EXP 2 demonstrated that broilers fed CON or 120ppm SAN increased d0-21 BWG as compared to 60ppm SAN (P=0.01). Also, birds fed CON had the lowest d0-21 FCR, with broilers fed 60ppm SAN having the highest FCR, and those fed 120ppm SAN being intermediate (P<0.01). No significant performance differences were observed in EXP 2 from d0-28, 0-42, or 0-56 (P>0.05); however, trends were detected for d0-28 FCR (P=0.052) and d0-42 BWG (P=0.07), wherein CON birds numerically reduced d0-28 FCR and numerically increased d0-42 BWG vs. broilers fed SAN. Lesion scores at d21 (P=0.09) and d49 (P<0.01) demonstrated a reduction in average scores for birds fed 120ppm SAN vs. those fed 60ppm SAN and CON. Overall, these data indicate that 120ppm SAN may help mitigate negative LCV effects and more research is warranted testing their use with LCVs within the same study.

Key Words: broiler, coccidiosis, phytogenic, sanguinarine

P307 Cloaca temperature and concentration of plasma metabolites in 15-week-old Lohmann LSL-Lite pullets upon challenge with a single dose of E. coli lipopolysaccharide when fed sources of α-linolenic acid and yeast bioactives (YB) from hatch Junhyung Lee*, Veronica Cheng, Elijah Kiarie *University of Guelph*

Modern egg-type pullets are exposed to various rearing stressors that compromise Immunocompetence. The objective of this study was to investigate the response of 15-week-old pullets to an *E. coli* lipopolysaccharide (LPS) challenge when fed sources of α-linolenic acid (ALA) and yeast bioactives (YB) from hatch. The ALA was from co-extruded full-fat flaxseed and pulse mixture (FFF; 1:1 wt/wt), and YB was derived from hydrolysis of whole yeast by β-1,3-glucan hydrolase. A total of 1,064 d-old Lohman LSL lite pullets were placed in conventional cages (19 birds/cage) based on body weight (BW) and allocated to 7 diets in a completely randomized design (n=8). The diets were: corn -SBM control, control + 1, 3, or 5% FFF, and control + 0.025, 0.05 or 0.1% YB. The birds had *ad libitum* access to feed and water from hatch to 16 weeks of age (woa). At 15 woa, 4 pullets per cage were weighed, 2 were intravenously injected with 1 mL of LPS (8 mg/kg of BW) and the other 2 equal volume of sterile saline. Body weight (BW) was monitored at 24, 72, and 168 h post-challenge. Cloaca temperature was measured at 3, 6, 9, 12, and 24 h post-challenge. At 6 and 12 h post-challenge, blood samples were taken in alternate pullets per cage. The statistical model had LPS, diet, time, and associated interactions as fixed factors. The LPS increased cloaca temperature (P<0.001) peaking (42.1°C) at 24 h and tended to reduce (P=0.06) BW at 168 h. The LPS reduced plasma albumin, glucose, gamma-glutamyl transferase (GGT), lipase, Na, K, and Cl increased plasma cholesterol, uric acid, bile acid, aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) (P<0.05). The interaction (P<0.05) between LPS and diet for plasma albumin, CK, K, and Cl was such that control birds exhibited lower creatine (CK) upon challenge with LPS relative to birds fed other diets. LPS birds fed 1% FFF and 0.05% YB showed higher plasma albumin than non-LPS cohorts. Non-LPS birds fed control, 1 and 3% FFF had higher plasma K than LPS cohorts. In general, FFF and YB exhibited linear and quadratic effects on plasma cholesterol, bile acid, AST, CK, GGT, GLDH, lipase, and K) (P<0.05). In conclusion, LPS elicited febrile and metabolic responses; provision of ALA and YB modulated some plasma metabolites in response to LPS.

Key Words: α-linolenic fatty acid, Yeast bioactives, Pullets, Immunocompetence

P308 The effect of supplementation of organic acid in drinking water on Pekin Duck growth and welfare Jessica Rocha*, Gregory Archer *Texas A&M University - College Station*

This study evaluated the effect of supplementing drinking water with organic acid on Pekin duck performance and welfare. Two treatments were tested with 9 replicate groups, each containing 126, day-of-hatch Pekin ducks. The treatments were: 1) unsupplemented water (Con) 2) organic acid supplemented water (OA). Body weight (BW) and feed consumption (FC) of birds from 1 to 35 days of age were measured for each pen and mortality corrected feed conversion ratio (FCR) was calculated. On d35, tibia ash, tibia breaking strength, digestive tract pH, and physical asymmetry scores were determined. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation. At d35, the CON treatment had lower (P = 0.001) BW (3.20kg) and higher (P < 0.001) FCR (1.561) than the OA treatment (3.52kg and 1.427). The CON treatment had higher (P = 0.005) asymmetry scores (2.49) than the OA treatment (1.99). The CON treatment had lower (P = 0.02) tibia breaking strength (24.7g) than the OA treatment (27.5g); however, no difference (P > 0.05) in tibia ash (57.5%) was observed. The pH of the proventriculus (4.75) and ileum (6.78) did not differ (P > 0.05) between treatments; however, the control had higher (P = 0.04) jejunum (6.31) and (P = 0.02) ceca pH (6.04) than the OA treatment (6.14 and 5.68). In conclusion, the OA supplementation in the water improved duck growth and feed efficiency and welfare.

Key Words: organic acid, performance, welfare, Duck

P309 Broiler breeder feed sanitation with a formaldehyde-based product and its impact on reproduction, feed and egg contamination, and offspring mortality Luis Avila*[1], Kelly Sweeney[1], Cheryl Schaeffer[2], Nicole Holcombe[2], Callie Selby[2], Enrique Montiel[2], Jeanna Wilson[1] *[1]University of Georgia Department of Poultry Science, [2]Antitox Corporation*

Due to increased demand for broiler chicks, broiler breeder operations search for dietary strategies that can encourage broiler breeder reproduction, improve hatchability and offspring performance. Microbial contamination of fertile eggs can be detrimental to hatchability and broiler chick growth performance. Formaldehydes have antimicrobial properties and have been used to sanitize layer feed to reduce the contamination of table eggs, although there is no evidence of its consequences on broiler breeder reproduction. The objectives of this experiment were to evaluate the effect of a formaldehyde-based feed sanitizer on broiler breeder hen reproduction, feed and eggshell contamination, incubation characteristics, and offspring early livability. At 21 wk of age, Ross 708 breeder pullets ($n = 508$) were placed in 6 floor pens with 3 Yield Plus males and fed a common pre-lay diet until 25 wk when they were assigned 1 of 2 laying diets: CTL = untreated control diet; or TRT = treated diet with a formaldehyde-based product (Termin-8 dry powder at 0.4% inclusion rate). A 2-step program was fed through lay: breeder I (wk 25 to 45), and breeder II (wk 46 to 60). Data were analyzed using a GLM PROC with SAS v 9.4 at a significance of $P \leq 0.05$, and tendencies declared when $0.05 < P \leq 0.10$. Treating broiler breeder feed did not affect overall hen reproduction or hatchability ($P \geq 0.222$). At wk 27 to 45, TRT hens had fewer embryonic losses due to % of live pips ($P = 0.042$) and had overall higher graded-quality hatched chicks ($P = 0.002$). TRT feed showed less presumptive aerobic bacteria, fungus, Enterobacteriaceae and *C. perfringens* compared to CTL feeds ($P < 0.001$). Similar to the feed analysis, the surface of nest-eggs from hens consuming TRT-feed showed reduced presumptive aerobic bacteria ($P < 0.001$) and tended to have less fungi contamination ($P = 0.061$). Overall chick mortalities with signs of yolk-sac contamination tended to be reduced when obtained from hens consuming TRT feed ($P = 0.099$), particularly from 60 wk-old hens ($P = 0.031$). Our results indicate that treating broiler breeder hen feed with a formaldehyde-based sanitizer reduced the microbial contamination of feed and eggshell surfaces, and positively impacted the quality and livability of the hatched chicks.

Key Words: broiler breeder, feed sanitation, egg contamination, formaldehyde, hatchery

P310 The effect of a yeast probiotic on the performance of Ross 708 broilers grown to 49 days of age Emily Jiral*[1], J. Padgett[2], Gregory Acher[1] *[1]Texas A&M University, [2]Phileo by Lesaffre*

This study evaluated the effects of various doses of a yeast probiotic on the performance of commercial male broilers. Five treatments were tested with 12 replicate groups, each containing 240, day-of-hatch Ross 708 straight run broilers. The treatments were: 1) Standard broiler diet program with 5% DDGs and no additional supportive gut health feed additives (NC), 2) NC diet +BMD at 50g/t in all dietary phases, 3) NC + Actisaf 0.5lb/t, 4) NC + Actisaf 0.75lb/t, 5) NC + Actisaf 1.0lb/t. All treatments were reared on used litter and Coccidiosis vaccinated at d0 with a 2x dose. Body weight (BW) and feed consumption (FC) of birds from 1 to 49 days of age were measured for each pen and mortality corrected feed conversion ratio (FCR) was calculated. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation. At d42, the Actisaf 0.75 and 1.0lb/t treatments weighed more (P < 0.05, 2.89 kg and 2.90 kg, respectively) than the NC (2.78 kg), however, no other differences (P > 0.05) in BW were observed. All of the Actisaf treat-

ments had lower (P < 0.05) FCR than NC starting at d14 and continuing through d35. At d42 only the two highest inclusion rates of Actisaf had lower (P < 0.05) FCR (1.556 and 1.553, respectively) than the NC (1.624). Finally, at d49 only the 1.0lb/t treatment had lower FCR (P < 0.05, 1.733) than the NC (1.774). In conclusion, the yeast probiotic Actisaf improved performance over the non-supplemented NC. The highest dose of 1.0lb/t had this improvement from starter phase and continued through the d49 illustrating that it can improve feed efficiency and thereby result in possible economic savings.

Key Words: Probiotic, Yeast, Broiler, Performance, Yield

P311 Effect of probiotics on jejunal immunity and microbiome in 10-day-old broiler chickens June Hyeok Yoon*[1], Sung Yong Joo[2], Ji Young Jung[3], Myunghoo Kim[2], Changsu Kong[1] *[1]Kyungpook National University, [2]Pusan National University, [3]Nakdonggang National Institute of Biological Resources (NNIBR)*

The objective of this study was to investigate the effect of various probiotics on the jejunal immunity and microbiome in 10-day-old broiler chickens. A total of 384 day-old Ross 308 male broilers were assigned to 6 dietary treatments with 8 replicates (8 birds/cage) in a randomized complete block design. Six dietary treatments consisted of: a basal diet without probiotics (NC), NC + commercial probiotics (PC), and NC supplemented with each of the 4 *L. paracasei* spp. (NSMJ15, NSMJ23, NFFJ04, or NKJ96). 5 g/kg of probiotics were supplemented at the expense of corn and all birds were not challenged. On day 10, 1 bird representing median body weight was selected within a cage. Jejunum tissues were used for jejunal immune cell isolation and jejunal contents were collected for microbiome analysis. Lamina propria (LP) were isolated from jejunum tissues. T-cells, B-cells, and antigen-presenting cells (APC) in LP were measured by flow cytometry analysis. Data were analyzed using GLM procedure of SAS. Treatment means between NC and supplemented groups were compared using contrast statements. Differential abundance of bacterial taxa was analyzed using Linear Discriminant Analysis Effect Size (LEfSe). NSMJ23 and NFFJ04 supplemented groups had increased (P < 0.05) Mono/Macrophage concentrations in jejunal LP, an indicator of APC, compared to NC group. Inverse Simpson index in NSMJ15 and NKJ96 supplemented groups showed a tendency to increase (P = 0.09 and P = 0.07) compared to NC group. Shannon index of PC group tended to decrease (P = 0.07) compared to NC group. LEfSe analysis indicated an increased abundance of *Peptostreptococcaceae* in NC group and more abundance of *Lactobacillus* in PC group. NSMJ23 supplemented group had more *Levilactobacillus*, and NFFJ04 supplemented group had more abundance of *Lacticaseibacillus*, *Clostridium*, and *Kocuria*. NKJ96 supplemented group had increased abundance of *Latilactobacillus* and *Sphingomonadaceae*. In conclusion, our results show that dietary NSMJ23 and NFFJ4 supplementation in the early stage of broilers might increase APC concentrations in jejunal LP. Supplementation of NSMJ23, NFFJ04, and NKJ96 in 10-day-old broiler might increase the abundance of *Lactobacillaceae*, a family that is beneficial for gut health and host growth.

Key Words: probiotics, immunity, microbiome, jejunum, broiler

P312 Anticoccidial effects of blueberry and cranberry pomace alone or in combination with a Lactobacillus isolate in broiler chickens Philip Mak*[1,2], Attiq Rehman[1], Carl Julien[3], Yan Martel-Kennes[3], Xianhua Yin[1], Elijah Kiarie[2], Moussa Diarra[1] *[1]Guelph Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), [2]Department of Animal Biosciences, University of Guelph, [3]Centre de recherche en sciences animales de Deschambault*

The objective of this study was to optimize benefits to broiler chicken performance and health using cranberry and blueberry pomace. Effects of a *Lactobacillus* (*L. reuteri*) isolate alone or in combination with wild blueberry and organic cranberry pomaces were evaluated using an *Eimeria* challenge model. A total of 480-day-old male broilers (Cobb 500) were distributed in 60 cages allocated to one of eight treatments: 1) Blank control (uninfected, unmediated); 2) negative control (infected, non-medicated); 3) positive control (infected, medicated with Zoamix and salinomycin); 4) 1% blueberry pomace (BLP); 5) 1% cranberry pomace (CRP); 6) *L. reuteri* (LAC); 7) 1% BLP+LAC; 8) 1% CRP+LAC. The LAC was administered by gavage daily from D7-D21 at 10^8 CFU/bird. Performance parameters and intestinal lesion scores were analyzed using both Chi square and CMH tests globally and by day. Longitudinal effects on broiler cecal microbiota composition diversity and community structure were analyzed using 16S rRNA sequencing. No treatment effects on body weight and average daily gain (ADG) were observed (P>0.05). Significant day effects were observed on lesion types (P<0.05), but no treatment effects were observed. At D7 Clostridia was significantly abundant in BLP fed-birds but the highest abundances of Bacilli and Gammaproteobacteria were observed in blank (P<0.05). At D21, the lowest abundance of Bacilli class was observed in CRP (31.9), BLP (30.3) and LAC (25.1) compared to non-medicated challenged (18.7) and blank (8.3). *Lactobacillus* was significantly more abundant in CRP (31.0) and BLP (29.5)(P<0.05). *Enterobacteriaceae* remained enriched in non-medicated but challenged and berry-fed birds. At D28 the *Streptococcaceae* and *Enterococcaceae* populations were significantly more abundant in the non-medicated but challenged birds compared to all other groups (P<0.05). At D35 berry fed-birds showed the highest abundance of *Faecalibacterium prausnitzii* compared to other groups. At each sampling day, Kruskal-Wallis and pairwise statistical tests using PERMANOVA on β-diversity showed significant difference between berry pomaces (CRP and BLP) fed birds and blank. This study showed that CRP or BLP and *L. reuteri* could be developed as alternatives to control *Eimeria* infection in broiler.

