2021 INTERNATIONAL POULTRY SCIENTIFIC FORUM

ABSTRACTS

January 25-26, 2021

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Table of Contents

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Dendy Keynote Lecture

KEYNOTE, Impact of COVID-19 in the broiler chicken industry Ashley Peterson* *National Chicken Council*

COVID-19 changed everything, everywhere and has impacted all of us in different ways. From masks and social distancing to home offices and virtual meetings, 2020 has certainly been a challenging year. We saw cleaners and disinfectants disappear from grocery shelves overnight. Paper products were scarce and when available, they were rationed. The food supply chain was also dramatically impacted as dining out options were limited, travel virtually came to a halt, and eating three meals a day at home became the norm. With the increase in preparing meals at home, grocery sales soared, and the supply chain struggled to keep up with demand. Amidst the ever-changing situation, the chicken industry relied on their essential frontline workers to restock grocery shelves and ensure that we all had safe and nutritious chicken. The chicken industry adjusted quickly by retrofitting production lines to help with social distancing, providing employees with PPE and temperature screenings, enhancing sanitation procedures, and many other measures to help keep employees safe while they were working to ensure that meat cases were stocked with chicken. While most of these changes were implemented in early Spring 2020, the industry has continued to provide a safe environment for essential frontline workers and to produce safe and wholesome chicken and chicken products. Though 2020 has presented many new challenges opportunities, the industry has and will continue to provide the number one protein of choice – chicken.

***Author presenting paper**

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Physiology, Endocrinology and Reproduction: Broilers

M1 Effect of Wooden Breast severity on myogenic regulatory factor and cytokine protein expression in 25- and 43- day-old broiler chickens Joshua Flees*GS, Katherine Meloche, Jessica Starkey *Auburn University Department of Poultry Science*

Previous research aimed at elucidating the etiology of Wooden Breast (WB) revealed increased collagen and macrophage infiltration as well as altered densities of muscle satellite (stem; SC) cell populations with different myogenic regulatory factor (MRF) expression profiles in breast muscles of severely affected broilers. The objective of the study was to use quantitative, multiplex, fluorescent Western blotting to determine the effect of WB severity on the protein expression of 3 MRF (myogenic differentiation factor 1; MyoD, paired box 7; Pax7, and myogenin) and 3 cytokines (interleukin-1β; IL-1β, interleukin-6; IL6, and interleukin-10; IL-10). Muscle (*Pectoralis major)* samples from birds with varying WB severity scores (0 = normal; 1 = mild; 2 = severe) were collected at 25 and 43 d post-hatch (n = 5 samples per score per age). Target proteins and total protein were quantified using Image Quant TL 8.1 software. Target proteins were first normalized to total protein in each lane and then set relative to WB score 0. Data were analyzed as a one-way ANOVA and least square means were separated using SAS (V9.4) PROC GLIMMIX and PDIFF for multiple means comparisons at $P < 0.05$. On d 25, MyoD protein expression was similar ($P = 0.7803$) and Pax7 protein was more abundant in WB-affected (score 1 and 2) birds compared with normal ($P = 0.0025$). Myogenin protein expression was greater in severe d 25 birds compared to mild ($P = 0.0073$). On d 43, MyoD protein abundance was decreased in WB-affected muscle compared with normal ($P = 0.0017$). However, Pax7 and myogenin expression were unaltered by WB ($P = 0.8392$). At d 25, IL-6 ($P = 0.0009$) and IL-10 ($P = 0.0063$) protein abundance was decreased in WB-affected compared with normal muscle, while IL-1β was unaffected ($P = 0.1071$). Yet, on d 43, expression of IL-1β, IL-6, and IL-10 was unaltered in WB-affected birds compared with normal ($P \geq 0.7064$). In conclusion, alterations in the expression of MRF suggests that muscle SC proliferation and differentiation capacities may be impacted in WB-affected broilers. In alignment with previous reports of increased macrophage density at d 25, cytokine expression was also altered in WB-affected birds.

Key Words: Wooden Breast, myogenic regulatory factor, cytokine, Woody Breast, broiler chicken

M2 Effect of Wooden Breast severity on autophagy pathway protein expression in 25- and 43-day-old broiler chickens Caroline Gregg*GS, Joshua Flees, Katherine Meloche, Jessica Starkey *Auburn University Department of Poultry Science*

The underlying cellular and molecular mechanisms leading to the development of the Wooden Breast (WB) myopathy in broilers have not yet been fully elucidated. Autophagy is necessary for the degradation and recycling of cellular components but it has yet, to our knowledge, to be studied in WB affected muscle tissue. The objective of this study was to use quantitative, multiplex fluorescent Western blotting to determine the effect of WB severity on the expression of 5 autophagy pathway proteins (Beclin1, autophagy related 5; ATG5, ubiquitin-binding protein; p62, lysosome-associated membrane protein 2; LAMP2, and microtubule-associated proteins 1A/1B light chain 3B; LC3B). Samples from the *Pectoralis major* muscle of broilers with varying WB severity scores (0 = normal; 1 = mild; 2 = severe) were collected at 25 and 43 d post-hatch (n = 5 samples per score per age). Target proteins and total protein were quantified using Image Quant TL 8.1 software. Target proteins were first normalized to total protein in each lane and then set relative to WB score 0. Data were analyzed as a one-way ANOVA and least square means were separated using SAS (V9.4) PROC GLIMMIX and PDIFF for multiple means comparisons at $P < 0.05$. On d 25, the expression of ATG5 tended to be lower in birds with mild and severe WB compared with normal birds ($P = 0.0640$). The protein expression of p62 on d 25 was decreased in severe compared to normal and mildly affected birds ($P = 0.0438$). Expression of LAMP2 protein was decreased in WB-affected compared with normal at d 25 ($P = 0.0026$). On d 43, Beclin1 protein was more abundant in WB-affected birds compared with normal ($P \geq 0.2380$). However, expression of ATG5, p62, LAMP2, and LC3B were unaltered by WB in breast muscle from 43-d-old broilers ($P = 0.0159$). Overall, expression of autophagy proteins from birds at 25 and 43 d-of-age was altered by WB. These results suggest that the degradation and recycling of cellular components may be disrupted in WB-affected compared with normal breast muscle. Further investigation of protein expression in various pathways is warranted to better understand the etiology of WB.

Key Words: Wooden Breast, Woody Breast, autophagy, protein expression, broiler chicken

M3 Effect of dietary 25OHD3 supplementation on broiler chicken ileal IL-10 and IL-17 protein expression Gerardo Abascal-Ponciano*GS, Samuel Leiva, Jessica Starkey, Charles Starkey *Auburn University Poultry Science Department*

Vitamin D signaling is important for intestinal homeostasis. Increased presence of vitamin D receptor in immune cells modulates cell phenotype and secretion of cytokines. Cytokines regulate inflammatory responses triggered by external stimuli. Interleukins are a large family of cytokines secreted by cells in the intestine and can modulate both pro-inflammatory (Interleukin 17; IL-17) and anti-inflammatory (Interleukin 10; IL-10) responses. The objective of the study was to determine the impact of dietary supplementation with 25-hydroxycholecalciferol (25OHD3) on the local immune response over time in broilers. A randomized complete block design experiment was conducted to evaluate the effect of post-hatch dietary 25OHD3 inclusion on IL-17 and IL-10 protein expression in ileum of broiler chickens from 3 to 21 d post-hatch. On d of hatch, male chicks (n = 480) were randomly assigned to raised floor pens. The experimental corn-soybean meal based treatments were: 1) common starter diet containing 5,000 IU of D3 per kg of feed (D3) and 2) common starter diet containing 2,240 IU of D3 + 2,760 IU of 25OHD3 per kg of feed (25OHD3) fed from d 0 to 21. From d 0 to 7, birds were housed with 4 birds per pen (0.05 m^2 per bird) and individually (0.21 m^2 per bird) from d 8 to 21. On d 3, 6, 9, 12, 15, 18, and 21, 12 birds from each treatment were euthanized and ileum tissue samples were collected and analyzed using quantitative, multiplex, fluorescent Western blotting. Target proteins were quantified using Image Quant TL 8.1 and expressed relative to total protein in each lane. Data were analyzed for ANOVA with SAS (V9.4) PROC GLIMMIX and least square means were separated with PDIFF at $P \leq 0.05$. Feeding 25OHD3 post-hatch decreased ileal IL-10 (anti-inflammatory) protein expression in 21 d-old broilers compared with D3 only ($P = 0.0190$). Broilers fed only D3 post-hatch had greater IL-17 (pro-inflammatory) protein expression in the ileum at 18 and 21 d-of-age ($P = 0.0412$) than those

fed 25OHD3. Dietary inclusion of 25OHD3 altered the expression of key inflammatory cytokines in the ileum of young broilers.

Key Words: 25-hydroxycholecalciferol, cytokine, interleukin, intestine, inflammatory response

M4 Optimization of electroporation mediated gene transfer for primary chicken cell cultures Gautham Kolluri[*1,2GS], Iqbal Hyder[1], Shahin Eghbalsaied[3], Avishek Biswas[2], Wilfried Kues[1] *[1]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, [2]ICAR-Central Avian Research Institute, [3]Islamic Azad University*

Electroporation otherwise called *electropermeabilization* is the non-viral mediated physical process of gene delivery and involves the application of external electric shock with defined strength, time, and frequency. Though, electroporation is commonly applied in mammalian cell culture experiments its use in chicken experiments is least defined. In chickens, electroporation is limited to in ovo application in embryos with specially designed electrodes. We have previously optimized the electroporation based transfection protocol for bovine and murine primary chicken cells. Here, we have attempted to validate the electro-transfection protocol for primary chicken embryo fibroblasts and fibroblast-derived cell lines (DF-1) using square wave bulk pulsing technology with pT2 Venus as a reporter gene. Electrotransfection efficiency as influenced by electroporation medium and pulsing conditions were assessed by cell viability, cell transfection rate, and Western blotting analysis of reporter protein. Dulbecco's modified eagles medium (DMEM) advanced with low serum content had a higher transfection rate (81.61%) in DF-1 and CEF cells followed by Opti-MEM Reduced Serum Medium without GlutaMAX, Opti-MEM supplemented with GlutaMAX and Neon E buffer. However, cells polarized in Neon E buffer have witnessed significantly higher deaths ($p<0.01$). DMEM knock out buffer induced a comparatively higher (59%) transfection rate than Neon E2 buffer but compromised cell viability. The lowest transfection and viable rate were observed with D10 medium and buffered saline in both DF1 and CEF cells. Pulsing strength of 300 v for 10 ms pulse and 10 s interval in Opti-MEM with GlutaMax was found to be the most efficient ($p<0.01$) with a higher transfection rate (88.4%) with maximum viability (53.8%) compared to 350 v at similar pulse conditions. The protein expression of the reporter gene (cargo size of 6.3 kbp) was also confirmed in both chicken cell cultures. These results suggest that electroporation can be used efficiently as a non-viral delivery in primary embryo fibroblasts and cell lines involving the studies related to loss and gain of gene function and CRISPR/Cas9 experiments.

Key Words: Electroporation medium, CEF, DF-1, cell viability, cell transfection rate

M5 Early post-hatch growth and development of broilers may be supported by reduced insulin-like growth factor binding protein expression in liver and muscle Lauren Vaccaro[*GS], Abigail Wilson, Emmaline England, Kyle Herring, Laura Ellestad *University of Georgia*

Vertebrate growth is regulated, in part, by insulin-like growth factor (IGF) 1 and 2 activation of their receptor, which is modulated by IGF binding proteins (IGFBPs). This study's objective was to examine gene expression of the IGF system in commercial broilers throughout the latter half of embryogenesis and early post-hatch. Breast muscle (*Pectoralis major)* and liver tissue were collected from males at embryonic day (e) 12, e14, e16, e18, e20, and post-hatch day (d) 0, d1, d3, d5, d7, d10, d14, and d21. Total RNA was extracted from 6 birds at each age (n=6). Expression of mRNA was determined by reverse-transcription real-time quantitative PCR, and target mRNA levels were normalized to 18S rRNA levels. Data were analyzed by one-way ANOVA, and *post hoc* means comparisons were made with the test of least significant difference when ANOVA indicated significance ($P\leq0.05$). Age-induced differences in expression were observed in both breast muscle and liver. *IGF1* in muscle decreased between e12 and e20, remained low through d1, increased again on d3, and remained high until d10 ($P\leq0.05$). Expression of *IGFBP2, IGFBP4,* and *IGFBP5* in muscle was higher during embryonic development and diminished after hatch ($P\leq0.05$). In liver, *IGF1* and *IGFBP4* expression was low during embryonic development and drastically increased after d3 ($P\leq0.05$). Liver expression of *IGFBP1*, *IGFBP3*, and *IGFBP5* was lowest just after hatch, between d0 and d3, but then began increasing to levels comparable to those observed during embryonic development ($P\leq0.05$). Levels of *IGFBP2*, on the other hand, steadily increased between e12 and d0, then decreased and remained low through d21 ($P\leq0.01$). The decrease in *IGFBP* expression in muscle after hatch could serve to enhance its sensitivity to endocrine or paracrine IGF signaling, permitting increased post-hatch growth of this tissue. The liver is the major producer of IGFBPs in circulation, which can prevent IGFR activation by sequestering IGFs. Thus, depressed hepatic expression of *IGFBPs* shortly after hatch suggests greater endocrine signaling in target tissues during this time. Reduced *IGFBP* expression in the two tissues between d0 and d3 suggests that enhanced IGF signaling could be necessary to induce early post-hatch growth and development in broilers.

Key Words: gene expression, embryonic growth, juvenile growth, insulin-like growth factors, binding proteins

Processing and Products

M6 Examining the use of Sodium Formate Salts to Reduce Salmonella Enteritidis Persistence in Broiler Litter Aidan Talorico[*GS], James Krehling, Kaicie Chasteen, Luis Munoz, Matthew Bailey, Dianna Bourassa, Kenneth Macklin *Auburn University*

Shedding nontyphoidal Salmonella in the feces can cause contamination of litter which can act as a reservoir. This study aims to examine the effects of sodium formate salts (SF) and sodium bisulfate (SB) on Salmonella Enteritidis (SE) persistence in broiler litter by using litter grab (LG) and boot cover (BC) sampling methods. Commercially sourced chicks were randomly spread out into 24 pens (25 birds/pen) and placed on litter that was seeded with a nalidixic acid resistant strain of SE from a previous seeder flock. Chickens were fed a standard starter, grower, and finisher diet and the flock was terminated at 45 days. Eight pens were assigned to SF, SB and control (C) treatment groups and they were applied the day before flock placement. The SF treatment was applied at 40 gallons/1000ft2 and SB was applied at 100lbs/1000ft2. LG sampling was performed at 4 & 7 days before flock termination, the day of termination, and 4 & 7 days after termination. Litter samples were performed by taking a handful of litter from under the feeder, under the water line, and between the two. BC samples were taken the day before and after flock termination and were performed by covering roughly 75% of the surface area of the pens. Collected LG samples were diluted, plated for enumeration and both sample methods were enriched in tetrathionate broth. Bacterial counts were log10 transformed prior to statistical analysis and both methods were assessed on the number of SE positive samples detected after enrichment. Statistical differences were determined using GLM with significance determined when $p < 0.05$, means were separated using Tukey's HSD. No significant differences ($P=0.086$) were observed between groups in LG bacterial counts, although a numerical difference was observed between the SF (0.05logcfu/g), C (0.20log cfu/g) and SB groups (0.35log cfu/g). For LG, the SF group had 2/40 positive which was significantly ($P<0.05$) lower than the SB group (14/40) with C (6/40) being intermediate. There were

no differences between any of the three in BC sampling . The SF treatment showed evidence of reducing SE compared to SB, although SF did not outperform the C group, there is a potential for use as an antimicrobial.

Key Words: Salmonella, Litter Treatments, Litter Sampling, Broilers

M7 Quality parameters of pet treats generated from chicken processing co-products Marc Presume*[1GS], Rigo Soler[1], Jorge Sandoval[1], Luis Avila[1], Moses Chilenje[1], Catherine Odom[1], Laura Garner[1], Amit Morey[1], Robert Mason[2], Eric Altom[2], Charles Starkey[1] *[1]Auburn University Poultry Science Department, [2]Balchem Animal Nutrition and Health*

Pet treat production in the United States has increased in the last few decades. This trend has influenced co-product markets since pet food manufacturers are looking for new and inexpensive ways to provide protein to pets. Broiler processing co-products such as liver and heart are excellent sources of protein but may be difficult to utilize due to high moisture and adverse textural characteristics. The objective of this experiment was to determine the characteristics of pet treats generated from chicken liver (CL) and chicken heart (CH) with sodium alginate and calcium lactate (ALGIN) as a structure forming component. Initially, CL and CH were ground and mixed to achieve the following 3 ratios: 25% CL:75% CH, 50% CL:50% CH, and 75% CL:25% CH. Subsamples of the 3 CL:CH combinations were then mixed with 2 dosages of ALGIN (0.5% of sodium alginate + 0.425% calcium lactate, and 1% of sodium alginate + 0.85% calcium lactate) to produce final batches of 6 treatments. Jerky slices (20-mm thick) were wrapped and refrigerated at 3°C for 48 h. Forty samples (25.4 mm × 63.5 mm) were dehydrated at 93°C for 2.5 h for cooking loss analysis. Ten raw samples of 25.4 mm × 25.4 mm were used for expressible moisture measurement, and 10 additional raw samples were used for pH measurement. Finally, water activity was assessed on 10 raw and 10 dehydrated samples. Data were analyzed for ANOVA using SAS PROC GLIMMIX and least square means were separated at $P \leq 0.05$. Cooking loss percentage decreased with increasing ALGIN dosage ($P < 0.0001$) but increased as CL proportions increased ($P < 0.0001$). Water activity of raw samples was not affected by ALGIN dosage ($P = 0.9174$), nor by CL and CH combinations ($P = 0.2845$). Water activity of dehydrated treats decreased with increasing CL proportions ($P < 0.0001$) but increased as ALGIN inclusion increased ($P < 0.0001$). Raw treat pH was not affected by ALGIN dosage ($P = 0.4942$), nor by CL and CH combinations ($P = 0.2703$). Expressible moisture of pet treats increased with increasing CL proportions ($P < 0.0001$) but decreased as ALGIN dosage increased ($P < 0.0001$). CL and CH proportions interacted positively with ALGIN inclusions thus demonstrating that these lower value organ co-products can be utilized to produce acceptable pet treat products.

Key Words: alginate, pet food, chicken processing co-products, liver, heart

M8 Effect of location and time on microbial load in a poultry processing wastewater stream Rachel Osborne*[UG], Amrit Pal, Ally Jackson, Montana Riggs, Matthew Bailey, Dianna Bourassa *Auburn University*

Poultry processing results in high outputs of wastewater containing organic materials that may be useful for controlled environment agriculture such as hydroponic systems. However, there is currently little data on the prevalence of pathogens in poultry processing wastewater (PPW) and more information regarding food safety implications of utilizing PPW is needed. This study aimed to determine the microbial load and presence or absence of *Salmonella*, *Campylobacter*, *E. coli*, and total coliforms at different locations and times within the PPW stream. On three continuous weeks, 100-mL raw wastewater samples (n=9/week; n = 27) were collected from a commercial poultry processing facility from three locations: post-screen, post-dissolved air flotation (DAF), and final effluent. Each week, one sample was collected at each location during sanitation, shift change, and post-chiller dump. *E. coli* and ISO total coliform counts were obtained for each sample and data were log transformed (Log_{10} CFU/mL). Water samples were enriched and analyzed utilizing the 3M MDS™ for prevalence of *Salmonella* and *Campylobacter*. Isolation was performed on samples indicated as positive. Eight samples were positive for *Salmonella* (4 isolates recovered, 4 viable but non-culturable) and 10 samples were positive for *Campylobacter* (all viable but non-culturable). Log count data were analyzed by PROC GLM of SAS using Tukey HSD for mean separation and prevalence data by PROC FREQ of SAS using Fisher's Exact Test. Levels of *E. coli* averaged 0.87 $\log_{10}$ CFU/mL and were not impacted by sampling location or time. Coliform levels were not impacted by sampling time, however, the final effluent had higher coliforms (4.12) than post-DAF (2.56) and post-screen (2.75, P=0.0415). Neither sample location nor time influenced *Salmonella* (30%) or *E. coli* (74%) prevalence. Post-screen *Campylobacter* prevalence (67%) was higher than final effluent (11%) with post-DAF intermediate (33%, P=0.0424). Samples taken post-chiller dump had a lower prevalence of coliforms (44%) than shift-change or post sanitation (100%, P=0.0047). These findings provide insight into necessary considerations for how PPW may best be repurposed in controlled environment agriculture applications in a manner compliant with food safety protocols.

Key Words: Salmonella, Campylobacter, coliforms, E. coli, poultry wastewater

M9 Prevalence of Salmonella, Campylobacter, and spoilage bacteria on broiler meat at different stages of commercial poultry processing Hudson Thames*[GS], Courtney Fancher, Mary Gates Colvin, Mika McAnally, Emily Tucker, Nikhil Nuthalapati, Li Zhang, Aaron Kiess, Thu Dinh, Anuraj Sukumaran *Mississippi State University*

Commercial poultry processing plants employ a series of antimicrobial applications to reduce pathogenic and spoilage bacteria on meat. The objective of this study was to determine the prevalence of *Salmonella* and *Campylobacter*, and the counts of spoilage bacteria at various stages of processing, in three commercial plants that employed different concentrations of peracetic acid (PAA). Post-pick, pre-chill, and post-chill carcass buffered peptone water (BPW) rinses and drumsticks were collected from three plants (same processor) and mechanically deboned meat (MDM; 25 g/sample) from two plants out of the three. Each plant was sampled three times and 10 of each sample type were collected each time. The drumsticks and MDM were homogenized with 225 mL of BPW. All rinsates were serially diluted in BPW and plated on to plate count agar, 3M™ Petrifilm™, MRS agar, and cetrimide agar to determine the counts of total aerobic bacteria, coliforms, lactic acid bacteria (LAB), and *Pseudomonas* spp., respectively. To isolate *Salmonella*, samples were pre-enriched in BPW, enriched in TT broth, and streaked on XLT4 agar. For *Campylobacter*, samples were enriched in 2X blood free Bolton's broth and streaked on Campy-Cefex agar. Data were analyzed by the GLIMMIX procedure of SAS 9.4 at a significance level of 0.05. For all the bacteria, there was a greater prevalence on post-pick and pre-chill samples, than on post-chill and drumstick samples ($P<0.001$). From pre-chill to post-chill, *Salmonella* and *Campylobacter* prevalence was decreased by 98.9 and 93.3%, respectively; while counts of APC, coliforms, and LAB were reduced by 3.7, 3.0, and 3.2 log, respectively ($P<0.001$). There were no differences in the levels/prevalence of any of the tested bacteria in MDM between the plants ($P\geq0.158$). In MDM samples, *Salmonella* and *Campylobacter* prevalence was 90 and 70% and the counts of APC, coliforms, and LAB were 3.0, 2.4, and 3.1 log, respectively. Viable counts of *Pseudomonas* were only detected at the post-pick step of one plant. The chiller PAA application decreased pathogenic and spoilage bacteria on carcasses in all plants, irrespective of the differences in PAA concentrations. However, measures to reduce the prevalence of *Salmonella* and *Campylobacter* in MDM may need to be re-evaluated.

Key Words: Salmonella, Campylobacter, Chicken, Processing, Peracetic Acid

M10 Early recovery of Salmonella isolation from naturally contaminated broiler carcasses through selective pre-enrichment broth Surendra Rasamsetti*GS, Tomi Obe, Nikki Shariat *Department of Population Health, Poultry Diagnostic and Research Center, University of Georgia*

Salmonella is a leading bacterial foodborne pathogen, causing over 1.3 million illnesses each year in the United States. Despite improvements to mitigate *Salmonella,* outbreaks linked to poultry meat still occur. Conventional *Salmonella* detection is time consuming as it requires a 24h pre-enrichment step in buffered peptone water (BPW) to resuscitate injured cells, followed by a 24h incubation in either Rappaport Vassiliadis (RV) or tetrathionate (TT) selective enrichment broths before plating onto indicator agar such as Xylose Lysine Tergitol 4 (XLT-4). The objective of this study was to reduce this time by increasing the selectivity of the pre-enrichment step. We evaluated the influence of selective components of RV and TT as additives to BPW to improve *Salmonella* recovery from commercial broiler carcasses. Drip line samples (n = 14) were collected from 500 sequential broiler carcasses post-pick at seven different commercial processing plants in the United States over a 4-month period. The samples were pre-enriched in BPW for a short time before addition of bile salts or malachite green and then incubated overnight at 37°C. Samples were streaked onto XLT-4 agar to identify presumptive *Salmonella* colonies. Isolated colonies were restreaked and confirmed to be *Salmonella* by serogrouping. As a control, samples were enriched in BPW alone and then sub-cultured into RV and TT broths in parallel before plating onto XLT-4. All 14 samples contained *Salmonella*, as defined by the control procedure, but only nine samples were *Salmonella* positive with direct plating after pre-enrichment in BPW only. Addition of either bile salts or malachite green resulted in 100% (14/14) recovery of *Salmonella* after pre-enrichment with direct plating (Fisher's exact test, $p = 0.0407$). There were differences in the effectiveness of these two additives: *Salmonella* was recovered in 86% (12/14) of samples when bile salts were present and in 29% (4/14) when malachite green was present. There were two samples where *Salmonella* was recovered in BPW+malachite green, but not in BPW+bile salts. This data demonstrates that addition of selective additives to the pre-enrichment step can result in recovery of *Salmonella* and reduces the time taken to detect *Salmonella* by 24 hours.

Key Words: Salmonella, broilers, pre-enrichment, selective enrichment, processing

M11 Trans-cinnamaldehyde nanoemulsion dip treatments rapidly inactivate Salmonella Enteritidis on eggs Jodie Allen*1GS, Brindhalakshmi Balasubramanian[1], Kimberly Rankin[1], Annie Donoghue[2], Indu Upadhyaya[1], Yangchao Luo[1], Abhinav Upadhyay[1] *[1]University of Connecticut, [2]USDA-ARS, PPPSR*

Salmonella Enteritidis (SE) is a major foodborne pathogen that causes enteric illnesses in humans, largely through consumption of contaminated eggs. Egg surfaces are contaminated with SE during oviposition, egg collection, or transport. Disinfectants such as chlorine are not completely effective in inactivating SE on eggs. Therefore, there is a need of developing novel disinfection strategies that are safe and effective in killing SE on eggs. Phytochemicals such as trans-cinnamaldehyde (TC) have been previously shown to exhibit significant anti-*Salmonella* efficacy, however, the low solubility of TC is a major hurdle in its adoption as a disinfectant.

This study investigated the efficacy of TC nanoemulsions (TCNE) dip treatments in killing SE on eggs at 32°C. TCNE were prepared by high-energy sonication using Tween 80 (Tw80) or Gum Arabic and lecithin (GAL). White-shelled eggs were spot inoculated (200 µl; attachment time 60 min) with a 4-strain mixture of SE (10^8 CFU/mL) followed by dipping of eggs in sterile deionized water (control) or water containing TC, or TCNE-Tw80, or TCNE-GAL at 0.06, 0.12, 0.24, 0.48% for 1, 3, or 5 min. Post washing, the eggs were transferred to Dey-Engley broth and the population of surviving SE on eggshell was enumerated by dilution and plating on XLD agar. All experiments had triplicate samples, repeated twice and analyzed using one-way ANOVA at $p<0.05$.

In case of baseline (inoculated eggs, not washed), ~6.5 log CFU SE/egg were recovered. After washing with water for 5 min (control), ~ 4.5 log CFU SE/egg were recovered indicating that water alone is not sufficient to completely eliminate SE on the eggs. In case of TC, only the highest concentrations (0.24 and 0.48%) were effective in reducing SE by at least 1.6 log CFU/egg as compared to control by 1 min ($P<0.05$). In contrast, all TCNE-Tw80 treatments were effective in killing SE by at least 2 log CFU/egg as early as 1 min ($P<0.05$). TCNE-GAL (0.06, 0.12%) were less effective in killing SE than corresponding TCNE-Tw80 treatments. TCNE-Tw80 (0.24, 0.48%) were the most effective treatments and reduced SE to below detection limit (2 log CFU/egg) by 1 min of treatment ($P<0.05$). Results suggest that TCNE could be used as an antimicrobial wash to disinfect SE contaminated eggs.

Key Words: Trans-cinnamaldehyde, Salmonella, Nanoemulsions, Food-grade emulsifiers, Eggs

M12 Influence of stunning method on broiler chicken serum corticosterone concentration Montana Riggs*GS, Jacob Vincent, Ileana Berganza, Ranjit Boyal, Jessica Starkey, Dianna Bourassa *Auburn University*

Consumer perspective on the importance of animal welfare has led to a shift in stunning methods in the poultry industry from electrical stunning to controlled atmospheric stunning. Carbon dioxide controlled atmosphere stunning involves multiple phases which gradually increase in carbon dioxide concentrations. In some controlled atmosphere stunning systems, oxygen is added in the early phase to reduce negative effects of carbon dioxide. Controlled atmosphere stunning also eliminates the need to handle conscious birds for shackling, reducing human to bird contact. Historically, circulating blood corticosterone has been used as an indicator of broiler stress response. To assess the impact of stunning methods on blood serum circulating corticosterone concentration, 30 broiler chickens were divided into 5 groups (n=6) and stunned using one of the following methods: 1) no stun, 2) low voltage pulse DC electrical waterbath, 3) captive bolt, 4) controlled atmosphere stunning with added oxygen, and 5) controlled atmosphere stunning without added oxygen. Following stunning, birds were immediately exsanguinated and blood samples (4 mL) were collected in heparin tubes during exsanguination then placed on ice. Corticosterone concentrations (pg/mL) were determined from blood serum using a competitive enzyme-linked immunosorbent assay (ELISA). Resulting corticosterone concentrations were then analyzed by one-way ANOVA of SAS with significance declared at $P \leq 0.05$. No significant differences in corticosterone concentrations were detected between birds stunned by controlled atmosphere stunning without added oxygen (506 pg/mL), controlled atmosphere stunning with oxygen (644 pg/mL), captive bolt (664 pg/mL), without stun (676 pg/mL), or electrically stunned birds (921.42 pg/mL, P=0.1735). These results indicate that the addition of oxygen during controlled atmosphere stunning did not impact the concentrations of circulating corticosterone. Anecdotal evidence of welfare improvements by addition of oxygen during controlled atmosphere stunning were not evidenced by corticosterone concentrations.

Key Words: controlled atmosphere stunning, corticosterone, poultry processing, electrical stunning

Pathology

M13 Effect of a Innocuous Prebiotic Powder as part of a palan for the control of septicemic processes caused by Pathogenic Escherichia coli in laying hens Hernán Morales-Alarcón[1], Carmen Sandoval-Jimenez[1], Hernando Morales-López[2], Andres Rodríguez-Ávila[*3] *[1]BioALFA de Colombia SAS, [2]Universidad de Caldas, [3]BioARA SAS*

Avian pathogenic Escherichia coli (APEC) infections cause acute and systemic diseases that generate significant economic losses in the poultry industry. The effect of an aerosolized innocuous prebiotic powder (IPP) was evaluated as part of a plan for the control of septicemic processes caused by E. coli (APEC) in laying hens between 2012 and 2019. The IPP was designed based on genetic consensus of E. coli strains present in poultry farms, which led to the identification of a set of APEC strains that were selected to be included in the formulation, these were supported by chitosan nano-spheres. Inoculation was carried out by dusting 3 million birds in growing houses from August 2018 to date. In total, two doses were applied in the growth period, separated by four weeks. Considering that the critical age of manifestation of the entity has a higher incidence between 18 and 55 weeks of age. The monitoring carried out during 2019 compared to 2018 in the production phase of the houses intervened with the IPP, shows the significant reduction in the number of antibiotic treatments applied within the framework of the control lots after the implementation of the IPP (P <0,05). Also, significant differences were found in the number of antibiotic treatments by group of age of the inoculated lots (P <0.05). This study shows that IPP is more than 42% effective and 73% effective in controlling septicemic processes caused by E. coli (APEC). This was verified with the reduction of antibiotic treatments, increases in egg production, reduction in mortality and improvements in the welfare of the birds.

Key Words: Innocuous Prebiotic Powder, E. coli, Chitosan, Antibiotic Treatments, Aerosolized

M14 Effects of Q-Biotic™ Bacillus subtilis on coccidiosis and Clostridium perfringens-induced necrotic enteritis Miguel Ruano[*1], Troy Lohrmann[1], Miloud Araba[1], Brian Dirks[1], Brett Lumpkins[2], Greg Mathis[2] *[1]Quality Technology International, Inc., [2]Southern Poultry Research, Inc.*

Three 28-day experiments were carried out to evaluate the efficacy of dietary inclusion of Q-Biotic™ *Bacillus subtilis* strain on body weight gain (BWG), feed conversion ratio (FCR) and mortality of Cobb 500 broiler chickens subjected to a mild, moderate or severe coccidiosis + *C. perfringens*-induced necrotic enteritis (NE) challenge. In each study, chicks were housed in Petersime Battery cages and randomly assigned to 4 treatments with 8 replicates and 8 birds per replicate. The experimental treatments were non-infected, non-supplemented control, infected, non-supplemental control (INSC), infected + leading commercial *Bacillus subtilis*, and infected + Q-Biotic™ *Bacillus subtilis* (Q-Biotic™ *B. subtilis*, QTI, Inc.). In experiment 1 and 2, NE was induced by daily oral inoculation of NetB producing *C. perfringens* on 19, 20 and 21 days of age, following exposure to a single oral dose of 5000 oocyts per bird of field *Eimeria maxima* given by gavage on day 14 of age. In experiment 3, NE was induced by oral inoculation of NetB producing *C. perfringens* on day 16 of age, following exposure to a single oral dose of 5000 oocysts per bird of an *E. maxima* vaccine (Advent®) given by gavage on day 9 of age. A moderate, severe and mild NE condition was induced in experiments 1, 2 and 3, as evidenced by significant reduction of 12.5%, 37% and no mortality, respectively. In experiment 1 and 2, NE resulted in significant reduction in BWG and significant increase in FCR and intestinal NE lesion score, while only FCR was significantly increased in experiment 3 due to NE. Broilers fed diets with Q-Biotic™ *B. subtilis* exhibited a significantly higher BWG, lower FCR, lower mortality, and lower NE lesion score in Experiment 1 and 2, and no significant differences in experiment 3, compared to the INSC treatment. In all three experiments, efficacy of Q-Biotic™ *B. subtilis* strain in reducing NE was comparable or better than that of leading commercial *B. subtilis*. In conclusion, dietary supplementation of the Q-Biotic™ *B. subtilis* significantly reduced the moderate and severe effects of NE induced in broiler chicks using a coccidiosis + *C. perfringens* challenge model, and restored performance of mild NE affected birds.

Key Words: Q-Biotic™ Bacillus subtilis, Broiler chickens, Eimeria Maxima, Clostridium perfringens, NetB

M15 Effects of Activo and Activo Liquid on broiler chickens under a Necrotic Enteritis challenge Miguel Barrios[*1], Kowsigaraj Palanisamy[2], Ajay Bhoyar[2] *[1]EW Nutrition, USA, [2]EW Nutrition GmbH*

Necrotic Enteritis (NE) costs the poultry industry over 2 billion USD every year. This disease has been a challenge in poultry for decades, and it has become even more relevant as chicken growers have transitioned to antibiotic-free production systems. Activo and Activo Liquid are composed of phytomolecules, which have shown antibacterial properties. Therefore, the objective of this trial was to determine the effects of Activo and Activo Liquid on broiler chickens challenged with a Necrotic Enteritis model. For this experiment, 1,400 Cobb 500 males were raised on floor pens with fresh pinewood shavings at production stocking density from1-42 days of age. There were 4 treatments with 7 replicates each and 50 birds per pen. The treatments were as follows: 1- Unchallenged Control, 2- Challenged Control, 3- As 2 + Activo (0-42 days: 150 g/MT), 4- As 2 + Activo (0-42 days: 100 g/MT) + Activo Liquid (days 12-14: 250 mL/1,000 L, 24 hours/day; days 19-21: 500 mL/1,000 L, 16 hours/day). On days 19, 20, and 21 birds in treatments 2-4 were challenged with a 10^8 CFU/mL *Clostridium perfringens* broth via feed inoculation. On day 21, 3 birds per pen were necropsied and scored for NE lesions. Fresh fecal samples were also collected to determine *Eimeria* spp. oocyst shedding. Birds and feed were also weighed on days 21, 35, and 42 to determine feed conversion ratio (FCR). Data was analyzed using the one-way ANOVA procedure of JMP 12. On day 21, NE scores were highest in challenged birds, while treatments 3 and 4 showed significantly lower (P<0.0001) scores. Mortality was significantly lower (P<0.04) in treatment 4 as compared to treatment 2. These differences disappeared on days 35 and 42. FCR was statistically similar among treatments 2-4 on day 21, while on day 42, treatments 3 and 4 were 10 and 5 points lower (P<0.01) respectively, than treatment 2. Lastly, *E. acervulina* oocysts were significantly lower (P<0.03) in treatment 4 as compared to treatment 2. Taken together, these results suggest that Activo and Activo Liquid may ameliorate the impact of NE on broilers. We hypothesize that the effects of Activo Liquid were particularly important in improving overall mortality. Further work is needed to understand the effects of these phytomolecules on broiler microbiota.

Key Words: Necrotic Enteritis, Broiler, Coccidiosis, Activo, Phytomolecules

M16 An investigation of the cause of wild turkey mortality in Mississippi Rachel Thiemann[*1], Martha Frances Dalton[2], Heidi Rose[2], Brittany Baughman[2], Adam Butler[3], Natalie Armour[1] *[1]Poultry Research and Diagnostic Laboratory, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [2]Mississippi Veterinary Research and Diagnostic Laboratory, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [3]Mississippi Department of Wildlife, Fisheries and Parks*

Lymphoproliferative disease virus (LPDV) is an exogenous *alpharetrovirus* which sporadically causes fatal lymphoid neoplasia in affected turkeys. Previous studies of wild turkeys (*Meleagris gallopavo*) in the United States have demonstrated widespread LPDV infection, and frequent co-infection with Fowl Poxvirus (FPV) and Reticuloendotheliosis Virus (REV). This study was conducted to better understand the causes of mortality in

Mississippi wild turkeys, and the relative importance of LPDV, FPV and REV in contributing to wild turkey mortality. Wild turkeys which died in the field or were euthanized due to illness were submitted to Mississippi State University's Poultry Research and Diagnostic Laboratory for necropsy examinations. Birds originated from seven counties across the state over the past four years and had been frozen prior to submission. At necropsy, a majority of birds had severe, proliferative cutaneous lesions on the head and neck, with diptheritic to proliferative oral and esophageal lesions in some birds. Samples were collected for molecular diagnostics (LPDV and REV PCR), histopathology and bacterial culture and isolation. External and internal parasites were collected in formalin for identification. Fowl Pox (cutaneous and/or diptheritic forms) was diagnosed in a majority of birds by identification of pathognomonic histologic lesions (including intracytoplasmic viral inclusion bodies). Parasitism (internal and external), Histomoniasis and bone fractures were additional diagnoses. Tissues were sent to the Southeastern Cooperative Wildlife Disease Study at University of Georgia for detection of LPDV and REV by PCR.

Key Words: Fowl poxvirus, Lymphoproliferative disease virus, Alpharetrovirus, wild turkeys, Order Galliformes

M17 Impact of volume, number of spray and nozzle type in vaccination accuracy with Infectious Bronchitis vaccine Massachusetts type Enrique Montiel* *Boehringer Ingelheim*

The accuracy of spray vaccination at one day of age using live-modified Infectious bronchitis (IB) vaccine Massachusetts type was studied. Groups of 50 one-day-old 50 broiler chicks were vaccinated with a frozen Infectious bronchitis vaccine, Mass type using the Spravac™ vaccinator as follows, 2 groups per treatment : a) 28 ml single spray at full or half dose using cone or fan nozzles b) 14 ml single spray at full or half dose using cone or fan nozzles; c) 7 ml single spray at full or half dose using cone or fan nozzles; d) 14 ml double spray for a total full or half dose after both applications using cone+cone, cone+fan or fan+ fan nozzles and e) 7ml double spray for a total full or half dose after both applications using cone+cone, cone+fan or fan+ nozzles. In a second study, 250 one-day-old commercial broilers were divided into 5 groups of 50 chicks each and vaccinated as follows: a) 14 ml single spray full dose using the cone nozzle; b) 7 ml single spray at full dose; c) 7 ml single spray at half dose; b) 7ml double spray for a total full dose using cone+cone nozzle; c) 7ml double spray for a total full dose using cone+fan nozzle; 0.03 full dose via eye drop. All chicks were placed in negative pressure isolation units and provided feed and water *ad libitum*. Three days after vaccination, five birds per group were humanely euthanized and tracheas were aseptically collected in tryptose phosphate broth (TPB), homogenated and inoculated into 10-day-old spf embryos for virus re-isolation. Results indicate that the 14 ml spray volume consistently showed the best re-isolation rates when giving in single or double spray or in two sprays of 7 ml each at full or half dose; the birds vaccinated with the cone nozzle had higher re-isolation rates when user alone in a single spray or used in double spray in combination with the fan nozzle as compared with the fan alone. When using the 28 ml volume single spray the cone and farm nozzle performed in a very similar manner. The re-isolation rates were lower for the single 7 ml and 28 ml spray spray at half dose. Results will be discussed.

Key Words: Spray, vaccination, bronchitis, spravac, Massachussetts

Environment, Management and Animal Well-Being: Environmental Impacts

M18 Later exposure to different perching design and materials affect the behavior and performance of White Leghorn chickens Sohail Ahmad*, Athar Mahmud, Muhammad Usman, Muhammad Waqas *University of Veterinary and Animal Sciences, Lahore, Pakistan*

Perching behavior is natural and evident in Red Jungle fowl, from which modern-day chickens originated. A positive impact of perching in the improvement of a bird's physical condition and increase bone strength. In this study, 180 40-week old Leghorn hens were randomly divided into 6 treatment groups having 3 replicates of 10 birds each according to Complete Randomized Design under 2 × 3 factorial arrangement. Treatments consisted of 2 perching material (wooden and plastic) and 3 perching shape (square, round, and triangle). Effect of perching material and shape were evaluated on productive performance, behavioral and welfare traits, egg characteristics, and heterophil/lymphocyte ratio for 14 weeks (40-54 weeks). Collected data were analyzed by factorial ANOVA using General Linear Model procedures in SAS software followed by Duncan's Multiple Range Test. Regarding perching materials, egg weight was higher in wooden perching materials than plastic. However, in the case of behavior, welfare, and egg quality trait non-significant results were observed. Regarding perching design, egg weight, production percentage, and feed conversion ratio per kg of egg mass of commercial layers differed significantly. Moreover, wing flapping and perching behavior were significant among different perching designs. Egg weight, shell thickness, albumen height, Haugh unit score, and egg volume differed significantly among perch designs. In conclusion, the provision of different perching designs positively influences productive performance, behavior, welfare, and egg quality traits in laying birds.

Key Words: Egg Weight, Haugh Unit, Shell Thickness, Welfare

M19 Trends in Mycotoxin Contamination in the United States Corn and Corn By-product Lan Zheng*[1], Chasity Pender[1], Erika Hendel[1], Paige Gott[1], Shelby Ramirez[1], Ursula Hofstetter -Schähs[2], Ganapathi Murugesan[1] *[1]BIOMIN America, Inc., [2]BIOMIN Holding GmbH*

Mycotoxins are fungal metabolites that may have detrimental effects on animal health. The classic signs such as reduced feed intake, impaired growth, and oral and intestinal lesions are used as indicators of exposure in the field; however, other costs of mycotoxicosis are often underestimated, including increased frequency and severity of disease via inflammation, immunosuppression, and modulation of the gastrointestinal environment. The objective of this study was to evaluate the mycotoxin levels in corn and corn DDGS from the US 2020 harvest and compare those with previous years. Twenty-seven new corn and 21 corn DDGS were submitted as of October 2020. Samples were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for mycotoxins within five major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), and zearalenone (ZEN). Data were analyzed using GLIMMIX procedure of SAS with fixed effect of harvest year. Over the past decade, the average B-Trich and FUM contamination level in corn has been significantly ($P < 0.05$) affected by harvest year. However, over the last 5 years, B-Trich levels have remained similar, whereas FUM was decreased ($P < 0.05$) from 2019 to 2020. Contamination levels in corn for Afla, A-Trich, and ZEN have not been statistically ($P > 0.05$) different over the past decade. Over the past 5 years, B-Trich, FUM, and ZEN contamination has been significantly ($P < 0.05$) affected by harvest year in corn DDGS samples. The B-Trich contamination level was decreased ($P < 0.05$) from 2019 to 2020, whereas FUM and ZEN contamination levels remained similar ($P > 0.05$). The risk profile of this crop year is likely to change as the sample pool expands. Mycotoxin risk of this harvest season is still coming into focus as the combination of hot weather, storm, and drought in summer 2020 has crops maturing earlier than usual, leading grain quality concerns including mycotoxins. Due to the continued risk of multi-mycotoxin contamination,

mitigation strategies are needed beyond adsorption, including biotransformation and support of the immune system and liver function.

Key Words: mycotoxins, corn, feed ingredients

M20 Evaluation of commercially manufactured animal feeds to determine presence of Salmonella, Escherichia coli and Clostridium perfringens Luis Munoz*GS, Wilmer Pacheco, James Krehling, Kaicie Chasteen, Aidan Talorico, Kenneth Macklin *Auburn University*

Animal feeds can serve as a vehicle for transmission of bacterial pathogens to farm animals. These pathogens are potentially harmful and can cause clinical or subclinical intestinal diseases to farm animals, which may lead to carcass contamination with foodborne pathogens during processing at the abattoir. The experiment was conducted to: (1) establish if feed ingredients or commercial animal feeds serve as sources of contamination of *Salmonella*, *E. coli* and *C. perfringens*, and (2) characterize an unknown group of bacteria belonging to the genus *Clostridium* identified during microbiological isolation process. One research feed mill and four commercial feed mills were evaluated. A total of 292 samples (132 ingredients and 160 feeds) were collected from 5 different locations within each feed mill: ingredient receiving, post mixing, post pelleting, post cooling and loadout. All samples were assayed for *Salmonella*, *E. coli* and *C. perfringens* using selective media. Resulting counts were $\log_{10}$ transformed and then analyzed using ANOVA, if significant (P<0.05), means were separated using Tukey HSD. Peanut meal and corn gluten meal were the ingredients with highest contamination of *Clostridium* spp. (CSC) (3.91 and 2.61, respectively), and peanut meal and corn meal for *E. coli* (ECC) (4.15 and 2.85, respectively). Soybean meal and distillers dried grains with solubles had the lowest contamination with CSC (0.42 and 0.20, respectively) and ECC (0.49 and 0.92, respectively). The stage of feed processing did not influence the degree of contamination with CSC (P>0.05). However, contamination with ECC was higher at the post mixing stage (3.03), but it was significantly reduced after the pelleting process (0.23). Recontamination of mixed feed with ECC after pelleting process was observed in 2/5 feed mills studied. *Salmonella* was not present in ingredients and feed samples evaluated during this project, but *C. perfringens* was present in some mixed animal feeds. Finally, the unknown bacteria found in mixed feeds were identified as *Clostridium argentinense*, which is ubiquitous to soil and has the potential to produce a botulinal-like toxin capable of inducing to disease in different animal species.

Key Words: Feed contamination, Pathogens in feed, Clostridium perfringens, Clostridium argentinense

M21 Shift in microbial composition of dust during growout of broiler chickens Amrit Pal*GS, Ally Jackson, Andrea Giron, Matthew Bailey, Aidan Talorico, James Krehling, Kenneth Macklin, Dianna Bourassa *Auburn University*

Dust found in poultry houses can be a source of microorganisms that can be disseminated in air and deposited on surfaces. This study aimed to evaluate the microbial composition of settled dust in a poultry house during growout. Bacteria in dust deposited during each week (wk) of growout (n=12/wk) and cumulatively throughout growout (n=12/wk) were analyzed. On the day of chick placement, all sampling areas were cleaned and sanitized. To sample dust within each wk, the sample area was cleaned and sanitized following each sample collection. Cumulative dust samples were obtained by sampling an area adjacent to the location sampled in the previous wk. Swab samples of dust were collected during the growout period (6 wks), by swabbing a 28 cm^2 area and analyzing for *Salmonella*, *E. coli*, coliforms, and aerobic plate counts (APC). *Salmonella* prevalence was analyzed following enrichment. Data were log transformed (Log_{10} CFU/28 cm^2) and analyzed by PROC GLM of SAS using Tukey HSD for mean separation. During each wk of growout, *E. coli* counts were highest at 4 wk (2.46) compared to 1 wk (1.12) with all other wks intermediate (P=0.0432). During 1 wk and 2 wk of growout, APC were lower (5.38–5.53) than subsequent wks (>7.12, P<0.0001). Coliform counts (2.02) did not differ by wk (P=0.5435).*Salmonella* was not detected during wk-by-wk testing. When dust was collected cumulatively throughout growout, *E. coli* (1.76) and coliforms (2.08) did not differ over time (P=0.1248, P=0.7919). APC steadily increased from 1 wk (5.38) to 5 wk (8.25) and remained at 8.11 at 6 wk (P<0.0001). Two cumulative dust samples at 2 wk and one at 3 wk were positive for *Salmonella.* Overall, these results indicate that on a wk by wk basis, 4 wk old broilers contributed the highest levels of *E. coli* and 4-6 wk old broilers contributed the highest levels of APC to settled dust. If dust was sampled cumulatively there was not an additive effect for *E. coli* and coliforms, but APC continued to increase throughout growout. *Salmonella* was present in dust at low levels that were only detected from 3/72 sample enrichments from cumulatively collected dust at 2 and 3 wk of age indicating that bird age may influence the ability to detect *Salmonella* in environmental dust samples.

Key Words: dust, poultry house, Salmonella, broiler, growout

M22 Prevalence of Clostridium perfringens in no antibiotics ever broiler farms in Mississippi Courtney Fancher*GS, Hudson Thames, Mary Gates Colvin, Li Zhang, Nikhil Nuthalapati, Aaron Kiess, Anuraj Sukumaran *Mississippi State University - Poultry Science*

Clostridium perfringens is the etiological agent of necrotic enteritis and gangrenous dermatitis, two diseases that cause significant mortality in broilers and consequent economic loss. A recent industry shift to antibiotic-free production has increased the incidence of these diseases in broilers. To our knowledge, there are no studies investigating how the shift to antibiotic-free production affects the prevalence of this pathogen in broilers. The objective of this study was to determine the prevalence of *C. perfringens* in different types of samples collected from commercial no antibiotics ever (NAE) broiler farms during different ages of the flock. Litter, feces, and cloacal swabs were collected from four NAE broiler farms on days 28 and 56 of one flock cycle. The samples were enriched in Reinforced Clostridial Broth by incubating at 37°C for 24 h anaerobically, then streaked on Oxoid™ Perfringens agar plates, and incubated anaerobically at 35°C for 24 hours. Round colonies with an opaque ring and black center were identified as *C. perfringens*. Up to four colonies were taken per sample for future analysis. Glimmix procedure of SAS 9.4 was used to analyze the data and statistical significance was determined at $P \leq 0.05$. A total of 734 isolates were obtained from 192 samples collected in the study. Irrespective of the age of flock and sample type, all 192 samples contained at least one colony identified as *C. perfringens* based on colony morphology ($P > 0.05$). In conclusion, there is a very high presumptive prevalence of *C. perfringens* in NAE broiler farms with the potential to cause necrotic enteritis and gangrenous dermatitis outbreaks. However, the isolates need to be confirmed using polymerase chain reaction to determine the exact prevalence. Moreover, further toxinotyping and virulence characterization are essential to assess the disease risk.

Key Words: Clostridium perfringens, broiler, poultry, no antibiotics, necrotic enteritis

M23 Comparison of DNA extraction methods in poultry litter and fecal samples to identify pathogenic bacteria using real-time PCR Sabin Poudel*1GS, Sadie White[2], Tianmin Li[1], Xue Zhang[2], Aaron Kiess[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Department of Food Science, Nutrition and Health Promotion, Mississippi State University*

Rapid and accurate detection of bacterial pathogens is crucial for disease diagnosis and reduce economic losses through effective prevention strategies in poultry. Real-time PCR assays are commonly used for rapid and accurate pathogen identification. However, the accuracy of this method is prone to PCR inhibitors, which are often present in poultry feces and litter samples. These inhibitors can affect the sensitivity of the assay or even lead to false-negative results. Therefore, selection of an appropriate

DNA extraction method is necessary for accurate subsequent molecular analysis. In this study, four DNA extraction methods [Phenol-chloroform (Phenol), silica membrane (Qiagen), salt precipitation (Wizard), and magnetic beads-based (MagMax)] were used to extract DNA from pooled litter and fecal samples. Real-time PCR was preformed to evaluate the presence of specific pathogens [*Clostridium spp* (*Clost*), *Staphylococcus spp* (*Staph*), *Escherichia coli* (*E.coli*), *Campylobacter spp* (*Campy*)] as well as the presences of a universal 16S rRNA gene. Threshold cycle (Ct) value data obtained from real-time PCR were analyzed using one-way ANOVA, significance was set at a $P \leq 0.05$ and means were separated using Fisher's LSD. DNA extracted from feces for 16S rRNA and *Staph* showed that MagMax had the lowest Ct compared to the other three methods. Ct for 16S rRNA was higher in the Phenol method compared to the MagMax method ($P \leq 0.05$) and had the highest Ct for *E. coli, Clost,* and *Staph* ($P \leq 0.05$). In litter samples, the Qiagen method had a lower Ct for *Campy* and *Clost* when compared to the MagMax method, whereas MagMax had a lower Ct for *Staph* ($P \leq 0.05$); the Phenol method had the highest Ct for *Campy* ($P \leq 0.05$). Litter DNA isolated using the Phenol and Wizard were not capable of amplifying *Clost* and *Staph*, and the Phenol method did not amplify *E.coli.* The real-time PCR results showed that the identification of bacteria is highly dependent on the DNA extraction method and sample type. It suggested using Qiagen or MagMax methods to extract DNA from poultry litter and feces for accurate pathogen detection . The DNA extraction method needs to be selected, based upon the nature of the sample and the bacteria to be tested.

Key Words: DNA extraction methods, Real-time PCR, poultry litter, poultry feces, pathogen detection

M24 Effect of sprinkling on water conservation, house environment, and broiler performance Jonathan Moon*[1GS], Jan DuBien[1], Reshma Ramachandran[1], Yi Liang[2], Tom Tabler[1] *[1]Mississippi State University, [2]University of Arkansas*

Numerous challenges face global poultry production including increasing demand for high quality animal protein and the need to adapt to environmental pressures such as heat stress and growing demands on natural resources (particularly water). During two summer flocks (May-July and Aug-Oct, 2020), a commercial sprinkler system used in combination with a cool cell system (SSCC) was evaluated against a cool cell only system (CC) in two commercial broiler houses (42 x 400 ft; 13 x 122 m) at Mississippi State University. Two lines of overhead sprinklers above outside feed lines spaced evenly 20 ft (6 m) apart and 7ft (2.1 m) above the litter surface intermittently applied controlled volumes of large water droplets onto birds. Hs 1 was SSCC house during the first flock. Cooling arrangements were reversed on the second flock, with Hs 2 being SSCC. Environmental data (temperature, humidity, litter moisture were collected periodically throughout the flocks. Performance data (feed conversion ratio (FCR), body weight, daily mortality, paw quality, water consumption, cooling water utilization) were calculated and recorded throughout the flocks. Data were analyzed as a Randomized Complete Block Design using SAS 9.4. Because there was no effect of house ($P>0.05$), treatments were compared across flocks. Despite higher average temperatures in SSCC than in CC of 3°F ($P=0.08$), average water usage by SSCC was 64% less than CC, and average humidity across flocks was lower in SSCC than in CC ($P=0.054$). Litter moisture tended ($P=0.11$-0.16) to be lower at several time points between and across flocks, but there were no differences in any of the other measurements. Final body weight was .1 lb higher and average FCR was 0.04 lower in SSCC, neither of which was significant; however rate of return was 0.155 cents per pound higher ($P=0.02$) on birds from SSCC. Although replication was an issue in this study, as is always the case with whole-house treatments, results are similar to those reported previously (Liang et al., 2014; Moon et al., 2020), and provide additional evidence that sprinklers in conjunction with cool cells allow comparable production, with lower water usage and higher rate of return. Future research should include optimum flock age to begin SSCC and CC cooling.

Key Words: Sprinkler, Water conservation, Welfare, Sustainability, Broiler

Environment, Management and Animal Well-Being: Stress Responses, Behavior

M25 Succession patterns of poultry litter microbiota after bird removal and PLT® application Jasmine Johnson[1], Benjamin Zwirzitz[2,3], Adelumola Oladeinde*[1,4], Marie Milfort[1], Samuel Aggrey[1], Albert Fuller[1], Torey Looft[5], Lilong Chai[1], Gregory Zock[1] *[1]University of Georgia, [2]University of Veterinary Medicine, [3]Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, [4]US National Poultry Research Center : USDA-ARS, [5]National Animal Disease Center : USDA-ARS*

Sulfate based acid amendments are used for treating reused litter between broiler chicken flocks and during grow-out for in-house ammonia abatement. These amendments reduce litter pH and inhibit NH_3 volatilization by converting NH_3 into nonvolatile NH_4^+. Research on the effects of acid amendments on litter microbiota is limited and are usually done in microcosms, which do not replicate natural environments. In this study, we determined the changes in bacterial populations present in reused litter during downtime (period after a flock was removed and before new broiler chicks were placed) and 24-h before and after Poultry Litter Treatment® (PLT) application – a sodium bisulfate ($NaHSO_4$) based amendment. We made use of nascent DNA sequencing technologies to characterize the litter microbiota, elucidating microbial shifts in litter samples with respect to downtime, litter depth, and PLT® application. During downtime (~18 days), the litter microbiota was dominated by *Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria* and bacterial species from the genus *Corynebacterium*. PLT® affected the microbiota in the top layer (10 cm) of reused litter topdressed with fresh pine shavings and resulted in an increase in *Escherichia coli* and *Faecalibacterium* spp., and a decrease in members of the phylum *Acidobacteria*. The higher *E. coli* abundance was correlated with an increase in culturable *E. coli* and litter moisture 24-h after PLT application. While the effect of acidifiers on ammonia reduction, bird performance and litter performance are well documented, its effect on litter bacteria is not well understood. Our results suggest that acidifiers may lead to a dysbiotic litter microbiota when topdressed with fresh pine shavings and requires further research.

Key Words: Poultry litter, Acid amendment, Downtime, Microbiota, Dysbiosis

M26 Effect of variable light intensity lighting program on dust-bathing behavior of commercially housed broilers Seong Kang*[1], Karen Christensen[2], Michael Kidd Jr[1], James Clark[2] *[1]University of Arkansas, [2]Tyson Foods, Inc.*

Light influences behavioral and physiological responses of birds. Previous variable light intensity studies indicate that when birds have a dual light intensity choice, birds consumed more feed in the lighter intensity area compared to lower intensity area. Interestingly their central welfare indicators suggest better central welfare in the variable light intensity treated birds. Aim of this study was to evaluate broiler's dust-bathing behavior by counting the evidence of dust-bathing in the commercial broiler houses in different lighting program. Day old broilers (Cobb 700, mixed sex) were

housed in four commercial broiler houses (12.8 m x 122 m, wood-shavings). Each quadrant of the house was placed with 4,800 chicks with all source flocks equally represented in each quadrant (section). Four lighting programs began on day 7 with 5 lux (5L) or 20 lux (20L) or natural light (NL, 480 lux) or variable light (VL, 2-5/40 lux) using LED lights on a 16L:8D photoperiod. VL house was about 40 lux light intensity over the feed lines and dimmer light intensity at the sidewalls (2-5 lux). NL house has a 60 cm wide strip of clear plastic that runs the length of the house from 1.2 to 1.8 m high on the sidewall and allows for natural light to enter. In each section of the house, dust-bathing holes as the evidence of dust-bathing behavior were counted at 23 days of age. Dust-bathing holes were counted in six parts of each section and number of holes per 10 square meters was determined. Differences among light treatment groups were analyzed using one-way ANOVA followed by mean separation using the Tukey's HSD test using JMP 14. Significance level was $p<0.05$. The number of dust-bathing holes in NL house (3.90 ± 0.49) was expectedly higher compared to those in 5L (1.27 ± 0.32) and 20L (1.29 ± 0.37) houses ($p<0.05$). In VL house, number of dust-bathing holes was 5.69 ± 0.87, which is significantly higher compared to those of NL houses as well as 5L and 20L houses ($p<0.05$). Result indicates that voluntary natural behavior, dust-bathing, of birds was stimulated by dual light intensity program as well as natural light program in the commercial broiler production conditions, suggesting a beneficial effect of the variable lighting program on broilers' natural motivation and exercise.

Key Words: variable light intensity, broilers, welfare, dust-bathing

M27 Choice lighting effects on wellbeing and health of broilers Michael Kidd*[1GS], Seong Kang[1], Karen Christensen[2], Sami Dridi[1] [1]*University of Arkansas,* [2]*Tyson Foods*

Light intensity, a key environmental factor, has multifaceted effects on broiler production including health and well-being attributes along with strong influences on performance. The aim of the present study is to evaluate mortality and lameness along with growth performance in a "choice" lighted environment, one light source placed on feeders to create low and high light areas within the house, compared to low and high intensity lighting programs. Choice lighting would allow birds to move between different light intensity areas within the same house. 1 day old broilers were placed in four industry houses (dimensions 42Wx400L)(n=4). Each house was divided in 4 sections with 4,800 birds/section. Lighting programs were as follows: High (26.16± 0.70 lux (lx)), low (6.16±0.16 lx), choice (two light intensity zones 40.4 /2.07± 0.04 lx) and natural (483.76±42.02 lx), which was used as a control. 1 individual treatment was assigned to each house. Light levels were determined using Extech LED light meter LT45. 50 zones in each house were randomly selected and measured before birds were placed to obtain the house average light intensity. Birds had ad libitum access to feed and water. Day 1 and 2 lights were max at ~60 lx, and light treatments began on day 3. Mortality was recorded at least 2 times daily with lame birds being recorded and culled. Birds were processed at day 56. No significant difference in mortality was discerned between the treatments. However, the choice lighting house was found to have 28%, and 23% less lame birds than the natural and low and high light houses, respectively. Lower percent of lame birds within the choice house could be the result of bird movement between light zones. Also, the choice house was more efficient with 4 points less FCR compared to the natural light house. FCR and daily weight gain (DWG) for each treatment are as follows: Choice FCR=1.93 DWG=0.1484, Natural=FCR 1.97 DWG=0.1461, Low FCR=1.95 DWG=.1480, High FCR=1.99 DWG=.1448. In conclusion, choice lighting demonstrated lower percent lameness and improved FCR compared to several conventional lighting programs, but more work is needed to determine choice lighting's efficacy in commercial broiler production.

Key Words: Welfare, Lighting, Lameness, FCR, Choice Lighting

M28 The effects of monochromatic light on the growth, egg production, egg quality, behavior, and hormone concentration of Hy-Line® W-36 laying hens Ishab Poudel*[GS], Aaron Kiess, Mary Beck, Pratima Adhikari *Mississippi State University*

A study was conducted to investigate the effect of blue and red light-emitting diode (LED) on performance, egg quality, and hormonal concentration of pullets and laying hens. A total of 1,000 Hy-Line® W-36 birds were raised in a cage-free housing system consisting of two identical groups under two lighting systems (blue-LED from 1 to 17 weeks of age and red-LED from 18 to 31 weeks of age). The experiment involved 2 treatments and 2 research trials that resulted in 2 identical experiments for replication. Data were analyzed as a randomized complete block with a split-plot in time design, where year was a blocking factor, room was considered as a main plot factor and weeks were considered as a split plot factor. Body weight was recorded every 2 weeks in the pullet phase and every month after birds started laying. Egg production was recorded daily. Egg quality, organ weight, tonic immobility (TI), heterophil/lymphocyte ratio, serum corticosterone, luteinizing hormone, melatonin, and bone parameters were analyzed every month once egg production started. Results showed that birds in the blue-LED had a significantly higher body weight during the pullet phase from 1 to 18 weeks of age (P=0.049). Higher relative egg yolk percentage (P=0.043) was observed in the red-LED, along with a lower albumen percentage (P=0.015). Lower relative spleen weight was observed in the red-LED as compared to the normal-LED (P=0.027). We observed a coccidia infection in the red-LED at 22 weeks of age during the first trial and a mite infestation in the red-LED at 24 weeks of age during the second trial, suggesting that lower spleen weight might have made the birds more susceptible to these infections. No difference was observed between the light treatment with respect to egg production, brain weight, TI, and hormone concentration. Exposing birds to blue-LED light in the pullet phase can increase the body weight and initiate early laying in hens. However, heavier body weight at the start of production has been associated with larger eggs throughout the lifespan of birds, more feed consumption and decreased longevity. Therefore, it is difficult to conclude that exposing birds to blue-LED in the pullet phase for improved growth would have a beneficial effect on overall egg production.

Key Words: Light-emitting diode light, blue-LED, red-LED, egg quality, egg production

M29 Sex differences in glucocorticoid and heterophil to lymphocyte ratio responses to ACTH in the adult Pekin duck Victoria Tetel*[1UG], Brooke Van Wyk[1,2], Gregory Fraley[1] [1]*Purdue University,* [2]*Hope College*

More often, poultry scientists are utilizing direct and indirect measures of stress hormones to monitor bird welfare. However, it has been clear that our understanding of the avian hypothalamic-pituitary-adrenal axis (HPA) is insufficient as evidenced by the many conflicting reports regarding stress responses, such as transportation stress, in poultry. It has long been assumed that the poultry HPA functions similarly to that of mammals. However, we now know that there are considerable differences in the avian HPA. In addition to corticotropin releasing hormone, the avian brain utilizes a second neurohormone, arginine vasotocin, and both are released from the brain to stimulate pituitary corticotropes (ACTH). Both glucocorticoids (GC) are synthesized from a common pathway that begins with cholesterol and pregnenolone. The synthesis of one of the glucocorticoids does not depend upon the synthesis of the other. The purpose of our study was to test the hypothesis that ACTH will stimulate both corticosterone and cortisol. To test this hypothesis, we injected intramuscularly artificial ACTH (cosyntropin; 0.0625, 0.031, 0.016 mg/kg or saline as control) into adult drakes and hens (N = 10/sex/dose). Both glucocorticoids (GC) were assayed in serum using previously verified ELISAs. Blood smears were also assessed for heterophil to lymphocyte ratios (HLR). Data were analyzed by repeated measures 3-way ANOVA with Fishers PLSD as an *ad hoc* test. We observed that both GC were secreted in significantly ($p = 0.0002$) different patterns in a dose response manner

compared to controls, and that there was a significant (p = 0.0001) sex difference in both GC compared to saline controls. Further, we observed that the high dose ACTH elicited a significant (p = 0.004) sex difference in the HLR response compared to controls. Our data suggest that ducks, at least, may utilize more than just corticosterone for physiological homeostasis. Further, the time course of stressor to release of GC and subsequent HLR response may be dependent upon sex. More detailed analyses of the HPA is necessary in all poultry species to better understand stress responses as we utilize biological bases for welfare assessments.

Key Words: corticosterone, cortisol, welfare, stress response

M30 Leg infrared surface temperatures and tibia morphology show quantitative differences in broilers with normal compared to abnormal gaits Anna Magnaterra[*1GS], Randy Mitchell[2], Zoie McMillian[1], Ashlyn Synder[1], Shawna Weimer[1] *[1]University of Maryland, [2]Perdue Foods LLC*

Broilers can express lameness-related behavioral indicators of leg health issues, creating welfare and management complications. Gait scoring (GS) is a useful measure to understand a bird's leg health and welfare status but gait scoring presents a serious challenge due to the subjectivity of the test. This brings a need for more quantitative measures of leg health. The objective of this study was to compare quantitative measures of leg health and bone morphology between broilers with normal and abnormal gaits. On d51, live and post mortem measures were collected from 120 randomly selected conventional broiler chickens from 20 pens (N=6 birds/pen). Live observations and measures were taken: GS (binomial scale, where 0 was a normal gait and 1 was an abnormal gait), body weight (BW), and an infrared thermal (IR) image of the legs. Post-mortem morphological measures of the right and left tibia were recorded: angle, total length, depth of the proximal and distal intercondylar areas, and width at 10%, 25%, 50%, 75%, and 90% of total length. Means and deltas of both legs and tibiae were calculated for each bird. Data was analyzed in JMP using a paired t-test for the fixed effect of GS (0, 1). There was no effect of BW on GS. The mean hock and shank IR temperatures were lower (P≤0.05) for GS 1 birds (hock=36.95°C, shank=36.29°C) compared to GS 0 (hock=37.36°C, shank= 37.01°C). Mean tibial width at 75% and 50% of total length were greater (P≤0.04) for GS 1birds (75%=14.31mm, 50%=10.43mm) compared to GS 0 (75%=13.89mm, 50%=10.10mm). There was a tendency for the mean tibial angle of GS 1 birds to be greater (P=0.06) than GS 0. The mean delta angle of the proximal tibial head for GS 1 birds was greater than GS 0 (GS 1Δ=6.19°, GS 0Δ=3.79°; P=0.001), while the mean delta depth of the proximal medial intercondylar area of GS 1 birds (0.36mm) was less than (P=0.03) than GS 0 (0.49mm). To conclude, IR leg temperatures and tibia bone measurements show potential as quantitative measures of broiler leg health. Direct morphological measures can provide valuable insight on physiological differences, which arise with increased GS, while mean IR temperature provides a quantitative indirect measure of leg health.

Key Words: Gait Score, Tibia, Morphology, Infrared thermography

M31 Impact of every-day versus skip-a-day feeding of broiler breeder pullets during rearing on body weight uniformity and reproductive performance Kelly Sweeney[*1GS], Carla Aranibar[1], Luis Avila[2], Jessica Starkey[2], Charles Starkey[2], Jeanna Wilson[1] *[1]University of Georgia Poultry Science Department, [2]Auburn University Poultry Science Department*

Genetic selection for increased growth rate in broilers makes feed restriction programs for broiler breeders essential to managing body weight, flock uniformity, and reproductive performance. A common feed restriction method is skip-a-day (SAD) feeding, which allows a large volume of feed to be distributed evenly through the house. The objective of this experiment was to compare the reproductive performance (20-45wk), body weight gain and uniformity of broiler breeders after being reared on a high fiber diet (3.8% crude fiber, 8% whole oats and 16% wheat middlings) fed every-day (ED) or SAD. The same developer ration and feed amounts were fed to both treatments. Day old Ross 708 pullet chicks (n=912) were randomly distributed into 4 floor pens (n=228/pen, 2pens/feeding treatment) covered in pine shavings. At 20wk of age all birds were weighed, and the coefficient of variation (CV) and average body weight was calculated for each treatment. Birds were then distributed into 10 lay pens (n=35 birds/pen, 5 pens/treatment) at 21.5wk of age where the pen was 2/3 raised slats and 1/3 litter. Light was increased from 8h to 15.25h at the time of the move, and birds were daily feed for the remainder of the study. Data were analyzed by SAS SLICE using a significance level of P<0.05. During lay, 25% of the birds from each treatment were weighed weekly to adjust feed and monitor body weight. At 21wk the ED fed pullets were more uniform (P=0.0007) than the SAD fed pullets. Eggs were collected daily and set for hatch every 4wk from 28 to 42wk of age. No significant difference in fertility, hatchability, or hatch of fertile was observed. The ED fed birds achieved first egg at 23.5wk of age while the SAD fed birds were 24.5wk at the start of lay. At 26wk of age the ED reared hens had higher egg production (P=0.04). Specific gravity was measured every 2wk from 30-40wk, with ED reared birds having better overall eggshell quality (P=0.02) and greater egg weight (P<0.0001). Feeding a high fiber diet on an ED basis during rearing, improved body weight uniformity in rearing, encouraged early lay, improved eggshell quality and increased egg weight. ED feeding has the potential to improve chick quality and broiler health with the larger egg size and better shell quality.