Key Words: broiler chickens, berry pomace, probiotics, cecal microbiota

P313 The effect of synbiotic supplementation on the ileal microbiota and short chain fatty acids concentration in broilers. Walid Al Hakeem*[1], Jeferson Lourenco[2], Emily Cason[1], Daniel Adams[1], Shahna Fathima[1], Bikas Shah[1], Revathi Shanmugasundaram[3], Ramesh Selvaraj[1] *[1]Department of Poultry Science, University of Georgia, [2]Department of Animal and Dairy Science, University of Georgia, [3]Toxicology and Mycotoxin Research Unit, USDA-ARS*

Synbiotic supplementation enhances beneficial gut microbiota and increases short-chain fatty acids (SCFAs) production in broilers. This study aimed to characterize the effect of Poultry star® supplementation on the ileal microbiota and ileal SCFAs concentration in broilers. 168-day-old broiler chicks were randomly allocated into two treatment groups: Control and Synbiotic. Each treatment was replicated in 6 pens (n = 6) with 14 chicks per pen. Poultry star® was supplemented in 0.5g/kg feed from the day of hatch. Ileal mucus and contents from each treatment were collected on days 28 and 35 for microbiome and SCFA analysis. The bacterial composition of the ileum was determined by sequencing the V1–V9 region of the 16S rRNA gene. Poultry star® supplementation didn't alter the alpha and beta diversity compared to the control group. On the phyla level, Poultry star® supplementation increased the relative abundance of *Proteobacteria* (P=0.05) on day 28 compared to the control group. On the family level, Poultry star® supplementation increased the relative abundance of *Enterococcaceae* (P=0.01) and *Enterobacteriaceae* (P=0.05) on day 28 compared to the control group. Moreover, on the genus level, Poultry star® supplementation increased the relative abundance of *Enterococcus* (P=0.01) and *Faecalibacterium* (P=0.06) on day 28 compared to the control group. While on day 35, Poultry star® supplementation decreased the relative abundance of *Ruminococcus* (P=0.06), and *Subdoligranulum* (P=0.06) compared to the control group. On the species level, Poultry star® supplementation increased the relative abundance of *Faecalibacterium prausnitzii* (P=0.06) on day 28 compared to the control group. While on day 35, Poultry star® supplementation decreased the relative abundance of *Enterococcus cecorum* (P=0.06) compared to the control group. Poultry star® supplementation increased (P=0.04) acetate concentration

in the ileum on day 35. In conclusion, Poultry star® supplementation increased acetate concentration and beneficial bacteria such as *Faecalibacterium prausnitzii* and decreased the relative abundance of *Enterococcus cecorum,* responsible for enterococcal spondylitis in broilers.

Key Words: Synbiotic, Feed additives

P314 Comparative efficacy of cranberry pomace and antibiotic growth promoters on growth performance of broiler chickens fed corn and soybean meal-based diet and challenged with Eimeria

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Cranberry pomace (CBP) is a rich natural and sustainable source of antioxidants and polyphenolic compounds that could serve as alternative to antibiotic growth promoters in poultry production. We compared supplementation of enzyme treated (ETCBP) and untreated (UTCBP) and AGP (bacitracin methylene disalicylate and narasin) on growth performance of broiler chicken challenged with *Eimeria*. The CBP (~25% DM) was procured from processor in frozen state. For preparation of ETCBP, thawed material was weighed, mixed with multi-enzyme supplement (1 kg CBP:2.5 g), placed in incubator shaker 24 h at 40˚C and, along with thawed UTCBP samples, dried at 60˚C and ground for feed preparation. A total of 960 d old male Ross 708 broiler chickens were placed based on bodyweight (BW in floor pens (20 birds/cage) equally distributed in four rooms and allocated to 6 diets (n=8). Diets were: 1) NC; no additives, 2) PC; NC + AGP, NC + 0.5 and 1% ETCBP or UTCBP. Diets were formulated for two phase feeding programs: starter (d0-14) and finisher (d 15-42). On d 14, all birds received 1 mL of 100,000 *E. acervulina,* 15,000 *E. maxima,* and 10,000 *E. tenella* culture via oral gavage. Feed, BW, and mortality were monitored on weekly basis for calculation of BW gain (BWG) and FCR. On d 42, 2 birds/pen were necropsied for breast yield. The statistical model had diet and block (room) as fixed factor and LSmeans were separated using Tukey. There was no diet effect (P>0.05) on growth performance in the stater (pre-challenge) and immediate post challenge (d 15-21) and in the overall (d 0-42) periods. However, on d 22-28, PC birds had higher (P<0.01) BWG and d 28 BW relative to birds fed other diets. The FCR was better (P<0.01) for PC birds than NC and 0.5% ETCBP birds. Overall, higher mortality (found dead or euthanized for non-viability) was observed for the NC and ETCBP birds than for PC and 0.5% UTCBP birds. The mortality was 9.0, 0.6, 4.5, 6.4, 8.8 and 10.2% for NC, PC, 0.5%UTCBP, 1.0%UTCBP, 0.5%ETCBP and 1.0%ETCBP, respectively. In conclusion, although diets did not influence overall growth performance, AGP performed better in acute phase. Thus, ETCBP birds exhibited higher mortality than AGP birds suggesting ETCBP supplementation was not as effective during an *Eimeria* exposure.

Key Words: Broiler chicken, growth performance, coccidiosis, cranberry pomace

P315 Effects of dietary supplementation with AlphaD3TM on laying hen performance parameters through an extended production cycle.

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Laying hen production cycles have been extended from 68 to 100 weeks in order to increase the number of eggs per hen lifetime. Extended cycles have economic and environmental sustainability benefits; however, skeletal integrity, eggshell quality, and hen-day egg production (HDEP) diminish with age. This is due to imbalances in Ca and P in diet as well as absorption and metabolism, which are regulated by vitamin D_3. We have found that dietary supplementation with AlphaD3™ (Iluma Alliance), a commercially available 1α-hydroxycholecalciferol product, appears to improve long bone mineralization between the onset of sexual maturity and early lay, and thereby may improve Ca and P balance in such a way that reduces issues associated with extended production. Therefore, this study sought to determine effects of dietary AlphaD3™ supplementation on laying hen production performance during an extended production cycle. A total of 750 Nick Chick hens (H&N International) were individually caged in 30 blocks of 25 hens each, and 15 blocks were fed a control diet and 15 blocks were fed a diet supplemented with AlphaD3 ™ (3.5 µg/kg) between 18 and 86 weeks of age (n=15). Hens were monitored for HDEP, feed conversion ratio (FCR)/dozen eggs, egg weight (g), and cumulative mortality (%). Data were analyzed by two-way ANOVA, and Fisher's LSD test was used when ANOVA indicated significance (P≤0.05). There was a diet-by-age interaction, where hens fed AlphaD3 ™ exhibited increased HDEP after 60 weeks of age [92.12%] relative to control [90.29%] (P≤0.05). As a result, FCR/dozen eggs displayed a near-significant diet effect (P=0.06) in that it was lower for AlphaD3 ™-fed hens. Egg weight also showed a diet-by-age interaction where hens fed AlphaD3™ had lower egg weights [63.5g] after 58 weeks relative to control [64.8g] (P≤0.05). Cumulative mortality was not affected by diet but was influenced by age, with greater mortality noted after 60 weeks (P≤0.05). These data suggest that AlphaD3™ supplementation improved HDEP and FCR/dozen after 60 weeks of age, potentially as a result of increased bone mineralization at the onset of lay. Thus, addition of AlphaD3™ has the potential to improve skeletal health and egg production during extended production cycles in laying hens.

Key Words: Laying hen, Extended production, Egg production, Calcium, Phosphorus

P316 Natural choline supplementation reduces steatosis in broilers

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This study aimed to evaluate choline supplementation in the diet of broilers under hepatic steatosis. The design was completely randomized, with 6 treatments of 6 replicates of 25 birds each, Ross from 30 weeks-old breeders with a weight of 45 g. Choline supplementation occurred under two sources, Choline Chloride 60% (CC) and Natural Choline (KPFC). The control treatment was not supplemented. The diets were supplemented with 400g/t, 800g/t, and 1200g/t of CC and 100g/t, 200g/t, and 300g/t of KPFC. The chicken starter diet was formulated with feedstuffs commonly used in Brazil. The diets were representative of local commercial formulation and calculated analysis met or exceeded nutritional guidelines according to the breed and age of the bird. Complete records of feed mixing, as well as test product inventories, were maintained. On day 42, liver samples measuring 1cm² were collected, coated in neutral talc, and immediately frozen in liquid nitrogen, being kept in an ultra freezer until processing. Subsequently, the samples were sectioned in cryostat microtomes with 20 µm thickness on histological slides, the slides were stained by Sudan IV stain for lipid quantification by a score from 1 to 4, where, 1: without areas of lipid quantification, 2: Few tissue regions; 3: Larger areas of lipid quantification and 4: Larger areas of lipid quantification. The areas with the presence of lipids showed a reddish color. Orthogonal contrasts were performed and compared by a 5% T-test. In contrast, KPFC reduced steatosis compared to CC doses (P<0.001; 400g/t CC vs 100g/t KPFC | P<0.001; 800g/t CC vs 200g/t KPFC | P<0.001; 1200g/t CC vs 300g/t KPFC). KPFC has an effective contribution to phosphatidylcholine. Additionally, KPFC has additional compounds, such as curcuminoids and catechins compounds. Curcuminoids have reported hepatoprotective action. Therefore, the results obtained, especially in the case of a significant reduction in hepatic steatosis, can be explained by the presence of curcumins and catechins in the composition of the natural source of choline. In

conclusion, KPFC supplementation is more efficient than CC in reducing hepatic steatosis in broilers from 1 to 42 days.

Key Words: curcuminoids, hepatoprotective, natural choline, Phosphatidylcholine, choline chloride

P317 Evaluation of cranberry pomace supplementation on egg production and metabolic responses in mid-lay Lohmann LSL lite hens Veronica Cheng*, Jessica Gasarabwe, Elijah Kiarie *University of Guelph*

Fruit pomaces, residuals of fruit processing are rich in nutrients and functional polyphenols that could be beneficial in poultry production. This study investigated the inclusion of various doses of cranberry pomace (CP) in a corn-soybean meal diet on egg production and quality. A total of 160 Lohmann LSL lite hens were placed in enriched cages (4 hens/ cage), allocated five diets in a complete random design (n=8) from 45 to 56 weeks of age (woa). The diets were: control (0.0%CP) formulated to meet specifications and test diets in which CP was incorporated at 0.5 1.0, 2.5 or 5.0% at the expense of corn. The hens had *ad libitum* access to feed and water. Egg were counted daily, feed intake (FI) weekly, and egg weight (EW) on bi-weekly basis. These data were used for calculation of hen day egg production (HDEP), egg mass (EM) and feed conversion (FCR). Egg shell thickness (EST), shell breaking strength (ESBS), yolk colour (YC), and albumin height (AH) were analyzed on all eggs collected on a single day in weeks 4, 8, and 10. Hens were weighed at the start and end of the experiment to determine body weight (BW) change. Two hens/ cage were randomly selected at the end of experiment, weighed, euthanized via cervical dislocation, liver excised and weighed. The data were analyzed using the PROC GLM procedure of SAS with diet, sampling time, and their interactions as fixed effects, cage was the experimental unit. Linear, quadratic, and cubic were applied for CP response. The calculated AME based on analyzed chemical composition was 2.97, 2.44, 3.36, 2.47, and 3.14 mcal/kg for the CP diets, respectively. The FI exhibited quadratic and cubic ($P \leq 0.019$) response to CP with highest FI (121 g/h/d) for 1.0%CP and lowest (113 g/h/d) for 0.0 and 5.0%CP birds. Feeding CP increased HDEP, and EM, linearly and non-linearly ($P \leq 0.026$) such that birds fed 1.0 or 2.5%CP had higher values than 0.0 and 5.0%CP birds. A quadratic response ($P=0.015$) was observed for the ESBS such that birds fed 1.0% CP produced the stronger eggshell. However, the EST showed a linear decrease ($P=0.030$). There were no ($P>0.05$) diet effects on FCR, YK, AH, BW and liver weight. In conclusion, 1.0 and 2.5% CP resulted improved egg production and eggshell quality whereas 5.0% CP reduced these parameters.

Key Words: cranberry pomace, Lohmann LSL lite, hens, egg production

P318 Characterizing the benefits of granulated-sodium butyrate supplementation on broiler chicken growth performance when fed antibiotic-free diets Anhao Wang, Rob Patterson*, Paul Garvey *CBS Bio-Platforms*

A total of 288 newly-hatched, mix-sexed Ross 708 chicks were used in a 36-d feeding study to characterize the benefits of a granulated-sodium butyrate product (SB) on growth performance. The birds were randomly assigned to one of six dietary treatments with 12 cages/ treatment and 4 birds (2 males and 2 females)/ cage. The experimental diets were corn-soy-based and antimicrobial growth promoter-free. Sodium butyrate was supplemented at 0, 125, 250, 500, 1000 and 2000 mg/kg (0, 125, 250, 500, 1000 and 2000SB), respectively. All diets also contained a commercially available phytase and a non-starch polysaccharide degrading enzyme. Performance was measured at 14, 24 and 36-d. The data were analyzed by one-way ANOVA; the Orthogonal polynomial contrasts were performed to test the linear, quadratic and cubic effects among the means. Supplementing SB improved broiler chicken body weight (BW) at 24-d (cubic, $p = 0.029$) and at 36-d (linear, quadratic and cubic, $p < 0.05$). Broiler chicken fed SB-supplemented diets had higher average daily gain (ADG) during 15 - 24 d (quadratic, $p < 0.001$) and 25 - 36 d (quadratic, $p = 0.042$). A linear improvement in average daily feed intake (ADFI) was observed in birds fed SB-supplemented diets during 25 - 36 d ($p = 0.013$) and 0 - 36 d ($p = 0.005$). However, SB did not affect ADFI during 0 - 14, 15 - 24 and 0 - 24 d of growth ($p > 0.05$). Feed conversion ratio (FCR) was improved quadratically by SB supplementation during 15 - 24 d and 0 - 24 d ($p < 0.001$). No treatment effects of SB on FCR was observed during 0 - 14, 25 - 36 and 0 - 36 d of growth ($p > 0.05$). Furthermore, 125SB-fed birds had higher ADG (54.8 vs 52.6 g/d; ANOVA, $p = 0.025$) and were heavier (2013.1 vs 1932.2 g; ANOVA, $p = 0.049$) than those receiving 0SB (control) diets at the end of 36-d experiment. Taken together, this study indicated dietary supplementation of a granulated-sodium butyrate product had positive effects on the growth performance of broiler chickens, and these improvements can be observed with a supplementation rate of 125 mg/kg.

Key Words: granulated-sodium butyrate, growth performance, broiler chickens, antibiotic-free diet

P319 Comparative Effects of Eubiotics and Antibiotic Feed Additives on Performance, Coccidial Lesion Scores and Fecal Clostridial Counts in Challenged and Disease-Free Broilers Luis Gomez*[1], Ken Bafundo[1], Bruce Johnson[1], James McNaughton[2] [1]*Phibro Animal Health Corporation,* [2]*AHPharma, Inc.*

A study conducted in broilers to evaluate the effects of eubiotic and antibiotic feed additives in improving gut health, which may lead to improved performance and reduction of the incidence of coccidia and fecal *Clostridium perfringens* (CP) counts. Two different challenge conditions were used in this test: a clean environment with new litter was used in each pen and no disease organisms were intentionally introduced, and a moderate disease challenge involving used litter that was known to contain *Eimeria acervulina* and *Eimeria maxima* oocysts, and the spores of CP. Dividers were used between pens to limit the cross contamination of pathogens from one to another. Eight treatments were used with 4 feeding programs: non-treated control, Magni-Phi® Ultra at 125 ppm (MPu); Bacitracin at 55 ppm (BMD), and a Phytogenic Blend (PB1) were administered in each of the 2 challenge environments. Each product was fed for the duration of the 42-day test. All broilers were vaccinated for coccidiosis at the hatchery (1d). Treatments were arranged in a randomized complete block design involving 12 blocks. Pens contained 55 Ross 708 broilers at the start of the study. Both, coccidia lesion scores were evaluated, and fresh fecal samples were collected for CP, on d 21. Performance was measured at 21 and 42d. The effects of disease challenge were evident: Control birds reared under challenge had higher lesion scores and fecal CP counts than those control birds reared without imposed challenge. Performance was affected similarly. Coccidia lesion scores and fecal CP counts were reduced by MPu and BMD. Under challenge conditions at 21 and 42d, the effects of BMD on body weights and feed conversion were evident, this response is likely related to reduction of CP and other intestinal bacteria that affect performance. Although it is clear that MPu is not as effective as BMD under challenge conditions, it significantly reduced CP numbers at d 21, thereby providing performance improvements that were competitive to BMD. Under the 2 challenge conditions used in this study, performance responses in the broilers fed MPu at the lowest feeding rate were better than those broilers in the PB1 and control groups. Future studies will include higher feeding rates of MPu and other challenge levels.

Key Words: Phytogenic, Intestinal Health, Poultry, Challenge

P320 Effect of protected sodium butyrate to protect broilers from Salmonella heidelberg Carlos del Cuvillo*[1], Mónica Puyalto[1], Hebert Silveira[2], Giselle da Silva Gallio[3], Juan José Mallo[1] [1]*Norel SA,* [2]*Natural BR Feed,* [3]*Nutrinzoo*

The aim of this trial was to assess the effect of a protected sodium butyrate additive on broilers challenged with *Salmonella heidelberg*. Five groups of fifty 1-day-old broilers were assigned to five treatments: negative con-

trol (NC) fed with a control diet without additive and not challenged, positive control (PC) fed with a control diet without additive, and three treatments with control diet supplemented with different doses of protected sodium salt of butyric acid (Gustor N'RGY®, NOREL SA) at 0.5 g, 1 g and 1.5 g per kg diet (NRGY0.5, NRGY1 and NRGY1.5 respectively). At day 3, 20% of birds (10 per treatment) were orally challenged with *Salmonella heidelberg* (6 log CFU/mL).

From each treatment, 20 birds were euthanized at day 14 and another 20 birds at day 28 for ceca sampling. One fragment from jejunum and ileum were also sampled from 12 birds among the euthanized at day 28. *Salmonella* counting was performed in ceca and villus length and crypt depth was measured in jejunum and ileum. Bacteriological data of challenged animals were analysed using the least squares mean statement of GENMOD procedure of SAS. Intestinal morphology parameters were analysed as a one-way ANOVA via SAS. *Salmonella* incidence was analysed using a Chi-squared analysis via SAS.

At day 14 NRGY at 0.5 g/kg was the only treatment that improved PC results (6.39a, 5.14b, 6.17ab, 6.50ab log CFU/mL for PC and NRGY at 0.5, 1 and 1.5 respectively, $p<0.05$). At the end of the study, all NRGY treatments tended to decrease *Salmonella* counts (4.55x, 2.73y, 3.59y and 2.65y log CFU/mL for PC and NRGY at 0.5, 1 and 1.5 g/kg respectively, $p<0.10$). The number of positive animals to *Salmonella heidelberg* at day 28 was significantly reduced with NRGY at 0.5 and 1.5 g/kg (20a, 15b, 19a and 15b for PC and NRGY at 0.5, 1 and 1.5 respectively, $p<0.05$). Villus length in ileum was significantly higher for all doses of NRGY when compared to PC ($p<0.05$), and there is a significant increase in villus height:crypth depth ratio in jejunum (6.58a, 5.62b, 6.12ab, 7.13a and 7.29a for NC, PC and NRGY at 0.5, 1 and 1.5 g/kg respectively, $p<0.05$).

Key Words: Sodium butyrate, broiler, Salmonella, Salmonella Heidelberg, Intestinal Morphology

P321 Feeding Varium® produces growth performance similar to feeding an antibiotic on-farm. Sara Johnston*, Eduardo Baggio, Fernando Blini, Sergio Candeo *Amlan International*

As poultry producers work to reduce the use of antibiotics in raising birds, replacements are needed to maintain health and growth performance. Several controlled studies have been reported comparing the feed additive Varium to antibiotics with positive results. Therefore, an on-farm study was conducted. The farms in the current study were in southern Brazil using a total of approximately 180,000 broilers. There were three farms with two poultry houses on each farm. The farms selected were from the high, average, and low range of production based on historical data. There were two treatments 1) Control – standard feed with a mycotoxin binder + the enramycin; and 2) Varium – the standard feed with the mycotoxin binder and the antibiotic removed and Varium (0.1%) added. The day-old-chicks supplied to the farms for the evaluation were from breeders of the same age. Every seven days approximately 1% of the birds in each barn were weighed and mortality for the week was tabulated. At each weigh date, except day 21, birds fed the Varium treatment were heavier than those fed the standard diet with an antibiotic. Age at slaughter was 47.29 days for the Control birds and 47.17 days for the Varium fed birds. However daily weight gain was 69.60 g for the Control birds and 70.62 g for the Varium fed birds. Weekly mortality, cumulative mortality, and transport mortality were all lower for the birds fed Varium. Overall feed conversion was 1.717 for the Control birds and 1.671 for the birds fed Varium; an advantage for birds fed Varium of 4.6 points. When feed conversion was adjusted to 45 days of age the feed conversion for the Control birds was 1.700 and 1.656 for the Varium fed birds, an advantage of 4.4 points for feeding Varium. Feeding Varium on farm resulted in broiler performance comparable to that achieved using the diet with antibiotics.

Key Words: Antibiotic, broiler, feed conversion, mortality, weight gain

P322 Effects of citrus flavonoids supplementation in feed on performance and oxidative stress of broiler chickens under heat stress conditions Nicha Rodsiatan[1], Igor Peñarrubia[2], Mar Serra[2], Javier Crespo[2], Alfred Blanch*[3], Yuwares Ruangpanit[1] *[1]Kasetsart University, [2]HTBA, [3]Addimus, Providing Trust, SL*

High heat stress has a major impact on poultry performance, oxidative stress being its direct consequence and the basis of the corresponding negative effects on performance and health. On the other hand, citrus flavonoids (e.g., hesperidin and naringin) are natural compounds with a strong antioxidant activity in the animal organism. This study was conducted to determine the effect of the addition of citrus flavonoids in feed on performance and antioxidant status in broilers under heat stress conditions. A total of 1,440 one-day-old Ross 308 male chicks were randomly allotted into 4 dietary treatments with 12 replicates of 30 birds: a control diet (T1) or the same diet supplemented with 500 ppm of bacitracin (T2), 300 ppm of citrus flavonoids (T3) or a mixture of 500 ppm of bacitracin + 200 ppm of citrus flavonoids (T4). The average environmental temperature and the average environmental humidity were 31 ± 3 °C and 70 ± 3%, respectively, through the experimental period. Bodyweight and feed intake were recorded at 10, 30 and 37 days of age. Body weight gain and FCR were calculated for each feeding phase (starter, 1-10 d; grower, 11-30 d; finisher, 31-37 d) and for the overall experimental period (1-37 d). The number of deaths was recorded daily to calculate mortality rate. On day 35 of age, two birds per replicate were randomly selected for malondialdehyde analysis in blood, as oxidative stress marker. The results were analyzed as a complete randomized design using General Linear Model procedure of Free Statistic Software, SAS®. Analysis of all data was conducted using Analysis of Variance and the Duncan's new multiple range test, statistical significance being stated at $P\leq0.05$. No significant differences ($p>0.05$) were found in body weight gain, feed intake, FCR, and mortality rate among dietary treatments during the starter, grower and finisher periods. However, birds fed T3 diet showed lower FCR and lower mortality ($P<0.05$) compared with the control group (T1) during the overall period (1 to 37 d). Furthermore, supplementation with citrus flavonoids alone (T3) reduced serum malondialdehyde ($P<0.05$). In conclusion, these results indicate that citrus flavonoids deserve to be considered in broiler feed formulation when birds face heat stress.

Key Words: citrus flavonoids, heat stress, oxidative stress, broilers

P324 Standardized citrus extract combination with probiotic to improve broiler chicken's performances Sekhou Cisse*[1,2], Abderrahim Mansour[3], Mohamed el Amine Benarbia[1,2] *[1]Nor-Feed SAS, [2]Labcom FeedInTech, [3]Sofabel SARL*

Probiotics are efficient to improve broiler chickens' growth performances. However, the cost of the efficient dose may be not competitive. Combin-ing them with prebiotics may be a solution to reduce the efficient dose of probiotics, thus making them more competitive. As natural source of prebiotic, citrus extract (CE) has already shown beneficial effect on broiler chickens' performances by modulating intestinal microbiota. In this study, we investigate the effect of reducing by 50% the dose of a commercial probiotic and associating them with Citrus extract prebiotics.

540 Arbor Acres one day old birds were divided into 3 groups. Each group contained 6 replicates of 30 birds.

- Group 1: CTL group: a standard diet without supplementation;
- Group 2: PRO group: supplemented with the manufacturer recom-mended dose of a commercial probiotic (200 ppm), added to water and fed with a standard diet;
- Group 3: SYM group: a standard diet supplemented with 80% of the recommended dose of citrus prebiotic (200 ppm) and 50% of the recommended dose of the same probiotic used in the PRO group (100 ppm).

Broiler chickens were reared to day 46 and were put under thermic stress at 30°C for 4 h per day, from day 26 to day 30. Growth performances were recorded weekly for each replicate. Statistical analyses were performed by analysis of variance (ANOVA) using GraphPad software.

Results showed that the final live weight of chickens from the SYM group was higher ($p < 0.05$, Anova) than chickens from CTL group. In addition, the average daily gain (ADG) of chickens from CTL group was lower than the ADG of PRO and SYM groups ($p < 0.05$, Anova). Moreover, chickens from SYM group had a lower FCR compared to chickens from the PRO group ($p < 0.05$, Anova) and CTL group ($p < 0.01$, Anova). No statistical difference was observed for mortality from the 3 groups.

The combination of 80% of citrus prebiotics recommended dose with 50% of commercial probiotic recommended dose has better effect in broil-er chickens' performances and is cheaper than probiotic supplementation alone at its recommended dose. According to these data, combining pre-biotics and probiotics could be a good way to optimize broiler chickens' growth performances while reducing the costs of probiotics supplementa-tion.

Key Words: Citrus extract, Prebiotic, Probiotic, Symbiotic, Performances

P325 In vitro and in vivo efficacies of a fatty acids esters association against Clostridium perfringens Clarisse Techer, Julie Castier, Nolwenn Bernard, Charlotte Raybaud, Claire Le Dain* *Mixscience*

Clostridium perfringens, a bacterial agent involved in necrotis enteritis, is a major pathogen encountered in chicken and turkey breedings. In a context of societal pressure regarding the use of antibiotics, various alternatives have been explored to fight against this microorganism. In this study, the minimum inhibitory concentrations (MIC) of around thirty compounds from different families (organic acids, short and medium chain fatty acids (SCFA-MCFA) and their derivatives (esters), plant extracts, essential oils) were evaluated against *C. perfringens*. Depending on the nature of the compounds, MICs varied from 4000 to 32 ppm. This first *in vitro* screening allowed to select the most promising candidates regarding their antibacterial potential. The efficacy of different formulations, based on fatty-acid esters, was then evaluated in an experimental model of necrotic enteritis with co-inculation of *C. perfringens* and coccidia *Eimeria* spp. A total of 550 days-old broilers were separated into five treatment groups: an infected and untreated control group (IUC), an infected and treated control group (ITC) receiving amoxicillin and three infected groups supplemented with different test formulas: a mixture of SCFA-MCFA esters at 1 kg/T of feed (PMA+), and this same mixture of esters at 5 kg/T (PMA++). Zootechnical performance, histology, parasites load and microbiota analyzes were carried out. Depending on the treatment, performance results showed significant differences versus IUC group, in particular PMA+ with +33 g of weight at 27 days (-0.02 pts in feed conversion ratio). For this group, effects on the intestinal mucosa and on the modulation of the microbiota were also observed, such as significant differences on the length of the intestinal villi and on the decrease in *Clostridium perfringens* load. Taking into account all these criteria and the different formulations tested, this trial showed that the PMA+ formula was the most promising for improving the growth and health of chickens in environment challenge by *C. perfringens*.

Key Words: antimicrobial, broiler, fatty acid esters, necrotic enteritis, gut health

P327 Antioxidant diversification in broiler diet, an effective strategy in heat stress conditions Paul Engler*, Cedric Vandenbossche, Mohammed BENARBIA *Nor-Feed SAS*

Vitamin E is one of the reference dietary antioxidant compounds used in animal nutrition. Its nutritional needs from all species associated to its antioxidant activity have led to its generic utilization. However, recent economical context of vitamin E prices and recurrent crisis have led the feed industry to try and find stable, reliable and efficient alternatives to partially replace vitamin E in the diet. Grape polyphenols have been ex-tensively studied for many years due to their natural antioxidant properties discovered in particular as part of the "French Paradox" study. This dis-covery has led to more and more research studying the possibility of sub-stituting vitamin E, a reference antioxidant in animal nutrition, by grape polyphenols.

The present trial, carried out in hot climate conditions in northern Africa, aimed at evaluating the optimization of the antioxidant cover of a broiler feed where part of the vitamin E provided in the premix was replaced by a standardized dry grape extract with a specific phenolic profile (SDGE, Nor-Grape®, Nor-Feed, France). 1300 day-old unsexed Arbor broilers were divided in 20 pens of 65 chicks each (10 pens/group) with a control diet (CTL group: diet with 75IU of vitamin E at all stages) and a NG group (20IU vitamin E + 5ppm of SDGE at all stages). Birds were raised for 30 days and weekly average pen mortality, growth, feed consumption, FCR and European Broiler Index (EBI) were recorded. Results were analyzed using SAS software.

No difference was observed between groups for the different zootechnical measurements (mortality, growth, feed consumption, FCR, $p>0.10$). Both groups performed equally and presented similar European Broiler Index which takes into consideration all zootechnical parameters.

The daily average temperature and relative humidity monitoring allowed for assessing the THI, an index of heat stress. Despite a moderate (THI>75) to severe (THI>79) heat stress for more than 80% of the trial period, no difference could be observed between groups for all studied parameters.

This trial thus shows that optimizing vitamin E through an efficient diver-sification of antioxidant sources with SDGE at a 1:11 rate has no nega-tive impact on the growth performances of broilers, even in suboptimal thermal conditions.

Key Words: Broilers, antioxidant, grape extract, polyphenols, vitamin E

P328 Multi-strain Q-Biotic® Bacillus and combination multi-strain Bacillus plus yeast cell wall BacPack® improve performance of turkeys under coccidial stress Miloud Araba*[1], Troy Lohrmann[1], Miguel Ruano[1], Brett Lumpkins[2], Greg Mathis[2] *[1]Quality Technology International, Inc., [2]Southern Poultry Research, Inc.*

Two experiments (EXP) were conducted to evaluate the effect of a multi-*Bacillus* strain (MBS; Q-Biotic® 3DP) and a multi-*Bacillus* strain plus yeast cell wall (MBSY; BacPack® Q3+1) on performance of turkeys under stress induced by a mixture of field *Eimeria adenoides* and *Eimeria meleagrimitis* (MEAEM*)*. In EXP1, 192 d old male Nicholas Select turkeys (MNST) were randomly assigned to 4 dietary treatments with 8 battery cages per treatment and 6 birds/cage and fed to 28 days of age (DOA). Treatments consisted of a negative control (NC; un-infected, un-supplemented), positive control (PC; orally infected with MEAEM *at 14 days of age,* un-supplemented), MBS (orally infected with MEAEM, 227 g MBS/ton feed), and MBSY (orally infected with MEAEM, 454 g DFM2/ton feed). In EXP2, 1080 d old MNST were assigned to 3 dietary treatments with 12 floor pens per treatment and 30 birds/pen and fed to 42 DOA. Treatments consisted of a control (un-supplemented), MSB (227 g/ton feed), and MBSY (454 g/ton feed). All birds in EXP2 were infected with MEAEM via feed at 14 d of age. BW and FI were recorded at start, 14 and 28 DOA in EXP1, and start, 14, 21 and 42 d in EXP2, and mortality daily. In EXP1, excreta were evaluated for oocyst shedding (OPG) at 19 DOA, and numerically scored for degree of coccidiosis at 21 DOA. OPG was evaluated at 14, 21, 28, and 35 DOA, in EXP2. In EXP1, PC reduced ($P\leq0.05$) BWG by 189 g, increased ($P\leq0.05$) mortality-corrected FCR by 25 points at 28 DOA, and increased fecal score from 0 to 1.88 (0-3 scale), compared to NC. Feeding MSB and MSBY alleviated ($P\leq0.05$) the adverse effects of the coccidial challenge on BWG by 74 and 65 g and on FCR by 9.5 and 12.5 points, respectively. Compared to PC, OPG was reduced by 35% and 55% when MSB and MSBY were fed, respectively. Fecal score was reduced by 40% (MSB, $P\leq0.05$) and 13% (MSBY), compared to PC. In EXP2, compared to the control at 42 DOA, MSB and MSBY improved ($P\leq0.05$) BWG by 113 and 148 g, and adjusted-FCR by 3.1 and 6.3 points, respectively. OPG was highest at 28 DOA and lowest at 35 d in control birds. Relative to the control, birds fed MSB and MSBY had 82% and 52% lower OPG at 28 d, respectively. The data showed that feeding MSB or MSBY can lessen the depression in turkey growth resulting from field coccidia.