Key Words: Broiler Breeder, Every-day, Skip-a-day, High Fiber, Uniformity

Metabolism and Nutrition: Enzymes, Feed Additives

M32 Growth performance and tibia ash responses of broiler chickens to phytate phosphorus levels and a novel consensus bacterial phytase variant doses during days 1 to 11 or 12 to 23 post hatching Olufemi Babatunde[*1], Abiodun Bello[2], Yueming Dersjant-Li[2], Olayiwola Adeola[1] *[1]Purdue University, [2]Dupont Animal Nutrition*

Two studies evaluated the effects of phytate P (PP) and a novel consensus bacterial phytase variant (PhyG) on growth performance and tibia ash of broilers during days 1 to 11 (trial 1) or 12 to 23 (trial 2) post hatch. In each of trial 1 and 2, diets included a nutrient-adequate positive control (PC) with 2.8 g PP/kg and negative control (NC) diets (diets reduced by 88 kcal/kg ME, 0.070% dig. Lys, 0.197% available P, 0.20% Ca and 0.048% Na vs PC diet). Broiler chicks were randomly assigned to 6 replicate cages (12 or 8 birds per cage in each trial 1 or 2) in a 3 × 5 + 1 factorial arrangement of treatments with 3 NC at 3 phytate levels [2.3, 2.8, or 3.3 g PP/kg for respective NC1, NC2, or NC3], 5 phytase levels (0, 500, 1,000, 2,000, or 4,000 FTU/kg) and one PC. In trial 2, birds were fed a common commercial diet from 0-11 days of age and test diets from 12 to 23 days of age. Birds fed the NC2 without phytase had lower ($P < 0.01$) body weight (BW), weight gain (WG), feed intake (FI), tibia ash, and a higher ($P < 0.01$) feed conversion ratio (FCR) as compared with birds fed the PC in each trial. With increasing PP levels, there was a linear decrease ($P < 0.01$) in BW by up to 4.5% in trial 1 or 4% in trial 2. There was a decrease ($P < 0.01$) in FI with increasing PP, but no effect on FCR and tibia ash in trial 1. In trial 2, increasing PP had no effect on FI or tibia ash but increased ($P < 0.01$) FCR of birds. Increasing phytase dose levels linearly improved ($P < 0.01$) WG of birds up to 28% in trial 1 or 26% in trial 2. Similarly, with increasing phytase doses, FI was linearly increased ($P < 0.01$) by

approximately 14 or 11% in trial 1 or 2, respectively. There was a linear decrease ($P < 0.01$) in FCR or a quadratic response ($P < 0.01$) with FCR of birds in phase 1 or 2, respectively, with the addition of phytase. There was a quadratic response ($P < 0.01$) with tibia ash of birds in trial 1 and 2 with increasing phytase dose levels. In conclusion, increasing PP concentrations had negative effects on the growth performance of broiler chickens. However, phytase supplementation mitigated this effect by improving P availability as observed with the growth performance and bone mineralization of broiler chickens in each trial, regardless of phytate levels.

Key Words: broiler chickens, growth performance, phosphorus, phytase, phytate

M33 Nutrient and energy utilization of broiler chickens in response to phytate phosphorus and phytase concentrations during the starter phase (day 1 to 11 post hatching) Olufemi Babatunde*[1GS], Abiodun Bello[2], Yueming Dersjant-Li[2], Olayiwola Adeola[1] [1]*Purdue University,* [2]*Dupont Animal Nutrition*

This study evaluated the nutrient and energy utilization of broilers fed diets containing varying phytate P (PP) levels and doses of a novel consensus bacterial phytase variant (PhyG) from day 1 to 11 post hatch. A total of 1,152 broiler chicks were randomly assigned to 16 dietary treatments arranged in a 3 × 5 + 1 factorial with 3 PP levels [2.3, 2.8, or 3.3 g/kg], 5 phytase doses (0, 500, 1,000, 2,000, or 4,000 FTU/kg) and one nutrient-adequate positive control at 2.8 g PP/kg (PC) diet in 6 replicate cages of 12 birds per cage. The negative control (NC) were diets reduced by 88 kcal/kg ME, 0.084% dig. Lys, 0.18% available P, 0.25 Ca, and 0.048% Na vs PC diet. Ileal digesta collected from birds on day 11 post hatching, and excreta collected during the last 3 days of the experimental period, were used to determine the apparent ileal digestibility (AID) and total tract retention (TTR) of nutrients, respectively. Birds fed the PC diet had greater ($P \leq 0.01$) AID of dry matter (DM), energy, P, Ca, Zn, AA and nitrogen corrected apparent metabolizable energy (AMEn) as compared with birds fed the NC2 diet without phytase. There was no interaction between PP and phytase levels in all responses. Increasing PP levels linearly decreased ($P < 0.01$) the AID of P and Ca by up to 23 and 22%, respectively, and elicited a quadratic response ($P < 0.05$) for the AID of Met, Thr, Trp, and Tyr. There were linear decreases ($P < 0.01$) in the TTR of P and Ca with increasing PP levels. With phytase supplementation, there were linear increases ($P < 0.05$) in the AID of DM, energy, nitrogen, P, Ca, and all amino acids in broiler chickens. However, there was no effect of phytase on the AID of Zn. Similarly, the TTR of P and Ca was increased ($P < 0.01$) by 62 and 86%, respectively, in broilers fed diets with added phytase at 4,000 FTU/kg as compared with birds fed NC without phytase. There was a linear improvement ($P < 0.05$) in the TTR of DM, N and Zn and a quadratic increase ($P = 0.05$) in the AMEn of birds fed diets with increasing phytase dose. In conclusion, increasing PP levels reduced utilization of nutrients in broilers while phytase supplementation improved the utilization of nutrients and energy in broiler chickens during the starter phase regardless of phytate levels.

Key Words: broiler chickens, nutrient digestibility, nutrient retention, phytase, phytate

M34 Effect of phytase level and form on broiler performance from 1 to 21 days of age J.B. Adkins*[1GS], K.M. Downs[2], J.P. Gulizia[1], M.S. Rueda[1], W.J. Pacheco[1] [1]*Auburn University,* [2]*Middle Tennessee State University*

Phytase supplementation in broiler diets reduces the amount of inorganic phosphorus sources needed to meet bird requirements by releasing bound phytate-P. This research evaluated the individual and combined effects of a coated and uncoated (heat stable) phytase on performance and bone mineralization in broilers. Two replicated studies were conducted using 240 day-old Cobb 500 male broilers per trial. For each trial, birds were assigned to 4 treatments with 4 replicate battery cage units per treatment (60 birds/trt) and grown for 21 d. Treatments included: (1) negative control (NC), (2) NC + 1000 phytase units (FTU) coated phytase (C), (3) NC + 1000 FTU uncoated phytase (U), and (4) NC + 500 FTU coated + 500 FTU uncoated phytase (CU). Body weight and feed intake were measured on d 14 and 21 to determine feed conversion ratio (FCR) and body weight gain (BWG). On d 21 for both trials, left tibias were collected (5/pen; 40/trt) for bone shear strength and tibia ash analysis. Data were analyzed using ANOVA and means were separated using Tukey's HSD. Body weight gain in birds fed C and U phytase forms were significantly different from NC (*P=0.012*). Combining phytase forms reduced BWG resulting in an insignificant difference from NC (*P>0.05*). When compared to NC, CU resulted in FCR differences at d 14 (CU=1.24, NC=1.29: *P=0.016*) and d 21 (CU=1.28, NC=1.34: *P=0.009*), while single phytase form inconsistently improved FCR compared to NC at d 14 (C=1.24: *P=0.016*, U=1.25: *P>0.05*) and at d 21 (C=1.30: *P>0.05*, U=1.28: *P=0.009*). No significant FCR difference was determined between CU and single form phytase sources. In both trials, no difference in mortality was observed. Phytase form did not affect bone shear strength (F_{break}) (C=410.1N, U=397.5N, CU=384.0N: *P>0.05*). Similarly, tibia ash did not differ between single and combined phytase forms (C=52.4%, U=52.1%, CU=52.6%: *P>0.05*). Bird performance was essentially unaffected by phytase form. Therefore, the results of this research suggest that combining phytase forms offers no distinct advantage to bird performance or bone mineralization.

Key Words: Phytase, Broilers, Tibia ash, Bone mineralization

M35 Effect of a commercial heat stable phytase with varying inclusion levels on broiler performance and tibia ash from 1 to 49 days of age J.P. Gulizia*[1GS], M.S. Rueda[1], F.K. Ovi[1], S.M. Bonilla[1], L.C. Smith[1], H.E. Reaves[1], M. Jackson[2], O. Gutierrez[2], W.J. Pacheco[1] [1]*Department of Poultry Science, Auburn University,* [2]*Huvepharma Inc.*

The majority of phosphorus (P) in plant-based feedstuffs is bound to phytate which renders it biologically unavailable to poultry. Phytate-P forms complexes with minerals and other nutrients leading to reduced broiler performance and nutrient utilization. The objective of this study was to evaluate the effect of OptiPhos Plus, which is an intrinsically heat stable form of phytase with varying inclusion levels on broiler performance from 1 to 49 d of age. A total of 1,200 d-old Ross x YPM 708 male broilers were randomly distributed in 40 floor pens assigned to 5 dietary treatments with 8 replicates per treatment. A positive control (PC) was formulated to contain 0.80% Ca and 0.40% avP (starter period 1 to 21 d), 0.76% Ca and 0.38% avP (grower period 21 to 35 d), and 0.70% Ca and 0.35% avP (finisher period 35 to 49 d). A negative control (NC) was formulated to contain 0.20% less Ca and avP compared to the PC at each feeding phase. Treatments consisted of a PC, NC, NC + 500, NC + 1000, or NC + 2000 FTU/kg of OptiPhos Plus. Feed intake and BW were determined at 21, 35, and 49 d of age and FCR corrected for mortality. On d 21, 4 birds/pen were euthanized and tibias were collected for % tibia ash determination. Data were analyzed using ANOVA procedure and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Broilers fed NC + 2000 FTU/kg of OptiPhos Plus had higher BW (2347 vs. 2211 g) and feed intake (3516 vs. 3349 g) than broilers fed the NC treatment at 35 days of age ($P<0.05$). BW also increased numerically with increasing phytase levels up to 2000 FTU/kg. At 21 days of age, broilers fed the NC without OptiPhos Plus inclusion had lower ($P<0.05$) tibia ash compared to the PC or NC 500, 1000, and 2000 FTU/kg of Optiphos Plus. Tibia ash increased numerically with increasing phytase levels. Overall, broiler performance improved with increasing levels of phytase and was significantly improved at 35 days with the addition of 2000 FTU/kg of OptiPhos Plus to the NC diet, and tibia ash was improved with Optiphos Plus inclusion.

Key Words: uncoated phytase, phosphorus, broilers, tibia ash

M36 Effect of phytase and cocci vaccines on broiler growth performance and bone mineralization Hanyi Shi*GS, Jinquan Wang, Woo Kyun Kim *University of Georgia*

An experiment was conducted to evaluate effect of phytase and cocci vaccines on growth performance and bone mineralization on broilers. The experiment was conducted in a 2 × 4 completely randomized factorial arrangement with 6 replicates per treatment and 10 birds each. Two factors were cocci vaccines and dietary treatments: 1) a positive control (PC; 0.90% Ca and 0.45% avP); 2) a negative control (NC; 0.75% Ca and 0.30% avP); 3) NC + 500 FTU/kg of phytase; 4) NC + 1500 FTU/kg of phytase. Data were analyzed using SAS by two-way ANOVA via GLM procedure. P<0.05 was considered as significant, and means were further separated using Duncan's Multiple Range Test. There were some interaction effects on growth performance. Unvaccinated birds fed NC diet showed lower (P=0.0403) BWG during d 0-21 period compared to unvaccinated PC birds, whereas supplementing 500 or 1500 FTU/kg phytase to unvaccinated birds reached to the same level as unvaccinated PC. Vaccinated birds fed PC, NC or 500 FTU/kg phytase diet showed significantly lower BWG compared to unvaccinated PC birds at d 21; however, supplementation of phytase at 1500 FTU/g to vaccinated birds improved BWG to the same level of unvaccinated PC group. During d 0-21 period, vaccinated birds supplementing 500 FTU/kg phytase in NC diet had lower (P=0.0473) FI compared to unvaccinated PC birds, whereas supplementing 1500 FTU/kg phytase in NC diet improved FI to the same level of PC. For bone ash, no interaction effect of phytase and cocci vaccines supplementation during d 21 was observed, but they both had a main effect. NC diet showed a negative effect on ash weight (P=0.0379), ash percentage (P<0.01) and ash concentration (P<0.01) at d 21 compared to PC, whereas supplementing phytase at 500 or 1500 FTU/kg in NC diet was able to improve these ash parameters to the same level as PC. In addition, vaccinated group showed a lower ash weight (P=0.0222), ash percentage (P=0.0215) and ash concentration (P=0.0142) compared to unvaccinated group at d 21. In conclusion, cocci vaccines decreased growth performance on broilers, whereas supplementing 1500 FTU/kg phytase mitigated the negative effects of growth performance. Supplementation of phytase at 500 or 1500 FTU/kg improved bone mineralization regardless of vaccination.

Key Words: phytase, cocci vaccines, broiler, growth performance, bone mineralization

M37 Effects of varying protein/amino acid level of diets with or without protease on 30-50 wk layer performance and egg production parameters Victoria Williams*1GS, Kelley Wamsley[1], Kevin Roberson[2], Pratima Adhikari[1] *[1]Mississippi State University, [2]CSA Animal Nutrition*

Exogenous protease supplementation may help to improve amino acid (AA) utilization in low crude protein (CP) diets. This study was conducted to determine the effects of reducing CP/AA in diets with or without a commercially available protease on performance and egg quality of laying hens in peak production. A 20-week study was conducted using 30 wk old 768 Hy-Line W-36 laying hens that were equally and randomly placed into one of 96 experimental units (raised-wire cages). There were 12 replications per treatment and the stocking density was 7.62 hens/m^2. Each replicate pen (8 hens) was fed one of 8 diets from the 4 AA level (100, 95, 90 and 85% of breeder recommendation) x 2 protease inclusion (0 or 0.012%). The adequate (100%) diet was based on corn and soybean meal and formulated on the basis of dig. Lys: dig. AAs (Met, Thr, Trp, TSAA, Ile and Val) to meet 100% of Hy-Line W-36 breed recommendation. Variations in CP/AA (95, 90, and 85% diets) were accomplished by reducing the 100% diet by 5, 10 and 15%, respectively. Data were subjected to statistical analyzes of variance (PROC GLM procedure, SAS 9.4). There was an AA levels x protease interaction for hen day egg production (HDEP; P=0.0120). Inclusion of protease in 100, 95, and 90% diets improved HDEP, whereas HDEP decreased at 85% AA + protease. Hens fed the 95% diet had the highest HDEP and those fed 85% diet without protease had the lowest HDEP. The average feed conversion ratio (FCR; kg feed/dozen eggs) was lower in 85% diets (1.57) compared to others (P=0.0019). Egg weight (EW) was significantly lower in hens that were fed the 85% AA diets (P<0.0001). Percent of defective eggs were significantly lower in hens fed 90% AA diet (P=0.0244) compared to all other AA levels. Throughout the experiment, there were no significant differences between any treatments for feed intake, Haugh unit, albumen weight, albumen %, yolk weight and yolk %. These data demonstrate that AA level impacts EW and % of unsaleable eggs, while inclusion of the tested protease does not. More research should be done to determine the practical application and economics of this protease inclusion. Future research can include investigating AA digestibility and feeding protease for a longer experimental period.

Key Words: amino acid, crude protein, egg weight, laying hen, protease

M38 Bacillus subtilis DSM 29784 improves broiler performance and welfare – a meta-analysis approach Ambre Caravati*1,2GS, Friedrich Rouffinau[3], Karoline Sidelmann Brinch[4], Adam Nelson[5], Lamya Rhayat[3], Estelle Devillard[3], Pascal Thiery[1], Amsa Timouni[2], Damien Prévéraud[1] *[1]Adisseo France SAS, Health by Nutrition, [2]Sup'Biotech, [3]Adisseo France SAS, Center of Excellence and Research in Nutrition, [4]Novozymes A/S, [5]Novozymes Biologicals, Inc.*

With the global objective to ban or reduce the use of AGP in poultry production, new alternatives such as probiotics increasingly come into consideration in the way of feeding animals. Animal welfare is another important concern for the poultry industry as well as a major consumer demand. The objective of the present study was to evaluate *Bacillus subtilis* DSM 29784 (BS27984) as an alternative to AGP. To that matter, broilers performance and welfare were assessed using a meta-analysis of experimental and field trials.

This meta-analysis involved 3 series of trials performed from 2015 to 2020. The first was based on 8 performance trials comparing the effect of AGP and BS27984 on 6,896 broilers. The second one is a compilation of 4 field trials where performance on 1,557,280 broilers was measured following the dietary inclusion of BS27984. Finally, the third one focused on welfare parameters (pododermatitis (FPD) and litter quality) scored on 347,360 broilers. After standardization of performance outcomes, statistical analyses were performed using multiple nonlinear regression.

Meta-analysis showed that BS27984 improved FCR by +1.76% relative to non-supplemented control, and was not significantly different from the AGP group (P>0.05). This improvement induced by BS29784 can be explained by an increase of BWG (+1.60%, P<0.001) without affecting the FI (-0.16%; P>0.05). Focusing on field conditions only, FCR and European Broiler Index were also enhanced by 2.38% and 3.14%, respectively, with the supplementation of BS27984. Finally, the probiotic supplementation resulted in an signifiicant improvement of litter quality and in a significant reduction of FPD scores compared to control birds.

BS29784 showed better performance due to an effect on intestinal health because the feed intake was not affected. BS27984 has been shown to enhance the host response and to modulate the microbiota leading to reinforce the intestinal barrier and controlling inflammation. Therefore, in controlled or field condition, BS27984 consistently improved the performance of the flocks associated with better animal welfare.

Key Words: bacillus subtilis, broiler, performance, welfare, meta-analysis

M39 Effects of a Direct Fed Microbial (DFM) on Broiler Chickens in Three Consecutive Experiments Exposed to Cyclic Heat Stress Albaraa Sarsour*1GS, Elizabeth Kim[2], Michael Persia[1] *[1]Virginia Tech, [2]DuPont Animal Nutrition*

Heat stress (HS) can be experienced within broiler production in the USA and this experiment was designed to determine the effects of a cyclic HS on broilers grown on built up litter over three consecutive flocks with and

without a direct fed microbial (DFM). Three consecutive experiments were conducted using built up litter to investigate the effects of a multi-strain DFM (3-strain Bacillus; Enviva ® PRO) fed to broiler chickens exposed heat stress from 28 to 35 d on broiler performance, cloacal temperature, ileal digestible energy (IDE), litter moisture and litter bacterial counts. Experiment treatments were set up as a 2 x 2 factorial with HS (6 hours at 33.3 to 35.0 C followed by 18 hours at 27.8 C from 28 to 35 d of age) and Thermoneutral (TN- 22.2 C continuously from 28 to 35 d) with or without the addition of DFM fed continuously throughout the experiment. In each experiment, 648 Ross 708 broiler chicks were assigned to 9 replicate pens of 18 broilers resulting in 162 broilers per treatment. Data were analyzed as a 2x2 factorial in JMP 14 using the ANOVA procedure ($P \leq 0.05$). Few interactions were noted overall, and only main effects are reported currently. As expected, cloacal temperature was increased ($P \leq 0.01$) with HS in all three experiments at both 28 and 35 d that correlated with a reduction in BWG from 28-35d ($P \leq 0.01$). The DFM did not improve BWG in the first experiment on clean pine shavings, however DFM treatment increased BWG ($P \leq 0.05$) in the second and third experiments on the built-up litter before HS was applied. Chick IDE was improved by DFM ($P \leq 0.01$) during the acute HS period at 28 d in all three experiments, however no effect ($P \leq 0.05$) was observed after 7 d of HS exposure. The treatment of broilers with DFM over two consecutive flocks resulted in a reduction of *E coli* in the litter ($P \leq 0.01$) in the second experiment when the chicks started on built up litter, but not when clean pine shavings were used. Overall, HS had a major impact on broiler performance and DFM treatment improved bird performance, IDE, and litter quality starting with the second flock that used built up litter.

Key Words: Heat stress, broiler, built up litter, digestibility

M40 Evaluation of Promitec essential oil products on a heat stress model that induce inflammation and gastrointestinal leakage in broiler chickens Jared Ruff*[1UG], Guillermo Tellez Jr.[1], Christine Vuong[1], Sami Dridi[1], Elizabeth Greene[1], Billy Hargis[1], Guillermo Tellez-Isaias[1], Blanca Martinez[2], Alvaro Uribe[2], Jaime Angel[2] *[1]Department of Poultry Science, University of Arkansas, [2]Promitec*

The objective of the present research was to assess dietary supplementation of three formulations of essential oils (EO) on performance, intestinal permeability, and bone strength during cyclic heat stress (HS) in broiler chickens. Day-of-hatch Cobb 500 male broiler chicks (n= 600) were obtained from a commercial hatchery. Upon arrival, all chickens were vaccinated with Coccivac®-B52, neck tagged, and randomly distributed into five groups. Group 1: Thermoneutral (TN) + control diet for starter, grower, and finisher with no antibiotics; Group 2: HS control + control diets; Group 3: HS + control diets supplemented with 37 ppm EO of Lippia origanoides (LO); Group 4: HS + control diets supplemented with 45 ppm LO + 45 ppm EO of Rosmarinus officinalis (RO) + 300 ppm red beetroot; Group 5: HS + 45 ppm LO + 45 ppm RO + 300 ppm natural betaine. Treatments were provided by Promitec Santander S.A. and included based on manufacturer's recommendations and analysis. Groups were allocated to twenty-four environmental rooms, five replicates per treatment for HS groups, and four replicates for the TN group, with 25 birds/replicate. The environment for TN chickens was established to simulate commercial production settings. For induction of HS, the heat stress treatment groups were exposed to cyclic HS, receiving 35°C for 12 h daily from day 7 to day 42. Performance and serum levels of fluorescein isothiocyanate-dextran (FITC-d), one hour after administration by oral gavage, were evaluated at d 21 and 42. Tibias were removed to assess breaking strength and total ash on d 21 and 42. A significant ($P < 0.05$) increase in chicken temperature was observed two h following the increase of environmental temperature in all HS groups, which persisted until the study's termination and was associated with decreased performance parameters compared with control TN chickens. At 21 and 42 d of age, chickens exposed to HS had impaired gut permeability and a significant reduction in bone mineralization compared to TN control chickens. However, all three EO treatments partially mitigated these adverse effects at statistically significant levels. These results suggest that the strategic use of some EO formulations during periods of stress, such as cyclic heat stress, could reduce adverse effects.

Key Words: chickens, enteric inflammation, heat stress, serum FIT-d, essential oils

M41 Phytogenomic and nutrigenomic effect of eucalyptus globulus labill. leaves extract in broilers Muhammad Kiwan Akram*[1GS], Saima Mahad[1], Anjum Khalique[1], Muhammad Ashraf[2], Sohail Ahmad[3], Usama Naeem[2] *[1]Department of Animal Nutrition, University of Veterinary and Animal Science, [2]Department of Pharmacology and Toxicology, University of Veterinary and Animal Science, [3]Department of Poultry Production, University of Veterinary and Animal Science*

Public health hazards and restrictions on subtherapeutic use of antibiotics as a growth promotors and other pharmaceutical enhancers in animal nutrition have opened avenues for the use of feed additives from natural origin, such as phytogenic substances. *Eucalyptus globulus* is the phytobiotics, which is reported to possess the antioxidant, antimicrobial and immune-modulatory properties. A total of 270 day old Cobb-*500* broilers were allocated to nine treatment groups having 3 replicates of 15 birds each. The dietary treatments consisted of un-supplemented corn-soya diet as negative control group, supplemented with antibiotic (0.5g Kg^{-1} Zinc bacitracin) and probiotic (1g Kg^{-1} Safmannan) as a positive control and three different levels (0.1%, 0.3% & 0.5%) of *Eucalyptus globulus* leaves extracts (EGLE). In-*vivo* genotoxicity, redox status and gut absorption were determined by COMET assay (genetic damage index GDI), 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity and d-xylose absorption test respectively, while in-*vitro* antioxidant activity was assessed by DPPH radical decomposition complimentary with total phenolic content (TPC), total flavonoid content (TFC) and total tannins percentage (TTP). EGLE had higher antioxidant activity in term of 50% inhibition concentration IC_{50} = 34.12 with TPC= 136.99mgGAg^{-1}, TFC= 229.85mgQEg^{-1} and TTP= 0.827 tannic acid equivalent. In comparison with the control groups, birds fed with the EGLE had higher serum DPPH radical decomposition ($P < 0.001$). The d-xylose absorption was higher in birds group fed with EGLE as comparison to control groups ($P \leq 0.05$). The least GDI was observed in birds fed with EGLE as compared to antibiotic and DMSO 20% (Positive control for GDI). Growth performance in terms of body weight, body weight gain, production number and feed conversion ratio were highly significant ($P < 0.01$) in birds fed with 0.3% EGLE compared to control groups. However, inclusion of 0.3% and 0.5% EGLE significantly improved redox status, gut absorption and growth performance in broilers with no DNA damage.

Key Words: Phytobiotics, gut absorption, In-vivo genotoxicity, Antioxidant, serum DPPH

SCAD

M42 Comparison of vaccination and medicated feeds for coccidiosis control in 0-6 week old turkeys Cameron Ellington*GS, Fernando Ruiz-Jimenez, Taylor Boyett, Rocio Crespo *North Carolina State University*

It has been reported that a full dose of commercial coccidia vaccine in turkeys is associated with mortality and poor performance. For this reason many producers deliver only partial vaccine doses. The goal of this study was to compare the effect of a full dose and a quarter dose of a commercial coccidia vaccine in Hybrid Converter female poults. .Two hundred and forty female poults were randomly allocated to one of 4 groups. Each group had 60 birds. Group 1 received a full dose of the vaccine at day of age. Group 2 received a quarter dose of the vaccine also at day of age. Groups 3 and 4 were received a medicated feed during the duration of the study. Group 3 was fed a lasalocid-medicated feed while group 4 was given zoalene-medicated feed. Weights, body temperatures, behavior and coccidia shedding were recorded. Results showed that following vaccination via oral gavage, coccidia in turkeys reaches peak shedding approximately 1 week after administration. The poults from the two vaccinated groups were overall less active compared to the two groups on medicated feeds and initially slower to gain weight. Interestingly Group 2 had higher oocyst counts, the birds were more depressed and their weight remained lower than the other 3 groups. Group 1 had average weights that were higher compared to one of the control groups by 5 weeks of age. No changes in rectal temperature were observed during the entirety of the study. In summary, vaccination with full dose is better for the performance of the turkeys. Further research is needed to clarify why a partial dose may be more harmful.

Key Words: coccidiosis, vaccination, turkeys, medicated feed, behavior

M43 Flow Cytometry-based enumeration and speciation of Eimerian coccidia affecting turkeys Taylor Boyett*GS, Javid Mohammed, Ravi Kulkarni, Rocio Crespo *North Carolina State University*

Coccidiosis is an economically important disease of poultry prevalent worldwide. The conventional method of identification, speciation, and enumeration of Eimeria oocysts in chickens and turkeys is manually done microscopically based on their size and shape. A fast, accurate and reliable diagnosis of Eimeria at the species level are both challenging and necessary. The objective of this study was to develop a non-antibody based diagnostic method for rapid, automated and objective speciation and quantification of Eimeria sp. in turkeys, using flow cytometry (FCM). Here, we employed FCM to identify and enumerate two species of Eimeria, E. adenoeides and E meleagrimitis, that are the most important coccidian species affecting turkeys (). Our preliminary work demonstrated that FCM can be used to identify E. adenoideas and E. meleagrimitis on their forward scatter (size) and side scatter (shape/granularity) patterns. The method was optimized by gating each of the two Eimeria species using a commercial vaccine as the reference using LSRII instrument. The gated populations were then sorted using MoFlo XDP cell sorter and the individual species identity was confirmed by PCR. Subsequent analysis was performed on various field samples to validate the method efficacy. The number of oocysts in the samples analyzed by FCM were verified using the manual microscopic counting McMaster modified method. The comparisons between the results of the conventional McMaster vs. FCM counting is currently underway. A key advantage of FCM is that it can be done quickly with larger sample numbers, enabling decisions to be made immediately, whereas other diagnostic methods are slower, labor-intensive, and subjective.

Key Words: Flow-cytometry, coccidia, eimeria, turkey

M44 Using Oxford Nanopore next generation sequencing technology to analyze ITS-1, 18S, and CO1 genes for identification of Eimeria parasites in poultry. Benjamin Jackwood*, Brian Jordan *University of Georgia*

Coccidiosis is a costly enteric disease of commercial poultry worldwide caused by a single-celled, parasitic protozoa of the *Eimeria* genus. The parasite reproduces rapidly both sexually and asexually causing significant damage to intestinal linings, and often mortality. The most common method for the identification and differentiation of *Eimeria* species is classical microscopy using morphometric characteristics (size, shape). While a simple technique, microscopy can be subjective, and the sensitivity is not ideal. Other identification methods, including parasite life-cycle features such as region of the gut parasitized, are subjective and debated. As molecular tools advance, using PCR may afford the opportunity to further characterize *Eimeria* samples past simple species differentiation. Therefore, the overall goal of this research is to produce high quality profiles from sequence data to promptly identify *Eimeria* strains. Three different genome regions have previously been used to differentiate species of coccidian on a molecular level; Internal Transcribed Spacer-1 (ITS1), Ribosomal 18s DNA (18S), and Cytochrome Oxidase 1 (CO1). *Pan-Eimeria* primers have been developed for these three gene regions, and provide a method to amplify all the sequences present in a mixed sample. Our laboratory is following these PCR reactions with Oxford Nanopore's Mk1B (MinIon) next generation sequencing (NGS) technology. The MinIon platform allows our laboratory to sequence samples as they are prepared, and the sequencing and bioinformatics can be performed in house. For all vaccines, all species predicted to be present (per manufacturer label) were identified for each gene target. These results show the applicability of the Minion system for differentiating *Eimeria* species in a mixed sample, and for the potential in diagnostic use.

Key Words: Eimeria, Coccidiosis, Sequencing, Oxford Nanopore, MinION

M45 Using FIJI Image J as a tool for counting and speciating Eimeria oocysts Fernando Ruiz-Jimenez*GS, Oscar Fletcher, Sesny Gall, Rocio Crespo *Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University*

Coccidiosis is one of the most common and expensive diseases of intensive poultry production worldwide. In past years, control was mainly focused on continuous medication with low levels of anticoccidial drugs, but due to the recent demand for birds raised without antibiotics (ABF), other methods of control such as vaccination have gained attention. The McMaster fecal egg counting technique is routinely used to monitor coccidia infestations in poultry flocks since it allows to estimate the number of oocysts in a gram of feces (OPG) and to speciate them according to their size. OPGs are critical to monitor the effectiveness and uniformity of coccidia vaccination and allow for the detection of issues in an early manner. OPGs can be tedious and time-consuming, which leads to an increased number of errors during the counting and speciation of oocysts. Therefore, the objective of this study is to introduce FIJI Image J, an open-source image processing program developed at the NIH, as a tool to semi-automate the OPG technique. The step-by-step process will be described and comparisons between the results of traditional vs. semi-automatic counting will be shown. Overall, FIJI Image J facilitated the OPG readings and offered comparable results to the traditional analysis, allowing to get accurate counting and speciation of coccidia oocysts in shorter amounts of time.

Key Words: coccidia, OPG, counting, speciation, oocysts

M46 Flow Cytometry as a tool for enumeration and speciation of chicken Eimeria oocysts Daniel Adams*GS, Raveendra Kulkarni, Javid Mohammed, Rocio Crespo *North Carolina State University, College of Veterinary Medicine, Department of Population Health and Pathobiology*

Coccidiosis is an apicomplexan parasitic disease that causes severe economic losses to the poultry industry worldwide through decreased growth performance, increased mortality, and treatment. The severity of coccidiosis infections widely varies based on the species of Eimeria present and the number of oocysts ingested by the bird; therefore, An accurate and speedy diagnosis of Eimeria at the species level are both challenging and necessary. In our previous research, we demonstrated that flow cytometry (FCM) can be used to identify different species of Eimeria, based on their forward scatter (size) and side scatter (shape/granularity) patterns. The objective of this study was to develop a non-antibody based diagnostic method for rapid, automated and objective speciation and quantification of Eimeria sp. in poultry, using FCM. To quantify the oocysts, a known amount of counting beads were added to the suspension. FCM method optimization was achieved by gating each of the four Eimeria species using a commercial vaccine as the reference using LSRII instrument. The gated populations were then sorted using MoFlo cell sorter and the individual species identity was confirmed by real-time PCR. Subsequent analysis was performed on various field samples to validate the method efficacy. The number of oocysts in the samples analyzed by FCM were verified using the McMaster modified method. The comparisons between the results of the traditional McMaster vs. FCM counting will be shown. A key advantage of FCM is that it can be done quickly, enabling decisions to be made immediately, whereas other diagnostic methods are slower, labor-intensive, and subjective.

Key Words: Coccidiosis, Broiler, Flow Cytometry, Eimeria, parasitology

M47 Fenbendazole resistance in Heterakis gallinarum, the vector of Histomonas meleagridis James Collins*1GS, Brian Jordan2, Andrew Bishop3, Ray Kaplan1 *[1]University of Georgia, Department Of Infectious Diseases, [2]University of Georgia, Department of Population Health and Department of Poultry Science, [3]Amick Farms*

Surveys show that *Heterakis gallinarum* is the most common nematode of chickens, and more importantly, the vector of *Histomonas meleagridis*, a protozoan parasite that causes blackhead disease. *Histomonas* is typically more pathogenic in turkeys, leading to high mortality, and while disease caused by *Histomonas* is less severe in chickens, it is known to reduce productivity. With no current treatments approved for *Histomonas*, control of *Heterakis* is the only means to reduce infections with *Histomonas*. Currently in the US, fenbendazole (FBZ) is the only drug labeled for treatment of *Heterakis*. Recently, we demonstrated resistance to FBZ in *Ascaridia dissimilis*, a related nematode of turkeys. Through collaboration with an industry veterinarian, we identified a farm with potential resistance and performed a controlled efficacy study to determine if the *Heterakis* on that farm were indeed resistant to FBZ. Treatment groups included a control, label, and a 2x dosage. 36 birds per group were placed at 1-day post hatch and were infected with 150 embryonated eggs via oral gavage at 3 weeks of age. Two weeks post infection treated birds were administered a minimum of either a label or 2X dose of FBZ in water for 5 days (SafeGuard® Aquasol, 1mg/kg BW). To ensure that all birds consumed the full intended dose at a minimum, the dosage was calculated using 1.25 times the average body weight. One-week post treatment, birds were euthanized, ceca removed, and parasites enumerated. Efficacy was calculated by comparing the total numbers of worms recovered from each treatment group to the numbers recovered in the non-treated control group. *Heterakis* worm counts were reduced by 33.96% and 36.63%, for the label and 2X dosages, respectively. The poor efficacy of FBZ against Heterakis, and the fact that efficacy was no better in the 2X dose group, provide strong evidence that like *Ascaridia*, *Heterakis* has also developed resistance for FBZ. Consequently, in houses infected with FBZ-resistant *Heterakis*, *Histomonas* will be able to cycle through the birds in an unrestricted manner. Further investigation is needed to determine the prevalence of resistance in *Heterakis* on chicken farms, but it is clear this has the potential to have a large-scale economic impact on the poultry industry.

Key Words: Heterakis, Fenbendazole, Resistance, Histomonas, Blackhead

M48 Avian Pathogenic Escherichia coli O Serogroup Epidemiology for Poultry Health Nicolle Barbieri*, Aline de Oliveira, Caleb Austin, Robert Pasko, Lisa Nolan, Catherine Logue *University of Georgia*

Colibacillosis caused by Avian Pathogenic *E. coli* (APEC) is a costly disease for the poultry industry worldwide, significantly affecting one of the world's cheapest sources of high-quality protein, resulting in mortality at the farm and carcass condemnation at slaughter. For years, geographic variation in APEC distribution has been known, with the most common *E. coli* serogroups representing >90% of characterized APEC which includes serogroups O1, O2, and O78. Currently, commercial vaccines are available specifically targeting these serogroups. Here, we assessed the use of an epidemiological based analysis to investigate emerging O serogroups that may be contributing to poultry disease.

Our research group has developed a PCR-based-assay that identifies the 13 most frequently found O serogroups (including O1, O2, O8, O18, O24, O25, O44, O78, O86, O88, O103, O117 O119, and O186). We found that among 72.2 % of isolates from diagnostic APEC, the most prevalent serogroups identified included O78, O24, O2, and O25 with a prevalence of 22.8, 14.3, 10, and 8.6% respectively. We also found that 25.3 % of the fecal isolates were positive for one of these 13 serogroups tested, with serogroups O8, O44, O18, and O186 (5.8, 5.6, 4.5 and 3.0% prevalence) being the most common among fecal isolates collected.

Epidemiological analysis of our data has allowed us to identify new emerging serogroups, such as O25 and O24 that were not previously associated with APEC. Interestingly we found O8 and O18 serogroups in fecal chicken isolates that are common serogroups found in *E. coli* associated with human urinary tract disease. Also, when we analyze the O types present in a production barn during grow out, we found there was a fluctuation in the O type prevalence, suggesting that *E. coli* population in the gut changes over time.

With better knowledge of the populations of microbiota present, we can better predict vulnerable flocks and identify strategies for colibacillosis control at the farm level. Having a better knowledge of the current APEC causing disease we can identify new potential targets for vaccine development which could potentially reduce losses for producers.