Key Words: Turkeys, Performance, Q-Biotic, BacPack, Coccidia

P329 A natural coccidiostat, Phylox Feed, improves performance, intestinal lesion scores, and fecal oocyst shedding of Eimeria-infected broilers San Ching*[1], LeAnn Johnston[1], Brett Lumpkins[2] *[1]Amlan International, [2]Southern Poultry Feed & Research, Inc.*

The poultry industry is looking for alternatives to ionophores or chemicals used to control coccidiosis because of resistance and growing restrictions on antibiotics. Phylox Feed containing multiple natural ingredients has been formulated for this purpose. Two studies were conducted to identify the effects of Phylox Feed on birds challenged with cocci oocysts. Both studies lasted 28-d, each used a total of 320 birds in 4 treatments and 8 replicates per treatment. In both studies, birds were challenged on day 14 with an oral challenge containing *E. acervulina* (100,000 oocysts), *E. maxima* (50,000 oocysts), and *E. tenella* (75,000 oocysts). On day 20 lesion scores, a 0-4 scoring system, on the infected regions were measured, and blood collected for serum analysis of total carotenoids. Fecal oocysts counts were measured on day 19, 20, 21, and 22. Growth performance criteria was measured weekly. In the first study the treatments were: 1) Control; 2) salinomycin; 3) Phylox Feed; and 4) nicarbazin with challenge in all treatments. In the second study treatments were 1) Control; 2) Control Challenged; 3) Phylox Feed Unchallenged; 4) Phylox Feed Challenged - no birds received any cocci vaccine or cocci medication in this study. In the first study, Phylox Feed, salinomycin, and nicarbazin improved ($P<0.05$) FCR both on d14-20 and d0-28 when compared with control group. Lesion score (d20) and total fecal coccidia oocysts counts (d19-22) in all treated groups also decreased ($P<0.05$) significantly. No difference of serum total carotenoids concentration was found between treated group and control group. In study two, the unchallenged Phylox Feed birds had the same FCR as the unchallenged control birds on d14-20 and d0-28. In challenged birds feeding Phylox Feed improved ($P<0.05$) FCR both d14-20 and overall. In challenged birds lesion scores also improved ($P<0.05$) in the Phylox Feed treatment. No difference of total fecal oocysts count

was found between Phylox Feed and control group. However, *E. maxima* decreased (P<0.05) when Phylox Feed was fed to the challenged birds. In conclusion these studies show Phylox Feed can be an alternative to ionophore or chemical coccidiostats to help prevent coccidiosis and restore bird productivity following an oocyst challenge.

Key Words: oocyst, coddidiosis, Phylox Feed, coccidiostat, Enterotec

P330 The effect of a Lactobacillus LB fermentate postbiotic on the performance of commercial male broilers Erik Eckhardt*[1], Janet Snow[2], Hilary Pavlidis[2], Gregory Archer[3] *[1]Adare Biome, [2]CSA Animal Nutrition, [3]Texas A&M University*

The objective of this study was to evaluate the effects of various doses of a Lactobacillus LB fermentate postbiotic (LB) on the performance and yield of commercial male broilers. A total of five dietary treatments with 15 replicates per treatment containing 24, day-of-hatch Cobb 500 male broilers each were tested. Dietary treatments were: 1) Standard 3 phase broiler diet program with no additional supportive gut health feed additives (NC), 2) NC diet + Maxiban at 0.50 kg/MT (PC), 3) NC + LB at 2.2g/MT (LB2), 4) NC + LB at 5.5g/MT (LB5), 5) NC + LB at 11.0g/MT (LB10). All treatments were reared on previously used litter and vaccinated at hatch with a 3x dose of COCCIVAC®-B52. Key performance metrics of body weight (BW), feed intake (FI), feed conversion (FCR), and livability (LIVE) were measured from D1 to 42 for each pen. On D43, 6 birds per pen were selected and processed for evaluation of meat yield which included carcass (WOG), breast, tender and leg quarter weights expressed relative to live weight. Depending on data type, data were analyzed using either the MIXED or GLIMMIX procedures of SAS® OnDemand for Academics with LSMEANS used for mean separation. At D42, no differences ($P > 0.05$) were observed between treatments in BW, FI, FCR, or meat yield. There were differences between treatments observed in BW CV% ($P < 0.0001$). The PC had the lowest BW CV% (9.01 ± 0.13) compared to all other treatments. The three postbiotic treatments also had lower BW CV% (avg. 9.55 ± 0.14) than the NC treatment (11.41 ± 0.16). While not statistically significant the 5.5g/MT LB postbiotic treatment numerically weighed 105g more and had FCR numerically lower by 0.036 points than the NC. In conclusion, none of the *LB* postbiotic doses nor the PC improved performance or yield over the NC. Supporting this conclusion is that all treatments had around 5% mortality including the NC. Despite this the 5.5g/MT treatment did result in numerical improvements in BW and FCR at D42 which could indicate an economic improvement by using the product at that dose.

Key Words: postbiotics, broiler, performance, yield, Lactobacillus

P332 Evaluating the effects of a synbiotic (probiotic + prebiotic) alone or in combination with a precision-biotic on broiler performance Chasity Pender*[1], April Levy[1], Shelby Corray[1], Gregory Archer[2] *[1]DSM Nutritional Products, [2]Texas A&M University*

The impetus to eliminate antibiotics from animal production has provided an opportunity to rethink the conventional approach to animal nutrition, health, and welfare and survey potential alternatives. The objective of the present study was to evaluate the effect of a synbiotic (probiotic + prebiotic; SYN) alone or in combination with a precision-biotic based glycan (PB) on the growth performance of broiler chickens. A total of 720 day-old Cobb 500 broiler chicks were placed in a completely ran-domized block design with 12 replicates pens per treatment (20 birds per pen) and fed corn-SBM based diets. The treatments consisted of a non-supplemented group (CON), supplementation with SYN (500 g/MT), or supplementation with SYN (500 g/MT) plus PB (500 g/MT; SYN+PB). Birds were raised on used litter in floor pens. Chicks and remaining feed were weighed at 0, 14, 28, and 42 days of age and average body weight, feed intake, and feed conversion ratio (FCR) were calculated. Data were analyzed as a one-way ANOVA using JMP with significance reported at $P < 0.05$. Means were separated using Tukey's HSD. Body weight was significantly improved in both SYN and SYN +PB compared to CON on days 14 ($P = 0.0002$), 21 ($P = 0.0005$), and 28 ($P = 0.01$). During the starter period (d1-14), FCR was significantly improved ($P < 0.0001$) in both treated groups compared to the control, with SYN+PB having sig-nificantly lower FCR than the other two groups. Feed conversion was also significantly improved in both supplemented groups in the d1-21 ($P < 0.0001$) and d1-28 ($P = 0.0007$) periods. Overall, these results suggest the supplementation of SYN and PB may be a beneficial strategy for aug-menting broiler performance in antibiotic-free production systems.

Key Words: broiler, synbiotic, prebioic, probiotic, performance

P333 Impact of Alterna® HTS on Histomonas meleagridis induced lesion scores and mortality in poults during a challenge Ashley Wagner*[1], Bertrand Medina[1], Ivan Girard[1], Jessica Suagee-Bedore[2] *[1]Probiotech International Inc., [2]Virginia Tech*

Histomonas meleagridis is a protozoan that is the etiological agent for his-tomonosis, also known as blackhead disease or histomoniasis, that result-ed in economic losses exceeding 2 million USD to the turkey industry in 2020. With limited pharmacological options to treat or prevent histomono-sis, natural alternatives have been investigated. Therefore, the objective of this study was to evaluate Alterna® HTS, a proprietary blend of botani-cals (Probiotech International Inc.), fed to turkeys during a histomonosis challenge. Day old hybrid female poults were randomly allocated to NC (no inoculation, no treatment), CC (inoculation, no treatment), or HTS (inoculation, Alterna® HTS at 1lb/ton). Four pens of 23 poults each were assigned to each treatment. Intracloacal inoculation (1 mL) occurred on day 15 containing 100,000 histomonads/mL. Post-inoculation mortality was measured daily and coupled with liver and cecal lesion scores. Scores were reported as a percentage of birds with lesions (Presence: visible sight of lesions; Absence: no lesions). Because CC did not reach 40% mortality, all remaining birds were euthanized and necropsied for liver and cecal lesion at d 35. Statistical analysis of count data was performed in SAS v.9.3 using Fisher's exact test. Post-inoculation mortality was slightly lower than expected based on previous trials using this inoculation protocol, and was not different ($P > 0.05$; NC: 12.5%; CC: 25%; HTS: 0%) between treatments. Cecal and liver lesion scores revealed little to no NC birds with

(cecal: 1.23%; liver 0%) lesions indicating the inoculation protocol as effective. Additionally, there were fewer NC birds with lesions ($P < 0.001$) than the inoculation treatments. There was a trend ($P = 0.08$) towards fewer liver lesion scores when fed HTS (10.2%) compared to CC (20.5%). A similar phenomenon was observed with cecal lesion scores ($P = 0.14$; HTS: 56.4%; CC: 60.3%). Combined the impact on mortality and lesion scores of poults inoculated with *H. meleagridis* may indicate a benefit of HTS during a challenge. Additional research is warranted with a larger sample size, and a prolonged study period to validate these initial findings.

Key Words: Histomonosis, Turkeys, Botanicals

P334 A triple strain probiotic demonstrates high siderophore activity: a new step towards the understanding of pathogen inhibition in poultry Antoine Meuter*, Line Skjoet Rasmussen *Chr. Hansen, A/S*

Iron is a vital nutrient for virtually all forms of life. The requirement for iron is based on its role in cellular processes ranging from energy generation and DNA replication to oxygen transport and protection against oxidative stress. Bacterial pathogens are not exempt from this iron requirement. However, these organisms must acquire iron from within their animal hosts in order to replicate and cause disease. Chr. Hansen probiotic strains produce siderophores and are screened for this feature. One known siderophore in Bacillus subtilis is called bacillibactin. Research was conducted to evaluate siderophore production in a new 3-strain Bacillus-based probiotic in comparison to several strains present in the poultry probiotic market. When siderophores are produced overnight by the Bacilli growth, they will compete for iron uptake, chelating the iron. This leads to the blue dye color changing to green-yellow. This color change can be measured in a spectrophotometer as a drop in absorbance when compared to an internal standard after 3 hours (delta T3-T0). All experiments were conducted in repeates of five, paired t-test was applied to the results and $P<0.05$ were considered significant. The results show that the two Bacillus subtilis (DSM32325 and DSM32324) strains have a significantly ($p<0,05$) superior siderophore production with delta T3-T0 of -0,2149 and -0,1395 respectively compared to most of the Bacillus sp. strains present in the poultry probiotic market (from -0,0809 to +0,5515). In addition, the Bacillus amyloliquefaciens strain (DSM25840) also exhibits a significantly ($p<0,05$) higher siderophore production (delta T3-T0 of -0,0776) than most of the comparative probiotics containing Bacillus subtilis (-0,0707 to +0,5515) and Bacillus velezensis strains tested in this assay (-0,061 to +0,0163). The capability of Chr. Hansen Bacillus triple-strains to produce superior siderophore activity represents an important property to compete for iron acquisition with pathogenic bacteria such as E.coli. By depriving them of this key element for their development and virulence properties, therefore they inhibit their growth (Harwood et al., 2018).

Key Words: bacillus, probiotic, inhibition, pathogen, siderophore

P335 Supporting normal function of the gastro-intestinal tract with effective probiotics Christophe Bostvironnois*[1], Jean Bodin[1], Antoine Meuter[1], Steve Lerner[2], Eric Sobotik[2] *[1]Animal Plant Health & Nutrition, Chr. Hansen A/S, [2]Animal and Plant Health & Nutrition, Chr. Hansen, Inc.*

It is well-known and thoroughly documented that effective probiotics support many of the normal functions of the gastrointestinal tract, specifically, the digestive, absorptive, barrier and immune functions. For decades, those investigating the efficacy of probiotics in poultry have reported improvements in variables of economic importance, such as feed conversion, average daily gain, morbidity, and mortality. It is our hypothesis that these types of differences measured in well-controlled, challenge-type studies or in field observation trials are due to a greater percentage of the birds in the probiotic-fed group simply being normal. As the percentage of normal, healthy birds increases in one group versus another, then all variables of economic importance should likewise improve. Concomitantly, variation among the birds in the probiotics-fed group should decrease. To test this hypothesis, we worked with a major global broiler integrator and fed 1.8M Ross birds from 72 flocks from the same complex, placed as hatched (males and females mixed); half receiving a daily feeding of GALLIPRO® Fit ($1.6x10^6$ cfu/g of feed) and other half only on the standard diets. Average age at market was 35,7 and 35,2 days respectively. Feed conversion ratio (FCR) was significantly improved (p:0.04; P-value for Bonett's test of homogeneity of variance). For FCR, livability and condemnation, the distribution of observations from the probiotics-fed group compared to the control group was shifted in an economically-positive direction and variation was reduced (FCR: 1.617 ± 0.074 vs 1.651 ± 0.106; livability: $96.12\% \pm 1.70\%$ vs $95.73 \pm 2.33\%$; and condemnation: $0.64\% \pm 0.39\%$ vs $0.86\% \pm 0.64\%$; GALLIPRO® Fit fed vs Control, respectively). Daily feeding of an effective probiotic such as GALLIPRO® Fit enables commercial broilers to digest and absorb nutrients in a normal manner, to maintain a robust microbiome, and to achieve as much of their genetic potential as their environment allows.

Key Words: bacillus, probiotic, microbiome, productivity, variation

P336 Effects of dietary supplementation of medium chain fatty acids on gut health in broilers challenged with Salmonella heidelberg Carlos del Cuvillo*[1], Mónica Puyalto[1], Hebert Silveira[2], Giselle da Silva Gallio[3], Juan José Mallo[1] *[1]Norel SA, [2]Natural BR Feed, [3]Nutrinzoo*

The aim of the trial was to assess the effect of sodium salt of medium chain fatty acids (MCFA) on broilers challenged with *Salmonella heidelberg*. Five groups of fifty 1-day-old broilers were assigned to five treatments: negative control (NC) fed with a control diet without additive and not challenged, positive control (PC) fed with a control diet without additive, and three treatments with control diet supplemented with different doses of sodium salt of MCFA (Dicosan®, NOREL SA) at 0.5 g, 1.0 g and 1.5 g per kg diet (DIC0.5, DIC1 and DIC1.5 respectively). At day 3, 20% of birds of PC, DIC0.5, DIC1 and DIC1.5 groups (10 per treatment) were orally challenged with *Salmonella heidelberg* (6 log CFU/mL).

From each treatment, 20 birds were euthanized at day 14 and 20 birds in day 28 for ceca sampling. One fragment from jejunum and ileum were also sampled from 12 birds among the euthanized at day 28. Bacteriological data were analysed using the least squares mean statement of GENMOD procedure of SAS. Intestinal morphology parameters were analysed as a one-way ANOVA via SAS. *Salmonella* incidence was analysed using a Chi-squared analysis via SAS.

No reduction of Salmonella count was observed. Challenge modified the microbiota composition for other bacteria and MCFA treatments helped to modulate the microbiota and reduce these changes. For *E. coli* colonisation, the counts were lower in the three DIC treatments than in PC, with a significant reduction in day 14 (7.05a, 8.10b, 7.12a, 7.33a and 7.43a log CFU/mL for NC, PC and DIC at 0.5, 1 and 1.5 g/kg respectively, $p<0.05$) and *C. perfringens* at day 28 (4.76a, 4.91a, 4.51ac, 3.94bc and 4.1bc log CFU/mL for NC, PC and DIC at 0.5, 1 and 1.5 g/kg respectively, $p<0.05$). As for intestinal morphology, DIC1 birds had significantly higher villus height:crypth depth (V:C) ratio in jejunum (6.58ab, 5.62b, 6.01b, 7.14a and 6.47ab for NC, PC and DIC at 0.5, 1 and 1.5 g/kg respectively, $p<0.05$) and the ratio tended to be higher in ileum (4.88x, 4.91x, 5.78y, 6.05y and 5.88y for NC, PC and DIC at 0.5, 1 and 1.5 g/kg respectively, $p<0.10$).

We can conclude that DICOSAN help challenged broilers to be more resilient, by the improvement of gut health modulating the cecal microbiota and enhancing the small intestine development.

Key Words: MCFA, broilers, microbiota, intestinal morphology, Salmonella heidelberg

P337 Effects of Phytogenic feed-additives on growth performance, carcass parameters, and meat quality of male broilers. Garrett Mullenix*, Sami Dridi *University of Arkansas*

The use of natural health-promoting feed additives in broiler production is prominent in the current NAE era. This experiment aimed to determine the effects of two phytogenic products (NUQO, France) on growth performance, gas emission, and meat quality in broilers. Day-old male Cobb500 chicks (n=1,995) were randomly allotted to one of 95 pens and fed a 3-phase feeding programs (0-14d, 15-28d, and 29-40d). The dietary treatments include a corn-soy basal Negative Control (NC), NEX100 (100g/MT), NEX75 (100g/MT for 0 to d28, then 75g/MT for 29 to d40), FLEX100 (100g/MT), and FLEX50 (50g/MT). Growth performance, gas emission, and processing parameters, were analyzed as randomized complete block design using a one-way ANOVA. Pen represented the experimental unit, with 19 replicates each. Data were analyzed using JMP Pro16 statistical analysis software (SAS Institute, 2022) and statistical significance was considered at $P < 0.05$.

At the end of the cycle, although it was not statistically discernible, all treatment improved FCRs. Of particular interest, NEX42 reduced feed intake, and increased processing body weight by approximately 46g/bird and improved breast weight by 19g. This was accompanied by an increased incidence of woody breast, but not white striping, though the difference was not statistically significant. Both FLEX treatments displayed more desirable yellow pigmentation in the breast fillet compared to the NC and NEX100 ($P<0.001$). NEX42 reduced both tibia and femur head necrosis, and reduced N_2O and CO_2 gas emission. Together, the data indicate that the supplementation of NEX and FLEX could be promising, however further in-depth investigations are warranted.

Key Words: NEX, FLEX, broilers, growth, gas emission

P338 Effect of the probiotic strain Enterococcus faecium 669 on intestinal colonization and influence on the microbiome composition of broiler chickens. Antoine Meuter*[1], Christophe Bostvironnois[1], Dorthe Sandvang[1], Eric Sobotik[2] *[1]Animal and Plant Health & Nutrition, Chr. Hansen, A/S, [2]Animal and Plant Health & Nutrition, Chr. Hansen, Inc.*

The intestinal tract of the newly hatched chickens plays a crucial role in immune cell formation and immune challenges. The interaction between pathogenic microorganisms such as *Escherichia coli (major* causative agent of early mortality and the host commensal microbiota. The aim of this study was to evaluate the effect of the probiotic strain *Enterococcus faecium* 669 after early life application on intestinal colonization and microbiome composition of broiler chickens. Chicks were inoculated by oral gavage to decrease individual variation to mimic the post hatch spray application. Inoculation dose was 4.2×10^8 CFU/bird, while the broiler chickens were fed ad libitum. Infectious bronchitis aerosol vaccine was used, and no other treatments were applied during the trial period. At day 4 and day 7 after hatch, 15 birds from each group (control and probiotic) were euthanatized, DNA from cecal sacks were purified and 16s RNA was sequenced. *Enterococcus faecium* 669 seemed to be present in caeca, as represented by an increase in Enterococcus in the treated birds. Furthermore, *Enterococcus faecium* 669 modulated the cecal microbiome by increasing the diversity of the microbiome and shaping the composition in a beneficial direction with fewer potentially pathogenic species and more potentially probiotic species. The increase in Enterococcus and other potentially beneficial bacteria could have happened on the expense of reduction in Bifidobacteriaceae. This modulation of the microbiome can translate into improved gut health and performance, as the *Enterococcus faecium* 669-treated chicks showed strong potential for counteracting infections due to competitive exclusion and improved immunity.

Key Words: probiotic, broiler, microbiome, colonization, diversity

P339 Effect of an energetic metabolism enhancer on laying hens' growth and performance Juan Miguel Ruiz Rodriguez*[1], Hasliza Abu Hassim[2], Annas Salleh[2], Octavi Colom[1] *[1]Igusol Advance S.A., [2]Universiti Putra Malaysia*

The study was conducted to evaluate the efficacy, of an energetic metabolism enhancer called SOLERGY added to replace palm oil, on body weight (BW), laying rate (LR), egg size (EW), average daily feed intake (ADFI), and visceral fat (VF) of laying hens up to 36 weeks old. A total of 380 Hyline Brown laying hens of 20 weeks age were distributed randomly to 5 treatments. Each treatment consisted of 8 replicates during a 16 weeks period. Treatments consisted of diets varying in palm oil content: 3 % palm oil control (CONT); 1.5 % palm oil and 0.15 % SOLERGY (SL15); 0 % palm oil and 0.3 % SOLERGY (SL30), 0 % palm oil and 0.6 % SOLERGY (SL60); 3 % palm oil and 0.067 % SOLERGY (CNSL) diets. Diets were formulated as CP 18 %, and isocaloric, 3,240 kcal/kg AME. But diets SL60 and CNSL, which contained an extra amount of energy. Solergy is marketed by the Spanish company Igusol Advance S.A., and it is composed of gluconeogenic and phytogenic compounds. The study was conducted at the facilities of the University Putra Malaysia, Malaysia. 4 weeks previous to the start of the study the birds were acclimated to control diet and housing. BW, and ADFI were measured weekly. LR and EW were measured daily and reported weekly. VF was measured on weeks 25^{th} and 36^{th}.

Data was analysed as per one-way analysis of variance by SAS software. Means were compared by post-hoc Tukey test. Significance is considered when $P < 0.05$. Results for CONT, SL15, SL30, SL60, and CNSL did not show significance differences among treatments ($P > 0.05$) for ADFI 104.1; 105.6; 105.8; 105.2; and 104.7 g respectively, LR 80.7; 82.8; 84.5; 83.9; and 81.6 %, EW 58.1; 57.9; 58.7; 58.7; and 57.8 g, or BW 1,557; 1,531; 1,523; 1,557; and 1,545 g. VF did not differ between groups on week 25. However, it did on week 36, being control group higher (9.9 g) than the others (5.9; 5.1; 5.1; 5.2). The fact that SL60 and CNSL showed low levels of VF suggests that SOLERGY triggers an energetic metabolism which avoids the synthesis of VF. It is concluded that the energy enhancer Solergy is capable of substituting up to 3 % of palm oil efficiently without affecting BW, ADFI, LR or EW parameters in Hyline Brown hens. The presence of Solergy on the diet decreases visceral fat regardless of diet energy concentration.

Key Words: Energy, lipid, fat, laying, Solergy

P340 Effects of various concentrations of supplemental biochar on ileal digestible energy and live performance of broilers during an 8-week production period Klint McCafferty*, Joseph Purswell *USDA-ARS Poutlry Research Unit*

An experiment was conducted to evaluate the effects of feeding various concentrations of a novel biochar product on apparent ileal digestible energy and growth performance of broilers during an 8-week production period. A total of 600 straight-run chicks (YPM × Ross 708) were feather-sexed and distributed into 30 floor pens (10 males and 10 females) and fed 1 of 5 dietary treatments. Dietary treatments consisted of various concentrations of supplemental biochar (0.0, 0.5, 1.0, 1.5, or 2.0%) in a 4-phase feeding program: starter (1 to 15 d of age), grower (16 to 29 d of age), finisher 1 (30 to 43 d of age), and finisher 2 (44 to 56 d of age). Birds and feed were weighed on a pen basis at 1, 15, 29, 43, and 56 d of age to determine live performance. At 15 and 29 d of age, 4 birds per pen (2 males and 2 females) were selected and euthanized for ileal digesta contents (Meckel's diverticulum to 2 cm above ileo-cecal junction) to determine apparent ileal digestible energy. At 15 d of age, broilers fed diets with 2.0% supplemental biochar had a 10% lower ($P < 0.05$) apparent ileal digestible energy than those fed the diet without supplemental biochar. However, at 29 d of age, no differences ($P > 0.05$) in apparent ileal digestible energy were observed. Similarly, no differences ($P > 0.05$) in broiler growth performance were observed throughout the experiment. Feeding

diets with supplemental biochar inconsistently affected apparent ileal energy digestibility but did not affect growth performance of broilers during an 8-week production period.

Key Words: energy utilization, feed additive, activated carbon

P341 Stacking feed and water additives to reduce Salmonella shedding in broilers Wendy Attuquayefio, James McNaughton* *AHPharma, Inc.*

Recent research has shown that saponins derived from *Quillaja saponaria* and *Yucca schidigera* (QY) and DFMs composed of *Bacillus* spores can improve gut health and mitigate pathogen proliferation in broilers. A factorial was employed to measure the effect of combining 125mg/kg QY with a *Bacillus* spp. DFM blend at 2 x 10^6 CFU/g in the finished feed with and without weekly dosing of 5ppm chlorine dioxide (ClO2) in the drinking water. An RCB design was employed to assign Ross-708 males (N=1600) to 40 pens containing 40 birds each (N=320 per treatment). The negative control (NC) group received no feed or water additives and no challenge. The positive control (PC) group received no feed or water additives but was challenged weekly from 0-42d with a 12h feed withdrawal followed by an oral gavage containing 1 x 10^6 CFU/bird of *Salmonella* Typhimurium (ATCC 53647). Additionally, a 50/50 w/w mixture of litter and feed was administered as the sole food source for 24h post-inoculation. Treatments included QY+DFM in the feed, ClO2 in the water, or their combination. Data were analyzed using the GLM to determine the effect of treatment on live performance and weekly *Salmonella* shedding. Dosing ClO2 once per week did not damage drinker line equipment, as is commonly reported with continuous administration of chlorine products. The NC group averaged a 42d body weight (BW) of 2613g, which was higher than all other treatment groups (P<0.05). The QY+DFM group's 42d BW averaged 1817g, the ClO2 group 1723g, and their combination 1870g, all higher than the PC group at 1530g (P<0.05). QY+DFM, ClO2, and their combination reduced weekly *Salmonella* shedding compared to PC (P<0.05). The CFU/g of *Salmonella* recovered was inversely related to bird age in all treatment groups (except for the NC group, which was *Salmonella* negative from 0-42d), indicating that the challenged broilers developed an innate immunity to the pathogen. This study demonstrates that a stack of products in the feed and water can effectively reduce *Salmonella* incidence in broilers subject to a severe experimental challenge from 0-42d.

Key Words: Saponins, DFM, Chlorine dioxide, Salmonella

P342 Effects of Yucca schidigera plant extract on the performance and blood gases of broiler chickens Marquisha Paul, Rebecca Delles*, Elizabeth Meza, Michael Ford, Tuoying Ao, Anthony Pescatore, Daniel Graugnard, Ronan Power *Alltech-University of Kentucky Nutrition Research Alliance*

A study was conducted to evaluate the effects of *Yucca schidigera* plant extract (YPE) on the performance, blood pH, gases, electrolytes, and metabolites of broiler chickens. An RCB design with 3 trt and 10 replicate groups/trt was used for this study. A total of 180, one day old, male byproduct breeder chicks were randomly assigned to 3 corn-soybean meal-based diets with the following supplemental levels of YPE: 1) 0 g/ton, 2) 60 g/ton, 3) 120 g/ton. Chicks were raised in cages (6 chicks/cage) with *ad libitum* access to water and feed for 21 days in an environmentally controlled room and 22L: 2D photo period. At the end of the study, whole blood was collected and immediately analyzed for pH, blood gases, electrolytes, and metabolites using Heska Element POC Blood Gas and Electrolyte Analyzer. Data were subjected to ANOVA and means were compared using Fisher's protected LSD test. Birds fed diets with 60 g YPE/ton had higher (P≤0.05) FI than those fed 0 g/ton. The body weight gain and feed to gain ratio were not different among trt groups. Blood pH and base excess of extracellular fluid was highest (P≤0.05) in birds fed 120 g YPE/ton, while functional oxygen saturation was the lowest (P≤0.05) in birds fed diets without YPE supplementation. Partial pressure of CO_2 and O_2 in blood was not affected by YPE supplementation level, yet bicarbonate concentration, total CO_2 concentration, and anion gap tended (P<0.10) to be affected by YPE supplementation. Blood potassium was lowered (P≤0.05) with YPE supplementation, whereas sodium, chloride and ionized calcium levels were similar. Blood glucose, lactate, hematocrit, and hemoglobin values were not significantly different among trt groups. In conclusion, this study revealed that supplementation of YPE in broiler diets resulted in higher feed intake, lower blood hydrogen ion concentration and a potentially higher capacity for blood oxygen binding.

Key Words: Yucca schidigera, broiler, blood gas, blood electrolyte, blood metabolite

P343 Mitigation of Salmonella Typhimurium in an in vitro broiler cecal culture with a yeast fermentate Dana Dittoe[1], Lindsey Wythe[1], Abe Scheaffer[2], Steven Ricke*[1] *[1]Meat Science and Animal Biologics Discovery, University of Wisconsin-Madison, [2]SweetPro LLC.*

ProBiotein, a yeast fermentate (YF), has demonstrated the ability to modulate the broiler gastrointestinal microbiota as a feed supplement. However, no data exists on the anti-*Salmonella* effect of this YF. Therefore, the objective was to evaluate the anti-*Salmonella* effect of ProBiotein in an *in vitro* cecal model. Over 4 separate trials, 42-45d-old broiler chickens (n=7-9) were euthanized, and ceca were aseptically collected. Ceca were transferred to an anaerobic chamber and diluted 1:3000 in anaerobic dilution solution (ADS). Diluted ceca were aliquoted (20 mL) to serum bottles containing 1.25% of a basal diet plus the following treatments: 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25% (wt/v) ProBiotein (YF). Cecal cultures were pre-acclimated (PA) for 24 h anaerobically at 37°C. After PA (0h), 200mL of a 10^8 CFU/mL NA strain of *S.* Typhimurium (64 µg/mL) was inoculated into the cecal cultures and allowed to incubate anaerobically for 24h at 37°C (24h). At PA, 0h, and 24h, the pH of the *in vitro* cultures was taken. At 0 and 24h, aliquots were taken to enumerate *Salmonella* via dot plating on XLD+NA. Data were analyzed in JMP Pro 15. The pH data were analyzed as a mixed effect model (random effect=trial). Microbiological data were reported as the change (24-0h) in *Salmonella* (Log CFU/mL) and analyzed as a mixed effect model (random effect=trial). Pairwise differences were determined using Tukey's HSD with a significance of P<0.05. There was a main effect of treatment and time (P<0.0001) on the pH of the *in vitro* cultures but not an interaction (P>0.05). As the concentration of YF increased, the pH decreased significantly (P<0.05). Those treated with 0.00% (6.60 pH) were different than those treated with 0.25% and above concentrations (6.43 pH) with those treated as 1.25% being significantly lower than that of 0.00, 0.25, 0.50 and 0.75% YF (P<0.05). As time progressed, the pH decreased (7.25, 6.25, and 5.45 pH; P<0.05). There was a main effect of treatment on *S.* Typhimurium with the addition of 1.25% (-4.87 Log CFU/mL) resulting in the largest change in *Salmonella* compared to those treated with 0.00 and 0.25% (-1.53 and -2.92 Log CFU/mL; P<0.05). These results demonstrate the potential of ProBiotein® as an in-feed anti-*Salmonella* broiler supplement.

Key Words: Salmonella, in vitro model, yeast fermentate, ceca

P344 Impact of a yeast fermentate on Salmonella Typhimurium control in an in vitro broiler intestinal model Dana Dittoe[1], Lindsey Wythe[1], Abe Scheaffer[2], Steven Ricke*[1] *[1]Meat Science and Animal Biologics Discovery, University of Wisconsin-Madison, [2]SweetPro LLC.*

Modulation of the broiler gastrointestinal (GIT) microbiota through the use of in-feed supplements has been an effective measure to reduce. Therefore, the objective was to evaluate the anti-*Salmonella* effect of varying levels of an in-feed supplement, a yeast fementate (YF), ProBiotein®, in an *in vitro* GIT model. Over 4 separate trials, 42-45d-old broiler chickens (n=7-9) were euthanized, and the GIT were aseptically collected. The collected GIT were transferred to an anaerobic chamber and jejunal digesta was diluted 1:300 in anaerobic dilution solution (ADS). Diluted je-

junal digesta were aliquoted (20 mL) to flasks (125 mL) containing 1.25% of a basal diet (250 mg) plus the following treatments: 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25% (wt/v) ProBiotein® (YF). Intestinal cultures were pre-acclimated (PA) to treatments for 24 h at 37 °C under microaerophilic conditions. Inoculation of 10^8 CFU/mL nalidixic resistant NA) strain of *S.* Typhimurium (64 mg/mg; 200 mL) occurred after PA (0h). Cultures were incubated for 24h at 37°C under microaerophilic conditions (24h). At PA, 0h, and 24h, the culture pH was taken. At 0 and 24h, *Salmonella* was enumerated via dot plating on XLD+NA. Microbiological data were reported as the change (24-0h) in *Salmonella* (Log CFU/mL). Data were analyzed in JMP® Pro 15 a mixed effect model (random effect=trial). Pairwise differences were determined using Tukey's HSD with a significance of $P<0.05$. There was an interaction of treatment x time ($P=0.002$) on the pH of the *in vitro* cultures. There were no differences in pH at PA, but at 0 and 24h, pH of treatments decreased with 0.25% of YF (5.80 pH) being greater than pH of 1.25% YF at 24h (5.35 pH). There was a main effect of treatment on *S.* Typhimurium with the addition of 1.25% (-3.60 Log CFU/mL) resulting in the largest change in *Salmonella* compared to those treated with 0.25% (-1.75 Log CFU/mL; $P<0.05$). Therefore, ProBiotein® (1.25%) was effective in reducing *Salmonella* in an *in vitro* intestinal model.