Key Words: APEC, Colibacillosis, O serogroups, escherichia coli

M49 Type 6 Secretion System plays a role in the virulence of Avian Pathogenic Escherichia coli (APEC) Aline de Oliveira*GS, Darby Newman, Nicolle Barbieri, Catherine Logue *University of Georgia*

Avian pathogenic *Escherichia coli* (APEC) causes extra-intestinal infections in production birds, known as colibacillosis, that can manifest as localized or systemic infections. Colibacillosis is one of the most common and costly diseases, impacting all facets of the poultry industry and causing heavy economic losses. Despite the identification of several pathogenicity determinants in APEC, the role of protein secretion in their pathogenicity remains undefined. The Type 6 Secretion System (T6SS) functions as a molecular syringe that secretes proteins into the external milieu or directly into the host cells. T6SS is associated with virulence mechanisms used by several bacterial pathogens. However, knowledge on the role of T6SS in APEC pathogenesis is limited. Here, we aim to elucidate the role of T6SS in APEC pathogenesis.

We have screened a collection of 454 APEC, 106 avian fecal isolates (AFEC) and 102 litter *E. coli* isolates for five T6SS1 genes including ef-

fector *hcp*, and uncharacterized structural components, *vasK*, *impG*, *evpB* and *impK*. Results show a significantly higher prevalence of these genes in APEC (6-35%) when compared to avian fecal (5-14%) and litter (1-11%) isolates, providing evidence that the T6SS contributes to virulence in APEC. To further characterize T6SS in APEC, we have created deletion mutants for *hcp, evpB*, and *impK* in APECO18. These mutants were assessed for biofilm formation, adhesion and invasion to cell lines, and resistance to predation by the social amoeba *Dictyostelium discoideum*. Expression assays to determine if T6SS genes are overexpressed in contact with host cells are ongoing. Deletion of *hcp, evpB*, and *impK* did not seem to have affected biofilm formation and resistance to predation by *D. discoideum*. However, deletion of *hcp*, which encodes an effector secreted by T6SS, affects the ability of APECO18 to adhere to and invade chicken fibroblasts DF-1. These findings suggest that the T6SS is a virulence factor that is involved in the interaction of APEC with its host.

Key Words: APEC, T6SS, pathogenicity

Metabolism and Nutrition: General Nutrition

M50 Effects of different proprietary feed brands on physical attributes of eggs from hens at the late laying phase in days of storage
Ireti Oludoyi*GS, Olugbenga Ogunwole *University of Ibadan*

There are several feed brands in the Nigerian poultry industry with each proprietor claiming products of similar or superior standard. The quality of feed fed to hens directly influence laid egg quality. Thus, effects of feed brands and duration of egg storage on physical attributes of eggs from hens at the late laying phase were assessed. Lohmann Brown layers (n=540) aged 59-week, were randomly allotted to six feed brands-A, B, C, D, E and F. Each treatment was replicated ten times and the hens were fed for 15 weeks. At week 74, 360 eggs (60/treatment, same day of lay) were randomly sampled and stored in ambient environment (temperature between 24 and 29°C and relative humidity of 73-84%). Egg physical characteristics were determined on 60 (one/replicate) eggs each at 0, 7, 14, 21 and 28 days of storage. The experimental design was a 6x5 factorial arrangement in a completely randomized design. Results observed showed no significant effect (p>0.05) of feed brands on egg weight, length, width and yolk diameter. Eggs of hens on B had higher yolk weight (16.11g) and deeper yolk color while those on brand A had significantly thicker (p<0.05) eggshell among the group. Hens fed C(77.69) and F(77.50) produced eggs with significantly higher (p<0.05) Haugh unit than E (75.07) while those laid by hens on A, B and D were similar (p>0.05). Egg weight, width and albumen weight reduced with increased egg storage duration. Only egg length was not altered by the egg storage duration. Effects of interaction of feed brand and egg storage duration on external and internal egg attributes was significant (p<0.05). Therefore, egg physical quality characteristics varied with the different proprietary feed brand in duration of storage.

Key Words: feed brands, physical quality, late laying phase, egg storage, hens

M51 Evaluation of particle size, feed form and pellet diameter on performance and nutrient digestibility of broilers at 39 d of age
Susan Bonilla*GS, Martha Rueda, Cleison de Souza, Jessica Starkey, Charles Starkey, Wilmer Pacheco *Department of Poultry Science, Auburn University*

The objective of this study was to evaluate the effect of corn particle size and feed form on broiler performance and nutrient digestibility from 1 to 39 d of age. A total of 1800 d old male Cobb 500 broilers were randomly distributed among 9 dietary treatments with 8 replicates per treatment and 25 birds/pen. The experiment consisted of a 3 × 3 factorial arrangement of 3 corn particle sizes (750, 1150 and 1550 µm) and 3 feed forms (mash, 3- and 4-mm pellets) provided from 1 to 39 d. Pelleted treatments were offered as crumbles from 1 to 17 d. Titanium dioxide (TiO2) was added as an indigestible marker (0.5%) from 27 to 39 d of age to determine nutrient digestibility. Feed intake and BW were determined at 17, 27 and 39 d of age and FCR corrected for mortality. Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Broilers fed 3- and 4-mm pellets had higher feed intake, BW and lower FCR (P<0.05) than broilers fed mash diets at 39 d of age. Broilers fed diets with 750 µm corn particle size had higher (P<0.05) BW than broilers fed diets with 1550 µm, but similar to broiler fed diets with 1150 µm. Corn particle size did not influence FCR at 39 d of age (P>0.05). Broilers fed 3- and 4-mm pellets had higher (P<0.05) apparent ileal digestibility (AID) of protein, energy and fat than broilers feed mash diets. Significant interactions were observed between feed form × corn particle size on AID of protein, energy, and fat. Corn particle size didn't influence AID of protein and energy when diets were fed as mash or 4 mm pellets; however, when broilers were fed 3 mm pellets, AID of protein and energy increased (P<0.05) as corn particle size increased from 750 to 1550 µm. Furthermore, AID of fat increased (P<0.05) in mash diets when corn particle size was reduced from 1550 and 1150 to 750 µm; however, in broilers fed pelleted diets AID of fat was unaffected by corn particle size. The results of this study demonstrated that pelleting improves broiler performance and nutrient digestibility and the optimum corn particle size is influenced by feed form.

Key Words: Pellet diameter, Cobb, nutrient digestibility

M52 Effect of conditioning temperature and pellet die compression ratio on pellet durability index
L.C. Smith*1UG, S.M. Bonilla[1], J.P. Gulizia[1], M.S. Rueda[1], F.K. Ovi[1], J. Escobar[2], J. Froetschner[2], W.J. Pacheco[1] *[1]Department of Poultry Science, Auburn University, [2]Elanco Animal Health*

Pellet quality influences performance and nutrient utilization of broilers and it is typically measured using either the ASABE method S269.5 or the Holmen tester. The objective of this study was to evaluate the effect of conditioning temperature and compression ratio (L/D), which is the relationship between die hole diameter and pellet die thickness on pellet quality. The experiment consisted of a 3 × 2 factorial arrangement of 3 conditioning temperatures (60, 75 and 90°C) and 2 L/D ratios (6 and 10). A corn-soybean meal based with 2.4% soybean oil addition was mixed in a twin shaft mixer and then steam conditioned and pelleted using a constant production rate of 0.7 ton/h with a 50-horsepower pellet mill. Two pellet dies with the same die whole diameter (3.175 mm), but with different die thickness (19.05 and 31.75 mm) were used in this trial. Pellet samples were collected throughout the run and immediately cooled. The following day pellet durability index (PDI) was analyzed using the ASABE method S269.5 with the addition of 5 hex-nuts to simulate pellet breakage during handling and transportation and with the Holmen NHP 100 (TekPro Ltd, Norfolk, UK) for 60 seconds. Data were statistically evaluated using the GLM procedure of JMP and means were separated by Tukey's HSD with statistical significance considered at $P \leq 0.05$. Increasing conditioning temperature improved (P<0.05) PDI when it was measured with both methodologies. In addition, increasing L/D ratio increased PDI (P<0.05) from 84.76 to 89.70% and 32.89 to 51.21% when measured with the ASABE method S269.5 and Holmen NHP 100 tester, respectively. Significant interactions were observed between conditioning temperature and L/D ratio on PDI (P<0.05) for both methodologies. Increasing conditioning temperature increased PDI (P<0.05) regardless of the L/D ratio, however the highest increase in PDI was observed when conditioning temperature increased from 75 to 90°C compared to 60 to 75°C particularly when diets were pelleted using a pellet die with a L/D ratio of 10. The results of this study demonstrated that increasing condi-

tioning temperature and L/D ratio improves pellet durability of corn-SBM diets, which can have a positive effect on broiler growth performance.

Key Words: Pellet quality, Conditioning temperature, Compression ratio

M53 Effects of acid-binding capacity of feed ingredients, water alkalinity and water acidification on nutrient digestibility and performance of broilers. Kyle Venter*[1,2GS], Thobela Nkukwana[1], Clara Angel[3], Peter Plumstead[2] [1]*University of Pretoria,* [2]*Chemunique International (Pty) Ltd,* [3]*University of Maryland*

Acid binding capacity (ABC) of a diet is the amount of hydrochloric acid in milliequivalents (mEq) required to lower the pH of 1kg of a feedstuff to pH 4.0. Each feed ingredient therefore contributes to the ABC of a complete diet and has the potential to alter gastric pH and digestive process. In a similar manner, water alkalinity is the capacity of water to resist acidification. This study objective was to evaluate effects of feed ABC, water alkalinity and water acidification on crude protein (CP), dry matter (DM) digestibility. The design was a 2x2x2 factorial with High or Low ABC diets; two sources of water with High or Low alkalinity; without, or with addition of a water acidifier. ABC of individual feed ingredients was determined prior to pre-starter (0-10d) and starter diets (11-21d) formulation. Diets met or exceeded commercial breed specifications but had High (862.09 mEq and 807.90 mEq) or Low (762.59 mEq and 731.34 mEq) ABC in pre-starter, and starter phases, respectively. Two sources of well water were identified as having an initial pH of 8.2 and 8.5, and alkalinity of 84 mg/L and 416 mg/L. Half of each water source was acidified until a pH of 4.0 was achieved. Male Ross 308 broiler chicks were placed in battery cages and treatments (Trt) assigned to 12 replicates of 12 birds/Trt. At 7 d and 21d; 6 birds/cage were euthanized, digesta from the terminal ileum collected, freeze dried, and DM and CP digestibility determined using TiO_2 as an indigestible marker. All data were analyzed using the Mixed procedure (SAS, 9.4). There was an effect of age (P<0.05) with respective CP and DM digestibility, being 79.16% and 64.13% at 7d and 82.08% and 68.85% at 21d. No effects (P>0.05) of water alkalinity or water acidification or two and three-way interactions of diet ABC, water alkalinity and acidification were observed. Diet ABC altered DM and CP digestibility at 7 and 21d (P<0.01). At 7d, birds fed High ABC diets had lower CP (78.4% vs. 79.88%) and DM digestibility (62.7% vs. 65.56%) vs those fed Low ABC diets. At 21d, CP digestibility was 81.39% and 82.78% for the High and Low ABC diets, while respective DM digestibility was 68.19% and 69.51%. These data suggest diet ABC, but not water alkalinity or acidification altered CP and DM digestibility in broilers.

Key Words: broiler, acid-binding capacity, alkalinity, digestibility

M54 The effects of maternal fish oil supplementation on offspring-broiler growth performance, body composition and bone microstructure at market age. Yuguo Tompkins*[1GS], Chongxiao Chen[1], Jeanna Wilson[1], Brynn Voy[2], Woo Kim[1] [1]*University of Georgia,* [2]*University of Tennessee*

Maternal supplement of fish oil has been shown to benefit the growth and development of offspring in humans and mice. In order to evaluate the effect of maternal dietary fish oil on growth performance, body composition, and bone quality in market-age broilers, breeder hens were fed the experimental diets containing 2.3 % soybean oil (SO) or fish oil (FO) for 4 wk. Fertile eggs were collected and incubated under standard conditions. A total of 240 one-day-old chicks from different maternal diet groups (SO group and FO group) were selected and allocated in 12 floor-pen and raised for 42 d. Growth performance was recorded at d 1, 14, 28 and 42. On d 42, 3 birds per pen were randomly selected for body composition measurement by a Dual Energy X-ray Densitometry (DEXA), and the femurs were collected for bone microarchitectural analyses by micro-CT. Bone marrow was collected to measure the expression of key osteogenic and adipogenic genes by quantitative real-time PCR Analysis. One-way ANOVA was performed, and means were compared by student's t-test (P<0.05).

The Maternal FO diet significantly improved the growth performance in offspring broilers at the finisher stage (p<0.05) but did not alter the feed conversion ratio. A higher percentage of body fat (p<0.05) was detected in the FO group by DEXA at d 1, but a higher percentage of lean mass was detected in the FO group at d 42 (p<0.05). The FO group had significantly lower cortical porosity percentage and pore volume (p<0.05) by microarchitectural analyses, and cortical bone mineral density was relatively higher (P=0.054). Moreover, maternal FO supplementation suppressed the expression of adipogenesis-related genes PPARγ, FABP4 and C/EBPβ, but did not significantly affect osteogenesis gene expression in the bone marrow. Cortical porosity and bone marrow adiposity are determinants of bone strength and mechanical competence that have adverse effects on bone quality. Based on those findings, the maternal fish oil intake not only benefits body composition in offspring broilers, but also decreased adipogenesis in the bone marrow that coupled with lower porosity on the cortical bone which indicated an improvement in bone quality.

Key Words: n-3 PUFA, maternal fish oil, body composition, market age broilers, bone health

M55 Effect of almond hulls on the growth performance, body composition, digestive tract weight and liver antioxidant capacity of broilers Jinquan Wang*[1GS], Caimei Wu[2], Fanbin Kong[1], Woo Kim[1] [1]*University of Georgia,* [2]*Sichuan Agricultural University*

The objective of this study was to evaluate the effect of graded increasing inclusion of almond hulls on growth performance, body composition, digestive tract weight and liver antioxidant capacity of broilers. A total of 420 one-day-old Cobb male chicks were randomly placed to five experimental treatments with six replicates of 14 birds each. Five treatments consisted of a corn-soybean meal control diet and four almond hull treatments containing 2.5, 5, 7.5 and 10% of almond hulls with 97.5, 95, 92.5 and 90% of control diet, respectively. Data were analyzed using one-way ANOVA model for a completely randomized design using the GLM procedure of SAS 9.4. Significant level was set at $P < 0.05$. Trend was taken at $0.05 < P < 0.10$. Feeding almond hulls at 10% reduced broiler body weight gain ($P \leq 0.048$) and increased FCR ($P < 0.01$) at d 0-7 and 0-14 period compared to the control group. Inclusion of almond hulls showed a quadratic effect ($P = 0.044$) on bodyweight gain and linear effect ($P = 0.045$) on FCR during d 0-19 of age. The numerically highest bodyweight gain was achieved when broilers fed the diet containing 5% of almond hulls at d 19 of age. As almond hull inclusion level increased, a linear ($P = 0.035$) effect was observed on the relative whole intestine weight, whereas a quadratic ($P = 0.086$) trend was observed on the relative gizzard weight at 19 d of age. Inclusion of almond hulls showed a quadratic ($P \leq 0.011$) effect on broiler body fat weight and fat percentage. Broilers fed diet containing almond hulls at 5% had a lower body fat weight ($P = 0.011$) and fat percentage ($P < 0.01$) compared to the control group. Inclusion of almond hulls also showed a quadratic ($P = 0.075$) trend on broiler body mineral content. Hepatic superoxidative dismutase was linearly ($P < 0.01$) increased as the increasing level of almond hulls in the diet. In summary, inclusion of almond hull showed a beneficial effect in intestinal development and liver antioxidant capacity. Moreover, broilers fed the diet containing 5% almond hull showed an optimum bodyweight gain with lower body fat weight and fat percentage, whereas up to 7.5% inclusion of almond hulls did not show any negative effects on growth performance.

Key Words: Almond hull, Broiler, Performance, Body composition, Antioxidant capacity

M56 Effects of pellet quality to on-farm nutrient segregation in broiler houses varying in feed line length Courtney Poholsky*[1GS], Daniel Hofstetter[1], Dariush Khezrimotlagh[2], John Boney[1] [1]*Penn State University,* [2]*Penn State Harrisburg*

Literature suggests that improving pellet quality (PQ) improves broiler production efficiency. The hurdles of feed manufacturing coupled with throughput demands often supersede the use of manufacturing techniques that improve PQ. Therefore, additional PQ benefits must be presented. The objectives of this study were to understand how pellets and fines segregate in feed lines differing in length and to measure amino acid (AA) and phytase segregation throughout commercial broiler houses. Four experiments (exp) were conducted and each exp included four replicate feed lines conceptually segmented into 8 regions, creating 32 systematic experimental units. A commercially formulated broiler finisher diet was manufactured using techniques to create either poor pellet quality (PPQ) or improved pellet quality (IPQ) feeds. Exp1 and exp2 were carried in out in 152-m houses containing four feed lines equipped with 192 feed pans per line. PPQ feed replicates were augered through house 1 while IPQ feed replicates were augered through house 2. Exp3 and exp4 were carried out in a 76-m house containing four feed lines with 95 feed pans per line. PPQ feed was augered through the four feed lines, samples were collected, and then the IPQ feed was handled in a similar manner. A total of 2,296 feed samples were collected across the four exp and were transported to Penn State University for pellet-to-fine ratio (P:F) determination. Pellets and fines were collected separately from the eight aforementioned regions per feed line in each exp and were used to determine P:F, pellet durability, AA concentration, and phytase activity. The feed metrics for each exp were evaluated using separate one-way ANOVA analyses. Student's t-tests were used to compare PQ means and feed line length means. Segregation of AA and phytase was apparent in exp1, demonstrated by varying concentrations of AA and phytase activity in the eight regions of the feed line ($P<0.05$). Phytase segregation was not apparent in exp2 ($P>0.05$). Threonine and phytase segregation occurred in exp3 ($P<0.05$). No evidence of nutrient segregation was observed in the feed lines for exp4 ($P>0.05$). These data suggest that investing in PQ improvements provides a more uniform distribution of nutrients throughout the house.

Key Words: pellet quality, nutrient segregation, amino acid, phytase

M57 Evaluation of different pellet durability index (PDI) methods on extruded feed Jorge Sandoval*[UG], Gerardo Abascal-Ponciano, Charles Starkey *Auburn University Poultry Science Department*

One of the most important physical factors to assess in thermally processed feed is the durability or ability of the pellet to withstand transport and handling without breaking. Durability is determined by subjecting the feed to a standardized durability test and is expressed by means of the pellet durability index (PDI), which is the percentage of a mass of pellets remaining intact with respect to the total mass of pellets. The objective of the study was to compare 2 variations of 2 methods commonly used to assess durability of feed (PDI). The Holmen Tester HNP100 (HT) circulates feed in an air stream at 75 PSI for the selected time, causing the pellets to collide with each other and the perforated surfaces of the test chamber causing them to break. For the HT method, 7 100-g replicate samples were analyzed for each tumbling time cycle (90 and 120 s). Another commonly used PDI method is the Tumbling Box (Seedburo Pellet Durability Tester; TB) method. For the TB method, 3 500-g replicate feed samples were analyzed with 5 19-mm hex nuts (HN) in the dust-tight enclosure to increase pellet destruction, while 3 replicate feed samples were analyzed without HN. All the samples were tumbled at 50 rpm for 10 minutes, collected, sieved using a 2-mm opening sieve, and weighed to estimate PDI. Data were subjected to ANOVA using SAS PROC GLIMMIX and multiple mean comparisons were performed using the PDIFF option at $P \leq 0.05$. No differences in PDI values were observed among HT and TB methods ($P = 0.8397$). However, feed samples analyzed using the HT with a 90 s cycle had higher PDI than those with a 120 s cycle ($P = 0.0003$). As expected, inclusion of HN in the TB method reduced the PDI compared with exclusion of HN ($P = 0.0019$). In conclusion, cycle time affects the PDI estimates when evaluating feed durability on the HT. The addition of HN when tumbling feed samples leads to lower PDI estimates when performing the TB method. While both methods and their variations are acceptable for use, it is important to consistently and correctly employ one procedure to ensure generation of PDI data suitable for comparison.

Key Words: Holmen Tester, Tumbling Box, pellet durability index, extruded feed

Metabolism and Nutrition: Vitamins and Minerals

M58 Effect of folic acid on the innate immune antiviral response in chicken fibroblast infected with IBDV Santiago Uribe-Diaz*[1GS], Alexander Yitbarek[2], Nauman Nazeer[1], Julian reyes[3], Marya Ahmed[1], Juan Rodriguez-Lecompte[1] [1]*University of Prince Edward Island,* [2]*University of Guelph,* [3]*Biogenesis Research Group, University of Antioquia*

Folic acid (FA) plays a role during cellular replication and also as immunomodulator. We aimed to evaluate the FA supplementation effect on 13 genes on chicken's fibroblast during an IBDV infection. DF-1 cells at 5x105 cells / well were supplemented with 3.96 mM FA by duplicates in 24 well plates overnight. Then, cells were infected with live IBDV vaccine at MOI: 1; the experiment was repeated three times. The expression of TLR-3, TLR-4, MDA5, TRIF, MyD88, IL-6, IL-12, IRF7, IFN-ß, OAS, PKR, Viperin, and RFC and IBDV-VP2 was evaluated by qRT-PCR at 0, 3, 6, 12, 24, and 36 hours post-infection. Statistical analysis used included a Proc mixed procedure of SAS, CT values, FA level, one level of MOI, and 6-time points. Results: Virus load at 6, 12, 24, and 36 hours was increased ($P<0.05$) in the presence of FA; Virus peak was reached at 24 hpi in both groups, followed by a significant decrease. FA upregulated ($P<0.05$) TRIF and MyD88 at 3, 12, and 24 hpi, with a very similar expression behavior for both molecules, reaching their peaks at six hpi and then rapidly decreasing in the non-supplemented group. TLR-3 was downregulated ($P<0.05$) at all sampling points consistent to viral load peaks; TLR4 and MDA5 expression were upregulated ($P<0.05$) at 12 and 24 hpi in the FA group, indicating their role as IBDV sensors. Cytokines IL-6 and IL-12 were upregulated ($P<0.05$) in the FA group at 12, 24, and 36 hpi associated with the viral load and the effect of FA on MDA5 expression. The IRF-7 had the same expression behavior of MDA5, IL-6, and IL-12, implying the upregulation through the same viral sensors. IFN-ß, OAS, and Viperin were upregulated ($P<0.05$), same as MDA5 expression pattern. However, PKR expression was downregulated. In conclusion, FA-treated chicken fibroblasts infected with IBDV increase FA cellular requirements, increasing the RFC demand and expression. FA supplementation can activate the innate antiviral immune response through the upregulation of TLR-4, MyD88, MDA5, proinflammatory cytokines IL-6 and IL-12, the antiviral cascade IRF7 and interferon-inducible antiviral effectors, OAS, Viperin, and PKR. Our results are inferring FA supplementation has an immunomodulatory effect in the presence of a modified live IBDV vaccine strain.

Key Words: IBDV, Fibroblast, Folic Acid, Innate immunity, gene

M59 Effects of the in ovo and dietary administration of L-ascorbic acid (L-AA) on the concentrations of L-AA in the eyes and nitric oxide in the serum of Ross 708 broilers Ayoub Mousstaaid*[1GS], William Miller[2], Katie Elliott[1], Saman Fatemi[1], Abdulmohsen Alqhtani[1], E. David Peebles[1] [1]*Mississippi State University,* [2]*Animal Ophthalmology Clinic*

Improvements in the performance, meat quality, and antioxidant capacity of broilers in response to supplemental L-ascorbic acid (L-AA) have been previously reported. However, its effects on the inflammatory response and eye L-AA concentrations of broilers are not well documented. Therefore, the objectives of the current study were to determine the effects of the dietary and *in ovo* administration of L-AA on the serum nitric oxide (NO) and eye L-AA concentrations of Ross 708 broilers at 0 and 14 d of age (doa). A total of 1,440 Ross 708 broiler hatching eggs were randomly assigned to treatments pre-specified as: non-injected (control); saline-injected (control); and 12 or 25 mg of L-AA in saline. At 17 d of incubation (doi), 100 µL solution volumes were administered using an Inovoject multi-egg injector. The pre-specified treatments were randomly represented in each of 6 replicate incubator tray levels. At hatch (21 doi), 9 male and 9 female chicks taken at random from each *in ovo* treatment replicate group were fed a commercial diet or a commercial diet supplemented with 200 mg/kg of L-AA from 0 to 14 doa. In a randomized complete block design, all treatments (4 *in ovo* x 2 diet) were represented in each of 6 replicate blocks of pens, and data were analyzed by two-way ANOVA. Serum NO concentrations were lowest ($P = 0.032$) in the 12 L-AA injected treatment. At 0 doa, eye L-AA concentrations were higher ($P < 0.0001$) in males than in females, and serum NO concentrations were numerically lower ($P = 0.085$) in females than in males. At 14 doa, eye L-AA concentrations were highest ($P < 0.0001$) in males that received 12 mg/ml of L-AA. These results indicate that female hatchlings exhibited lower levels of inflammation than male hatchlings, and the *in ovo* injection of 12 mg of L-AA elevated male posthatch eye L-AA concentrations and reduced the inflammatory response of male and female broilers.

Key Words: Broiler, inflammatory response, in ovo injection, L-ascorbic acid, L-AA concentrations

M60 Effects of dietary Ca concentration on the P digestibility and performance of broiler chicks fed various sources of dietary P with and without phytase supplementation Cooper Fritzlen*[1GS], Mike Bedford[2], Michael Persia[1] [1]*Virginia Tech University,* [2]*AB Vista*

An experiment was conducted to determine the effects of high dietary calcium (Ca) on broiler chicks fed low phosphorus (P) diets from either nonphytate (nPP) or phytate P (PP) with or without phytase. Newly hatched broiler chicks were housed in raised-wire starter batteries (65.8 in^2) for 16 d. The 12 treatments were replicated 6 times using 8 chicks per cage that resulted in a total of 576 Cobb 500 chicks. The 3x2x2 factorial arrangement included 3 P treatments (0.225%nPP, 0.225 + 0.1%P from nPP and 0.225 + 0.1%PP), 2 concentrations of Ca (0.9 vs.1.7%Ca) and either (0 or 1,000 FTU of phytase). Body weight gain (BWG), feed intake, feed conversion and mortality were calculated over the 16d period. On 16d, all remaining chicks were euthanized and the right tibia was analyzed for tibia ash weight (TAW) in mg/tibia and expressed as a percentage (TAP). All data were analyzed as a 3x2x2 factorial using ANOVA in JMP 14 ($P \leq 0.05$). There was an interaction between P x phytase as both nPP and PP increased BWG to a greater extent in diets without phytase than when phytase was supplemented ($P \leq 0.05$). This response is possibly associated with increased feed intake as both P and phytase would help mitigate the Ca:P imbalance with low P feeding. When TAP was considered an interaction between Ca x P was found, as both nPP and PP equally increased TAP and TAW in the 0.9%Ca fed birds, but in bird fed high Ca diets PP was not as effective as nPP ($P \leq 0.05$). These data suggest that higher concentrations of Ca may have a more detrimental effect on PP utilization when compared to nPP utilization. There were Ca x phytase interactions for BWG, TAP and TAW ($P \leq 0.05$). The high dietary Ca reduced BWG, but this reduction was minimized with the addition of phytase. This suggests that phytase is driving BWG possibly through increased feed intake as a result of phytase releasing more P than Ca, possibly improving the Ca:P ratio that the bird experiences. Interestingly in the bone ash data, the results are reversed as phytase response was reduced in the presence of higher concentrations of Ca ($P \leq 0.05$). Overall, the multiple interactions of Ca, P and phytase indicate a complex relationship among the three factors that require balancing both growth and skeletal health considerations.

Key Words: phosphorus, calcium, broiler performance, tibia ash, phytase

M61 Non-phytate phosphorus requirements of egg laying pullets from 0-3 weeks of age and carryover effects of phosphorus deficiency of pullets at 6, 9, 12 and 18 weeks of age Carlos Granghelli*[1], Cooper Fritzlen[2], Cristiane Araújo[1], Lúcio Araújo[1], Michael Persia[2] [1]*University of São Paulo (FMVZ-USP),* [2]*Virginia Tech*

Phosphorus is an important structural mineral in both cell membranes and hydoxyappitite that also has physiological and enzymatic roles in the body. The objective of this study was to determine the non-phytate phosphorus (nPP) requirement in 0-3-wk-old laying hen pullets using body weight gain (BWG), mortality corrected feed conversion ratio (FCRm), tibia ash percent (TAP), tibia ash grams (TAG) and bone mineral content (BMC) and to use the same response criteria to understand the longer-term effects of early phosphorus deficiency on pullets at 6, 9, 12 and 18 weeks of age. A total of 1260 Hy-Line W36 pullets were sourced from a commercial hatchery and were distributed in a RCBD resulting in 7 dietary treatments (0.15; 0.22; 0.29; 0.36; 0.43; 0.49 and 0.53 % of nPP and 1.0 % Ca) fed across 6 replicate cages that started with 30 chicks per cage. After the 3-wk P requirement feeding period, all birds were maintained in their respective treatment groups, but switched to common industry-based diets with all diets (0-18 weeks formulated to be isocaloric and isonitrogenous. At 3, 6, 9, 12 and 18 weeks of age, 6, 6, 3, 3, and 2 pullets per pen, respectively, were euthanized for whole-body Dual-energy X-ray Absorptiometry analysis and tibia ash determination. Linear and quadratic broken-lines were used to estimate nPP requirements of 0-3 wk old pullets using BWG, FCRm, TAP, TAG, and BMC. At 6, 9, 12 and 18 weeks of age ANOVA was used to determine differences among dietary treatments. The nPP requirements of 0 to 3-wk-old pullets was 0.31 and 0.40% nPP for BWG; 0.27 and 0.38% nPP for FCRm; 0.31 and 0.39% for TAP; 0.31 and 0.41% for TAG; 0.36 and 0.42% for BMC using linear and quadratic broken lines, respectively. Low concentrations of dietary nPP fed over the 0-3 wk period reduced BWG at 6 wk of age ($P \leq 0.05$). All measurements after 9 weeks of age were not different among any of the dietary nPP treatments ($P > 0.05$). These data indicate that the nPP requirement for pullets from 0 to 3 weeks is between 0.27 and 0.42% nPP dependent on model and response criteria used, and that there are no long-term effects of low phosphorus feeding when pullets are provided an adequate nPP diet after about 6 weeks of feeding.

Key Words: non-phytate phosphorus, requirement, pullet, tibia ash, recovery

M62 The effect of oil type, oil quality, and vitamin E supplementation on the performance, relative fat and liver percentages, and the apparent nitrogen and crude fat utilization in 21-day-old broiler chickens Richard Adefioye*[GS], Merlin Lindemann, Michael Ford, Anthony Pescatore, Sunday Adedokun *University of Kentucky*

This objective of this study was to determine the effect of oil type, oil quality, and vitamin E supplementation on the performance, relative liver and fat weight, and the apparent total tract utilization of nutrients in 21-day-old broiler chickens. The experiment used 384 day-old male by-product Cobb breeder chicks in a completely randomized design with 8 treatments consisting of 8 replicates of 6 birds per replicate cage in a factorial arrangement of treatments of 2 oil types (corn vs. soy oil), 2 oil quality (fresh corn oil; PV = 3 $meqO_2/kg$ and fresh soy oil; PV = 4 $meqO_2/kg$ vs. oxi-

dized corn oil; PV =104 meqO_2/kg and oxidized soy oil; PV =109 meqO_2/kg) and 2 levels (0 and 150 ppm) of dietary vitamin E (mixed tocopherols containing 55-75% γ-tocopherols) supplementation. Specifically, the treatments groups were 1 – Corn oil + 0 ppm of vitamin E; 2 – Corn oil + 150 ppm of vitamin E; 3 – Corn oil (oxidized) + 0 ppm of vitamin E; 4 – Corn oil (oxidized) + 150 ppm of vitamin E; 5 – Soybean oil + 0 ppm of vitamin E; 6 – Soybean oil + 150 ppm of vitamin E; 7 – Soybean oil (oxidized) + 0 ppm of vitamin E; 8 – Soybean oil (oxidized) + 150 ppm of vitamin E. Oxidized oils resulted in lower ($P < 0.05$) weight gain and feed efficiency but did not affect relative fat and liver percentages and total tract utilization of nutrients. Also, oxidized oils alone reduced nitrogen utilization ($P < 0.05$) and this effect was more pronounced in birds fed corn oil ($P < 0.05$). Vitamin E supplementation alone reduced crude fat and nitrogen utilization ($P < 0.05$) but did not affect growth performance and relative fat and liver percentages. From the results of this study, the dietary supply of highly oxidized oils has a deleterious impact on the growth performance and nitrogen and crude fat utilization in 21-day-old broiler chickens. The supplementation of γ-tocopherols did not exert any beneficial effect on the performance and utilization of nitrogen and crude fat.

Key Words: Vitamin E, Oil quality, broilers, oil type, oxidized oils

M63 Effects of source and level of trace minerals in the diet on growth performance and carcass quality of broilers from 1 to 45 days of age Lewis Aguirre*[1], Álvaro de Juan[1], Marisol Castillo[2], Silvia Peris[2], Gonzalo Mateos[1] *[1]Departamento de Producción Agraria, Universidad Politécnica Madrid, [2]Novus Europe A.A./N.V*

The objective of this research was to study the effects of the concentration of Cu and the source of Zn, Cu, and Mn [chelated organic (OTM) vs. inorganic (ITM)] in the diet, on performance, carcass quality, and foot pad lesions (FPD) in broilers from 1 to 45d of age. Mineral sources were ZnO, $CuSO_4$, and MnO for the ITM, and Mintrex Zn, Cu and Mn (Novus International Inc.) for the OTM groups. One-day-old Ross-308 male broilers were randomly allotted to 36 floor pens in groups of 18 with an initial BW of 47.2 ± 0.21g. All diets (0 to 14d, 15 to 28d, and 29 to 45d of age) were based on soybean meal and wheat, with level and source of Zn, Cu, and Mn being the only difference. 4 treatments were arranged as a 2×2 factorial, with 2 sources of Zn, Cu, and Mn (OTM vs. ITM) and 2 levels of Cu (10 vs. 17ppm); with 9 replicates per treatment. Data were analyzed as a completely randomized design with sources of Zn, Cu, and Mn and level of Cu as main effects, using the GLM procedure of SAS for performance traits and the CATMOD procedure of SAS (SAS Institute, 2004) for carcass, FPD, and hock burns measures. The experimental unit was the pen for growth performance measurements and the individual bird for carcass quality traits. From 1 to 45d of age birds fed the OTM diets had better FCR than birds fed the ITM diets (1.544 vs. 1.584; $P< 0.05$). At the end of the experiment average BW of the birds was 3935g, independently of dietary treatment. At 45d of age, FPD and hock burns incidence decreased ($P< 0.001$) with OTM supplementation and with the inclusion of the higher level of Cu in the diet ($P< 0.05$). In this regard, an interaction between main effects was detected ($P< 0.001$); the incidence of FPD was decreased when using 10ppm Cu vs 17ppm as organic chelated source ($P< 0.05$), but not as inorganic source ($P> 0.05$). Dietary treatment did not affect the incidence of bruises and scratches or of wooden breast and white stripping. However, in the OTM group the incidence of white stripping was reduced with 10ppm Cu inclusion but not with 17ppm inclusion ($P< 0.05$). Data show that the inclusion of minerals in an organic chelated form improved feed conversion ratio from 1 to 45d of age and reduced the incidence of FPD and hock burn lesions at slaughter at 45d of age.

Key Words: Minerals, Broilers, Performance, FPD

Metabolism and Nutrition: Amino Acids

M64 Determining the optimal digestible isoleucine to lysine ratio in Ross x Ross 708 male broilers from 14 to 28 days of age Andrew Brown*[1GS], Jason Lee[2], Kelley Wamsley[1] *[1]Department of Poultry Science, Mississippi State University, [2]BIO Business for North America, CJ America Inc.*

Due to the continuous genetic improvement of high yielding broiler strains, the reevaluation of essential amino acids is important. Previous research investigating the starter phase utilizing Ross x Ross 708 males determined the optimum ratio for dIle:dLys to be 70%, which is higher than traditionally used ratios (67% dIle:dLys); however, the optimum dIle:dLys ratio for the remaining phases has yet to be reevaluated. Thus, the objective of this study was to evaluate the d14-28 dIle:dLys ratio of Ross x Ross 708 male broilers. A total of 2,400 male chicks were obtained from a commercial hatchery, equally allocated to 96 pens, and fed a common starter diet (70% dIle:dLys) from d0-14. At d14 pen weights were equalized. Experimental diets were created from a common deficient corn and soybean meal-based diet containing a dIle:dLys of 53%, after manufacture half of this diet was retained for the creation of the summit diet (83% dIle:dLys) by crystalline Ile addition. The remaining 5 experimental diets ranged from 58 to 78% dIle:dLys and were obtained by blending different proportions of deficient and summit diets. A practical control diet was formulated to 68% dIle:dLys and batched separately. There were 12 replications/treatment; birds were individually weighed, and feed intake was recorded on d28 to determine average BW, BW gain (BWG), bird uniformity, feed intake/bird, % mortality, and feed conversion ratio (FCR). All % dIle:dLys requirements were estimated using quadratic regression (95% of the asymptote; QR), as well as linear and quadratic broken line models (LBL;QBL). For BW and BWG, the QR model suggests the optimum ratio to be 68% dIle:dLys ($P<0.05$; R^2=0.78, 0.80). When utilizing the LBL model, the ratio was estimated to be 62% ($P=0.02$; R^2=0.88), while the QBL estimated the ratio to be 65% ($P=0.02$; R^2=0.86). Additionally, for FCR, the QR model estimated the ratio to be 68% dIle:dLys ($P=0.04$; R^2=0.80) while the LBL model estimated the ratio to be 67% ($P=0.06$; R^2=0.76) and the QBL model estimated it to be 70% ($P=0.07$; R^2=0.73). These data are an important step in continued evaluation of the optimum dIle:dLys ratio of Ross x Ross 708 male broilers to optimize performance; future research should continue this work for the remaining grow-out phases.

Key Words: Isoleucine, Grower, Optimal Ratio

M65 Effects of Mushroom Stump Waste (MSW) inclusions to broiler diets on amino acid digestibility and d1-21 performance Logan Erb*[GS], Courtney Poholsky, Alyssa Lyons, John Boney *The Pennsylvania State University*

Mushroom cultivation in Pennsylvania produces 63.9% of all U.S. white mushrooms and subsequently generates a waste stream of inutile mushroom stumps. This waste stream was processed to create a Mushroom Stump Waste (MSW) product. Two experiments were conducted to evaluate the efficacy of MSW. The objectives were to determine the nutrient profile and optimal inclusion of MSW in broiler diets based on performance measurables and amino acid (AA) digestibility. In Experiment 1, 12 Single Comb White Leghorn roosters were arranged in a randomized complete block design (RCBD) where eight roosters were precision fed corn or MSW. Four non-fed control roosters accounted for endogenous nitrogen loss. Excreta was collected 24-h post feeding to determine True Metabolizable Energy corrected for nitrogen (TMEn). The TMEn of MSW (1,173 kcal/kg) and proximate analyses were used to formulate diets with 0, 1, 3, and 5% MSW. In Experiment 2, four treatments were randomly assigned to 8 blocks forming a RCBD. The experimental unit

was one raised wire cage of 10 broilers. On d18, titanium dioxide was added to the diet at 0.20%. On d21, the distal ilea of five birds per pen were extracted and their contents flushed with distilled water. Following lyophilization, pooled digesta samples were analyzed for AA concentration to calculate digestibility coefficients. Day 21 results indicate birds consuming 1% MSW improved live weight gain (LWG) by 57.6 g per bird compared to birds fed 5% MSW. Birds fed 0% or 3% MSW were intermediate for LWG (P=0.0492). Birds fed 0, 1, or 3% MSW had improved FCR compared to birds fed 5% MSW (P=0.0010). Body weight (BW) followed a similar trend as LWG (P=0.0617). Neither mortality nor feed intake were affected by MSW inclusion (P>0.05). The MSW inclusion did not affect AA digestibility; however, trends in cysteine (Cys) and methionine (Met) digestibility were apparent (P<0.10). Birds consuming 0% MSW improved Cys digestibility by 9.2% compared to birds consuming 5% MSW. Birds fed 1% or 3% MSW were intermediate (P=0.0548). Additionally, Met digestibility trends suggest reduced digestibility when MSW was included at 3% or 5% (P=0.0662). These data support MSW inclusion in broiler diets up to 3% without detriment to performance or AA digestibility.

Key Words: Mushroom, TMEn, digestibility

M66 The impact of soybean meal particle size on milling efficiency, broiler digestibility, and subsequent performance Alyssa Lyons*GS, Logan Erb, Courtney Poholsky, Paul Patterson, John Boney *Pennsylvania State University*

Ingredient particle size (PS) affects digestibility and subsequent poultry performance. The objectives of this study were to determine the effects of reducing soybean meal (SBM) PS on hammermill energy use, broiler performance and apparent ileal amino acid digestibility (AIAAD). In experiment 1, four hammermill screen sizes were used to reduce extruded SBM PS. Three replicate batches of SBM were passed through each hammermill screen, totaling 12 batches of SBM. Hammermill motor amperage was recorded every second throughout each replicate. Each screen represents a treatment (trt). The screen sizes were 7.9 mm (trt A), worn 5.6 mm (trt B), new 5.6 mm (trt C), and 2.4 mm (trt D). Nine replicate PS analyses were conducted per trt. In experiment 2, 480-day-old Ross 708 male broilers were housed in battery cages with 12 replicate cages per trt. Treatments were arranged in a randomized complete block design with a cage of 10 broilers serving as the experimental unit. Birds were fed a mash starter diet from 0-10d and a mash grower diet from 10-20d. On d21, three birds per cage were euthanized via cervical dislocation and contents of their distal ilea flushed into a cup using distilled water. Feed and digesta samples were analyzed for titanium dioxide and amino acid concentrations. At the mill, electrical current draw by the motor (amps) and power usage (watts) were similar for trts A, B, and C while trt D drew the most current (P<0.0001) and power (P<0.0001). The PS was similar for A (891.85 µm), B (861.12 µm), and C (735.41 µm); however, D generated the smallest PS (463.88 µm) (P=0.0163). Soybean meal PS did not affect LWG, BW, or FCR from d1-21. Overall (d1-21) FI was highest for trts A and B and lowest for trt D. Treatment C was intermediate (P=0.0494). Soybean meal PS affected both essential and non-essential amino acid digestibility (P<0.05). The essential AIAAD was similar for trts A, B, and C and lowest for trt D (P<0.0001). The non-essential AIAAD followed a similar pattern (P<0.0001). A lack of performance differences in the presence of AIAAD differences warrants more research where birds are fed a pelleted diet from hatch to market weight.

Key Words: particle size, soybean meal, hammermill, digestibility, amino acid

M67 Effects of graded concentrations of threonine on growth performance and gut integrity of broiler chickens under a mild necrotic enteritis infection Ana Villegas*1GS, Juliano Dorigam[2], Jundi Liu[1], Todd Applegate[1] *[1]Department of Poultry Science, University of Georgia, [2]Evonik Operations GmbH*

The impact of dietary threonine (Thr) in maintaining the intestinal structure and mucosal barrier integrity has been previously demonstrated. Less well studied is the utilization and requirement of Thr under enteric disease where mucin integrity is impaired. The objective of this experiment was to investigate the effects of Thr during the grower phase (14 to 28 d of age) on growth performance and gut health of broiler chickens under a mild necrotic enteritis infection. Four isonitrogenous and isoenergetic diets were formulated via the diet-dilution technique to provide 0.58%, 0.63%, 0.68%, and 0.73% SID Thr while SID Lys was maintained 1.00%. Additionally, unchallenged groups were fed with diets with two Thr: Lys ratios (0.68 and 0.63) at 1.00% and 1.10 % SID Lys. All diets were formulated to contain 1.00% SID Lys to be second limiting amino acid, and a control diet contained 1.10% SID Lys to be adequate in Lys for confirmation. The necrotic enteritis challenge model consisted of one dose of coccidial vaccine (Coccivac B52®) at d of age and *Clostridium perfringens* (#CP6, *netb* positive) at d 14, 15, and 16. Performance (BW, feed intake, and feed-to-gain) was recorded on d 14 and 28. At d 17, NE and coccidiosis specific lesion scores, *Clostridium perfringens* enumeration, FITC-dextran (MW=4 kD) intestinal permeability, excreta oocyst counts, and intestinal morphology were measured. Dietary Thr supply did not alter growth performance nor intestinal health parameters measured. Nevertheless, birds challenged presented with elevated trend in NE mortality (P = 0.081) and increased severity of macroscopic (P = 0.0039) and microscopic (P = 0.0081) necrotic enteritis lesions. In conclusion, dietary Thr concentration during the grower phase (14 to 28 d of age) did not affect growth performance nor gut characteristics measured under a mild necrotic enteritis challenge. The necrotic enteritis challenge directly affects the gastrointestinal tract with consequences for Thr's absorption and utilization, which could explain the lack of effect of extra dietary Thr supplementation.

Key Words: necrotic enteritis, intestinal integrity, amino acid, threonine

Physiology, Endocrinology and Reproduction: Broilers

T1 Woody breast myopathy broilers show age-dependent adaptive differential gene expression in Pectoralis major and altered in-vivo triglyceride kinetics in adipogenic tissues Pramir Maharjan*, Antonio Beitia, Jordan Weil, Nawin Suesuttajit, Katie Hilton, Justina Caldas, Cole Umberson, Diego Martinez, Byungwhi Kong, Casey Owens, Craig Coon *University of Arkansas*

A study was conducted to understand the differentially expressed genes in *Pectoralis major* under woody breast (WB) myopathy condition in a high yielding broiler strain using RNA-sequencing at the growing (d 21) and finishing (d 42 and d 56) grow-out period. Follow-up study was conducted to understand the *in-vivo* triglyceride (TG) synthesis (d 49) occurring in adipogenic tissues using deuterium oxide (^2H_2O) as a metabolic tracer. Results indicated the top physiological systems affected in myopathy broilers were related to the musculo-skeletal system (d 21, 42, and 56) and cardiovascular system (d 42 and 56). Ubiquitin-specific proteases are expressed higher in myopathy broilers at d 21 (OTUD1) and d 42 (SACS) that potentially indicated higher degradation of muscle protein occurring. Genes related to transcription factors and muscle cell differentiation (ZNF234, BTG2) and muscle growth (IGF1) were up-regulated with myopathy broilers suggesting concurrent muscle regeneration occurring. The expression of PYGB and MGAM genes related to carbohydrate transport and metabolism were down-regulated at d 42 which indicated that the WB affected myofibers could be at nutrient-deficient state; whereas, the nutrient-deficient physiological state of cells seemed to be counteracted by up-

regulation of genes related to carbohydrate (ALDOB, GPD1L2) at d 56. There was a reduced ($P< 0.05$) *in-vivo* TG synthesis in liver of the myopathy broilers (0.123 %/hr) compared to non-myopathy broilers (0.197 %/hr). The majority of TG synthesized in liver with myopathy broilers could conceivably be delivered to *P. major* (rather than to abdominal fat pad storage) to fulfil the increased energy need of muscle cells (via TG lipolysis and fatty acid (FA) oxidation). The increased utilization of FAs in the WB affected muscle could result in reduced secretion of FAs into blood circulation leading to sub-optimal availability of FAs for re-esterification for TG synthesis in liver. Results indicated that myopathy broilers at later age (d 56) of grow-out period were synchronously going through adaptive physiological processes as feedback responses to adverse cellular states.

Key Words: woody breast myopathy

Teaching, Pedagogy, Extension

T2 Indian poultry industry vis-à-vis COVID-19: A situation analysis report Gautham Kolluri*, Jagbir Tyagi *ICAR-Central Avian Research Institute*

Originating in Wuhan city, Hubei province of China and rapid spread to multiple countries, the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has emerged as a novel public health emergence. During early February, spread of misinformation and rumors driven by the fear of linking chicken meat and eggs in the transmission of Coronavirus disease (COVID-19) among human population is witnessed in India. This resulted drastic reduction in consumption of poultry products with subsequent fall in demand thereby prices. The COVID-19 driven lock down during March in the country has further accentuated the crippling poultry industry due to arrest of logistics, destruction of eggs and birds. Here we have analyzed the impact of COVID-19 on poultry industry and showed the realistic flow of events that resulted in its economic fallout by disruption of poultry protein chain during pandemic crisis. Primary data was collected from respondents (N=65) randomly representing different poultry segments through a short structured questionnaire and responses were recorded from January to May 2020 via personal voice calls and social media platforms. Secondary data for egg, broiler and maize prices were retrieved from real time marketing platforms. Price trends for day old chicks, feed, production cost, sale price, change in percent price trends, price index, were calculated for layer and broiler entities. Descriptive statistics (one way ANOVA and Student's T-test) was used to analyze the data by SPSS and means were compared using Duncan's post hoc test. Percent change in price variation was recorded negative as 36, 43, 129 and 21.3 from Jan-Feb, Feb-Mar, Mar-Apr and Jan-Apr respectively. The lowest egg prices were recorded as USD 2.04 (Pandey, 2020) against the production cost of USD 5. Results pertaining to % change in price fluctuation showed a negative trend of 36, 43, 129 and 21.3 from Jan-Feb, Feb to Mar, Mar-Apr and Jan-Apr respectively. Prices index for egg and broiler revealed highest consumer demand and consumption pattern ($p<0.05$) in January with gradual reduction till April. Resultant COVID-19 loss in egg industry was less realized than broiler counterparts. The projected loss caused due to these events for the Indian poultry industry is around USD 3053 million.

Key Words: COVID-19, Lockdown, Economic impact, Poultry industry, India

Processing and Products

T3 Comparative whole-genome analysis of Campylobacter jejuni strains isolated from retail Chicken in Mississippi Sabin Poudel*[1], Tianmin Li[1], Mark Arick[2], Chuan Hsu[2], Aaron Kiess[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University*

Campylobacter jejuni (*C. jejuni*) is one of the leading foodborne pathogens causing human gastroenteritis. Consumption of contaminated poultry products is considered a major contributing factor for human *Campylobacter* infection. This study aimed to characterize and compare the genome sequences of 3 *C. jejuni* isolates isolated from broiler carcass and giblets collected from Mississippi's retail stores. The whole genome sequencing (WGS) was conducted by using both a long-read sequencing approach with a Nanopore GridION sequencer (Oxford Nanopore Technologies, Oxford, UK) and a short-read sequencing approach with a Illumina HiSeq X-Ten sequencer (Illumina, San Diego, CA, USA). The complete genome sequences of MS2005, MS2058, and MS2074 had a genome sizes of 1,768,497 bp, 1,842,026 bp, and 1,629,343 bp, respectively. Multilocus sequence typing analysis showed that MS2005 and MS2058 belong to ST 2132 and MS2074 belongs to ST 1047. The virulence factors (VF) were analyzed using VF database (VFDB). There was high variation in the O-linked flagellar glycosylation VF for MS2005, MS2058, and MS2074 which possessed 18, 16, and 11 virulence genes, respectively. MS2058 lacks *maf1* and *maf4*; MS2074 lacks *maf1*, *maf4*, *maf6*, *neuB2*, *neuC2*, *ptmA*, and *ptmB* genes. MS2058 only had the Cysteine acquisition VF. MS2005 strains lacked the *fliL* gene from the Flagella VF. The antimicrobial resistance genes (AMRs) were analyzed using the Resistance Gene Identifier (RGI) web portal. MS2005, MS2058, and MS2074 had AMRs to macrolide, fluoroquinolone, cephalosporin, fusidic acid; MS2005 and MS2058 had AMRs to aminoglycoside and nucleoside. MS2058 and MS2074 had AMRs to tetracycline and penam, respectively. Phenotypic antimicrobial susceptibility testing was performed using the disk diffusion technique for 8 antibiotics from 7 classes . Out of the three isolates, two (MS2058 and MS2074) showed a multi-drug resistant to 3 and 4 antibiotic classes, respectively, but MS2005 did not show resistance. Comparative analysis of WGS sequence of *C. jejuni* isolates, revealed a notifiable diversity in genetic composition, virulence gene, and AMRs. This information can be used for epidemiological investigations and to identify potential antigens for development of subunit vaccines via reverse vaccinology.

Key Words: Campylobacter jejuni, whole-genome sequencing, virulence factor, antimicrobial resistance, retail broiler meat

T4 Comparative study on the effect of latic acid as decontaminant in chicken carcass Natalia Malagutti, Simone Schmoeller, Darwem Araujo, Sandra Heidtmann, DANIELA MENGARDA* *BRF S/A*

The effect of lactic acid on the treatment of decontamination effects and quality attributes of chicken carcass was investigated. Three (3) different concentrations of lactic acid (2%, 3% and 5%) has been studied at two (02) steps in the process (step point 1 - after the defeathering and step point 2 - after final chiller). This research and all experiments have been done at industry as a pilot trial, considering the running variables from the chicken process. The chicken industry was running about 10.000 birds/ hour, and average carcass weight around 2.3 kg/bird and full plant dedicated in a automatically cut line. The method of application of lactic acid was sprays, in a specific cabinet, on the surface of chicken carcass. The lactic acid has been diluted in the water and controlled during all the time of experiments. The sampling was about 30 chicken carcasses at each point, during 7 days of production for each lactic acid concentration. The variables studied were, total plate count (TPC) and Enterobacteriaceae by rinsing method

for microbial analysis and also sensorial attributes has been variables considered in this research. All treatments reduced TPC and Enterobacteriaceae in both steps were added, being about 1,5 log cfu/g reduction on step 1 (after defeathering) and 0,5 log cfu/g ate the step number 2 (after water chiller). Lactic acid at were effective for reducing TPC and Enterobacteriaceae, with no significant difference between them. Also, varying the concentration from 2% up to 5% did not vary significantly ($p<0,05$) the reduction on microbiology results in both steps in the process. However, the sensorial quality attributes as Appearance, color, smell, and overall acceptability of treated chicken cuts has been changed, significantly, being not acceptable at 5% lactic acid concentration for skin on wings. For skinless cuts as chicken breast and inner fillets no changes has been notified although for thigh and drumsticks skin on cuts, the lactic acid had affected the sensorial aspects, but not significantly as being not acceptable. Overall, we could say that lactic acid application in a convenient and simple decontamination technique for hygienic and wholesome chicken production.

Key Words: lactic acid, decontamination, quality, chicken, processing

T5 Effects of Immersion Chiller Conditions on Peracetic Acid Half-Life Rates Daniel Sabo*, Stephanie Richter *Georgia Tech Research Institute*

The poultry industry relies on antimicrobials to control microbial contamination, commonly peracetic acid (PAA). The dependence on PAA demonstrates the need to understand PAA's chemical decay rates and stability within immersion chillers. Anecdotal reports state that PAA decays rapidly in the presence of high organics. Organics found in the chiller are in the form of: total suspended solids (TSS), total dissolved solids (TDS), fats, oils, and greases (FOGs). The exact contributors and concentrations that causes PAA's rapid decay have not been well documented, to date. Additional reports indicate that pH also effects PAA stability. This research project looks to determine the effects of organic carbon and pH on PAA stability. Two initial studies were conducted using simulated chiller water (SCW) and industrial main chiller water (IMCW), both adjusted to a pH of 8.6. SCW mirrored concentrations similar to IMCW in TSS, TDS, COD, and FOGs. Industry obtained PAA at 22% concentration was used in testing. PAA concentration was measured using two colorimetric adsorption device kits. For all tests, initial PAA concentrations targeted 75 ppm. PAA was added to test water and mixed for sixty seconds before concentration was measured. This measurement represented t=1. Studies measured PAA concentration at six time points (1, 5, 15, 30, 60, 90, 120 minutes) at a pH of 8.6. PAA decomposition in potable water acted as a control group for decay rate comparison. Results from t=5 minutes for SCW showed 58% PAA ppm remained from t=0, while IMCW showed an average of 65% PAA remained (*P-value* ≤ 0.05). In the presence of organic carbon, at a pH adjusted to 8.6, PAA half-lives measured approximately 10.8 and 18.9 minutes for the SCW and IMCW respectively. These results indicate a compounding effect of pH and organic loading on PAA concentration and stability within a chiller. PAA may not be at target concentrations as carbon content within chillers increases throughout a processing shift.

Key Words: PAA, Chiller, antimicrobial, peracetic acid

T6 Particalized eggshell membranes (PEM) and its functional hydrolysates for cosmetics and biomedical applications. Garima Kulshreshtha*[1], Ling Wu[1], Tamer Ahmed[1], Ty Diep[2], Maxwell Hincke[1,3] *[1]Department of Cellular and Molecular Medicine, Fculty of Medicine, University of Ottawa, [2]Burnbrae Farms, 830, Rue Lanoie, [3]Department of Innovation in Medical Education, Faculty of Medicine, University of Ottawa*

Eggshell membranes (ESM) comprises a fibrous meshwork of highly cross-linked protein, which function as both physical and chemical barrier to maintain egg quality and protect the developing embryo in fertilized eggs. In addition to proteins (~90%), it is also composed of a small amount of lipids (~3%) and carbohydrates (2%, mainly hyaluronic acid, HA). Proteomics analysis of ESM revealed >500 proteins including collagens, glycoproteins, avian beta-defensins, and lysozyme responsible for various biological functions including anti-microbial, anti-inflammatory, anti-wrinkle, and anti-oxidant activities. However, commercial utilization of ESM for these biological functions is restricted due to insolubility of ESM fibers. Hence, in our current study, we developed a novel chemical-free and environmentally friendly method of ESM extraction to produce particalized eggshell membrane (PEM) with enhanced anti-bacterial, anti-inflammatory, and anti-oxidation bioactivities for cosmetics and biomedical applications. A novel top-down method was developed to produce particalized ESM (PEM) from table eggs, in a size range approaching nanoscale, by a combination of cryo-milling / cryo-grinding, emulsifying, and freeze-drying processes. This method is subject to intellectual property disclosure. Statistical analyses were carried out using GraphPad Prism software (San Diego, CA, USA). The statistical significance of the data was determined by student's t test, one-way or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, with a $P \leq 0.05$ taken as a value of significance. PEM exhibited a size-dependent bactericidal activity ($P<0.05$) against skin associated bacterial pathogens including Gram-positive *Staphylococcus aureus* and bacteriostatic effect against Gram-negative *E. coli* and *Pseudomonas aeruginosa*. A significant ($P<0.05$) dose-dependent anti-inflammatory activity was observed for LPS-induced NO production by RAW 264.7 macrophages, for smaller size PEM particles (<15 μm and <53 μm). Bioactivity of PEM particles in various sizes was further enhanced by chemical hydrolysis. Our results demonstrate that alkaline hydrolysis of PEM resulted in enhanced anti-oxidant activity ($P<0.001$), as compared to untreated PEM in various sizes. These findings suggest that PEM and its functional hydrolysates have a great potential for the development of a new high value biomaterial for cosmetics and biomedical applications.

Key Words: Eggshell membranes (ESM), Particalized eggshell membranes, antimicrobial activity, anti-inflammatory activity, anti-oxidant activity

SCAD

T7 Results of an infectious bronchitis virus surveillance program in broiler chickens Brian Jordan*[1], Abigail Reith[2] *[1]The University of Georgia, [2]Zoetis*

Control of infectious bronchitis virus (IBV) is attempted by using modified live vaccines that are mass applied in the hatchery. IBV vaccines are usually serotype specific, meaning these vaccination programs vary based on the field challenge strains of each complex. Cross protection between serotypes is limited and the need for accurately matched vaccination programs to field challenge is imperative. In order to match the vaccination program, the serotypes circulating in flocks must be identified. A current tool for IBV identification is quantitative real time RT-PCR. This assay can detect specific serotypes, as well as provide a relative viral load in each set of samples tested. A comprehensive IBV qRT-PCR surveillance program was started by the PDRC to assess what IBV serotypes were actively circulating in flocks. Samples from broiler flocks from 25+ days of age were collected from multiple complexes spanning the south and southeast. Samples were tested using a universal IBV qRT-PCR assay, and then with Massachusetts, Connecticut, Georgia08, DMV/1639, Arkansas, Delaware/Georgia98, and Georgia13 specific qRT-PCR assays. It has typically been thought that IBV vaccine clears quickly, but analysis of these

results shows that recovering vaccine 25-35 days post vaccination is common. Other viruses, such as the variant DMV/1639, were also detected in specific locations, indicating that certain farms or complexes may be the reservoirs for this virus. Over time, many samples that were positive with the universal IBV assay tested negative with all the serotype specific assays, indicating either a novel virus or a known virus that has mutated may be circulating in some flocks. Sequencing attempts so far have been unsuccessful for these samples, but still ongoing. With this knowledge, it is clearly important to closely monitor vaccination application and takes to ensure proper vaccination, as well as monitor circulating field viruses even if no clinical disease is present to better understand the IBV load in an area.

Key Words: Infectious bronchitis virus, surveillance, variant

T8 Evaluation of the pathogenesis and transmission of live bird market H2N2 avian influenza viruses in chickens, Pekin ducks, and Guinea fowl Jongseo Mo*, Sungsu Youk, Christina Leyson, Erica Spackman, Mary Pantin-Jackwood *USDA Southeast Poultry Research Laboratory*

H2N2 subtype low pathogenic avian influenza viruses (LPAIVs) have persisted in live bird markets (LBMs) in the northeastern states since its first identification in early 1990s. Continued isolation of the H2N2 LPAIVs prompted us to examine the pathogenicity, infectivity, and transmissibility of the virus in three avian species widely available in LBMs. We compared two H2N2 viruses, one from 2018 and one from 2019. This second virus has a NA stalk deletion, a genetic trait that has been previously associated with adaptation to gallinaceous species. Three different doses of each virus were used to inoculate chickens, Pekin ducks, and Guinea fowl. Contact birds of each species were added to the virus-inoculated groups. No clinical signs were observed in any of three species. Based on serology and virus shedding, only the chickens inoculated with the high dose of both viruses became infected, with no difference in infectivity between the two viruses and no transmission to contacts. In contrast, the 2019 H2N2 virus showed higher infectivity than the 2018 virus in Pekin ducks and Guinea fowls. In terms of transmissibility, similar levels of oropharyngeal (OP) and cloacal (CL) virus shedding was detected in the high dose groups of both H2N2 viruses. However, both Pekin ducks and Guinea fowl inoculated with the medium and high dose of 2019 H2N2 virus shed virus for longer, consequently transmitting viruses to contact birds, whereas in the 2018 H2N2 virus counterpart, no transmission occurred in Pekin ducks and transmission was only observed in the high dose group of Guinea fowl. These results show that both of the H2N2 viruses examined were better adapted to Pekin ducks and Guinea fowl compared to chickens, suggesting that the NA stalk deletion has a role in the increased virus adaptation observed with the 2019 virus in Pekin ducks and Guinea fowl. The increase in viral fitness observed with the later H2N2 virus in these two bird species warrants continued surveillance for this virus in LBMs.

Key Words: Avian influenza, Live bird market, chicken, Guinea fowl, Pekin duck

T9 Infectivity, transmission, and genomic changes in turkeys and chickens infected with low and highly pathogenic H7N3 avian influenza viruses from the turkey farm outbreaks in North and South Carolina in 2020 Mary Pantin-Jackwood, Sungsu Youk, Miria Criado, David Suarez, David Swayne, Christina Leyson* *Southeast Poultry Research Laboratory*

In March 2020, an outbreak of H7N3 low pathogenicity avian influenza virus (LPAIV) occurred in turkey farms, affecting nine premises in North Carolina and one in South Carolina (SC). Highly pathogenic avian influenza virus (HPAIV) was subsequently confirmed on a second turkey premise in SC. The objective of this study is to investigate the pathobiology of these H7N3 viruses in turkeys and chickens, specifically focused on infectivity and transmission. One HPAIV and two LPAIV viruses were used. One LPAIV isolate (LP1) had a full-length neuraminidase (NA) gene whereas a second LPAIV isolate (LP2) had a deletion at the NA stalk region. The clinical signs and mean death time for the HPAIV were typical for HPAIV's in gallinaceous species, with most infected birds succumb to death by 3 days post inoculation. No disease was observed in turkeys and chickens exposed to the LPAIV's. Regardless of which H7N3 virus was used, virus infectious doses were lower in turkeys compared to chickens. Moreover, transmission in turkeys was observed at medium and high virus doses, whereas in chickens, transmission only occurred at the highest dose. These results might indicate that the three H7N3 viruses are more adapted to turkeys compared to chickens. Between the LPAIVs, no differences in infectious dose and transmission was observed in chickens. However, in turkeys, LP2 had a lower infectious dose compared to LP1, suggesting that the NA-stalk deletion might be associated to adaptation of the virus in turkeys. To further investigate the role of genomic changes in adaptation of the H7N3 viruses to these two gallinaceous species, we performed whole genome sequencing on swab and tissues collected from the bird experiments and then identified single nucleotide variations (SNV) in each sample. These data collectively contribute to our knowledge of the mechanisms that drive adaptation of avian influenza viruses to poultry and their impact on the pathogenesis, epidemiology, and evolution of the viruses.

Key Words: avian influenza, pathogenesis, transmission, virus genome sequencing

T10 Pathogenic characterization of same genotype avian reovirus variants based on histopathology Sofia Egana-Labrin*[1GS], Carmen Jerry[2], Charles Corsiglia[3], Beate Crossley[2], Rodrigo Gallardo[1] *[1]University of California, Davis, School of Veterinary Medicine, [2]University of California, Davis, California Animal Health & Food Safety Laboratory System, [3]Foster Farms*

Avian reovirus (ARV) continues causing negative impacts to the poultry industry worldwide. One of the reasons is the high variability of its genome allowing the generation of variants that escape from current preventative strategies. Variant strains differ in antigenicity and pathogenicity if compared with classical strains, used in commercial vaccines. Despite the increased detection of ARV variant strains, their pathobiology remains unclear and current genotyping does not provide enough pathological insights. Through this investigation, we aim to better understand the pathobiology of ARV variants belonging to the same genotype and compare them with conventional ARV strains. We focused on microscopically induced lesions in tendon, heart, bursa of Fabricius, and thymus. We hypothesized that different histopathological outcomes will be found in variant strains belonging to the same genotype. Two ARV variants typed as Sigma C genotype 1 and an S1133 vaccine were used in this experiment. One-day-of-age (DOA) specific-pathogen-free chickens were distributed into four groups, including a mock-inoculated control group, in BSL-2 facilities. At 1-DOA half of the chickens under each treatment were inoculated via subcutaneous (SC) in the right footpad with 100µl containing 1x10^5 embryo infective dose 50% of the respective inoculum. The other half of chickens under each treatment remained non-inoculated and served as contact birds to evaluate their horizontal transmission effectiveness. Necropsies were performed at 8, 15, 21, and 28 days post-challenge (DPC), and the above-mentioned organs were collected for histopathology. Microscopic lesions were evaluated and scored in tendons, hearts, bursas, and thymuses. Compared with contact groups, chickens inoculated with ARV strains showed microscopic differences in tendon, heart, bursa, and thymus ($p<0.05$). Bursas and thymuses from inoculated groups presented different lymphoid depletion scores for each of the ARV tested strains, time post-inoculation, and challenge ($p<0.05$). In conclusion, isolates grouped in the same genotypic group present similar distribution through

the organism of infected chickens and can present not only different pathological responses but also different levels of horizontal transmission.

Key Words: avian reovirus variant, tenosynovitis, pericarditis, lymphoid depletion, histopathology

T11 Clinical characteristics and neutralization patterns of avian reovirus field isolates from Mississippi Rebecca Mackey*[1GS], Alejandro Banda[1], Candy Zhang[2], Lifang Yan[2], Holly Sellers[3] *[1]Poultry Research and Diagnostic Lab, [2]Veterinary Research and Diagnostic Lab, [3]Poultry Diagnostic and Research Center*

Avian reovirus was isolated from over 100 diagnostic cases in Mississippi over the past four years. Most of the isolates were from three to four-week-old broilers, with a few cases in breeders. Clinical signs exhibited include lameness (75%), mortality (20%), intestinal or size-uniformity issues (16%), reovirus co-infections (16%), and neurological signs (2.5%). Genotyping a subset of samples by the S1 gene indicates that Mississippi isolates have representatives in all six reovirus genotypic clusters, with cluster 4 being the most common, followed by genotypes 1, 5, and 2. Although reovirus variants exhibited genetic similarity of 50% or less with commercial vaccine strains, neutralization tests showed that antiserum against the vaccine strain was capable of neutralizing these variants *in vitro*.

Key Words: avian reovirus, virus neutralization

T12 Evaluation of a cell line adapted Infectious Laryngotracheitis Virus (ILTV) strain for in ovo and/or spray vaccination Daniel Maekawa*[1GS], Sylva Riblet[1], Patrick Whang[1], Ivan Alvarado[2], Maricarmen García[1] *[1]University of Georgia, [2]Merck Animal Health*

In an effort to produce more stable live attenuated vaccines for infectious laryngotracheitis virus (ILTV), deletion of genes related to virulence has been extensively pursued. Among viral genes associated with virulence but non-essential for viral replication *in vitro* is the open reading frame C (ORF C). In a previous study, the protection efficacy of an ILTV recombinant strain with deletion of the ORF C gene (BΔORFC) was comparable to the tissue culture origin (TCO) vaccine. As rHVT-LT *in ovo* vaccination is a common practice in the poultry industry, the development of live attenuated strains that can be also administered *in ovo* is being considered as a possible vaccination strategy against the disease. Therefore, the objective of this study was to evaluate the safety and protection efficacy of an LMH cell line adapted BΔORFC strain when administered *in ovo* and/or spray with and without a rHVT-LT vaccine. Vaccination with the BΔORFC strain either alone or in combination with a rHVT-LT vaccine did not affect hatchability and only minimal signs of respiratory distress were recorded for groups of birds that received the BDORFC strain via spray. After challenge, all vaccinated groups showed reduction in clinical signs and viral load in the trachea. However, a decrease in challenge virus replication and viral load comparable to the non-vaccinated/non-challenge group was only observed for groups of chickens that received dual vaccination of rHVT-LT (IO) + BΔORFC. Consequently, compared to rHVT-LT or BΔORFC when administered alone, the dual vaccination strategy improved protection against challenge.

Key Words: Infectious laryngotracheitis (ILT), Vaccination, Protection

T13 Avian macrophage responses to virulent and avirulent isolates of Clostridium perfringens Raveendra Kulkarni*[1], Carissa Gaghan[1], John Prescott[2] *[1]North Carolina State University, [2]University of Guelph*

Necrotic enteritis (NE) in poultry is an economically important enteric disease caused by *Clostridium perfringens*, an anaerobic toxin-producing and spore-forming Gram-positive bacterium. Recent withdrawal of in-feed antibiotic supplements in poultry has caused a significant spike in NE and the global annual losses due to NE are estimated around $6 billion. The pathogenesis of NE involves intestinal damage and necrosis caused by *C. perfringens* toxins and enzymes. There is evidence that unique NE-causing strains which possess signature NE-associated virulence genes including *netB* and that the immunity against NE is associated with virulent (but not avirulent) strains of *C. perfringens*. Although NE pathogenesis is moderately well studied, avian cellular immune responses to *C. perfringens* and their secretory proteins is poorly understood. In the present study, we used *C. perfringens* isolates that varied in their NE virulence capacity to investigate both cell-associated and cell-free secretory proteins-induced macrophage responses in-vitro. The findings showed that macrophages treated with two virulent isolates and their secretory proteins had significantly up-regulated transcription of interferon (IFN)-γ cytokine and toll-like receptor (TLR)-2 pathogen-sensing immune genes, when compared to avirulent C. perfringens isolate. Furthermore, macrophage production of nitric oxide and surface expression of MHC-II antigen presenting molecule was also found significantly increased when treated with virulent *C. perfringens* compared to avirulent isolate. In summary, our study suggested that *C. perfringens* and their secretory proteins can activate chicken macrophages and that their activation is associated with the virulence potential of these bacteria.

Key Words: Clostridium perfringens, Necrotic enteritis, Macrophages, Immune response, Chickens

T14 Mitigation efficiency of dietary medium chain fatty acid blend supplement on cecal colonization by Salmonella Enteritidis in broiler chicks Yewande Fasina*[1], Nicholas Evans[2], Temitayo Obanla[1], Emily Kimminau[2], Adam Fahrenholz[3] *[1]North Carolina A&T State University, [2]Purina Animal Nutrition, [3]Prestage Department of Poultry Science, North Carolina State University*

Identification of alternative prophylactics to in-feed antibiotics for the control of enteric Salmonella colonization remains a priority for the poultry industry. Medium chain fatty acids (C6-C12) are potential alternatives due to their bactericidal activities. An experiment was conducted to evaluate the efficacy of a medium chain fatty acid blend in reducing cecal colonization by *Salmonella* Enteritidis (SE) in broiler chicks. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to 4 treatments. Treatment 1 (CON) consisted of chicks given unmedicated corn-soybean meal (SBM) basal without any additive. Treatment 2 (MCFA) consisted of chicks given unmedicated corn-SBM basal into which the medium chain fatty acid blend was added at 2.5 g / kg diet. Treatment 3 (CON-SAL) and Treatment 4 (MCFA-SAL) consisted of chicks that were given diets similar to CON and MCFA treatments respectively, and were each orally inoculated with nalidixic acid-resistant SE concentration of 7.22×10^8 colony-forming units (CFU) /mL at 1 day of age. Each treatment had 6 replicate cages, with each cage housing 10 chicks. Chicks had ad libitum access to feed and water throughout the 21-day experiment. On d 3, 7 and 14, ceca were aseptically collected and contents were plated for the enumeration of SE on xylose lysine tergitol 4 (XLT4) agar supplemented with 50 µg/mL nalidixic acid. Indicators of chick growth performance were also recorded on d 7, 14, and 21. These included body weight, body weight gain, feed intake, and feed conversion ratio. Results showed that the ceca of chicks not challenged (CON and MCFA) were negative for SE throughout the experiment. Among challenged treatments, cecal SE was higher for CON-SAL chicks ($P < 0.05$) compared to MCFA-SAL throughout the experiment. Growth performance indicators were similar ($P > 0.05$) among treatments on d 7 and 14. It was concluded that the dietary medium chain fatty acid blend supplemented at 2.5 g / kg diet (i.e. 0.25%) reduced cecal SE colonization in broiler chicks during the first 2 weeks of life, without any adverse effect on growth performance.