Key Words: intestinal model, yeast fermentate, Salmonella, in vitro

P345 Describing the ileal microbiota development in broilers when fed varying levels of a yeast fermentate product Lindsey Wythe[1], Dana Dittoe[1], Abe Scheaffer[2], Steve Ricke*[1] [1]*Meat Science and Animal Biologics Discovery Program, Department of Animal and Dairy Science, University of Wisconsin,* [2]*Harvest Fuels Inc*

Yeast fermentate (YF) products have been shown to improve broiler performance by modifying the gastrointestinal microbiota. The objective was to evaluate a YF product, ProBiotein® (PB), on the ileal microbiota composition of Ross 308 males fed PB-supplemented diets for 42 days. Starter (d0-14), grower (d14-28), and finisher (d28-42) diets were offered *ad libitum* and consisted of an industry-standard basal diet (con, 0.0PB) supplemented with: 0.2% (0.2PB); 0.5% PB (0.5PB); or 0.75% PB (0.75PB). Chicks were randomly assigned to batteries using an RCBD on d 0 ($N = 400$, $n = 10$, $k = 4$, block = 2[DD1]) and moved to floor pens on d14. All birds receiving the same treatment in a given block (battery) were combined into 1 floor pen ($n = 50$) to maintain the RCBD. On d 14 and 42, ileal digesta was collected ($n = 10$). Genomic DNA were extracted, and the V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq. Data were analyzed in QIIME2-2022.2. Main effects and interaction of treatment and day on α and β diversity were explored using ANOVA and ADONIS ($P £ 0.05$). Pairwise differences were determined using Kruskal-Wallis (KW; α) or ANOSIM (β; $Q £ 0.05$.). The treatment × day interaction on significantly different taxa were evaluated with ANCOM. All main effects and interactions were significant for α and β diversity ($P < 0.05$). However, while KW was significant for treatment effect and treatment × day interaction ($P < 0.05$), there were no significant pairwise differences ($P > 0.05$). Day was not significant through KW, but was for both Jaccard and Weighted UniFrac, with diversities different between d 14 and d 42 ($P < 0.05$). The treatment × day interaction was significant for both Jaccard and Weighted UniFrac; Jaccard revealed differences between treatments, while Weighted UniFrac revealed greater differences over time than between treatments ($Q < 0.05$). At the genus level, 18 taxa were significantly different in relative abundance, including *Lactobacillus*, *Butyricicoccus*, and *Weisella* ($W > 96$). Day was the driving factor in the differences, but treatment magnified these effects ($P < 0.05$). In conclusion, ProBiotein® was shown to influence the microbiota within the ileum and may be a useful feed additive in promoting microbial diversity in broilers.

Key Words: Microbiota, Yeast Fermentate, Ileum, Ileal Microbiota, 16s rRNA gene

P347 Oregano essential oil improves performance and survivial rate in turkeys challenged with Eimeria spp., Cochlosoma anatis or Histomonas Chongxiao Chen[1], Anna-Lena Beckmann[2], Thilo Borchardt*[2] [1]*University of Georgia,* [2]*Dostofarm GmbH*

Intestinal diseases caused by bacteria, viruses or protozoa have an impact on turkey health. Protozoa are capable of severely reducing immunity, making poultry more susceptible to secondary infections, negatively affecting performance and profitability.

Three in vivo studies aimed to determine the effect of dietary Oregano essential oil supplementation on growth and feed conversion in turkey poults challenged with either *Eimeria spp*, *Cochlosoma anatis or Histomonas meleagridis*

Eimeria challenge: 3 treatment groups included 20 poults per replicate with 8 replicates each. Groups 2-3 were vaccinated with 2x Immucox T at day 0. In group 1, non-vaccinated turkey poults treated with Amprolium served as negatiove control. cTreatment group 3 was supplemented with 25 g/t Oregano essential oil in feed. Results: animals receiving dietary Oregano oil showed significantly improved feed conversion, compared to negative control and vaccinated control (1.61 vs. 1.70 vs. 1.72, $p = 0,033$) From week 0-8.

Cochlosoma challenge: 3 treatment groups included 8 animals per replicate with 6 replicates each. In group 1, non-infected animals served as a negative control. Groups 2-3 were infected with *Cochlosoma anatis.* Treatment group 3 was supplemented with 22.5 g/t Oregano essential oil in feed. Results: animals receiving dietary Oregano oil showed signifi-

cantly improved feed conversion, compared to infected control (1.95 vs. 1.67, p = 0.038). Weight gain was also significantly improved (465g vs. 540 g, p=0.048) 14 days after infection.

Histomonas challenge: 4 treatment groups included 10 animals per replicate with 6 replicates each. In group 1, non-infected animals served as a negative control. Groups 2-4 were infected with *Histomonas meleagridis.* Treatment group 3 and 4 were supplemented with 37.5 g and 150g of Oregano essential oil in feed respectively. Results: animals receiving dietary Oregano oil at 150g/t of feed showed significantly improved feed conversion, compared to infected control (2.09 vs. 1.68, p = 0.007)14 days after infection.

Conclusion:The three in vivo studies confirm the performance and health promoting effect of standardized natural Oregano essential oil in turkey production.

Key Words: Oregano essential oil, Turkey, Eimeria spp, Histomonas meleagridis, Cochlosoma anatis

Metabolism and Nutrition: General Nutrition

P348 Influence of feed form and corn particle size on growth performance and meat quality of broiler chickens Basheer Nusairat*[1], Mohammad Qaddoura alhaj[1], Wilmer Pacheco[2], kamel Mahmoud[1] *[1]Jordan University of Science and Technology, [2]Auburn University*

The objective of this trial was to evaluate the effect of coarse corn inclusion and feed form on growth performance, meat quality, litter moisture and organ weights of broilers raised to 35 days. A total of 616 one-day-old mixed-sex Ross 308 broiler chicks were randomly distributed in 28 pens and assigned to 4 treatments with 7 replicates per treatment and 22 birds per replicate. Diets were corn-SBM-based and contained 2 levels of coarse corn (0 and 5%(starter)/10% grower) and were fed either as mash or crumble (starter)/pellet (grower)). The fine corn particle size was 924 µm and the coarse corn particle size was 1241 µm. Birds were fed starter (1-14 d) and grower (15-35 d). Body weight, feed intake, mortality adjusted FCR, and litter moisture were measured weekly. Processing, parts yield, and organ weights were determined on d 35. Data were analyzed as 2 × 2 factorial arrangement of (coarse corn inclusion x feed form) using GLM procedure of SAS to evaluate main effects and interactions. Tukey's HSD test was used to separate means and statistical significance considered at $P < 0.05$. The inclusion of coarse corn during starter and grower period and pellets produced higher BWG ($P<0.05$) at 35 days compared to fine corn and mash, respectively. Feeding pellets improved ($P<0.01$) FCR compared to mash diets (1.79 versus 1.63). Furthermore, broilers fed pelleted diets had higher ($P<0.001$) breast fillet compared to broilers fed mash diets (511 versus 435 g). Pelleted diets increased litter moisture at 35 d ($P<0.05$) compared to mash diets (40.2 vs 47.1%). Organ development (gizzard, proventriculus, duodenum jejunum, and ileum weights) was not influenced by treatments. It can be concluded that the inclusion of 5% and 10% coarse corn during the starter and grower improved broiler performance, while feeding pelleted diets improved BWG and FCR compared to feeding mash diets.

Key Words: Broiler, particle size, feed form, organs

P349 Determining an ideal dietary combination of starch, amino acids, and oil levels during the grower phase of broilers infected with coccidiosis Julianna Jespersen*[1GS], Juliano de Paula Dorigam[2], Rose Whelan[2], Maci Oelschlager[1], Kaitlyn Sommer[1], Bradley Gorenz[1], Robin White[3], Ryan Dilger[1] *[1]University of Illinois, [2]Evonik Nutrition & Care GmbH, [3]Virginia Tech*

Due to negative perception of anti-microbial use, alternative methods to ameliorate coccidiosis in broilers are being explored. One method is through optimizing dietary nutrients to support immune function and minimize disruptions in growth performance. In this study, we determined an optimal combination of dietary starch, amino acids (AA), and oil to elicit maximal growth performance in broilers experiencing a coccidiosis challenge. A total of 1,152 male Ross 308 broiler chicks were housed in floor pens and randomly assigned to 1 of 10 experimental treatments. Birds received a common starter diet d 0-10 and experimental grower dietary treatments d 10-24. The 10 experimental grower diets followed a simplex lattice design {3, 3} consisting of 3 basal diets formulated to have either the highest starch (454g/kg), AA (120%, 1.33% standardized digestible Lys), or oil (102g/kg) level and mixed in 4 equally spaced levels for each component (0, 0.333, 0.667, 1). The mixture of the 3 basal diets enabled varying densities of AA (80-120% of recommendation by AMINOChick®), metabolizable energy (ME), and starch:oil contributing to ME. Feed was provided *ad libitum* throughout the experiment. Bird and feeder weights were collected on d 0, 10, and 24. On d 11, chicks were orally inoculated with a commercial vaccine (CocciVac® B-52) at 3-times the manufacturer's recommendation based on body weight to induce an immune response to live oocysts. Surface responses based on growth performance were generated to determine an ideal combination of dietary nutrients during the grower phase for coccidiosis-infected broilers. High dietary starch (454g/kg) with both low AA (80%) density and oil (23g/kg) content reduced ($P < 0.05$) growth performance, eliciting the lowest weight gain (583g) and highest feed conversion ratio [(FCR), 2.01]. AA supplementation ranging from 93-107% of recommendations (depending on ME level) and low starch:oil (4) was advantageous to growth performance. High oil inclusion (94g/kg) improved ($P < 0.05$) FCR (1.44) and, although feed intake reduced, weight gain was not impaired. The determined optimal combination of dietary starch, AA, and oil can be utilized by the poultry industry to reduce future losses due to coccidiosis infections.

Key Words: broiler, coccidiosis, amino acids, metabolizable energy, performance

P350 The impact of retention time during the conditioning process of a starter diet on broilers grown to 21 days Rachel Strobeck*[1UG], Abigail McConnell[1], Cecilia Broadwater[1], Joseph Gulizia[2], Wilmer Pacheco[2], Kevin Downs[1] *[1]Middle Tennessee State University, [2]Auburn University*

The purpose of this research was to assess the effects of three retention times (RT) during conditioning on broiler performance, nutrient digestibility, and apparent metabolizable energy (AME) from 1 to 21 d. A total of 288 Cobb 500 byproduct males were distributed randomly into 18 battery cages, with 6 replicate cages per treatment and 16 birds per cage. A corn-soybean meal-based diet was mixed using a twin shaft mixer. After mixing, diets were steam conditioned at 82°C with three retention times (40, 80, and 120 s), pelleted through a 4.0-mm pellet die, cooled using a counterflow pellet cooler, and crumbled. Body weight and feed consumption data were collected at 7-d intervals. Feed conversion was corrected for mortality. Replicate fecal samples were collected from each cage on d 20 and 21 for AME determination. At the end of the study, 5 birds per cage (30 birds per treatment) were euthanized and ileal digesta collected. Data were analyzed as a completely randomized design, with battery cage representing the experimental unit, using the GLM procedure of SAS to assess treatment main effects. Means were separated using Tukey's HSD and statistical significance considered at $P \leq 0.05$. Likewise, linear regression analysis was conducted using the REG procedure of SAS. There were no treatment effects for BW on d 7 ($P = 0.169$), 14 ($P = 0.655$), or 21 ($P = 0.391$). Further, d 1 to 7 ($P = 0.469$), 1 to 14 ($P = 0.223$), and 1 to 21 ($P = 0.708$) feed consumption; nor d 1 to 7 ($P = 0.271$), 1 to 14 ($P = 0.347$), and

1 to 21 (P = 0.530) FCR showed treatment differences. However, d 14 to 21 FCR was improved at the highest RT compared to 40 s (1.34 vs. 1.40 g:g; P = 0.026). Conversely, d 1 to 14 FCR displayed a linear relationship approaching significance (P = 0.062), with FCR increasing with increasing RT. Apparent ileal nutrient digestibility and AME data is still being collected and not presented here. Overall, there was little evidence that retention time substantially impacted bird performance during the starter period.

Key Words: Conditioning, Retention time, Broilers

P351 Plasma, digesta, and litter attributes in broiler chickens fed different protein sources in the first 10 days of life Anastasia Tsementzis*[1], Anderson Maina[1], Colin De Cloet[1], David Trott[2], Lee-Anne Huber[1], Elijah Kiarie[1] *[1]University of Guelph, [2]Wallenstein Feed & Supply Ltd.*

Starter diets are often formulated with specialty highly digestible protein feedstuffs (SPF) and/or antibiotic growth promoters (AGP) to bolster early growth and development. However, implications of such approaches on metabolism, intestinal ecology, and litter quality are not well characterized. We evaluated the impact of incorporating select SPF and AGP in the starter and subsequent impact on plasma biochemistry, digesta pH and concentration of short chain fatty acids (SCFA), litter chemical composition, and foot pad lesions in broiler chickens. Day old Ross 708 male chicks were placed in 48 floor pens (35 birds/pen) and allocated to six iso-caloric and nitrogenous starter diets (n=8). The diets were: 1) NC, corn and SBM, 2) PC, NC+ Bacitracin Methylene Disalicylate and Narasin, 3) FSBM, NC+ further processed SBM, 4) SPC, NC+ soy protein concentrate, 5) PM, NC+ pork meal and 6) BSFLM, NC+ black soldier fly larvae meal. Birds transitioned to a common grower (d 11 to 25) and finisher (d 25 to 49). On d 10 and 28, 5 and 2 birds per pen, respectively, were bled for plasma and necropsied to access gizzard, ileum, and ceca digesta. Litter samples were taken between d 46 and 48, and foot pad lesions scored on d 49. Diet was the fixed effect in statistical analyses and LSmeans were separated using Tukey method. On d 10, NC and SPC birds had higher (P=0.01) plasma uric acid (PUA) than BSFLM birds. The PUA was 471, 557, 489, 538, 454 and 404 µmol/L for PC, NC, FSBM, SPC, PM and BSFLM, respectively. Concentration of plasma gamma-glutamyltransferase (GGT) in PC birds was similar to SPF birds but lower (P=0.02) than for NC birds. On d 10, FSBM and PM birds had lower (P=0.01) ileal digesta pH than PC birds, however, ceca digesta pH of PC birds was lower (P=0.02) than for birds fed other diets. Ceca digesta acetic acid in PC birds was lower (P=0.02) than for SPC birds. Litter was drier (P=0.004) for FSBM and PM birds than for NC birds. However, litter for NC birds had lower (P=0.03) ammonia than for PC, FSBM, PM and BSFLM birds. Diets had no (P>0.05) effects on d 28 plasma biochemistry, digesta and foot pad lesion score. In conclusion, metabolism, and intestinal ecology effects of incorporating SPF and AGP in starter were transient.