Key Words: Medium-chain fatty acid, Salmonella Enteritidis, Nalidixic acid, Cecal colonization, Broiler chicks

T15 Effectiveness of organic zinc with or without a live Salmonella vaccine for reduction of Salmonella infantis in broilers to processing Charles Hofacre*[1], Marco Rebollo[2], Duarte Neves[2], Matthew Jones[1], Virginia Baxter[1], Roy Berghaus[3] *[1]Southern Poultry Research Group, Inc., [2]Zinpro Corporation, [3]The University of Georgia*

According to the CDC, Salmonella is the second foodborne illness agent reported (30% of cases). Poultry meat accounts for 12% of these cases. There were three treatments: no treatment, Availa-Znä only (80 ppm) or Availa-Zn (50 ppm) plus MeganVac 1 day 1 and day 14. The challenge was oral gavage of 15 birds per pen (direct) at 32 days of age with *S. infantis*. Each pen had 25 birds total with non-challenged (indirect) tagged and dyed. The samples collected were 42-day bootsocks (environment), ceca 43-day, whole bird carcass rinse 43-day (hot rehang), and liver/spleen (43-day). A typical 8-hour feed withdrawal was performed prior to catching and processing. Salmonella prevalence and enumeration were performed with tetrathionate, then XLT-4. Enumeration was by micro most probable number (MPN) method of Berghaus, et al., 2013. Five horizontal (indirectly challenged) birds/pen and five direct challenged were processed for carcass/rinses and ceca collected from each. Also, liver/spleen from each bird was cultured to determine presence of S.I. in internal organs. The S.I. prevalence from hot rehang carcasses for the Availa-Zn only in the direct challenged had significantly lower S.I. (80%) versus untreated (96%). There were no significant differences in S.I. number (MPN/ml) of carcass rinsate between treatments. S.I. prevalence in ceca of indirect challenge were numerically reduced in Availa-Zn plus vaccine (91.8%), Availa-Zn (81.6%), and challenge (93.3%). The ceca of horizontal challenge MPN/g had a similar trend in Availa-Zn plus vaccine (0.69^a), the Availa-Zn (1.24^a), and untreated (1.59^a). The liver/spleen S.I. prevalence in untreated ($47.4\%^a$), Availa-Zn ($35.4\%^a$), and Availa-Zn plus vaccine ($30.3\%^a$). Serogroup C Salmonella are difficult to reduce in broilers in the processing plant. Availa-Zn alone did significantly reduce the C1, *S. infantis,* carcass rinse prevalence of direct challenged broilers. When all the data is combined, there is a clear benefit of the Availa-Zn alone or an additive effect with the vaccine for reduction in liver/spleen prevalence and number as well as ceca prevalence and number (MPN/g). This study also demonstrated the Availa-Zn in the feed at 50 ppm did not negatively affect the MeganVac 1 live vaccine.

Key Words: Salmonella infantis, carcass rinse, live vaccine, organic zinc

Metabolism and Nutrition: Amino Acids

T16 Reduced dietary crude protein as a strategy for sustainable egg production Balachandar Jayaraman*[1], Ferry Poernama[2], Tito Wibowo[2], Pradeep Krishnan[1], Girish Channarayapatna[1] *[1]Evonik (SEA) Pte., Ltd. Singapore, [2]PT Japfa Comfeed Indonesia TBK*

A study was conducted to determine the effects of feeding reduced crude protein-amino acid (CP-AA) balanced diets on production performance and egg quality of commercial laying hens. A total of 960 Lohmann Brown laying hens were randomly allocated into three dietary treatments (10 replicates per treatment, 32 birds per replicate). Dietary treatments consisted of three different CP levels, high CP (17.5%; High CP), medium CP (16.2%; Med CP), and low CP (15.2%; Low CP) diets. In High CP diet, only DL-Methionine (Met) and L-Lysine (Lys) were supplemented. In Med CP diet, in addition to Met and Lys, threonine (Thr) and tryptophan (Trp) were also supplemented. In Low CP diet, besides Met, Lys, Thr, and Trp, L-Valine and L-Isoleucine were supplemented. Corn-soybean meal-based diets were formulated to meet nutrient requirements according to Lohmann breed standards for the laying phase and provided in mash form. The total duration of the study was 16 weeks (24 weeks to 40 weeks). Feed and water were provided *ad libitum* throughout the experimental period. Response criteria measured were hen-day egg production (HDEP), feed intake, egg mass, feed conversion ratio (FCR) and Haugh unit score (HUS). Data were analyzed as one-way ANOVA using SAS software version 9.4. Mean values were compared by Tukey's test and statistical significance was declared at $P < 0.05$. Performance parameters like HDEP and feed intake were unaffected ($P > 0.05$) when dietary CP was reduced from 17.5% to 15.2%. Reduction of dietary CP from 17.5% to 16.2% did not affect FCR and egg mass; however extreme reduction of CP negatively impacted the above performance parameters. HUS as an indicator of egg equality was unaffected ($P > 0.05$) by the dietary CP levels. The income over feed cost (IOFC) for the Lohmann-Brown laying hens fed Med CP diet was the highest followed by high CP diet and low CP diet. Low CP diet could also be a sustainable solution when next limiting AA are commercially available at cost-effective price. In conclusion, the results from this study indicate that dietary CP reduction could be an effective nutritional strategy to improve sustainability of the egg industry.

Key Words: crude protein, amino acid, performance, egg quality, laying hens

T17 Glycine equivalents in reduced crude protein diets for sustainable broiler production Balachandar Jayaraman*[1], Ferry Poernama[2], Tito Wibowo[2], Pradeep Krishnan[1], Girish Channarayapatna[1] *[1]1Evonik (SEA) Pte Ltd, [2]PT Japfa Comfeed Indonesia TBK*

A study was conducted to determine the effects of maintaining digestible glycine equivalents (Gly_{equi}) in reduced crude protein (CP) diets on production performance, carcass characteristics and intestinal coccidiosis lesion scores in broilers. A total of 1,904 Lohmann Indian River male broilers were randomly distributed into 4 dietary treatments (14 replicates/treatment; 34 birds per replicate). Graded CP reduction of 4 treatments were as follows: Starter - 21.5, 20.75, 20 and 20% with digestible Gly_{equi} (1.262%) adjusted to the level of 21.5% CP, and for finisher- 19.8, 19.3, 18.2, and 18.2% with digestible Gly_{equi} (1.184%) adjusted to the level of 19.8% CP. Corn-soybean meal-based diets were formulated to meet nutrient requirements according to Lohmann Indian River breed standards for the dietary phases and provided in pellet form. Broilers were fed a common pre-starter diet and experimental diets were fed from d 11 to 35. Feed and water were provided *ad libitum* throughout the experimental period. Response criteria measured include performance parameters [average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), mortality corrected FCR, production efficiency factor (PEF)], and carcass characteristics (percent carcass yield, breast meat, and abdominal fat). Intestinal coccidiosis lesion scoring for *Eimeria acervuline*, *E. maxima*, and *E. tenella* were also measured. Data were analyzed as one-way ANOVA using SAS software version 9.4. Mean values were compared by Tukey's test and statistical significance was declared at $P < 0.05$. Dietary CP reduction from high to low during the experimental period tend to decrease ($P = 0.065$) overall body weight gain. However, birds fed low CP diet to meet dietary Gly_{equi} had similar BWG compared to high CP diet. Although FCR was negatively ($P < 0.05$) impacted with dietary CP reduction, maintaining Gly_{equi} in low CP diet favored similar FCR as high CP diet. Reducing dietary CP and maintaining Gly_{equi} did not affect ($P > 0.05$) PEF, carcass parameters and intestinal coccidiosis lesion scores. In conclusion, results from this study indicate that dietary CP reduction and maintaining digestible Gly_{equi} levels in starter (1.262%) and finisher (1.184%) diets could be an effective nutritional strategy for sustainable broiler production.

Key Words: crude protein, Glycine equivalents, performance, carcass characterisitics, broilers

T18 Effects of dietary Methionine + Cysteine levels on Laying hen production post peak performance from 39 to 51 weeks of age in different breeds Lieske Van Eck, Dinabandhu Joardar*, Kim Ton, Arturo Garcia, Elisangela Guaiume, Sarah Goodgame, Syrena Powell *Cargill Animal Nutrition - North America*

The study investigated effects of increasing levels of dietary True Fecal Digestible methionine + cysteine (TFD M+C) on laying performance from 39 to 51 weeks of age. A total of 648 laying hens were allotted in a completely randomized design in a 6 x 2 factorial arrangement, with six levels of dietary TFD M+C (0.420, 0.450, 0.480, 0.511, 0.571 and 0.668%) fed to two breeds (ISA brown and Dekalb white). Each dietary treatment was replicated 6 times with 9 hens per experimental unit. Egg production was measured daily and egg weights and feed intake weekly. The results showed linear and quadratic interaction (P<0.05) effects on BW at week 43, 47 and 51, where BW was higher with increasing TFD M+C levels, with a stronger response in brown hens compared with white hens. Both brown and white hens linearly increased FI (and M+C intake) as TFD M+C levels increased, with a stronger response in brown than white hens (linear interaction (P<0.05) in all periods). There were linear and quadratic interactions (P<0.05) on egg production in all periods, where brown hens increased lay percentage as TFD M+C levels increased, whereas white hens showed high egg production also with low TFD M+C levels. Therefore, the tested TFD M+C levels were not low enough to find an optimum for egg production in white hens. Both breeds showed linear and quadratic (P<0.05) improvement in FCR with increasing levels of TFD M+C. The broken-line model was used for determination of requirement for total M+C levels (analyzed) and TFD M+C/Lysine (formulated ratio). During 45 to 51 weeks of age, break-point of M+C (analyzed) and TFD M+C:Lysine ratios (formulated) for egg weight were estimated at respectively 0.499% (EW=60.3g, R^2=0.58, P<0.05) and 0.761% (EW=60.5g, R^2=0.59, P<0.05) in white hens and 0.643% (EW=62.1g, R^2=0.39, P<0.05) and 0.991 (EW=62.3g, R^2=0.41, P<0.05) for brown hens. In conclusion, egg weights increased as a response to higher dietary TFD M+C levels, regardless of breed, with a higher break-point and absolute egg weights in brown hens compared to white hens.

Key Words: Laying hens, Methionine + Cysteine, Dose response, Brown, White

T19 Effect of added fat and available lysine levels on growth performance of male turkeys Elisangela Guaiume*, Rachel Abner, Arturo Garcia, Sarah Goodgame, Dinabandhu Joardar, Allan Xue *Cargill Animal Nutrition*

The study investigated single and interactive effects of added dietary fat and available Lys on growth performance and breast meat yield of 2 breeds (A and B) of male turkeys fed from 0 to 21 weeks of age. A total of 1,584 male turkey poults were randomly assigned to 4 dietary treatments replicated 12 times (6 per breed) containing 33 birds per replicate pen. Treatments consisted of two Lys levels (High: HL and Low: LL) and two levels of added fat (High: HF and Low: LF) in a 2 x 2 factorial arrangement. At the end of the trial, 5 birds per pen were labeled to collect data of carcass characteristics. Body weight, feed consumption, mortality, carcass and breast weights were recorded for live performance and breast meat yield calculations. There was no interaction between fat and lysine levels. Overall, birds fed HF diets improved BWG (P < 0.05) compared with those fed LF diets. Similarly, FCR improved (P < 0.05) by 0.12 points in turkeys fed HF diets. There were no differences observed in the breast meat yield. However, breed A had greater (P < 0.05) carcass weight, breast meat weight, and breast meat yield compared with breed B. There was a breed x fat interaction observed, where breed A had greater ADG (0.346 versus 0.358 lbs/ day, respectively for LF and HF) and FBW (50.61 versus 52.38, respectively for LF and HF). In summary, feeding HF diets improved weight gain and feed efficiency, while decreased feed intake. The breed x fat interaction indicated the performance response to dietary fat level in breed A was greater than breed B.

Key Words: Lysine, Fat, Breast meat yield, Turkeys

Metabolism and Nutrition: General Nutrition

T20 Changes in corn starch due to drying temperatures affect post-grinding particle size distribution and broiler nutrient digestibility and energy utilization Edgar Oviedo-Rondon*, Hernan Cordova, Andres Ortiz, Yilmar Matta, Juan Hoyos-Torres, Gherly Buitrago-Mejia, Juan Martinez, Miguel Chico, Jose Yanquen *North Carolina State University*

Maize drying temperatures (DT) modify the starch structure, kernel fragmentation during grinding, and these effects may vary according to kernel hardness. The effects of DT (35, 80, and 120°C) for two corn hybrids, varying in kernel hardness (average and hard) were evaluated on amylose:amylopectin ratio (AM:AP), damaged starch, particle size geometric mean (d_{gw}) and standard deviation (S_{gw}) post grinding, nutrient digestibility, and AMEn. Each corn type was hammermilled with a tip speed of 2,808 m/min through a 12-12 screen combination. A total of 480 d-old male-chicks were randomly distributed among 96 cages in four Petersime battery units. The apparent ileal and total tract digestibility of CP and starch, as well as AMEn were determined using titanium dioxide as inert marker at 16 d of age. Data were analyzed in a completely randomized design with a 2x3 factorial arrangement of treatments. Pairwise correlations and regression analyses were conducted. The interaction of kernel hardness and DT affected (P<0.001) AM:AP and damaged starch. The d_{gw} was negatively correlated (P < 0.001) with AM:AP (r = -0.68) and damaged starch (r = -0.65). The S_{gw} was positively correlated (P < 0.001) with damaged starch (r =0.91). Damaged starch was negatively correlated (P < 0.001) with AMEn for average corn kernel (r = -0.64) and hard corn kernel (r = -0.47). The d_{gw} was correlated (P < 0.01) with AMEn (r = 0.39) for hard corn kernel. The S_{gw} was correlated (P < 0.001) with AMEn of both average (r = -0.58) and hard corn kernel (r = -0.45). The AMEn was reduced (P < 0.001) when AM:AP increased (r = -0.51) for the average corn kernel. In contrast to chicks fed diets based on harder kernel, the AMEn was positively correlated with AM:AP (r = 0.55). The CP total tract digestibility had (P < 0.001) a strong correlation (r = 0.92) with AMEn. The AMEn for average hardness corn = 1,279.8 – 957.1*AM:AP + 6.9*Starch ileal dig. + 23.6*CP total tract dig; R^2 = 0.92. The AMEn for hard corn kernel = 805.5 + 29.0 *CP total tract dig. + 0.6*d_{gw} of corn; R^2 = 0.82. In conclusion, DT modified starch properties and especially the starch-protein matrix. This response varied according to corn kernel hardness affecting fragmentation during grinding, nutrient digestibility, and energy utilization.

Key Words: Amylose:amylopectin, damaged starch, AMEn, particle size, protein digestibility

T21 Sole and combine: Improving the performance of intensively grown native chicken fed with Giant swamp taro and Wild yam root meal as energy sources Albino Taer* *Surigao State College of Technology*

Native chickens are in demand, but the scarcity of feed resources and high feed costs hindered the successful production. Hence, the use of giant swamp taro (GST) and wild yam (WY) meal evaluated the performance of native chicken under intensive farming. Ninety (90) heads of unsexed native chicken fed with different 50% sole and combine root meal inclusion diets distributed randomly to five treatments in three replicates, with six birds each in a completely randomized design. Dietary treatments were: T_0 (commercial), T_1 (corn-based), T_2 (50% GST), T_3 (50% WY) and T_4 (50% GST+WY). The GST, WY meal, and their combinations replaced maize

on a quantitative (w/w) basis by 50% from the total weight of maize in a corn-based diet. Feed intake, body weight and mean weight gain gathered bi-weekly while carcass assessment gathered on the harvest. Commercial diet recorded the highest feed intake (4,008.18g) and lowest for WY diet (3,619.53g) $P<0.05$. Also, the commercial diet found the highest final weight, mean weight gain, and specific growth rate values than the rest of the treatments. Better feed conversion ratio (3.30) noted for commercial than did the GST diet (4.30) $P<0.05$. Significant slaughter weight, nick, and thigh ($P<0.05$), while similar results for dress weight, dressing percentage, breast, back, and organ weights across treatments ($P>0.05$). The sole GST and sole WY diets negatively influenced the performance of chickens; the combined GST+WY diet inclusion as a substitute for maize resulted in improved growth and carcass performance comparable to commercial and corn-based diets.

Key Words: root crops, native chicken, formulated diets, anti-nutritional, energy substitute

T22 Variability in the nutrient content of feed ingredients compromises profitability; a case for improved sampling methodology
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Lerman and Bie (1975) concluded that improper sampling technique is a major component of ingredient variability. Nevertheless, few poultry studies report the sampling technique used, nor is the potential economic cost discussed. However, feed accounts for more than 65% of live production costs of poultry production, thus, accurate feed formulation is vital. Protein is an expensive and crucial macronutrient component of poultry diets; thus, the extent that variation in protein in feed ingredients affects expected performance and profits in poultry diets was modelled.

Wheat-SBM-canola meal-based starter, grower, finisher and withdrawal diets were formulated and profitability modelled using EFG Broiler Model software (EFG Software, 2020). The variability (coefficient of variation; CV) in crude protein of the components of Australian poultry diets were estimated from Moss (2020), and simulations were performed to estimate the likelihood a diet would fall below recommendations using Excel 2016, NORMINV function (10,000 simulations/diet). All prices are in $USD. Within withdrawal diets formulated to 19.2 g/kg crude protein from book values, there is approximately a 10% probability (or one in 10 diets) that the dietary crude protein content will fall below 182 g/kg, and diets may fall as low as 162 g/kg crude protein; which was modelled to lower the gross margin from $15.17/m^2 ($1.01/bird/cycle) to 5.62/m^2 ($0.37/bird/cycle) − a reduction in profits of 63%. Therefore, it is possible to incur a difference of up to $19,086 in gross margin from one cycle of 30,000 broilers by simply overestimating the nutrient content of feedstuffs. Thus, identifying the most accurate way to sample, and improving the understanding and implementation of proper sampling methodology, is of great importance for the poultry industry.

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Key Words: ingredient, variation, crude protein

T23 Mycotoxin contamination predisposes for bacterial chondronecrosis with osteomyelitis lameness in broilers
Jundi Liu*[1], Shelby Ramirez[2], Douglas Rhoads[3], Nnamdi Ekesi[3], Amer Hasan[3], Samuel Rochell[3], Ganapathi Murugesan[2], Barbara Doupovec[4], Todd Applegate[1], Adnan Alrubaye[3] *[1]University of Georgia, [2]BIOMIN America Inc, [3]University of Arkansas, [4]BIOMIN Holding GmbH*

Bacterial chondronecrosis with osteomyelitis (BCO) lameness is hypothesized to occur when bacteria enter the bloodstream via weak intestinal barrier and colonize within micro-fractures in the growth plates of fast growing bones eventually leading to lameness. Mycotoxins (MTX), such as deoxynivalenol and fumonisin have been shown to weaken the intestinal barrier and have immunosuppressive effects. Thus, this trial was aimed to test whether dietary MTX may be a predisposing factor for BCO lameness in broilers. A total of 720 Cobb 500 male chicks were allotted to 1 of 4 treatments in a 2 × 2 factorial design with 2 flooring types [wire (WF) and fresh litter (LF)] and 2 MTX contaminations [low (L-MTX) and high (H-MTX), mainly deoxynivalenol and fumonisin] with 6 pens per treatment and 30 birds per pen from d 0–14 and 25 from 14–57. On d 56 birds and feeders were weighed to calculate body weight gain and feed conversion. Starting on d 21, birds were walked within pen daily to assess lameness. On d 42 and 56, intestinal permeability was assessed via recovery of fluorescein isothiocyanate dextran (FITC-d; 3 – 5 kDa) marker from serum 1 h post after gavage with 8.32 mg FITC-d/kg BW. On d 57, all birds were necropsied and lameness assessed. Lameness and mortality percentage were analyzed with the GLIMMIX procedure of SAS using a binomial distribution and FITC-d (ng/ml) were analyzed using a Gaussian distribution. There was no interaction ($P>0.05$) between flooring and MTX level for overall lameness and FITC-d recovery (d 42 and 56). However, birds fed H-MTX had numerically ($P>0.05$) increased lameness (70.4%) compared to birds fed L-MTX (52.8%) and birds on WF had numerically ($P>0.05$) increased lameness (74.7%) compared to birds on LF (47.5%). On d 56, birds on LF (14.6 ng/ml) had greater ($P<0.05$) sera concentrations of FITC-d compared with birds on WF (4.7 ng/ml). Birds fed H-MTX (13.4 ng/ml) had greater ($P<0.05$) FITC-d compared with birds fed L-MTX (5.9 ng/ml). Therefore, WF was a suitable lameness model in broilers and feeding HMX numerically increased lameness compared with L-MTX fed birds. Further, higher concentrations of dietary MTX increased intestinal permeability. Thus, MTX may be a predisposing factor to BCO lameness by reducing intestinal barrier integrity.

Key Words: mycotoxin, deoxynivalenol, fumonisin, BCO, intestinal permeability

Metabolism and Nutrition: Vitamins and Minerals

T24 Negative impact of a disproportionally high level of dietary 25-hydroxyvitamin D3 on the performance and meat yield of Ross 708 broilers
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Supplemental dietary 25-hydroxyvitamin D_3 ($25OHD_3$) at 2,560 µg/kg of feed under commercial conditions has been shown to improve performance and meat yield of broiler. Also, dietary $25OHD_3$ (crystalline form) has not shown a negative impact on chicken health when it was fed at 10 times higher than 2,560 µg/kg. The objectives of this study were to determine the effects of up to 7 times (7X) higher than commercial level of dietary $25OHD_3$ (69 µg/kg of feed) on the performance and breast meat yield of broilers. Eighteen male chicks were randomly assigned to each of 30 pens within each of 2 dietary treatment. Treatments were: commercial diets containing 8 µg /kg of vitamin D_3 (control) for the starter (0-14 d of age (doa)), grower (15-28 doa) and finisher (29-42 doa); or 483 (7x) µg/kg of $25OHD_3$ for the starter, 276 (4x) µg/kg of $25OHD_3$ for the grower,

and 34.5 (0.5 x) µg/kg of $25OHD_3$ for the finisher dietary phases. The BW, BW gain (BWG), feed intake, and feed conversion ratio of the birds were determined in each dietary phase. At 14, 28, and 39 doa, the breast meat yield of one bird within each of the 30 replicate pens per treatment were weighed and then sampled for determination of the weights of their pectoralis major (P. major) and pectoralis minor (P. minor) muscles. Breast yield was calculated by adding the values of the P. major and P. minor muscles. A randomized complete block experimental design was used and all data were analyzed using a one-way ANOVA. From 14 to 39 doa, the dietary $25OHD_3$ decreased (P<0.0001) broiler performance and increased total mortality (P<0.0001) in comparison to control treatment groups. In addition, birds that received the dietary $25OHD_3$ had a significantly lower P. major (P<0.0001) and breast meat yield (P<0.0001) from 14 to 39 doa as compared to those bird fed the control diet. These results indicate that the disproportionally high level of dietary $25OHD_3$ resulted in detrimental effects on the performance and meat yield of broilers. A reduction in both performance and meat yield of broilers in response to the elevated level of dietary $25OHD_3$ may have been due to vitamin D_3 toxicity, which can result in association with the overproduction of the active form of vitamin D_3, leading to impaired growth

Key Words: 25-hydroxyvitamin D3, Breast meat yield, Broilers, Performance, Vitamin D3 toxicity

T25 Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from hatch to day 10 Carrie Walk*[1], Zhenzhen Wang[2], Shikui Wang[2], Jinlong Wu[2], Jose-Otavio Sorbara[3], Jingcheng Zhang[2] *[1]DSM Nutritional Products, UK, [2]DSM Nutritional Products, Animal Nutrition Research Center, [3]DSM Nutritional Products*

An experiment was conducted to determine the standardized ileal digestible (SID) calcium requirement of Arbor Acres Plus, male broilers from hatch to day 10. Broilers (n = 1,152) were obtained at hatch and allocated to one of six diets in battery pens to day 10. There were 12 birds/pen and 16 pens/diet. The diets consisted of a nutrient adequate positive control (PC) or formulated to contain graded concentrations of SID Ca at 0.60, 0.50, 0.40, 0.30 or 0.20%. Available P (avP) was maintained at 0.48% in all diets, including 0.19% avP expected from 3,000 FYT/kg of phytase. Data were subjected to an analysis of variance and the model included diet and block. Means were separated using orthogonal contrasts to compare the PC vs all SID Ca diets and linear and quadratic effects of SID Ca. There was no effect of graded levels of SID Ca on FI or BWG. Birds fed diets formulated using SID Ca ate (P < 0.05) and gained (P < 0.05) more compared with birds fed the PC. Mortality corrected FCR improved (linear, P < 0.05) as the SID Ca concentration in the diet increased from 0.20 to 0.60%. Tibia ash percent was not different (P = 0.24) between birds fed the PC or diets formulated using SID Ca. Tibia ash percent was greatest in birds fed 0.50% SID Ca and lowest in birds fed 0.20% SID Ca (quadratic, P < 0.05). Apparent ileal digestibility (AID) or retention of Ca or P was greater (P < 0.05) in birds fed the diets formulated using SID Ca compared with the PC. The AID or retention of Ca was similar in birds fed 0.60 or 0.50% SID Ca and increased as SID Ca decreased to 0.20% (quadratic, P < 0.05). The AID of some amino acids was greater (P < 0.10) in birds fed diets formulated using SID Ca compared with the PC. Broilers fed 0.50% SID Ca had the greatest AID of Glu, Pro, Leu, or Phe and this decreased as SID Ca decreased to 0.20% (quadratic, P < 0.10). Regression equations, developed using bone ash, apparent P retention, AID Glu, Pro or Leu, and livability suggest the SID Ca requirement of Arbor Acres Plus broilers from hatch to day 10 was 0.53, 0.48, 0.47, 0.52, 0.48, or 0.42, respectively. This corresponds to an SID Ca to available P ratio of 1.1 to 0.88.

Key Words: amino acids, broiler, digestible calcium, phosphorus, phytate-free

T26 Polyherbal choline supplement as replacement of betaine in broiler diets Javier Gonzalez*[1], Jean Leroux[1], Angela Atkinson[1], Christophe Alleno[2] *[1]Nuproxa Switzerland Ltd., [2]Zootests SAS, Animal Innovation Services*

Betaine (Bet) and choline chloride (CC) are used as methyl donors in broiler feeds. Biocholine® (Nuproxa Switzerland; Indian Herbs), is a polyherbal (PH) composed of *Azaridachta indica* and *Trachyspermum ammi*, with metabolic regulation properties used to substitute choline chloride in poultry diets. In some regions, Bet is used to replace CC in broiler feeds. Thus, the objective of this study was to evaluate if the PH product could replace Bet in broilers diets. The study was conducted at Zootests SAS, France where 3000 day-old male broilers (Ross 308) were randomly and evenly distributed into 10 treatments with 10 replicates per treatment in floor pens. The duration of the study was 42 d. Diets were based on corn, wheat and soybean meal. The control feed (T1) was not supplemented with methyl donors or the PH product. Treatments 2 to 10 included different levels of supplementation (g/ton) of Bet (200 and 400), CC (480 and 960) and PH (100, 200 and 400) and combinations of the above (PH 100+Bet 200 and PH 100+CC 480). Statistical analysis was performed using SAS. For quantitative traits, a linear mathematical model was used. Normality of the traits was tested with residuals of Shapiro test and evenness of variances with Bartlett test. Fixed effects were tested using ANOVA. When significant differences (p<.05) were observed between the diets, LSD test was performed. Results showed that the highest BWG at 42 d was obtained by broilers fed with PH (400 g/ton) and Bet (400 g/ton) with no difference (p>.05) among these treatments. Group of broilers fed with CC were intermediate in BWG. The lowest FCR at 42 d was achieved by broiler fed the combination of PH 100 g/ton + Bet 200g/ton, followed by the groups fed Bet and PH at 200 g/ton of feed each, with no differences (p>.05) between the above treatments. The highest FCR were observed in broilers supplemented with CC and the control group. The results of BWG showed that the PH product can fully replace Bet and CC in broiler diets. The response on FCR indicated that the combination of PH with Bet might have a synergistic effect that needs further investigation. In conclusion, the PH product Biocholine® can be a substitute for Bet in broiler diets with no detrimental effect on performance.

Key Words: broilers, choline, betaine, polyherbal, Biocholine

T27 Effect of different selenium sources on egg production and selenium deposition in aged laying hens under heat stress Robert Shirley*[1], Adam Davis[2], Casandra Juzaitis-Boelter[2], Jana Brackenridge[1], Michele De Marco[3] *[1]Adisseo USA, Inc., [2]University of Georgia, Poultry Science Department, [3]Adisseo France S.A.S.*

The aim of this study was to determine the effect of different selenium (Se) sources on egg production and Se deposition of ageing layers under heat stress. Three different sources of Se were compared: sodium selenite (SS), selenium yeast (SY) and pure hydroxy-selenomethionine (OH-SeMet). A total of 720 HyLine WD-36 laying hens were fed a corn-SBM-DDGS layer diet that was supplemented with 0.3 ppm Se from either SS, SY or OH-SeMet, from 41 to 71 weeks-of-age. Each treatment was provided to 12 blocks of cages, with 10 cages/block and 2 hens/cage. Average daily hen house temperature was 30.1°C, and the average humidity was 52.3% throughout the experiment. Standard production parameters were recorded, and egg and liver sample were collected for Se analyses. Data were analyzed with one-way ANOVA, with the treatment as the fixed effect. Means were separated using a Tukey post-hoc test (P<0.05). Hen body weights and feed consumption did not differ between the three treatments (P>0.119). On a per-hen-basis, over the 30-wk experimental period, hens fed the SS, SY and OH-SeMet produced a total of 179, 182 and 184 eggs, respectively (P=0.036), and 177, 180 and 182 total marketable eggs/hen, respectively (P=0.022). Egg weight and specific gravity were not different between the treatments. Comparing the feed consumption per dozen eggs over the 30-week period, feeding SS, SY and OH-SeMet resulted in the respective feed conversions of 1.656, 1.641 (P>0.05), and 1.603 (P<0.012).

Evaluating the Se content of whole eggs from 47 to 71 weeks, OH-SeMet showed the highest egg Se concentrations (1.72 ug/g DM; P<0.0001), significantly higher compared to both SS and SY (1.11 vs. 1.16 ug/g DM, respectively). Similarly with liver samples that were collected at the end of the trial, feeding hens OH-SeMet resulted in higher Se levels (2.18 ug/g DM; P<0.001) compared to SS and SY fed hens (1.77 vs. 1.78 ug/g DM, respectively). In conclusion, this study indicates that feeding pure OH-SeMet significantly enhances Se transfer into the egg and liver, and appears to be a viable solution that alleviates the detrimental effects of heat stress upon performance in ageing laying hens.

Key Words: selenium, hydroxy-selenomethionine, laying hens, heat stress, aging

Metabolism and Nutrition: Enzymes, Feed Additives

T28 Seaweeds: a sustainable source of feed supplements in poultry production Garima Kulshreshtha[*1], Alan Critchley[2], Maxwell Hincke[1,3] *[1]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa,, [2]Verschuren Centre for Sustainability in Energy and Environment, Cape Breton University, [3]Department of Innovation in Medical Education, Faculty of Medicine, University of Ottawa,*

Poultry production is an important economic sector in modern, global agriculture. Canada produces about 9 billion eggs/year. There has been an increasing interest in the market potential for functional feed, with added-value associated with the improvement in health of chickens and reduced use of antibiotics in poultry. The global market for animal feed supplements was USD 37.83 billion in 2019 and is projected to grow to USD 44.5 billion by 2023. Most recently, various types of seaweed have been utilized as dietary supplements in the poultry industry with a major goal to improve efficacy of feed and bio-conversion for the most cost-effective production of commercially important meat and eggs, with an added benefit of improving poultry health. The global annual seaweed harvest was valued USD 11.7 billion in 2016 for various commercial applications. Seaweeds feed additives function as sources of complex carbohydrates with prebiotic activities, in addition to pigments and polyunsaturated fatty acids, which impart beneficial effects on animal health and productivity. Sulphated polysaccharides from seaweeds are also a significant source of anti-microbials which can serve as an alternative to antibiotic growth-promoters in farm animals. Recent research developments from both *in vivo* and *in vitro* studies (Kulshreshtha et al. 2014 Poult. Sci., 2016 Front. Microbiol., 2017 Front. Microbiol and 2020a JMSE) provide promising evidence to support the utilization of specific seaweeds and their derived compounds to modulate the gastrointestinal microbiome and short chain fatty acids (SCFAs) within the gut. In addition, blends of seaweeds and/or combinations of seaweeds can work in synergy as feed supplements in animal diets to provide beneficial health outcomes. Our analyses (Kulshreshtha et al. 2020b JMSE) suggest that the utilization of specific seaweeds is a sustainable feed source for poultry production, which improves growth, performance, gastrointestinal flora, disease-resistance, immunity and overall health of laying hens/broiler chickens. However, more research is necessary to develop novel functional formulations with enhanced biological activities, which can be achieved by using robust extraction strategies, such as cryomilling, enzymatic and chemical hydrolysis, microwave-assisted extraction and microbial fermentation. Use of innovative strategies to develop new feed resources from selected seaweed supplements with enhanced efficacies will play a vital role as the global poultry industry adjusts to an antibiotic-free future.

Key Words: Seaweed feed supplements, Poultry, Prebiotics, antimicrobial, microbiome

T29 Use of a natural antimicrobial to replace organic acids and formaldehyde in feed for laying hens and productive performance David Díez[*1], Júlia Pié[2], Carlos Domenech[1], Connie Gallardo[3] *[1]Biovet S.A, [2]Innovative Feed & Technological Additives (IFTA), [3]Universidad Científica del Sur*

Disbalances of the gut flora are frequent in livestock and negatively affect performance. Organic acids (OA) and formaldehyde (FO) are used to control the presence of pathogens in the feed and maintain the balance of the digestive flora. However, they have some disadvantages. Alquermold Natural (AMN) is a safe plant-based solution that eliminated feed microbial contaminations and has a positive effect on the digestive flora. An experiment was conducted in laying hens to determine the efficacy of AMN to improve performance in comparison to OA and FO. 224 Dekalb Brown were raised for 14 weeks (15-17 weeks in rearing,18-29 weeks in laying) and distributed following a completely randomized design into 4 groups with 8 replicates each. All groups received the same basal diet without antimicrobials with the following products: control group with no additional products, a group with AMN at 0.5 kg/t, a group with OA at 1 kg/t, and a group with 33% FO at 2 kg/t. Performance was evaluated weekly. Significance was considered if P<0.05. AMN obtained numerically (P>0.05) better results in homogeneity during rearing. In week 17, weight gain significantly improved in all treatments compared to the control, and AMN obtained significantly better weight than OA and FO. In the laying period, AMN obtained higher (P<0.05) laying rate and eggs produced, and lower (P<0.05) feed conversion compared to the control and FO. AMN obtained significantly better results than OA for all three parameters most weeks. The other weeks, AMN still obtained numerically better results than OA. Improvement with AMN was also significant in egg quality, with lower percentages of dirty and broken eggs compared to the other groups. No significant differences were observed in mortality. Overall, formaldehyde obtained the worst parameters, which were worse than the control. In conclusion, AMN significantly improved productive performance and egg quality in laying hens in the rearing and laying phases. OA obtained the second-best results, while FO seemed to exert a negative impact on performance and egg quality. This suggests that AMN is a natural, safe, and effective solution for feed preservation and pathogen control in layers, which is correlated with lower egg contamination and more food safety.

Key Words: layers, botanical, biocide, performance, feed additives

T30 Use of Coneb (botanical) alone and in combination with coccidiostats in experimentally challenged broilers with Eimeria spp. Julia Pie[*1], Sudipto Haldar[2], Tushar Chowdhury[3], David Diez[4], Carlos Domenech[4] *[1]Innovative Feed & Technological Additives (IFTA), [2]Agrivet Consultancy P Ltd, [3]Doctor's Agro Vet Ltd, [4]Biovet S.A.*

Coccidiosis is a serious threat for the poultry industry and conventional mechanisms to control it are becoming obsolete. Coneb (CNB) is a plant-based additive that boost gut local immunity and can replace and complement coccidiostats in broilers.

Objective: An experiment was conducted to evaluate the effect of CNB alone and in combination with salinomycin (SAL) against conventional coccidiostats in experimentally challenged broilers.

Methods: 240 male broilers raised for 35 days were distributed into 8 groups with 6 replicates each. The experiment involved two groups without anticoccidials: uninfected control (NC) and infected control (PC). The other groups were supplemented with CNB or SAL alone, combined (CNB+SAL) and in two shuttle programs (CNB-SAL and SAL-CNB). Another group received diclazuril and narasin (DIC-NAR) in a shuttle program. All groups except NC were challenged with 8000 field-isolated

oocysts of *Eimeria* spp. at day 18 of age. Productive performance was evaluated and statistically analyzed. Significance was considered when P<0.05.

CNB+SAL and DIC-NAR improved daily gain between days 22-35 compared to PC (P<0.05). All supplements obtained numerically better weight than PC (between 152 and 87 g more) and improved feed conversion rate (FCR), between 4.5% and 2.8%, compared to PC (P<0.05). No significant differences were observed between supplements, although CNB+SAL obtained the best FCR among the supplemented groups, followed by SAL-CNB. Liveability was similar between groups (P>0.05): Sal and SAL-CNB obtained the best results, followed by CNB either alone or on top. Oocysts were lower in all supplemented groups compared to PC (P<0.05), obtaining similar results to those of the NC.

Conclusion: The challenge significantly decreased performance in the untreated control (PC), but all supplemented groups could prevent the negative consequences of the challenge: they obtained significantly better daily gain and feed conversion, and numerically better liveability, compared to PC. Results show that CNB is effective to prevent coccidiosis, either alone, on top or in shuttle programs with conventional coccidiostats. Moreover, CNB has additional advantages: it is completely natural, does not create resistances or they need a withdrawal period either.

Key Words: natural coccidiostat, botanical, shuttle program, coccidiosis, poultry

T31 Phenylpropanoid and vanilloid oleoresins ameliorate growth performance in conventional broilers Carl Julien*[1], Bertrand Medina[2], Ivan Girard[2] *[1]Centre de recherche en sciences animales de Deschambault (CRSAD), [2]Probiotech International Inc.*

Focusing on antimicrobial alternatives in poultry production, spices have been studied and have shown promising impact on growth performance in non-medicated feed. Since positive effects on gastric function, gut integrity, inflammation and immunity have been reported, spices might also be useful in conventional production, where antimicrobials are used. A total of 1,440 Ross 308 male broiler chickens were assigned to four dietary treatments: basal diet (control); basal diet supplemented with 25, 35 or 50 g/t of botanicals based on phenylpropanoid and vanilloid rich oleoresins (phenylproanoids:vanilloids ratio=3) granulated in a mineral matrix (Probiotech International Inc). The diets were offered ad libitum to 8 replicates of 45 birds from 0 to 34 days of age. 125 g/t nicarbazin (0-20 d) followed by 100 g/t monensin sodium (20-34 d) and 55 g/t bacitracin methylene disalicylate (BMD) (0-34 d) were used as anticoccidials and antibiotics to assess the oleoresins in conventionally raised broilers. Body weights and feed intakes were recorded on day 10, 20 and 34, and mortality rates were reported per period. The oleoresins dose-dependently increased body weight at the end of the trial on day 34 for all doses (P<0.05), with the greatest effect with 50 g/T (+0.07 kg (+2.7%), P=0.004), compared to untreated broilers. The higher dose increased average daily gain (ADG) during the starter period (0-10 d) (+78 g/d (+3.4%), P=0.033) and all doses increased ADG during the finishing period (20-34 d) (P<0.05), compared to untreated birds. Feed conversion ratio (FCR) was improved in birds fed the higher dose during the starter period (0-10 d) (−0.03 (−2.7%), P=0.005) and 35 g/t oleoresins improved FCR overall (0-34 d) (−0.02 (−1.6%), P=0.017). Mortality rates were not significantly affected by the oleoresins; however, the higher dose (50 g/t) fell just short of statistical significance to decrease mortality during the finishing period (20-34 d; −1.4%, P=0.12). Overall, 25-50 g/t phenylpropanoid and vanilloid rich oleoresins in feed showed improved growth performance in a dose-dependent manner in chicken conventionally raised with antimicrobials. Further investigations of biomarkers of gut integrity and activity could point out the mechanisms.

Key Words: feed additives, spices, broilers, conventional

T32 A comprehensive meta-analysis on the effect of feeding spray-dried plasma on broiler performance E.L. Krabbe[1], C. Marinelli-Martins[2], J. Campbell*[3], R. Gonzalez-Esquerra[3] *[1]EMBRAPA Swine and Poultry, [2]AAC&T Research Consulting LTDA, [3]APC, LLC*

Spray-dried plasma (SDP) modulates immunity, improves gut health, gut functionality, and performance in chickens (Campbell et al., 2019). A meta-analysis on the effect of cumulative SDP intake (g/bird) on BWG, feed intake, FCR and livability of broilers was performed. Performance was expressed in percentage as the difference between each SDP treatment performance relative to the control (i.e., ΔBWG = (BWG SDP – BWG Control)/BWG Control * 100). An extensive search of the scientific literature yielded 29 trials with 92 SDP vs Control comparisons. SDP intake was estimated from the SDP levels used in each treatment and its corresponding feed or water intakes. The inverse-variance method for quantitative continuous variables, weighted by the number of replicates per study, was used. Studies were divided into challenge (i.e. by a pathogen, heat stress, or if mortality was ≥10% for any treatment) or non-challenge conditions. The effect of SDP intake from trials feeding SDP in the first feeding phase only (starter only) was assessed. Four data sets were analyzed: overall (all data), challenge, unchallenge, and starter only. The impact of SDP intake was assessed at the end of the first starter phase or at the end of the trial when ended at ≥ 32d of age (slaughter age). Overall, feeding SDP improved ΔBWG, ΔIntake, ΔFCR & ΔLiv during the starter period (P<0.05). Challenged birds had greater ΔBWG (6.1 vs 3.6%) & ΔIntake (4.5 vs -0.2%) response vs unchallenged. At slaughter age, SDP improved all parameters. Greater responses were seen in broilers under challenge vs those unchallenged. Feeding SDP in the starter diet only, resulted in similar improvements when compared to overall estimates indicating that most of the value of feeding SDP in broilers is realized when fed in the first few days of life. During the starter phase, feeding 3g of SDP/bird increased ΔBWG by 4.0 vs 3.7%; ΔIntake by 4.9 vs -0.7%; ΔFCR by 0 vs -2.0 & ΔLiv by 0 vs 0.2% for broilers under challenge vs unchallenged, respectively, as estimated by regression equations. At slaughter age, ΔBWG by 2.0 vs 2.3%; ΔIntake by 0.5 vs 0.2%; ΔFCR by -5.2 vs -2.5 & ΔLiv by 2.1 vs 1.3% for challenge vs unchallenged broilers, respectively. The main improvement observed with SDP at slaughter age was ΔFCR, ΔBWG & ΔLiv.

Key Words: Meta-analysis, Spray-dried plasma, Performance, Livability, Broilers

T33 Effect of 1-d old chicken quality and spray-dried plasma supplementation in broilers J. Arce-Menocal[1], C. Lopez-Coello[2], E. Avila-Gonzalez[2], J. Polo[3], J. Campbell*[3], R. Gonzalez-Esquerra[3] *[1]Universidad Michoacana de San Nicolás de Hidalgo, [2]Universidad Nacional Autónoma de México, [3]APC, LLC*

Spray-dried plasma (SDP) modulates immunity, improves gut health, gut functionality, and overall performance in chickens (Campbell et al., 2019). 1d-old chicks of low quality underperform at processing age. The effect of feeding SDP to chicks of different quality was assessed. Cobb 500 1d-old chicks were weighed individually at arrival and divided into groups of 36.6 or 44g of BW on average. Groups T1 to T4 had access to feed and water about 12h after hatching, and groups T5 & T6 were fasted an additional 12h (24h in total). Birds were fed iso-nutritional diets with 0 or 2% SDP replacing soybean meal from 0 to 7d of age, and a common dietary program thereafter (T1= 36.6g + Ctrl; T2= 36.6g + SDP; T3= 44g + Ctrl; T4= 44g + SDP; T5= 36.6g + SDP + 24h; & T6= 36.6g + SDP + 24h). Each group had 10 floor pens of 50 birds each. Data were analyzed as a Factorial 2x3 design. At 7d of age, SDP improved BWG, intake and FCR in 36.6 and 44g birds but more noticeably in 36.6g birds. At 42d of age, SDP improved intake (P=0.053) and BWG (P<0.05) vs Ctrl in 36.6g (T2= 2962 vs T1= 2881g of BWG) greater than in 44g birds (T4= 2906 vs T3= 2896g of BWG). No differences in FCR were found at 42d but this parameter was particularly good in all birds (1.54 on average). Fasting 36.6g birds for 24h (T5 & T6) reduced performance at all ages and SDP did not

affected performance in this group. Feeding SDP improved carcass weight and yield by 70g & 1.48%, respectively. These improvements were larger in 36.6g birds (144g & 2.75%, respectively) rather than 44g birds (41g & 1%, respectively) or vs 36.6g + 24h (24g & 0.68%, respectively). At 15d, SDP increased villi number from 42.3 to 44 per 1000 µm2 ($P<0.03$) and absorptive area per µm2 from 9.75 to 10.61 ($P<0.0001$) in the duodenum; and ileum absorptive area from 6.91 to 7.44 per µm2 ($P<0.01$). At 42d, cold carcass skin pigmentation (*b; yellowness), increased with SDP in all groups (41.86 vs 42.76; $P=0.06$). In conclusion, feeding SDP improved performance at 7d and 42d, and carcass parameters with greater responses observed in 36.6g vs 44g chick. Small chicks fasted for 24h showed the lowest intake and BWG of all groups and were unable to respond to SDP. Overall, SDP improved gut development and gut functionality regardless of chick quality.

Key Words: Chick quality, Spray-dried plasma, Performance, Carcass, gut morphology

T34 The efficacy of combined xylanase and direct-fed-microbial in commercially-raised broilers. Rasha Qudsieh*[1], Yun-mei Lin[1], Basheer Nusairat[2], Jeng-Jie Wang[1] *[1]BioResource International Inc., [2]Department of Animal Production, College of Agriculture, Jordan University of Science and Technology*

In the poultry industry, it is common to utilize various enzymes and other feed additives in poultry feed to accommodate the rising demand for safe and affordable proteins and maintain a balance between production, animal wellness, and environmental stewardship. The combined supplementation of xylanase and direct-fed-microbial (DFM) to improve gut health and reduce formulation energy is gaining attention. The objective of this study is to evaluate the efficacy of endo-xylanase and multi-strain *Bacillus* spp. (EnzaPro®, BioResource International Inc.) supplemented to reduced-energy corn-soy-based diets, based on performance, litter moisture, and carcass traits of broilers raised in the production environment to 42 days. A total of 7,500 Cobb 430 broiler chicks were randomly assigned to 1 of 3 dietary treatments with 100 floor pens per treatment and 25 birds per pen. The 3 dietary treatment were: (PC) Standard diet with ME at 3000, 3100, and 3150 kcal/kg in starter (1-14 d), grower (15-28 d), and finisher (29-42 d), respectively; (NC) Negative control with 100 kcal/kg less ME than the PC in all phases; and EnzaPro (EP) supplemented in the NC diet at 100 g/MT. An ANOVA analysis was performed, and the significance was accepted at $P < 0.05$ for all parameters. EnzaPro improved ($P<0.05$) BWG by 103 grams and FCR by and 3 points compared to NC at 42 days of age, while mortality was reduced ($P<0.05$) by 42 %. There were no differences in litter moisture between the treatments. Carcass and parts yield harvested at 42 d were comparable between treatments. Results suggested that adding EnzaPro at a rate of 100g/MT in 100 kcal/kg energy-reduced mash corn-soy broiler diets can improve growth performance and reduce the overall feed costs.

Key Words: Xylanase, DFM, FCR, ME, Antibiotic free

T35 Effects of dietary enzymes on apparent metabolizable energy and ileal amino acid digestibility of broiler chickens fed diets low in energy Michael Persia*[1], Nathaniel Barrett[1], Brian Glover[2], Stephanie Block[2], Dave Heilig[2], Milan Hruby[2] *[1]Virginia Tech, [2]Archer Daniels Midland Company*

An experiment was conducted to determine the effects of two enzymes on the performance, energy digestibility, and ileal amino acid digestibility (iaad) of low metabolizable energy diets when fed to broiler chickens from d of hatch until 16 d. Positive (PC; 3,065 kcal/kg) and negative control (NC; 2,955 kcal/kg) corn-soybean meal diets were formulated. The enzymes (E1 and E2) used were added to the NC diet and are both multi-enzyme non-starch polysaccharide enzyme complex products from *Trichoderma reesei* fermentation. Each diet was fed to 24 replicate cages of 9 broiler chicks (58.5 in^2/bird) resulting in a total of 864 chicks. Feed intake (FI), body weight (BW), and mortality corrected feed conversion ratio (FCRm) were calculated over the 16-d period. After 14 d, excreta trays were cleaned and lined with coated paper for collection of fresh and clean excreta on d 16 for AMEn. On d 16, all remaining chicks were euthanized and the distal half of the ileum was flushed with water to collect contents and pooled by replicate for iaad. Performance, AMEn, and iaad were analyzed using SAS 9.4 ($P \leq 0.05$). Means were separated using a protected LSD test. Initial BW, final BW, and FI did not differ. As expected, FCRm was reduced in the PC fed birds in comparison to the NC fed birds ($P \leq 0.05$), although enzyme treatment did not improve FCRm from the NC fed birds. In agreement with dietary formulation, the PC resulted in a higher AMEn in comparison to the NC, although the absolute difference (42 kcal) was lower than expected ($P \leq 0.05$). Supplementation of the NC with either E resulted in an improvement of the AMEn above the NC (39 and 67 kcal/kg for E1 and E2; $P \leq 0.05$) and similar to the PC. Enzyme 2 increased the ileal digestibility of Asp, Thr, Ser, Pro, Gly, Ala, Cys, Val, Met, Tyr, Lys and His with E1 resulting in an intermediate improvement (except Cys) between E2 and the NC ($P \leq 0.05$). Overall iaad of essential and total amino acids was increased by 1.65 and 1.98 % points for E1 and 4.19 and 4.96 % points for E2, respectively. These data suggest that both enzymes improved the AMEn and iaad when added to broiler diets low in energy over the starter phase of production. In addition, E2 iaad suggested a 2.54% improvement over E1 and a 4.19% increase overall.

Key Words: enzyme, AMEn, ileal AA digestibility, broiler

T36 Effects of Original XPC versus a Refined Functional Carbohydrate product on broiler performance Jeff Firman*[1], Hilary Pavlidis[2], Timothy Johnson[2], Derek Petry[2] *[1]Missouri Contract Poultry Research, [2]Diamond V*

The objective of this study was to evaluate the comparative efficacy of feeding Original XPC™ (XPC), a *Saccharomyces cerevisiae* fermentation product; a commercially available Refined Functional Carbohydrate product (RFC); and a control diet containing no comparable feed additive (CON) on the performance of commercial broiler chickens grown to 35 days of age. A total of 1,440 Ross 708 male broiler chicks were divided into 3 treatment groups at hatch, with 24 replicate pens per dietary treatment each containing 20 chicks. Additives were fed from Day 0-35, with dietary inclusion of XPC at 1.25 kg/MT in the starter diet (Day 0-14) and 0.625 kg/MT in the grower (Day 14-28) and finisher diet (Day 28-35), and RFC at 0.50 kg/MT in all diet phases (Day 0-35). Pen body weights and feed intake were measured at Day 0, 14, 28, and 35 of age. Feed conversion ratio (FCR) and livability were assessed from Day 0-14, 0-28, and 0-35. BW, feed intake and FCR data were analyzed in SAS using the MIXED function with livability data analyzed using the GLIMMIX function. Data were analyzed fitting treatment and block as fixed effects and LSMEANS were calculated. Statistically significant differences in BW ($P = 0.046$) were observed at Day 35, where XPC-fed birds were significantly heavier than RFC-fed birds (2.021 vs 1.932 kg), with CON being statistically intermediate (1.979 kg). For FCR, statistically significant results were observed for Day 0-35 ($P = 0.002$), where XPC-fed birds had significantly lower FCR (1.491 points) than both CON- or RFC-fed birds (1.528 and 1.549 points, respectively). No statistically significant differences were observed at any timepoints for feed intake or livability. Overall, feeding Original XPC at the stepdown dose from 1.25 kg/MT in the starter and 0.625 kg/MT in the grower and finisher diets resulted in improvements in key performance parameters to 35 days of age compared to the birds fed a negative control diet and those fed a diet containing a commercially available RFC product.

Key Words: broiler, performance, RFC, Saccharomyces cerevisiae

T37 Efficacy of a dietary phytogenic formula on the performance, intestinal morphology and microbiota of broiler chicken after experimental challenge with Eimeria spp. Vasilios Tsiouris[1], Ilias Giannenas[*2], Eleftherios Bonos[3], Elias Papadopoulos[4], Ioanna Stylianaki[5], Erasmia Sidiropoulou[2], Diamanto Lazari[6], Ganguly Bhaskar[7], Ioanna Georgopoulou[1] *[1]Unit of Avian Medicine, Clinic of Farm Animals, School of Veterinary Medicine, Faculty of Health Sciences, [2]Laboratory of Nutrition, School of Veterinary Medicine, Faculty of Health Sciences, [3]Department of Agriculture, School of Agriculture, University of Ioannina, [4]Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, [5]Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, [6]Laboratory of Pharmacognosy, School of Pharmacy, Faculty of Health Sciences, [7]Ayurvet Limited*

The current study aimed to investigate the effects of dietary supplementation with a phytogenic formula on the performance of broilers challenged with coccidian oocysts. 150 day-old Ross-308 chicks were housed in cages and randomly allocated to 5 groups. Group Areceived a standard diet without anticoccidials and served as negative control, group B a standard diet without anticoccidials and challenged with *Eimeria*, group C the anticoccidial salinomycin at 60 mg/kg feed and challenged with *Eimeria*, while groups D and E, both challenged with *Eimeria spp.* oocysts, a phytogenic formulation [AV/CPP/12 (M/s Ayurvet Limited, India)] based on plants like *Holarrhena antidysenterica, Berberis aristata, Syzygium aromaticum, Polygonum aviculare,* and *Allium sativum*, at two different rates 1 and 2 g/kg feed. The broilers were challenged at a moderate level with 3.5×10^4 *Eimeria acervulina*, 7.0×10^3 *Eimeria maxima* and 5.0×10^3 *Eimeria tenella* oocysts at 14 days of age. Body weight, feed intake and feed conversion ratio were recorded weekly. One week post inoculation, the extent of bloody diarrhea, oocysts numbers and mortality along with intestinal lesions were evaluated. On the same day, viscosity and pH of jejunal and ileal contents were also measured and samples were taken for histopathology evaluation. All data were subjected to one-way analysis of variance with pairwise comparisons of marginal linear predictions for parametric ones and Kruskal-Wallis test for nonparametric ones. The phytogenic formula improved growth performance comparably equally to salinomycin against *Eimeria* and reduced oocyst shedding. Viscosity and pH values of intestinal contents were not affected. Lesion score was higher in challenged control group, whereas salinomycin and both the groups that received AV/CPP/12 presented lower macroscopic lesions. Villus height was greater in jejunum of the groups receiving the phytogenic formulation or salinomycin as compared to the non-challenged birds, which in turn was even greater than control infected group. Goblet cell enumeration showed higher numbers in the phytogenic groups. In conclusion, the phytogenic formula exerted a substantial improvement on growth performance and intestinal health of *Eimeria* infected birds.

Key Words: broiler, Eimeria spp, experimental challenge, performance, polyherbal formula

T38 The effect of phytogenic compounds via feed or drinking water on the performance, intestinal morphometry and microbiota of broiler chicken Vasilios Tsiouris[*1], Erasmia Sidiropoulou[2], Ilias Giannenas[2], Tilemachos Mantzios[1], Konstadinos Kiskinis[1], Tsiftsoglou Olga[3], Diamanto Lazari[3], Ioanna Stylianaki[4], K. Ravikanth[5], Ioanna Georgopoulou[1] *[1]Unit of Avian Medicine, Clinic of Farm Animals, School of Veterinary Medicine, Faculty of Health Sciences, [2]Laboratory of Nutrition, School of Veterinary Medicine, Faculty of Health Sciences, [3]Laboratory of Pharmacognosy, School of Pharmacy, Faculty of Health Sciences, [4]Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, [5]Ayurvet Limited*

This study investigated the *in vivo* effects of two phytogenic formulations on the performance and intestinal ecosystem in broiler chicks. 450 Ross-308 male one-day-old chicks were provided by APSI PINDOS and raised for 35 days on litter in pens. There were 3 dietary groups; the control group was fed commercial diets (starter, grower, finisher) based on corn and soybean meal without antibiotics or anticoccidials. The second (HERB-F) group received the same diets further supplemented with the AV/ADC/26; herbal remedy at the level of 250 g/tn of feed. It is a phytogenic antidiarrheal and gut function modulator containing *Aegle marmelos, Punica granatum, Plantago ovata*, essential oils and electrolytes. The third group (HERB-W) received AV/ADC/46 in drinking water (0-2 wks: 2.5 mL; 2-4 wks: 5.0 mL; 4-5 wks: 10.0 mL per liter of drinking water). AV/ADC/46 contains phytogenic essential oils rich in eugenol, thymol and cineol as gut function modulator and growth promoter. All chicks were vaccinated on hatchery against infectious bronchitis, Newcastle and Gumboro disease. Feed and water were offered to birds *ad libitum*. Temperature, relative humidity and lighting was monitored throughout the trial. The performance of experimental groups was evaluated at the end of the trial (day 35). The HERB-F and HERB-W treatment improved ($P<0.05$) final body weight compared to Control group (2618.4 and 26620.7 *vs* 2461.3 g, respectively). Water intake showed that HERB-W treatment had higher ($P<0.05$) water intake compared to both Control and HERB-F groups. Feed conversion ratio was better in HERB-W group (1.827) compared to HERB-F group (1.833) and control group (1.877). Intestinal morphometry showed that although villus height was similar among the groups, crypt depth was lesser in HERB groups compared to control. Intestinal microflora determination also showed that total anaerobic and lactobacilli loads were higher in both HERB supplement groups compared to control group. According to the results, the use of phytogenic products can improve performance of chickens raised without antibiotic or anticoccidial drugs.

Key Words: Phytogenic compounds, broiler chicken, water intake, performance

T39 Evaluating the effects of a novel phytogenic feed additive on performance of broilers compared to a chemical coccidiostat during a necrotic enteritis challenge Chasity Pender[*1], Shelby Ramirez[1], Raj Murugesan[1], Lan Zheng[1], Brett Lumpkins[2] *[1]Biomin America, Inc., [2]Southern Poultry Feed & Research, Inc.*

Transition to antibiotic-free broiler production has driven a need for the poultry industry and researchers to seek non-antibiotic solutions for disease challenges including necrotic enteritis. The objective of this study was to evaluate the effects of a novel phytogenic feed additive formulation on performance of broilers during a necrotic enteritis challenge as compared to a commercially available chemical coccidiostat. A total of 800 day-old Cobb 500 chicks were randomly allocated to one of four treatment groups, which consisted of a non-challenged control, a challenged control, a challenged group supplemented with a chemical coccidiostat (zoalene, 125 ppm), or a challenged group supplemented with Digestarom® PEP-Y (125 g/MT). Each group consisted of eight replicate pens with 25 birds per pen. Birds were raised on used litter and on days 19, 20, and 21, $1\times10^{8-9}$ CFU of *Clostridium perfringens* were administered via the feed. The trial was conducted over a 42-day period. Data were analyzed using the GLIMMIX procedure of SAS with significance reported at $P < 0.05$. The challenge significantly impaired bird performance in the challenged control as seen by a 5.6% reduction in final body weight ($P < 0.05$) and a 9 point increase in overall FCR ($P < 0.05$) compared with the non-challenged control. Final body weight and FCR were significantly improved ($P < 0.05$) in the zoalene and Digestarom® PEP-Y supplemented groups compared to the challenged control and were similar to each other as well as the non-challenged control. Though not statistically significant, mortality was numerically improved in the zoalene and Digestarom® PEP-Y supplemented groups compared to the challenged control (0.0% and 2% vs. 4.5%, respectively). Overall, Digestarom® PEP-Y performed similarly to a chemical coccidiostat and could be another valuable tool in the toolbox to combat necrotic enteritis in broilers.

Key Words: phytogenic, necrotic enteritis, antibiotic free, coccidiostat, poultry

T40 Factorial effects of phytate phosphorus levels and novel consensus bacterial phytase variant doses on ileal inositol phosphate degradation and phytate, phosphorus, and calcium digestibility in day 23 of age broilers Abiodun Bello*[1], Trine Christensen[2], Yueming Dersjant-Li[1], Olufemi Babatunde[3], Olayiwola Adeola[3] *[1]DuPont Nutrition & Biosciences, [2]DuPont Nutrition & Biosciences Aps, [3]Department of Animal Sciences, Purdue University*

The interactive effects of phytate P and novel consensus bacterial phytase variant (PhyG) were evaluated on ileal inositol phosphates ($InsP_{3-6}$) concentration (conc), $InsP_6$ disappearance ($InsP_6D$) and digestibility of P (Pdig) and Ca (Cadig) in broilers. A total of 768 Cobb 500 male broilers (8 birds per cage) were fed a commercial diet from d 0-11. From d 12 to 23, the birds were fed a nutrient-adequate positive control diet (PC) or one of 15 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.07% dig. Lys, 0.198% available P, 0.20% Ca and 0.048% Na) diets with 3 phytate P levels and 5 PhyG doses in a 3×5+1 factorial arrangement and with 6 replicates per diet. The diets were based on corn-soybean meal with 0.5% titanium dioxide and polished rice and rice bran were used to maintain phytate P levels at 0.23% (NC1), 0.28% (PC and NC2), and 0.33% (NC3). Each NC was supplemented with PhyG at 0, 500, 1,000, 2,000, and 4,000 FTU/kg. On d 23, ileum digesta were sampled per pen and while only the 15 NC diets were analyzed for $InsP_{3-6}$ conc and $InsP_6D$, all diets were analyzed for Pdig and Cadig. Mainly, data were analyzed for 3×5 factorial arrangement using the fit curve function of JMP 14.1. Increasing phytate P level linearly increased (P<0.05) $InsP_6$ conc from 0.588 to 1.051 to 1.557% with similar effect for $InsP_3$-$_5$ conc, but linearly decreased (P<0.05) Pdig from 58.8 to 50.0 to 41.7%, with similar effect on Cadig and $InsP_6D$. Increasing PhyG doses linearly decreased (P<0.05) $InsP_6$ conc (2.23, 1.41, 1.08, 0.49, and 0.12%) and increased (P<0.05) $InsP_6D$ (31.1, 59.1, 69.2, 86.1, and 96.2%), Pdig (32.7, 44.2, 52.1, 57.4, and 64.1%) and Cadig (33.2, 37.8, 42.1, 44.9, and 47.5%) for the 0, 500, 1,000, 2,000, and 4,000 FTU/kg doses, respectively. At each phytate P level, $InsP_6$ conc was linearly decreased (P<0.05) while each of $InsP_6D$ and Pdig linearly increased with increasing PhyG dose. Also, each of $InsP_{3-5}$ conc inconsistently decreased with increasing PhyG dose at each phytate P levels. Overall, increasing dose of the novel phytase from 500 to 4,000 FTU/kg linearly increased $InsP_{3-6}$ degradation to increase $InsP_6D$, Pdig, and Cadig at each phytate P level. Phytate P level in diet should be considered for ideal phytase dose needed for optimal phytate degradation.

Key Words: broilers, digestibility, inositol phosphates, phytate phosphorus, phytase dose

T41 Salmonella diversity and antimicrobial resistance behavior: the feed mill a source? Yadir Mauri Villarreal[1], Rocio Paredes Barbosa[2], Martha Pulido-Landinez*[3] *[1]Unidad de Posgrado Investigación y Desarrollo Maestría en Biotecnología Molecular. Universidad de Guayaquil, [2]AGROAVILAB S.A., [3]Mississippi State University*

The identification and control of Salmonella sources in a vertical integration are basic steps to be included in a comprehensive control program. Feed as potential contributor to Salmonella diversity in poultry farms must be considered as important. The antimicrobial resistance exhibited by Salmonella isolated from ingredients and feed are important on terms of the current fight against antimicrobial resistance. A total of 1960 samples collected from a feed mill were analyzed for *Salmonella sp* presence. After detection, Intergenic Sequence Ribotyping (ISR) and antimicrobial sensitivity test (AST - disk diffusion method), were performed. A total of 50 isolates of *Salmonella sp* were selected after isolation from ingredients, feed, and drag swabs from surfaces. Descriptive statistical analysis was performed to stablish the frequency of identification of serotype and AST patterns by source category. The bacterial susceptibility results were analyzed using the software WHONET 5.6 to stablish the MICs to inhibit the 50% and 90% of the organisms (MIC50, MIC90), the MIC range for each antimicrobial, and the percentage of resistant, intermediate, and susceptible isolates (% RIS multifile analyses). Ten different serotypes were identified. The serotypes with the highest isolation percentages by category were: raw material: Soerenga (30% soybean, 10% corn); Equipment: Soerenga (20% pelletizer, 10% hopper) and Mbandaka (20% chiller, 10% pelletizer). Feed: Uganda (10% prestarter, 10% starter, 10% grower, 5% finisher), Infantis was isolated from all feed types in low percentage. Transport: Kentucky (10% truck, 20% truck driver's cabin) and Mbandaka (10% truck, 10% driver's cabin). AST results showed differences between serotypes and among isolates of the same serotype. Of a total of 50 isolates, 30 (60%) were multidrug resistant (resistance to 3 or more antimicrobials), 18 isolates (36%) did not present multi-resistance, and only 2 isolates were pansusceptible. The detection of 10 different serotypes and the high variation in AST results within this group of Salmonella isolates demonstrated the importance of conducting periodic evaluations in the feed mill to monitor epidemiological trends, and the role of the feed mill in a Salmonella comprehensive control program.

Key Words: Salmonella, Feed mill, Antimicrobial resistance, Monitoring programs, ISR

T42 Effects of Phylox™ on performance, fecal oocyst shedding and intestinal lesion score of Eimeria-infected broilers: A meta-analysis of 4 experimental coccidiosis trials Hongyu Xue*, San Ching, Sara Johnston *Amlan International*

Phylox™ is a natural anticoccidial feed additive, consisting of a select blend of bioactive phytochemicals. Recent research suggests the product has potential as an alternative to ionophores to help prevent coccidiosis while maintaining gut health and bird productivity. Four *in vivo* trials were conducted to evaluate the effect of Phylox supplementation. The birds in these four trials were challenged with a coccidial inoculum containing single or multiple common *Eimeria* field isolate strains and fed various feed additives, including Phylox at 70 g/MT, for 20 to 28 days. Data from all four studies were combined in a meta-analysis to focus on the effect of Phylox versus the non-supplemented control. Data from all studies were tested for homogeneity, pooled, and analyzed by the random-effects model of Comprehensive Meta-Analysis Version 2.0 software with study as a random effect. Parameters for analysis included: weight gain during post-challenge phase (Days 14-20), feed conversion ratio (FCR) post challenge (Days 14-20), cumulative fecal oocyst count (Days 19-22) and coccidial lesion score (Day 20). The meta-analysis revealed that broilers supplemented with Phylox at 70 g/MT had significantly greater weight gain (187 vs 161 g; P = 0.0003) and better FCR (2.275 vs 2.872; P = 0.0009) than the control birds during the post challenge phase. Phylox treatment also decreased cumulative fecal oocyst count (4.585 vs 4.779 $\log_{10}$; P = 0.00005) and reduced coccidial lesion score (1.982 vs 2.426; P = 0.0004). In summary, Phylox significantly improved broiler performance post *Eimeria* spp. challenge, reduced fecal oocyst output and improved coccidial lesion score.

Key Words: coccidiosis, coccidiostat, antibiotic alternative, ionophore, broilers

T43 Effects of 3 Bacillus strains based probiotic supplementation on E.coli and Clostridium Spp. caecal counts and performance of broilers Jean-Christophe Bodin*[1], Khanittha Saikhlai1[1,2], Nattapong Choomkasian[1,3] *[1]Bangkok Animal Research Center Co., Ltd., [2]Bangkok Animal Research Center Co., Ltd, [3]Animal Supplement & Pharmaceutical Co., Ltd.*

This trial was designed to evaluate the effect of a 3-strain *Bacillus*-based probiotic (GalliPro® Fit) supplementation in diets on performance of broilers from newly hatched to 42 days of age. 768-day-old Cobb male chicks were randomly distributed in 32 pens (24 birds/pen) allocated to 2 dietary treatments: T1 (Basal diet without probiotics) and T2 (T1 + GalliPro Fit at 1.6 x10^6 CFU/g of feed). For T1 and T2, Basal diet used during the breeding phases were formulated on Cobb nutritional recommendations, no Antibiotic Growth Promotor (AGP) was added and Salinomy-

cin was used as a coccidiostat. Feed and water were provided *ad libitum*. Body weight (BW), Feed Intake (FI), and Feed Conversion Ratio (FCR) were measured at Days 10, 14, 21, 28, 35 and 42. On day 28, 3 birds from each pen (48 birds / treatment) were selected for the collection of caecal sacks. The bacteria counts for *E. Coli* and *Clostridium* spp. of cecal contents were determined. The results were analyzed by one-way ANOVA, P ≤ 0.05 were recognized as statistically significant. Pen means were used as the experimental units for the analysis. Despite high performing birds, the addition of GalliPro Fit improved significantly (P ≤ 0.05) BW from day 21 (+ 17 g) throughout day 42 (+66 g) compared to the broilers receiving Basal diet. FCR was significantly decreased (P ≤ 0.05) for birds supplemented with GalliPro Fit with a -0.03 point average reduction. Mortality for both treatments was very low with no significant difference. At day 28 of age, the microbial counts (log CFU/g) in caeca showed significant reduction for *E.coli* (-14%) and *Clostridium* spp. (-18%) for broilers which received Basal diet + GalliPro Fit (1.6 x10^6 CFU/g of feed). Even if not all *E.coli* and *Clostridium* spp. can be considered pathogenic, observed reduction helped to drop the pressure of pathogenic challenges. The current study shows that GalliPro Fit can significantly reduce potential pathogens in caeca and can effectively improve broiler performance. The positive response on performance was likely due to GalliPro Fit's influence on the microbiome composition of the birds and the moderation of *E.coli* and *Clostridium* spp. at day 28. Animal energy could be spent on growth rather than managing pathogenic challenges.

Key Words: Bacillus, E. coli, Clostridium, Productivity, Microbial

T44 A Trichoderma reesei enzyme tool-box generates soluble XOS from corn and improve performance of corn fed broilers Nelson Ward*[1], Roselina Angel[2], April Levy[1], Eduardo Della Pia[3], Morten Tovborg[3] *[1]DSM Nutritional Products, [2]University of Maryland, [3]Novozymes A/S*

The dominant hemicellulose in corn is arabinoxylan (AX). However, corn AX differs from most other cereal AX by its complexity which includes higher arabinose- and distinct level of glucuronic acid branching. Hence, corn AX is sometimes referred to as glucuronoarabinoxylan (GAX). Solubilization of corn GAX to soluble arabinoxylan oligomers (AXOS) may have effectively benefit such as direct nutritional improvements due to decaging and improved gut functionality due to prebiotic feeding of butyrate-producing microbes in the lower tract.

We have characterized a commercial feed enzyme product (MultiGrain®) from a specific strain of *Trichoderma reesei* known for its comprehensive carbohydrase genome. Proteomic analysis shows a rich diversity of enzymes targeting the carbohydrates found in corn. They include GH10 and GH11 xylanases hydrolyzing the xylan backbone, arabinofuranosidase, xylosidase and α-galactosidase with debranching activity on arabinofuranosyl, xylose and galactose sidechains of xylan, as well as acetyl xylan esterase and glucuronoyl esterase which increases NSP solubility by hydrolyze carbohydrate esters. In vitro incubation corn with MultiGrain® shows generation of soluble AXOS. Immunofluorescence confocal microscopy visualized and confirmed solubilization of the insoluble corn GAX following enzyme treatment.

In an in vivo experiment, MultiGrain was supplemented to a corn-based diet and fed to broilers over a 32-day period to evaluate body weight (BW), feed intake (FI), and FCR. Birds were fed a common control during the initial eight (8) days post-hatch. Three treatments (trt) were randomly assigned with 8 reps/trt in a battery trial: (1) positive control (PC) for all nutrients (NRC, 1999); (2) negative control (NC) formulated to be 131 and 134 kcal/kg lower in metabolizable energy than PC for grower (d 9 to d 28) and finisher (d 29 to d 32), resp.; (3) NC + MultiGrain at recommended level. D 32 BW and FCR (but not FI) differed (P<0.05) between PC and NC, while the addition of MultiGrain improved FCR (P<0.05) over the NC by 2.4% and BW by 4.7%. BW of PC and NC + MultiGrain-fed birds did not differ (P>0.05). These data indicate that MultiGrain effectively improves the utilization of corn-based diets fed to broilers.