Key Words: broiler chick, metabolism, intestinal ecology, specialty starter proteins

P352 Growth performance of broilers in response to increasing concentration of multiple mycotoxins in contaminated corn Allison Blomme*[1GS], Khairy Jenkins[2], Kara Dunmire[1], Haley Otott[1], Charles Stark[1], Chad Paulk[1] *[1]Department of Grain Science and Industry, College of Agriculture, Kansas State University, [2]Department of Animal Sciences, College of Agriculture and Environmental Sciences, North Carolina Agricultural and Technical State University*

Mycotoxins in grains are a result of mold or fungal growth from environmental stressors and cause detrimental impacts to poultry production. Thus, the objective of this experiment was to determine the effects of increasing concentration of a combination of mycotoxins on broiler growth performance. It was hypothesized that increasing mycotoxin concentrations would decrease broiler growth performance. A total of 250 one-day old male broilers (Cobb 500; initial BW 41.8g) were used in an 18-d study. Broilers were housed in 3 Petersime batteries with *ad libitum* access to feed and water. Treatments were randomly assigned to 1 of 50 cages within location block, resulting in 10 cages per treatment with 5 broilers per cage balanced by BW. For this experiment, sourced contaminated corn contained 8.2 ppm fumonisin (FUM), 8.0 ppm deoxynivalenol (DON), and 551 ppb zearalenone (ZEA). Dietary treatments consisted of 0%, 25%, 50%, 75%, 100% of the mycotoxin contaminated corn replacing non-contaminated corn. Resulting complete diet mycotoxin concentrations were 1.5 ppm, 1.4 ppm, 2.3 ppm, 2.9 ppm, and 3.9 ppm for FUM; <0.6 ppm, 1.0 ppm, 1.4 ppm, 2.3 ppm, 3.0 ppm for DON; and <51.7 ppb, 94.5 ppb, 180.5 ppm, 294.6 ppb, and 364.1 ppb for ZEA, respectively. Data were analyzed as a completely randomized design with cage as the experimental unit using the GLIMMIX procedure of SAS 9.4 (Cary, NC). Results were considered significant at $P \leq 0.05$. Total body weight gain decreased from 514g to 460g, and total feed intake decreased from 580g to 549g with increasing mycotoxin concentration from the 1.5 ppm FUM, <0.6 ppm DON, <51.7 ppb ZEA diet to the 3.9 ppm FUM, 3.0 ppm DON, 364.1 ppb ZEA diet (linear, P =0.005 and P = 0.047, respectively). The increase in mycotoxin concentration also produced a poorer feed conversion ratio from 1.13 to 1.20 (linear, P = 0.014). In conclusion, increasing concentrations of mycotoxins in broiler feed negatively impact body weight gain, feed intake, and feed conversion even when the mycotoxin levels are below acceptable limits for the industry.

Key Words: Broiler growth, Broiler nutrition, Mycotoxins, Mycotoxin contamination

P353 Hemp Seed Cake increases Fatty Acids, Enhances Gut Health but does not transfer Cannabinoids in Eggs and Tissues of Laying Hens Fausto Solis*[1], Kasula Rajasekhar[1], Byron Shaffer[2], Frank Connett[2], Chris Barrett[2], Rodney Cocker[2], Eric Willinghan[3] *[1]Wenger Animal Nutrient & Technology Innovation Center, The Wenger Group, [2]Kreider Farms, 1461 Lancaster Rd,, [3]Winfield Veterinary Consulting, Inc.,*

Background: Hemp seed and hemp seed products such as Hemp Seed Cake (HSC) have been shown to increase unsaturated fatty acid (FA) profile in eggs, including linoleic acid, and α-linolenic fatty acids known to increase egg weight and better human health respectively. However, the use of hemp products in animal feed is still a concern due to the potential residues of the of Δ-9 tetrahydrocannabinol (THC), a psychoactive substance present in the hemp plant. No significant published research is available on the effect of dietary HSC on fatty acids profile and cannabinoids residues in organs and tissues of laying hens. Objectives: the objectives of this study were to determine the effect of dietary HSC on the level of fatty acid composition, systemic, tissue, organ, gut health and bone mineralization and cannabinoid transfer in eggs of commercial laying hens. Materials and methods: Eight hundred (800) caged Bovans white hens in lay at 30 weeks of age were distributed into 4 treatments of 200 hens per treatment based on inclusion levels of hemp seed cake (HSC) at 0%, 10%, 20% and 30% levels of inclusion. Each treatment group comprised of had 8 cages of 25 hens each that served as per replicates. The observations per protocol were made over a timeline of 16 weeks following that precedes a 3-week acclimation phase. Results: HSC feeding to commercial laying hens increased (P<0.05) the levels of polyunsaturated fatty acids including linoleic and linolenic acids in eggs and abdominal fat. A significant trend of reduction in moisture excretion over the control with HSC feeding was noted. The levels of cannabinoids residues in eggs, blood, breast meat, body fat, liver, kidneys and spleen were below the detectable level. There was not effect on tissues and organ health parameters. Conclusion: The results of this study confirm that HSC fed to laying hens increased deposition of polyunsaturated fatty acids, but did not contribute THC or cannabinoid residues in eggs, internal organs or body tissues.

Key Words: hemp,, HSC, Eggs, Ttetrahydrocannabinol, fatty acids

P354 Energy and nutrient utilization of corn varieties differing in endosperm hardness and dried at two temperature levels, evaluated by two methods in broilers Joaquin Cabanas-Ojeda*, Nicolas Mejia-Abaunza, Paula Lozano-Cruz, Valmiro Lima Aragão Neto, Muhammad Ali, Maria Camila Alfaro-Wisaquillo, Gustavo Quintana-Ospina, Lina Penuela-Sierra, Edgar Oviedo-Rondon *Prestage Department of Poultry Science, North Carolina State University*

Corn variety and drying temperatures (DT) have been reported to affect independently broilers' nutrient digestibility and performance efficiency. This study evaluated the interactive effects of these factors on nutrient digestibility and energy utilization by the total and partial collection methods. Corn of hard and average endosperm hardness (62.83 *vs* 60.59% vitreousness) were planted under similar agronomic conditions. After harvest, each variety was mechanically cleaned and dried at 120 and 35°C. Additionally, twelve samples per corn treatment were ground in a Retsch mill to 1 mm and analyzed with NIRS using PNE calibration curves. Thereafter, 280 one-day-old male Ross 708 broilers were placed in 40 cages, with 7 chickens per cage. All chickens were fed a common standard starter diet in crumbles until 14 days. One basal grower diet was formulated along with four test diets that were produced using the basal substitution methodology by replacing 30% of the basal diet with each corn type. Using this methodology, the mineral and vitamin premix were equal across test and basal diets. Titanium dioxide was used as an indigestible marker to measure digestibility and energy utilization. Total excreta were collected for 72 h after five days of adaptation. Data were analyzed in a 2 x 2 factorial arrangement of treatments with kernel hardness and DT as the main effects in a randomized complete block design. No interaction effects ($P>0.05$) were observed on nutrient utilization. Although no significant effects were detected with the total collection methodology ($P>0.05$), DT effects were detected on the coefficient of apparent digestibility of DM (CADDM), protein digestibility, AME, and AMEn with the partial collection method. A difference of 1.09 and 2.44% in CADDM and protein digestibility were observed in favor of broilers-fed corn dried at high DT ($P<0.001$). This corn also had 41 and 35 kcal more AME and AMEn ($P<0.05$), respectively, than corn dried at 35ºC. The agreement between total and partial collection methods was above 98%. The NIRS results had a correlation coefficient of 0.36 ($P<0.05$) with the *In vivo* methodology. In conclusion, higher CADDM, protein digestibility, AME, and AMEn were obtained with corn dried at 120ºC independently of kernel hardness.

Key Words: Corn hardness, drying temperature, digestibility, broiler

P355 The effects of varying temperatures and times in a hot water bath on trypsin inhibitor activity Erika King*[UG], Victoria Ayres, David Hobbs *Tennessee Tech University*

Feed and feed manufacture are the greatest cost when rearing poultry. These necessities contribute to approximately 60 to 70% of the total cost of production. Soybean meal is rich in high quality protein and is the primary protein source in poultry diets throughout the world. However, soybean meal also contains antinutritional factors such as trypsin inhibitor, which has been shown to impede the activation of gastrointestinal proteolytic enzymes and negatively affect protein digestion. Heat-processing of soybean meal has been shown to reduce trypsin inhibitor. Specific feed manufacture techniques may also denature any remaining trypsin inhibitor complexes and improve dietary protein digestibility. The current study aims to identify optimal feed manufacture techniques to reduce trypsin inhibitor activity found in soybean meal. Soybean meal was obtained from the local co-op (Cookeville, TN). Two-gram samples of soybean meal were then submerged in a hot water bath and subjected to either 70 or 90° C for either 0, 15, 30, 60, 120, 240, 360, or 480s, mimicking practical conditioning temperatures and times found in commercial feed mills. Trypsin inhibition was then calculated using methods described by Liu et al., 2019 and 2021. Each temperature and time combination was repeated in triplicate. Treatments were arranged in a 2 (conditioning temperature) x 8 (conditioning time) factorial. Trypsin units inhibited (TUI)/mg sample decreased when soybean meal was subjected to 90°C, compared to 70°C ($P = 0.0128$). However, TUI/mg sample was comparable when soybean meal was subjected to heat for either 0 or 480 s ($P = 0.0003$). This study demonstrates that TUI/mg sample may be reduced with increased temperatures.

Key Words: Trypsin inhibitor, Conditioning temperature, Conditioning time, Soybean meal

P356 Omega-3 fatty acid-rich fish oil reduced growth performance loss and improved gut barrier integrity during Eimeria infection Yuguo Tompkins*, Hanyi Shi, Venkata Sesha Reddy Choppa, Woo Kyun Kim *University of Georgia*

Coccidiosis caused by *Eimeria spp.* has caused a substantial economic loss in the poultry industry. The objective of the present study was to test the effects of dietary supplementation of n-3 PUFA-enriched fish oil diet on growth performance, intestinal immune response, and bone characteristics in broilers challenged with *Eimeria spp*. A total of 576 14-day-old broilers were randomly distributed according to a completely randomized design in a 3x2 factorial arrangement, including two different diets supplemented with either 5% fish oil or 5% soybean oil; Three *Eimeria* spp. infection levels, including non-challenge control, the low *Eimeria* challenge dose, and the high challenge dose. Daily feed intake, growth performance, and body composition were measured. Gut permeability was measured after 5 days of post-infection (dpi). On 6 and 12 dpi, samples were collected to analyze gut health parameters, bone health parameters, and immune response. The means were subjected to two-way ANOVA, and the main effects (diets and challenge doses) and their interactions were considered. In the case of significant differences, Tukey's test was further conducted to test differences among sample means.

Results showed that *Eimeria* infection significantly decreased growth performance, increased gut lesion, altered intestinal morphology, and caused intestinal lesions. For diet variables, the fish oil supplement did not alter the total antioxidant level in the serum compared with the soybean oil diet but increased feed intake during the early infection stage (0-6 days post infection: DPI). Fish oil diet groups showed a trend of increase in body weight gain compared to the soybean oil groups under the *Eimeria* infection condition. Fish oil supplements significantly reduced gut permeability and reduced the intestinal lesion over the jejunum/ ileum junction compared to soybean oil. In conclusion, the dietary supplements of fish oil benefit gut health and growth performance under *the Eimeria* infection during the early infection stage, and short-term use of fish oil during the early infection stage can be a nutritional strategy to minimize growth performance loss during coccidiosis.

Key Words: Fish oil, Eimeria, coccidiosis, gut integrity, growth performance

P357 Mycotoxin contamination trends in United States corn grain: 2018-2022 Paige Gott*[1], Lan Zheng[1], Chasity Pender[1], Ursula Hofstetter-Schahs[2], April Levy[1] *[1]DSM Nutritional Products, [2]DSM Austria GmbH*

Mycotoxins are secondary fungal metabolites which may affect animal health and productivity. These naturally occurring metabolites are found in various feed ingredients worldwide. Classic signs of mycotoxicosis in poultry include reduced feed intake, diarrhea, as well as oral and intestinal lesions. Assessing clinical signs alone can underestimate the costs of mycotoxin-associated effects such as decreased nutrient digestibility, impaired gut integrity, and altered immune response. The objective of this study was to compare mycotoxin contamination levels of the 2022 corn crop with previous year trends. Samples submitted from the United States were screened via liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for six key mycotoxin groups: aflatoxins (Afla), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone, and ochratoxin A. The 2022 crop year data were compared to the prior four harvest years using GLIMMIX procedure of

SAS with harvest year as fixed effect and sample as the experimental unit with significance reported at $P \leq 0.05$. Inclement weather during spring of 2022 delayed planting in many areas and subsequently 2022 harvest was delayed which resulted in a limited number of samples (n = 44) having been screened by time of abstract submission. Therefore, the contamination profile of the 2022 crop will likely change as the sample pool expands to include more samples originating from the Midwest. Contamination levels in corn for the tested toxin groups have remained consistent over the survey period. Preliminary results indicate that 91% of the samples were contaminated with at least one mycotoxin (vs. 90% in 2021). Aflatoxin occurrence increased in 2022 compared to 2021 and 2020 crops (41 vs. 7 and 5%; $P < 0.05$). Occurrence of FUM was higher compared to 2021 and 2022 (86 vs. 64 and 64%; $P < 0.05$) while B-Trich was numerically lower over the same period (36 vs. 69 and 58%). Initial results of the 2022 US corn survey indicate mycotoxin occurrence and contamination levels are similar to or higher (Afla and FUM) than the prior crop years and justify continued surveillance to monitor mycotoxin risk is poultry and livestock.

Key Words: mycotoxins, corn, trichothecenes, fumonisins, United States

Metabolism and Nutrition: Vitamins and Minerals

P358 Effect of a polyphenol feed supplement used as antioxidant replacement of vitamin E in broiler diets Karsten Kjeldsen*[1], Grete Brunsgaard[1], Klaus Männer[2] *[1]R2 Agro A/S, [2]Freie Universität Berlin*

Polyphenols are secondary metabolites of plants and comprise flavonoids, tannins and pro-anthocyanidins. A basic feature of phenolics is their significant antioxidant activity. The aim of this study conducted at Freie Universität Berlin was to determine the effect of a polyphenol supplement (PS) and vitamin (vit) E *(all-rac-α-tocopheryl acetate)* in different dosage on body weight gain (BWG), Feed conversion ratio (FCR) and the antioxidant status in blood and meat samples of broilers. PS (Cabanin™CSD from R2 Agro) contains parts from citrus, grape, blackcurrant and tannic acid. A total of 525 one-day old Cobb 500 male broiler chicks were randomly assigned to five treatment (T) groups with 7 replicates: T1: 15 ppm vit E in Starter (St) (0-2 wks.) and in grower (Gr) diet (3-5 wks); T2: 105 ppm vit E in St diet and 65 ppm vit E in Gr diet; T3: 60 ppm vit E + 90 ppm PS in St diet and 40 ppm vit E + 50 ppm PS in Gr diet; T4: 15 ppm vit E + 180 ppm PS in St diet and 15 ppm vit E + 100 ppm PS in Gr diet; T5: 105 ppm vit E + 1000 ppm PS in St diet and 65 ppm vit E + 1000 ppm PS in Gr diet. The feed was a basal starter and grower diet based on maize, wheat, and soya beanmeal and was identical for all groups besides the addition of vit E and PS. From d21 to d35, room temperature was 28°C (82°F) to induce mild heat stress. Overall BWG at d35 for T1: 2305g; T2: 2311g; T3: 2356g; T4: 2372g; T5: 2397g ($P \leq 0.05$). Feed intake was unaffected between groups. Overall FCR for T1: 1.44; T2: 1.43; T3: 1.40; T4: 1.39 ($P \leq 0.05$); T5: 1.36 ($P \leq 0.05$). Statistical data were analysed using ANOVA. Statistical difference is compared to T1. At d35 blood and breast meat samples were taken from one average bird from each replicate group for determination of lipid peroxidation (Sigma kit) for measurement of thiobarbituric acid-reactive substances (TBARS). TBARS in blood, nmol/l for T1: 23.0; T2: 20.5; T3: 19.4; T4: 16.8 ($P \leq 0.05$); T5: 17.7 ($P \leq 0.05$). TBARS, nmol/l in refrigerated meat at d7 for T1: 0.43; T2: 0.37; T3: 0.32 ($P \leq 0.05$); T4: 0.33 ($P \leq 0.05$); T5: 0.29 ($P \leq 0.05$). The polyphenolic supplement was able to replace vitamin E as antioxidant by improving broilers FCR and, based on TBARS measurement, were dispersed, retained, and remained functional in blood plasma and in refrigerated meat.

Key Words: Polyphenols, vitamin E, feed conversion ratio, antioxidant activity, TBARS

P359 Effects of the in ovo administration of L-ascorbic acid on the tissue L-ascorbic acid concentrations, plasma nitric oxide and mineral concentrations, and tracheal histomorphology of Ross 708 broilers subjected to elevated levels of atmospheric ammonia Ayoub Mousstaaid[1], Saman Fatemi*[1], April Levy[2], Joseph Purswell[3], Hammed Olanrewaju[3], Brittany Baughman[4], Kaylin McNulty[4], Patrick Gerard[5], Edgar Peebles[1] *[1]Mississippi State University, Department of Poultry Science, [2]DSM, [3]USDA-ARS, [4]Mississippi State University, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, [5]Clemson University, Department of Mathematical Sciences*

Effects of the *in ovo* injection of L-ascorbic acid (L-AA) on tissue L-AA concentrations, plasma nitric oxide and mineral concentrations, and tracheal histomorphology of Ross 708 broilers subjected to elevated atmospheric ammonia (NH_3) levels after hatch were investigated. The four *in ovo* treatments were: non-injected (control), saline-injected (control), or saline containing 12 or 25 mg of L-AA. The *in ovo* treatments were applied at 17 days of incubation by injecting a 100 µL volume of each pre-specified treatment into the amnion. At hatch, 12 male chicks were randomly allocated to each of 12 replicate battery cages belonging to each treatment group. The cages were arranged in a randomized complete block design within a common room. All birds were exposed to 50 ppm of NH_3 through 35 days of posthatch age (doa) and the concentration of NH_3 in the room was recorded every 20 seconds. At 0, 7, 14, 21, and 28 doa, one bird from each cage was randomly selected for determinations of liver and eye L-AA concentrations at 0, 7, 14, 21, 28 doa; plasma nitric oxide concentrations at 0, 14, 21, and 28 doa; and plasma calcium and trace mineral concentrations at 0 and 21 doa. Tracheal histomorphology evaluations were performed at 0, 21, and 28 doa. All posthatch data were analyzed using one-way ANOVA and a repeated measures analysis was used to analyze lesion score data. All treatment differences were considered significant at $P \leq 0.05$. There were no significant treatment differences for plasma nitric oxide and mineral concentrations, as well as liver and eye L-AA concentrations at any of the time periods. However, the *in ovo* injection of either 12 or 25 mg of L-AA significantly (P=0.010) decreased tracheal attenuation incidence at 0 doa in comparison to the non-injected or saline-injected control groups. Furthermore, tracheal inflammation was significantly (P=0.033) reduced at 28 doa in response to the *in ovo* injection of 12 mg of L-AA compared to the non-injected or saline-injected control groups. These results indicate that the *in ovo* injection of L-AA resulted in a decrease in tracheal inflammation when birds were subjected to elevated atmospheric NH_3levels.

Key Words: Inflammation, In ovo injection, L-ascorbic acid, Ammonia, Tracheal histomorphology

P360 Effects of the in ovo administration of the Marek's Disease vaccine alone or in combination with the in ovo and dietary administration of supplemental 25-hydroxyvitamin D3 on the innate and adaptive splenic gene expression of 41-day-old Ross 708 broilers Saman Fatemi*[1], ayoub Mousstaaid[1], April Levy[2], Sabin Poudel[1], Ishab Poudel[1] *[1]Mississippi State University, Department of Poultry Science, [2]DSM Nutritional Products*

Either the dietary or *in ovo* administration of 25-hydroxyvitamin D_3 ($25OHD_3$) promotes the innate and adaptive immune responses of broilers. However, the possible synergistic effects of both the dietary and *in ovo* administration of $25OHD_3$ in conjunction with the Marek's Disease vaccine (MDV) have not been previously reported. The objectives of this study were to determine effects of the MDV alone or in combination with the *in ovo* and dietary administration of $25OHD_3$ on the expression of splenic genes linked to the innate and adaptive immune responses of Ross 708 broilers. Live embryonated hatching eggs were randomly assigned to one of the following 4 *in ovo* injection treatments at 18 d of incubation. 1) Non-injected; 2) MDV alone; or MDV in combination with either 3) 1.2

($25OHD_3$-1.2), or 4) 2.4 ($25OHD_3$-2.4) μg of $25OHD_3$. Hatchlings from each *in ovo* treatment were subsequently assigned to the one of the following posthatch dietary treatments: 1) commercial diet containing 250 IU of vitamin D_3 /kg of feed (control); or 2) commercial diet supplemented with an additional 2,760 IU of $25OHD_3$ /kg of feed (Hy-D diet). At d 40, whole spleen samples were collected from 1 bird per pen for determination of the expression of genes linked to innate (TLR-3, TLR-4, TLR-7, and TLR-21) and adaptive (INF-α, INF-β, INF-γ) immunity. A randomized complete block experimental design was used, and all data were analyzed using two-way ANOVA. Treatment differences were deemed significant at $P \leq 0.05$. A significant dietary x *in ovo* treatment interaction was observed for INF-α, in which the expression of INF-α was up-regulated ($P = 0.03$) in response to $25OHD_3$-1.2 *in ovo* treatment in the Hy-D diet, but there was no significant difference between the *in ovo* injection treatments in the control diet. Furthermore, the expression of INF-β was greater in birds that received 1.2 μg of $25OHD_3$ ($P = 0.03$) in comparison to all other treatments. It is well documented that both INF-α and INF-β promote the production of viral antibodies. In conclusion, the dietary and *in ovo* administration of $25OHD_3$ may be involved in the stimulation of the local humoral immune response of broilers, which could be related to activation of the INF-α and INF-β genes in the spleen.

Key Words: 25-hydroxyvitamin D3, Broiler, In ovo administration, Immunity, Marek's Disease vaccine

P361 Evaluation of transgenic Chlamydomonas reinhardtii for carotenoid and phytase production Alessandro Rocchi*[1UG], Beth Ahner[2], Mohammed Yazdani[2], Indira Tjorkorda[2], Walter Bottje[1] *[1]Department of Poultry Science, University of Arkansas, [2]Department of Biological and Environmental Engineering, Cornell University*

Chlamydomonas reinhardtii is a well-studied model algal species used in biological engineering and has been transformed to increase carotenoids and phytase expression. Carotenoids are a precursor to the antioxidant vitamin A. The phosphorous in corn and soybeans is predominantly found in phytic acid that requires phytase as a feed additive since phytase is not found in the g.i. tracts in poultry. In the present study, two genes, *AppA2* and *ORANGE* (for phytase and carotenoid expression, respectively) were transfected into a single strain of *C. reinhardtii.* To accomplish this, an existing transplastomic strain of *C. reinhardtii*, with increased phytase production (*AppA2*), underwent a secondary nuclear transformation for increased carotenoid production using glass beads for a mechanistic transformation. Transformed algae were then plated onto Tris-acetate-phosphate (TAP) agar plates containing hygromycin for single colony isolation. The transformants were then grown in liquid TAP media and evaluated for carotenoid content and response to light stress. From the results, transformants 3-6, (labeled as POr3-6), were shown to have carotenoid levels similar ($P > 0.05$) to that in the *ORANGE* gene transformed *C. reinhardtii* (OrHis19) and higher ($P < 0.05$) carotenoid levels than that of the cell wall mutant (CwM) and phytase producing strain (PBM2F2 - *AppA2* gene only). Levels of carotenoids (mg/g dry weight) were between 1.29 and 1.96 for the CwM, PBM2F2, and OrHis19 strains and ranged between 1.75 and 2.02 mg for the Or transformants (POr3-6). Further studies are needed to determine if the second transformation affected phytase production. This research was part of an undergraduate internship program awarded to A. Rocchi by Cornell University and USDA NIFA SAS # 2019-69012-29905.

Key Words: microalgae, carotenoids, phytase, feed additives

P362 Effect of in-feed inclusion of monovalent copper on performance, intestinal lesion score, and oocyst count in coccidiosis challenged broiler chickens Nasima Akter*[1], Thi Dao[1], Afsana Jahan[1], Alip Kumar[1], Shubiao Wu[1], Sukirno Sukirno[1], David Cadogan[2], Alessandra Monteiro[3], Ian Cockshott[3], Amy Moss[1] *[1]University of New England, [2]Feedworks Pty. Ltd., [3]Animine*

Avian coccidiosis is caused by several species of protozoa *Eimeria,* that damage intestinal tissue, resulting in decreased production and suppressed immune responses. Nutrition-based strategies have been implemented to control avian coccidiosis and copper (Cu) has been explored as a potential candidate, because of its antimicrobial properties. Thus, the aim of this experiment was to determine if the in-feed supplementation of monovalent copper (100 ppm of Cu) can assist broilers to lessen the severity of coccidiosis challenge, in comparison to a negative control (unchallenged, NC) and positive control (challenged, PC) group, offered standard industry wheat-soybean meal-based diets. A total of 216, day-old Ross 308 males (six replicates, 12 birds/pen) were allocated to one of the three dietary treatments. Birds were offered starter (d 1 – 10), grower (d 10 – 21) and finisher (d 21 – 35) diets. Birds in the challenge treatments were dosed with *E. maxima* and *E. acervulina* (Eimeria Pty.) oocysts in 1 mL sterile phosphate-buffered saline (PBS), while un-challenged birds were dosed with 1 mL PBS on d 14. Birds had unlimited access to feed and water in an environmentally controlled facility. Fecal collection was performed daily from d 17 to 28 for coccidial oocyst counts. Feed intake, weight gain and FCR were calculated for each dietary phase. Four birds/pen were slaughtered on d 21 to assess intestinal lesion scores. There was no significant effect of the treatments on performance in grower and finisher phases. Intestinal lesion scores of duodenum (2.8 vs 1.9; $p>0.05$), jejunum (0.88 vs 0.83; $p>0.05$) and ileum (0.83 vs 2.54; $p>0.05$) of Cu treated group were similar to that of positive control group on d 21. The challenge had the greatest impact on fecal oocyst count on d 21: *E. maxima* oocyst count (300 vs 650; $p=0.017$) was significantly reduced in birds fed Cu compared to the PC group on d 21. A similar pattern was seen for *E. acervulina* oocysts count (3650 vs 11466; $p=0.089$) on d 21. Though it was not statistically significant, the number of fecal *E. acervulina* oocyst was numerically lower. Overall, the Cu supplementation in broiler feed had potential to reduce the number of fecal *E. maxima* and *E. acervulina* oocysts in Eimeria infected birds.

Key Words: monovalent copper, Eimeria, oocyst, broiler, lesion score

P363 Evaluation of the stability of Vitamin A acetate concentrates mixed in a vitamin-trace mineral premix over a 56-day high temperature and humidity storage stress Rosana Hirai*[1], Daniel De Leon[1], Macey Randig-Biar[1], Austin Silva[1], Ervey Sanchez[1], Audrey McElroy[1], Christopher Bailey[1], Nicolas Martinez[2], Adebayo Sokale[2], Lawrie Music[2] *[1]Department of Poultry Science, Texas A&M University, [2]BASF Corporation*

Literature on Vitamin A stability in vitamin-trace mineral premixes is limited. The goal of this project was to determine the stability of Lutavit® A 1000 NXT (A-1000), a vitamin (Vit) A acetate product stabilized with butylated hydroxy toluene (BHT) as antioxidant. This product was tested against 3 others commercial Vit A sources currently from the market. According to material safety data sheets, Sources C and D were stabilized with ethoxyquin, while Source B used BHT as the stabilizing antioxidant. The four Vit A sources were blended into a Vit-trace mineral premix, completely devoid of Vit A, designed to put oxidation stress on the Vit A beadlets. For storage treatment, 2 kg of each of the 4 test premixes plus a negative control (NC), not containing any Vit A concentrate, were placed in closed paper sacks, and stored at two different conditions: Low Stress (LS) storage at 4°C or Heat Stress (HS) at 35°C and 60% Relative Humidity (RH). Temperature and humidity were monitored from 0-56 days of incubation. Premix samples were collected and assayed for Vit A content at 0, 28, and 56 days of storage. The experimental designed used was a 2×4 factorial with main effects of temperature and Vit A sources. The NC diet was treated separately as it was devoid of Vit A. Means were separated by Duncan's test and P-value was set as ≤0.05. Based on the analytical results, all 4 premixes had

a Vit A content of ~4,000 KIU/kg at d0 (P=0.353). Analysis LS premixes indicated they were stable at d56 except for Source D (P=0.000). However, the HS premixes were not stable when analyzed at d56 (P=0.000). Heat-stressed premix with Source A-1000 retained the most Vit A content (2,447 KIU/kg) at d56 compared to the other three Vit A sources. In conclusion, A-1000 had better stability than Sources C and D of Vit A over a 56-day high temperature and humidity storage stress, and there was no significant difference between A-1000 and Source B.

Key Words: Vitamin A acetate, stability, heat stress, butylated hydroxy toluene, vitamin-trace mineral premix

P364 Effect of different levels calcium and phosphorus and their interaction with bone darkening on refrigerated and frozen storage cooked chicken leg quarters. Luis Guzman*[GS], Amit Morey, Laura Garner, Charles Herron, Aftab Siddique, Katherine Sierra, Bet Wu, Micah Black *Auburn University*

Frozen bone-in leg quarters of US-grown broilers are exported worldwide but are subject to complaints associated with darkening of the bones (tibia and femur) after cooking. The purpose of this study was to evaluate the effect of dietary phosphorus (P) and calcium (Ca) on discoloration of tibia and femur of frozen and fresh chicken leg quarters after cooking. Broilers (n=144, 7-wk 7-8 lbs live wt) were fed with 12 diets containing different ratios of P and Ca respectively (P:Ca ratio: Starter 0.55 to 0.56, Grower 0.45 to 0.56, Finisher 0.38 to 0.56, Withdrawal 0.32 to 0.56). Slaughtered broilers were deboned 12-14 hours post-slaughter and the whole, bone-in leg quarters (n=2/broiler) were divided into two groups (right and left quarter). The right leg quarters (n=144) were placed under refrigeration (4°C) for 16 h while the left quarters (n=144) were frozen at -18°C for 14 days and thawed for two days. Leg quarters were cooked in a convec-tion oven until the internal temperature reached 165°F. After cooking, the quarters were cooled, and the bone was separated apart and analyzed for color LAB color space (lightness (L*), redness (a*), and yellowness (b*)). Data was analyzed using SAS Studio® statistical software with Tukey HSD with significant differences in the means at P<0.05. Dietary treat-ments did not affect (P>0.05) the lightness value of tibia either among the fresh or frozen samples. However, significant color (L* a* b*) differences (P<0.05) were observed between fresh and frozen bones from different dietary treatments. Redness (a*) value was more present in the frozen condition for the femur meanwhile the yellowness (b*) value in the fresh condition for the tibia.

Key Words: bone, chicken, color, quality, storage

P365 Evaluation of boron supplementation to a semi-purified diet fed to broiler chicks on growth performance, tibia mineral composition, and apparent total tract digestibility of nutrients. Tyler Chevalier*, Sunday Adedokun, Merlin Lindemann *University of Kentucky*

The objective of this study was to evaluate the effects of boron (B) supplementation on the performance, tibia mineral composition, and apparent total tract digestibility (ATTD) of nutrients in 22-day-old broiler chickens. The experiment used 448 day-old male by-product Cobb chicks in a randomized complete block design with 7 treatments consisting of 8 replicates of 8 birds per cage. The 7 dietary treatments consisted of a semi-purified basal diet with graded levels of added B (0, 1, 2, 5, 10, 25, 50 mg B/kg of diet) from sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 11.34% B) fed for 22 days. Three birds/cage were euthanized on d 22 to determine tibia bone breaking strength (BBS) and mineral composition. Data were analyzed using the GLM procedure of SAS with the cage as the experimental unit and the model containing terms for treatment and replicate. Orthogonal polynomial contrasts were performed to evaluate the linear and quadratic effects of increasing levels of dietary B. Additionally, a pre-planned single degree of freedom comparison between the control (0 ppm B) and B treatments (1, 2, 5, 10, 25, and 50 ppm B) was conducted. No effects ($P > 0.05$) of increasing B supplementation were observed for BW, ADG, ADFI, FCR, or ATTD of N, energy, or P. Calcium ATTD tended to decrease linearly ($P = 0.09$) with increasing dietary B levels. However, although not significant, tibia BBS numerically increased when B was supplemented at levels ≥ 5 ppm B. Additionally, there was a tendency for a linear increase in tibia ash percentage ($P = 0.09$) with increasing B supplementation and, as B supplementation increased, tibia Mg content had a tendency for a quadratic response ($P = 0.08$). Furthermore, birds fed diets supplemented with B had a greater tibia Mg concentration ($P < 0.01$) compared to birds fed the control diet. In conclusion, increasing dietary B supplementation to a semi-purified diet resulted in no adverse effects. The inverse effects of B supplementation on selected mineral ATTD observed while bone measures improved suggest that B supplementation may al-ter the efficiency of Ca and Mg utilization. However, further research is needed to better understand the relationship between B, Ca, and Mg.

Key Words: Boron, Broilers, bone, minerals

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