Key Words: corn, non-starch polysaccharides, carbohydrase, enzymes, performance trial

T45 Type of studies for FDA registration of modified live therapeutics Kathryn Kruziki, Yiannis Kaznessis* *General Probiotics, Inc.*

Clostridia perfringens is the causative agent of necrotic enteritis in chicken. Necrotic enteritis results in daily losses of 1% in poultry production facilities. Necrotic enteritis causes an estimated $6 billion in annual productivity losses globally. Additionally, foodborne Clostridia perfringens infects over 1 million people in the US annually.

The FDA's Veterinary Feed Directive is resulting in antibiotics being phased out from livestock production. The incidence of necrotic enteritis is expected to rise because of the withdrawal of antibiotics.

A new technology is needed for farmers to raise healthy poultry free of foodborne pathogens.

Our core competency is the precise engineering of advanced probiotics using synthetic biology techniques. Our probiotics are engineered for performance against pathogens inside the GI tract of animals. We have demonstrated high performance against of Salmonella enterica, Clostridia perfringens, Campylobacter jejuni in poultry, and E. coli, Lawsonia intracellularis and Streptococcus suis in swine. In our most recent experiment, we observed a 67% decrease in the mortality rate caused in broiler chickens by necrotic enteritis, when the birds were administered our antimicrobial cellbots.

We will present the agile research and development of live biotherapeutics against Clostridia perfringens. We will present results of animal trials demonstrating the effectiveness and safety of this technology. Based on these results, the FDA CVM granted us a waiver of fees to open an investigational new animal drug file. We will detail the unique development path for registering our product with the FDA CVM. To our knowledge this a first for a probiotic-based technology.

Key Words: live therapeutics, necrotic enteritis

POSTER SESSIONS

Teaching, Pedagogy, Extension

P1 What is the poultry industry really seeking in college graduates?: A perspective study to assess key traits and competencies important to poultry industry management personnel J.D. Scott*[1], K.M. Downs[2], J.P. Gulizia[3] *[1]University of Georgia, [2]Middle Tennessee State University, [3]Auburn University*

A perspective online survey instrument was constructed to identify certain skills and traits, including leadership behaviors and competencies, perceived important for career success by those in various management levels of the poultry industry. The survey instrument was comprised of 26 questions separated into two sections. Twenty-one questions assessed the importance of personality characteristics and competencies, and the remaining 5 evaluated the effectiveness of industry recruitment activities. A Likert scale (1=not at all important to 5=extremely important) was used to evaluate responses. Principal respondent targets included those in human resources, sales/marketing, live production and processing, and

complex management. Some industry veterinarians and nutritionists were also targeted. Emails with survey access link and instructions were sent to broiler, table egg, and turkey industry management personnel in AL, AR, CA, FL, GA, IA, MI, MO, MS, NC, OH, OK, PA, and TN. Respondents remained anonymous. Response rate was approximately 40%. Of the total responses, a majority of participants classify as working in live production (43.1%) or as a complex manager (12.3%). Participants (n=65) selected work ethic (4.75), teamwork ability (4.57), initiative to work (4.52), leadership ability (4.41), and problem-solving ability (4.37) as the 5 most important characteristics/competencies. Additionally, participants indicated knowledge of general science (2.97), a student's undergraduate major (2.64), a student's GPA (2.44), knowledge of a foreign language (2.15), and completion of undergraduate research (1.79) as the 5 least important characteristics/competencies. Respondents identified individual university contacts (3.59), departmental career fairs (3.28), and guest speaking opportunities (3.21) as the most important ways for student engagement. Preliminary data suggests undergraduate studies should focus on developing leadership skills and problem-solving abilities, and promoting an initiative to work and working in teams. Further, emphasizing relationships between higher education and industry can expand opportunities for graduates to enter the poultry industry. This study is ongoing with further expansion into additional states and continued response collection.

Key Words: Undergraduate Education, Poultry Industry, Teaching, Recruitment

P2 Small flock programming during a pandemic Maegan Perdue*[1,2], Jonathan Moyle[3], Enrique Escobar[1] *[1]University of Maryland Eastern Shore, [2]University of Maryland Extension, [3]University of Maryland*

During the late winter and early spring, the University of Maryland Poultry Extension team provides new and existing small flock owners educational programming. This programming usually takes place at farm and feed stores, which growers frequent to purchase chicks and supplies. However, due to the COVID-19 pandemic, in-person programming was suspended so educators decided to put together a series of online sessions to educate small flock owners on important topics such as: biosecurity, predators, hatching eggs, meat production and egg production. There were eight small flock sessions as part of this series and each session was taught at two different times, mid-day and evening in order to reach as many growers at possible. In addition to poultry, the Backyard Farming series included sessions on small ruminants, cattle and hay. University of Maryland Extension faculty taught all of the sessions with 342 people participating in the poultry sessions. While the program targeted local growers, the majority of those that participated lived outside the state of Maryland. Results from a follow-up survey found that 84% of participants indicated that the COVID-19 shutdowns affected their decision to participate and almost 95% indicated that the information they learned would help keep their animals healthier. A further 76% reported that they had already made changes to how they manage their birds based on information received, while 18% more stated they planned to. As all those that participated in the poultry sessions reported that the information was valuable, it appears that online educational programs is a viable option when face-to-face meetings are prohibited.

Key Words: education, poultry, small flock, extension

P3 Changes in broiler industry of China, Japan, and South Korea following the COVID-19 pandemic Soobin YOON[1], Seungjun SHIN*[2], Sang-Hyon OH[3] *[1]Amerikor International Foods, [2]PPS, Agriculture Corporation, [3]University of Maryland Eastern Shore*

This study is to evaluate the changes in broiler prices and/or poultry industry of China, Japan, and South Korea following the COVID-19 pandemic. The data used in this study were collected from the websites of Ministry of Agriculture and Rural Affairs of the People's Republic of China, Japanese Ministry of Agriculture, Forestry and Fisheries, Statistics Bureau of Japan, Korean Ministry of Agriculture, Food and Rural Affairs, Statistics Korea from 2017 to 2020. In China, chicken producers face a challenge of oversupply following the African swine fever outbreak in 2018. This oversupply has been further exacerbated by rising chicken feed prices. These two phenomena have resulted in a decline of chicken prices. Due to school closings in response to the COVID-19 pandemic, the demand for boilers has fallen. This fall in demand was met with a subsequent fall in price value, which stands 57% lower than the highest price of boilers recorded in October 2019. Japan was one of the first countries worldwide to be impacted by COVID-19. Unlike other Asian countries, Japan didn't prioritize efforts to shut down industries/workplaces and enforce social distancing. As a result, in Japan, the price of chicken thighs has risen compared to the previous year by 10-15%. But, this price rise has had a lower impact on Japan's domestic market compared to other countries. Due to the declining demand for meat products in Japan, the inventory of all meat products, including poultry, has risen. In South Korea, the COVID-19 pandemic has instigated an increased demand for the delivery of chicken foods, causing a spike in poultry consumption. A recent survey done by the Korean Rural Development Administration between June 30 and July 24 asked 1,110 men and women of ages 20-69 how often they consumed chicken. The results showed that 70% of households consumed chicken more than once a week. This is a 5.6% increase compared to 2017. The annual chicken consumption per adult increased by 1.2kg for a total of 15.76kg. If the impact of COVID-19 was not factored into the annual consumption calculation, it is estimated that the annual consumption would be 15.22kg. This means that overall poultry consumption has increased by 4.69% due to COVID-19. Home delivery of chicken foods has increased by 11.5%.

Key Words: COVID, Broiler, China, Japan, Korea

P4 Physical and physicochemical aspects of woody and normal condition in broiler breast meat Sang-Hyon OH*, Euyeon NOH, Byungrok MIN *University of Maryland Eastern Shore*

This study is to investigate the difference between woody (WB) and normal (NB) conditions in broiler breast meat using physical and physicochemical aspects. Woody breast is an issue of chicken meat quality arising from a muscle abnormality in the chicken, in which the chicken breast meat becomes more rigid and sometimes paler in color with inferior texture. The frequency of this defect in Europe is over 30% and some have argued that similar levels may exist in the US. Given that chicken with WB are of poor economic value and are discriminated by the consumers, producers face significant economic hardship under the current production paradigm. One hundred samples were used in this analysis (WB: 50; NB: 50), which were obtained from a local processing plant one day after harvest and sorted based on WB scoring (0 and 1 for NB while 2 and 3 for WB). The meat was subjected to physical and physicochemical analyses: pH, color (L*, a*, b*), cooking yield, and texture (firmness and compression energy were used for raw meat while shear force and energy for cooked meat). The textural properties of raw and cooked meat were measured using a texture analyzer and digital palpation device, and the variables considered in the measurements included oscillation frequency, stiffness, elasticity, mechanical stress relaxation time, and the ration of deformation and relaxation time. To examine the properties of cooked meat, meat was vacuum-packed, placed in boiling water, and taken out when the internal temperature reached 74°C. One way ANOVA was used to analyze the data as a statistical model. The least-squares means of the following variables were significantly different between WB and NB ($p<0.01$): WB score (1.98 vs 0.48), pH (5.99 vs 5.89), cooking yield (60.98 vs 75.49; %), b* (8.68 vs 6.78), firmness (20.18 vs 8.93; N), compression energy for raw meat (60.52 vs 26.41; N·s), cooked L* (78.66 vs 81.04) and b* (15.72 vs 14.52), shear force (12.87 vs 9.91; N) and energy (184.1 vs 125.7; N·m) for cooked meat, stiffness (603.4 vs 565.8; N/m), and elasticity (1.40 vs 1.55). These results collectively show that the physical characteristics of

WB differ from NB. If some of these physical characteristics can be quantified in live birds, it can be used for the genetic selection program.

Key Words: Woody, Physical, Physicochemical, Broiler, Breast

P5 Simulation study to control inbreeding for selection experiments on woody breast Hye Rin JEON[1], Seungjun SHIN[2], Sang-Hyon OH[*3] *[1]Ewha Womans University, [2]PPS, Agriculture Corporation, [3]University of Maryland Eastern Shore*

This study was to compare the results of the simulated selections based on the Optimum Genetic Contribution theory (OGC) under different conditions over 10 generations, which uses relationships among individuals as weighting factors. In a selection study, the experimental population should be maintained as a closed herd, which may cause high levels of inbreeding over time that are unavoidable due to no introduction of new genetic sources, also often associated with decreased performance known as inbreeding depression. One method to increase selection response enough while minimizing inbreeding is selecting individuals by weighting estimated breeding values with average relationships among individuals. This simulation study was designed to figure out which mating plan would show proper breeding values while controlling inbreeding assuming that a selection study would be done on the woody breast in broilers starting with 500 males and 500 females as a foundational population. From the 2nd generation, various selection plans were considered in each sex, which were the top 10, 20, 50, and 100 males selected, and the top 100 and 200 females selected every generation. Each female bird was assumed to have 10 eggs. The algorithm is as follows: 1) Identify the individual having the best EBV; 2) Calculate average relationships between selected and candidates; 3) Select the individual having the best EBV adjusted for average relationships using the weighting factor k; 4) Repeat process until the number of individuals selected equals number required. Three different weighting values (k=0, 1, 2) were used, which made a total of 24 different conditions compared (4×2×3). The additive genetic variance of breast meat was 1.134. Mendelian sampling terms were also considered when the breeding values were generated. Results showed that higher k value (k=2) controlled effectively inbreeding and maintained consistent increases in selection response. Differences in breeding values among selection plans with OGC and by EBV only were 4% on average; however, the average rate of inbreeding (0.1) was controlled by 27% after 10 generations. These results indicate that OGC can be used effectively in a short-term selection program with a relatively smaller number of populations.

Key Words: Simulation, Inbreeding, Optimum Genetic Contribution, Selection, Woody Breast

Physiology, Endocrinology and Reproduction: Broilers

P6 Effects of intensive genetic selection for growth efficiency on gene expression associated with calcium and phosphorus metabolism during embryonic and early post-hatch development in broilers Manuel Arango[*GS], Laura Ellestad *University of Georgia*

Genetic selection in commercial broilers has improved growth performance, though an increase in leg disorders due to poor bone density has occurred. The active form of vitamin D_3 [1,25$(OH)_2D_3$], parathyroid hormone (PTH), and calcitonin (CALC) are key hormonal regulators of Ca and P homeostasis, and the kidney plays a major role in this process by regulating vitamin D_3 metabolism and mineral balance. This study sought to elucidate mechanisms of Ca and P metabolism in kidney that may have been affected by genetic selection. Kidney was collected on embryonic days (E) 14, 16, 18, 20 and post-hatch days (D) 0, 1, 3, 5, 7, 10, and 13 from Cobb 500 and Athens Canadian Random Bred (ACRB) birds representative of broilers from the 1950s (n=6). Levels of mRNA for enzymes involved in vitamin D_3 conversion and hormone receptors regulating Ca and P balance were measured by RT-qPCR. Data were analyzed with 2-way ANOVA followed by the test of least significant difference. Levels of mRNA for 25-hydroxylase, which converts vitamin D_3 to 25$(OH)D_3$, were higher in ACRB at E20 and in Cobb at D3, while 24-hydroxylase, which deactivates 1,25$(OH)_2D_3$, was higher in Cobb at D3, D10, and D13. Vitamin D receptor and its transcriptional partner retinoid-x-receptor α were higher in ACRB at D7 and higher in Cobb at D13 ($P \leq 0.05$). While PTH receptor 1 (*PTH1R*) did not differ between groups ($P > 0.05$), *PTH3R* exhibited a main effect of line and was higher in ACRB ($P \leq 0.05$). Expression of CALC receptor was higher in ACRB at E16 and D7, while Cobb was higher at D13 ($P \leq 0.05$). Our results suggest that post-hatch deactivation of 1,25$(OH)_2D_3$ in the kidney of Cobb broilers could occur at a higher rate than in ACRB. Further, ACRB may have increased sensitivity to circulating 1,25$(OH)_2D_3$ on earlier ages relative to Cobb. Kidney sensitivity to PTH may also be higher in ACRB, which could enhance production of 1,25$(OH)_2D_3$ and Ca resorption. Altered sensitivity to CALC across developmental stages may also result in differing Ca excretion between the lines. Results indicate that genetic selection has influenced hormonal pathways regulating Ca and P metabolism, and it appears that enhanced sensitivity of legacy birds to these hormones at earlier ages may be crucial for formation of better skeletal structure.

Key Words: Vitamin D3, parathyroid hormone, calcitonin, gene expression, skeletal health

Physiology, Endocrinology and Reproduction: Layer or Broiler Breeders

P7 Metabolizability coefficients of dry matter, crude protein and crude energy of different soybean meals for broilers Lorrayne de Paulo[1], Alison Gouveia[1], Deibity Cordeiro[1], Natiele de Oliveira[1], Marcos Café[1], José Stringhini[*1,2] *[1]Departamento de Zootecnia, Escola de Veterinaria e Zootecnia, Universidade Federal de Goias, [2]CNPq research fellowship*

Soybean meal is a key ingredient for animal feed, containing high quality protein and energy contente that is not completely used by birds. Its nutritional composition can be influenced by factors such as soybean cultivar, soil type, latitude, environmental conditions, amount of antinutritional factors, oligosaccharides and non-starch polysaccharides and the processing method used. The quality of soybean meal reflects the ability of animals to metabolize the nutrients present in this ingredient. Therefore, the objective of this study was to evaluate the metabolizability coefficients of dry matter (DMMC), crude protein (CPMC) and crude energy (CEMC) of broilers fed different commercial soybean meal. The design used was completely randomized (IHD), with five treatments, five replicates containing eight birds per experimental unit, cobb500 males®. The treatments consisted of: reference diet (RD); RD + 40% soybean meal A; RD + 40% soybean meal B; RD + 40% soybean meal C; RD + 40% of soybean meal D. The analysed chemical and quality values for soybean meals A,B,C and D were, respectively: 44.3; 44.6; 42.1 and 45.0% for crude protein; 0.05; 0.03; 0.07 and 0.03 ΔpH for urease; 83.3; 83.8; 79.9 and 87.8% of KOH solubility; 37.8; 33.4; 26.8 and 33.6% of protein dispersibility. For the determination of the metabolizability coefficients of the experimental treatments, the methodology of total excreta collection was used in the period from 14 to 21 days of age. The variables analyzed were submitted to analysis of variance (ANOVA) and Tukey test (5%) was used to compare

the soybean meals tested. The values calculated for DMMC, CPMC and CEMC showed no significant difference between them. The values calculated for the DMMC were: 69.70%, 71.12%, 71.42%, 70.69% for bran A, B, C and D, respectively. The values calculated for the CPMC were: 57.78%, 56.94%, 60.00%, 57.81% for bran A, B, C and D, respectively. The values calculated for CEMC were: 74.67%, 74.84%, 74.86%, 74.51% for bran A, B, C and D, respectively. It was concluded that the commercial bran evaluated in the region have similar values for metabolizability coefficients of dry matter, protein and crude energy.

Key Words: animal feeding, ingredients, metabolism, nutrients

Processing and Products

P8 Classification efficiencies of Support Vector Machines (SVM) and Backpropagation Neural Networking (BPNN) in the categorization of chicken breast muscle myopathies Aftab Siddique*[1GS], Jaroslav Valenta[2], Alice Smith[1], Amit Morey[1] *[1]Auburn University, [2]Czech University of Life Sciences, Prague, Czechia*

Big data analytics classification methods such as artificial neural networks and support vector machines (SVM) are being increasingly used in the manufacturing sector. Bioelectrical impedance (BIA) data from raw poultry meat affected with myopathies such as woody breast (WB) white stripping (WS) and spaghetti meat (SM) is highly complicated especially due to the overlap of these myopathies on breast fillets and manual assignment of fillet categories. Previous research has shown that traditional statistical techniques such as ANOVA, regression among others are insufficient in the classification of myopathy affected fillets using BIA. There is a need to use SVM and BPNN to classify raw poultry breast myopathies using their bioelectrical impedance patterns such that the technology can be beneficial for the poultry industry in detecting the myopathies. Freshly deboned (3-3.5 h post-slaughter) deboned breast meat (n=100 x 3 flocks) were analyzed for 32 breast myopathy categories as follows: visual WS (0-normal; 1-mild; 2-moderate; 3-Severe) x hand-palpation WB (0-normal; 1-mild; 2-moderate; 3-Severe) x SM (presence and absence). BIA data (resistance and reactance) was collected on each fillet, the equipment's algorithm calculates protein, fat, and water index. Data were analyzed using SVM and BPNN with 70:30:: training: test data set. Chicken breast fillets (n=300) were classified into 23 of the 32 myopathy categories with 12% of the fillets categorized as normal WB x normal WS x absence of SM followed by 10% of the fillets with moderate WB x Mild WS x absence of SM. SM was most prevalent in fillets with normal and mild WB and WS. Compared to BPNN, SVM classified WB with an accuracy of 71.04 % for normal (data for normal and mild merged), 50% for moderate, 81.48 % for severe WB. Compared to SVM, BPNN training model accurately (100%) separated normal WB fillets with and without SM demonstrating the ability of BIA to detect SM. Models for other overlapping categories were trained but could not be tested due to less amount of data in those categories. Supervised learning algorithms such as SVM and BPNN can be successfully implemented in industries for detection of broiler breast muscle myopathy using bioelectrical impedance.

Key Words: Support Vector Machine, Back Propagation Networking, Woody Breast, Spegetti Meat, Breast Myopathies

P9 Predicting the Salmonella food safety issues as a result of disruption in cold chain during the last mile in supply chain. Charles Herron*[GS], Amit Morey *Auburn University*

Proper management of cold chain during the delivery of temperature sensitive products such as raw poultry may be critical in maintaining food safety. The doors of the refrigerated trucks may be left open while loading and unloading shipments leading to temperature abuse of raw poultry and growth of foodborne pathogens such as *Salmonella*. Research was conducted to determine and predict the effects of cyclic temperature abuse conditions during supply chain on the growth of *Salmonella* Typhimurium during two scenarios. The cyclic temperature abuse scenarios were as follows for 24 hours: Scenario 1: 2 hours at 4°C followed by 2 hours at 25°C and Scenario 2: 2 hours at 4°C and 1 hour and 25°C. *Salmonella* Typhimurium (35 µg/mL nalidixic acid resistant) was cultured for 18 h at 37°C (10^8 CFU/mL), serially diluted and inoculated in brain heart infusion broth (247.5 m/L) taken in a flask to obtain a concentration of 2.5-3 log CFU/mL. Temperature probes were placed in the center of the broth via holes that were drilled in the flask's screw cap to record the temperature of the broth. The flask was placed in an incubator programmed to alternate temperatures according to the scenario being tested. The flask was taken out of the incubator at 0, 6, 12, 18, and 24 hours and sampled (1 mL), serially diluted, spread plated on XLT4 with 35 µg/mL on nalidixic acid and incubated at 37°C for 24 h to determine the *Salmonella* count (log CFU/mL). The experiment was repeated four times. *Salmonella* growth data was analyzed determine the best fit curve and develop a predictive equation. Exposure to 2 hours at 4°C followed by 2 hours at 25°C resulted in an average of 17 hours above 10C in the span of 24 hours. The growth of *Salmonella* entered the log phase at 6 hours in the study which corresponds to 1.5 temperature abuse cycles. *Salmonella* levels reached ~ 5 log CFU/mL after 6 cyclic temperature abuse cycles. *Salmonella* growth can be predicted using the 2nd order equation $y = 0.0034x^2 + 0.004x + 2.3839$ ($R^2 = 0.9809$). Reducing the amount of temperature abuse at 25C from 2 hours to 1 hour (Scenario 2), did not initiate *Salmonella* growth in the broth. The study demonstrates the importance of cold chain management to ensure food safety at the last mile.

Key Words: Cold Chain Management, Food Safety, Salmonella Growth, Temperature Abuse, Poultry Products

P10 Differences in carbon dioxide concentrations in flasks containing media inoculated with Campylobacter spp. and incubated aerobically or anaerobically. Arthur Hinton Jr*[1], Nelson Cox[1], Arturo Levican[2] *[1]U. S. National Poultry Research Center, Agricultural Research Center, U. S. Department of Agriculture, [2]Pontificia Universidad Catolica de Valparaiso*

Campylobacter are pathogens associated with poultry. Since these bacteria require CO_2 for growth, the objective of this study was to compare CO_2 concentrations in containers with media inoculated with *Campylobacter* and incubated aerobically or anaerobically. Media composed of beef extract, tryptose, soluble starch, sodium bicarbonate, sodium lactate, and agar was prepared then inoculated with *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, or *Campylobacter lari*. Control flasks were not inoculated. Media was transferred to flasks with vented caps, vented caps covered with Parafilm, or plug-sealed caps; then incubated at 37C for 48 h in aerobic (21% 0_2, 0.04% CO_2, 78% N_2) or anaerobic (5% CO_2, 10% H_2, 85% N_2) atmospheres. The ppm of CO_2 in flasks headspace was measured with a Sampling Data Logger carbon dioxide meter. Significant differences in CO_2 concentrations were determined using GraphPad InStat statistical software.

Results indicated that CO_2 concentration was dependent on type of flask closure, incubation atmosphere, and inoculation with *Campylobacter*. CO_2 concentration after incubation ranged from 100 ppm in vented flasks to 31,600 ppm in plugged flasks. Higher CO_2 concentrations were detected in all vented flasks incubated anaerobically than in flasks incubated aerobically due to atmospheric exchange in these flasks. Conversely, there was no significant difference in CO_2 concentrations in plugged flasks incubated aerobically or anaerobically due to seals that prevented atmospheric exchange, while Parafilm flasks were intermediate in ability to prevent atmospheric exchange. Furthermore, when incubated aerobically, control

plugged flasks had higher CO_2 concentrations than vented or Parafilm flasks which indicated that the growth medium released CO_2. However, higher CO_2 concentrations were detected in plugged flasks inoculated with *Campylobacter* than in control flasks, which indicated that some CO_2 in the inoculated flasks was produced by the bacteria. Findings indicate that the medium can produce levels of CO_2 to stimulate growth of *Campylobacter*, then more CO_2 is produced as the bacteria grow in the media. These findings provide evidence of why this medium can support *Campylobacter* growth in closed containers incubated aerobically.

Key Words: Campylobacter, carbon dioxide, aerobic, anaerobic

P11 Validation of veriPRO Salmonella Q assay a real-time PCR assay for the quantification of Salmonella in ground turkey meat Laura Garner[*1], Charles Herron[1], Aftab Siddique[1], Adam Joelsson[2], Heath LaFevers[3], John Mills[3], Amit Morey[1] *[1]Auburn University, [2]Invisible Sentinel, Inc., [3]bioMérieux, Inc.*

Rapid methods for quantification of *Salmonella* species can be a useful tool in determining product disposition for poultry processors. The *veriPRO Salmonella Q* assay is a real-time PCR (RT-PCR) assay that can quantify *Salmonella* from raw poultry in less than 4 hours, compared to days using classical microbiological methods. To compare these methods, ground turkey meat was shipped overnight to Auburn University from a commercial processor. The meat was homogenized and divided into sterile filter whirl-pak bags (n=24; 125 g) and 5x buffered peptone water was added to each individual sample. Samples were randomly divided equally among treatments, non-inoculated control, 10 cfu/g meat, 100 cfu/g meat, and 1,000 cfu/g meat. Samples were inoculated with *S*. Typhimirium at the appropriate target levels and homogenized. Aliquots of each sample were used for the *veriPRO Salmonella Q* assay (40 ml), a modified MPN (mMPN) method (333.3 ml), direct plating in duplicate (0.2 ml) and remaining aliquot was enriched at 42˚C for 24 h. The *veriPRO Salmonella Q* assay utilizes an enrichment free sample prep paired with a multiplex detection method targeting a highly conserved pan-*Salmonella* genetic signature and an internal amplification control on the GENE-UP®. Traditional MPN methods were screened for positives with the SLM2 assay (GENE-UP®) for the mMPN tubes. Enriched tubes (3 each) 100 ml (10 g), 10 ml (1g), 1 ml (0.1g), 0.1 ml (0.01 g), and 0.01 ml (0.001 g) aliquots from the sample were incubated at 35˚C for 20 h and positive tubes were identified using the SLM2 assay on the GENE- UP. Entire experiment was repeated three separate times. *Salmonella* species counts were calculated and statistically analyzed. The levels of *Salmonella* species from the *veriPRO Salmonella Q* assay were 97% in agreement within 1 log of the mMPN method with no statistically significant bias (Bias=0.371, T-Test=0.14). Utilizing a rapid method of *Salmonella* species quantification could allow processors to make risk based decisions on product disposition.

Key Words: Salmonella quantification, Most probable number, Real-time PCR, Ground turkey meat, No enrichment

P12 Inhibition of Salmonella growth in the presence of 2-phenylethylamine, 2-hydroxybenzoic acid and 4-hydroxybenzoic acid Hung-Yueh Yeh[*], John Line, Arthur Hinton, Jr., Hong Zhuang *USDA ARS USNPRC*

Salmonella is the leading bacterial pathogen that causes foodborne illnesses worldwide. Consumption of contaminated poultry products is considered as a major source of infection. Many strategies for interventions have been explored to reduce and/or eliminate this pathogen during poultry production and processing. In this communication, 31 chemicals coated in the EcoPlates™ were used to assess the functional diversity of bacterial communities in the poultry meat samples and screen for their effects of these chemicals on the overall growth of the bacteria. Findings showed that the overall rates of bacterial growth patterns of all 31 chemicals in the plates followed a Gompertz model curve. Among 31 chemicals tested, several chemicals could not be metabolized by the bacteria in the communities from the poultry meat samples. Spot assays showed of the 31 chemicals tested, 2-hydroxybenzoic acid, 4-hydroxybenzoic acid and 2-phenylethylamine could inhibit growth of *Salmonella* isolated from poultry meat samples. The potential of these three chemicals as food preservatives requires for further investigation.

Key Words: Bacterial community, Salmonella, 2-Hydroxybenzoic acid, 4-Hydroxybenzoic acid, 2-Phenylethylamine

P13 Meat of broiler chickens fed fruit (acerola) meal Elisa Helena Ponsano[*1], Joselaine de Oliveira[2] *[1]Unesp Univ Estadual Paulista - Faculty of Veterinary Medicine, [2]Unip Universidade Paulista - Faculty of Nutrition*

The co-product derived from acerola juice production holds many bioactive compounds and fibers and might be used for feeding instead of being discarded and cause environmental pollution. Following this point of view, we aimed at feeding broilers with acerola meal (AM) made of peels and seeds and then investigate the changes in the chemical features of the meat. Two hundred male Cobb 500 broilers were raised (water and feed ad libitum) in a brick shed acclimatized by adiabatic evaporative cooling system with negative pressure ventilation for 42 days and slaughtered according to the national guidelines (procedures were ethically approved). Chicks were fed one among four isoenergetic corn-soy based diets (5 replicates/10 birds): NC (negative control) - without butylated hydroxytoluene (BHT); PC (positive control) - with BHT; and two diets with either 5% or 7.5% AM and without BHT. Performance data were recorded during the experiment. Proximate composition, cholesterol and fatty acids (FA) profile were determined in breast and thigh meat and the results were analyzed by ANOVA and Tukey`s test at 5% probability. The productive parameters were not influenced by the experimental diets. Except to protein in breast, that was higher with AM at 7.5% as compared to PC, no other bromatological parameter differed among the treatments in both cuts. On the other hand, AM diets provided approx. 34% less cholesterol in the breasts and 25% less in the thighs. Regarding the FA, AM in both concentrations decreased saturated fatty acids (SFA), increased polyunsaturated fatty acids (PUFA) and decreased the n-6/n-3 fatty acids ratio, making chicken meat very attractive for maintaining the consumers` health, since the replacement of SFA by PUFA in the diet can reduce the risk of atherosclerosis and cardiovascular diseases. In addition, adequate ratios of n-6/n-3 FA in diets can guarantee the balance in the processes of coagulation and inflammation. It was concluded that the use of AM in broiler chickens` feed maintained (or improved) the bromatological composition and positively altered the meat lipid pattern without compromising the performance of the animals, making it a viable option for feeding broilers and producing heathier meat.

Key Words: proximate composition, cholesterol, fatty acids profile

Environment, Management and Animal Well-Being: Environmental Impacts

P14 Flying insects as vectors for Salmonella spp. around chicken production facilities Ahmet Buyukyavuz*, Paul Dawson *Clemson University*

The objective of this study was to determine the incidence of flying insects carrying Salmonella using PCR/gel electrophoresis. Three chicken production facilities in the eastern, middle and western part South Carolina were studied. Fly traps were placed inside and outside chicken houses and 100 meters away from the closest point to a house in 8 locations in the north, northeast, east, southeast, south, southwest, west and northwest quadrants surrounding the production facility. Chicken feces samples were collected inside the chicken house by pressing a sterile swab on 6 feces in each house. Samples were collected every two weeks then all samples were tested for *Salmonella* spp. Two replications at three different farms in SC were conducted over 3 summer months. Insects were identified by a trained entomologist and divided into the predominant types of insects captured as houseflies, blowflies, Vespidae, darkling beetles and flesh flies. Collected insects were transferred in 15 ml sterile tubes and crushed using sterile forceps, then 0.1% peptone water was added according to the weight of insects, (9 ml 0.1 % peptone water for one-gram fly) and vortexed. Serial dilutions were prepared for the total aerobic counts and Salmonella plate count. 0.1 ml of inoculum was dispensed into 10 ml of Rappaport Vassiliadis and incubated at 42°C for 48 hours. The broth was sub-cultured by streaking on to plates of XLT-4. Agar which previously prepared and incubated at 35°C for 18-24 hours. Colonies were confirmed with PCR and presumptive positive Salmonella isolates were evaluated using pulsed-field gel electrophoresis (PFGE) for definitive serotyping. All farms had at least one Salmonella positive sample but in two of the six overall replications, no Salmonella positive samples were found. In locations were Salmonellae were found in the chicken droppings, Salmonella was also found in houseflies 100 meters from the farm over 90% of the time on farms where Salmonella was found in the house.

Key Words: Salmonella, Poultry house, flies, production

P15 Evaluating roller swabs and drag swabs for Salmonella spp. detection Hannah Haiderer*GS, Aidan Talorico, Matthew Bailey, James Krehling, Kenneth Macklin *Auburn University*

Salmonella is a leading cause of foodborne illnesses, a major reservoir is poultry. Its detection in broiler houses is an important step to help reduce contamination. Two trials were conducted using different litter sampling methods those being: drag swabs and roller swabs. The first trial was conducted at the end of a 40 pen, 35- day broiler experiment in which all of the birds were challenged with a Nal/Novo resistant strain of ended *Salmonella* Enteritidis. The subsequent trial was conducted using the same litter; however birds were not challenged and they were raised to 42 days. For both trials, the collection methods were as follows: Drag swabs were dragged over the litter in a zig zag pattern and then returned to the original tube. Roller swabs were rolled over 75% of the pen surface and then transferred to an empty sterile bag. Collected samples were then assessed on a +/- basis by enriching in tetrathionate broth containing a 2% iodine solution for 48 hours @ 37°C and then streaked onto Xylose Lysine Tergitol 4 Agar containing Nal (100µg/ ml) and Novo (15µg/ ml) (XLT4+). Collected data was analyzed using Pearson chi-squared test at a $P<0.05$ significance. The results of trial 1 showed that the drag swabs (93.75%) were significantly more effective ($P<0.05$) in recovering *Salmonella* than roller swabs (80.63%). The results of trial 2 showed that the roller swabs were significantly ($P<0.05$) more effective in detecting *Salmonella* than drag swabs. With roller swabs having a 22.65% recovery, while drag swabs had a 6.25% *Salmonella* recovery. Overall, trial 1 had higher *Salmonella* prevalence than trial 2. Drag swabs were more sensitive in detection when *Salmonella*, was present in high numbers whereas roller swabs were more sensitive in *Salmonella* detection when this pathogen was present at lower levels. The results from both trials were that drag swabs had a 55.52% *Salmonella* recovery and roller swabs had a 56.48% *Salmonella* recovery; overall there was no significant difference between the two methods. Roller swabs were simple, worked well and were more efficient to use in a research pen setting, however under commercial poultry house settings they may not be appropriate.

Key Words: Salmonella, Detection, Drag Swab, Roller

P16 Phenotypic and genotypic antimicrobial resistance characteristics of avian pathogenic Escherichia coli isolated from broilers Tianmin Li*1GS, Anuraj Theradiyil Sukumaran[1], Wen-Hsing Cheng[2], Aaron Kiess[1], Li Zhang[1] *[1]Department of Poultry Science, Mississippi State University, [2]Department of Food Science, Nutrition and Health Promotion, Mississippi State University*

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in poultry. Management of APEC infections is challenging due to the emergence of antimicrobial resistant strains in the poultry industry. This study aimed to investigate the genotypic and phenotypic antimicrobial resistance characteristics of APEC isolates from 0 to 42-day old male broilers. Thirty-seven APEC isolates recovered from broiler heart (n = 12), liver (n = 10), spleen (n = 11), and yolk sac (n = 4) samples were tested for the presence of 14 genes conferring resistance to ampicillin (*blaTEM*), gentamycin [*aac(3)-VIa*, *aac(3)-VIb*, and *aph(3')-Ia*], streptomycin (*aadA*), tetracycline (*tetA*, *tetB*), trimethoprim (*dfr1*, *dfr7*, *and dfr17*), quaternary ammonium compounds (*qacEΔ*), quinolone antibiotics (*qnr*), sulfonamide (*sul1*), and class 1 integrase (*intl1*). Antimicrobial susceptibility testing was performed using the Sensititre NARMS panel (YCMV4AGNF, ThermoFisher Scientific, Waltham, MA), and the results were interpreted using the FDA NARMS/CLSI guidelines. Overall, 32 APEC isolates carried resistance genes against 3 or more antimicrobial classes, among which 14 isolates showed multidrug resistance phenotypes. Among the isolates that showed phenotypic resistance against tetracycline (n = 23), gentamycin (n = 17), ampicillin (n = 16), streptomycin (n = 11), trimethoprim (n = 6), and quinolone antibiotics (n = 0), 22,17, 5, 9, 11, and 6 isolates carried at least one of the tested genes against the respective antimicrobials. In conclusion, phenotypic patterns to tetracycline and gentamycin correlated well with those obtained by genotyping; however, phenotypic patterns to the rest of the antimicrobials were not identical to their genotype for the same antimicrobial agents. Plausibly, the antimicrobial resistance is directed by the genes not tested in this study. Future research should include a more comprehensive list of the resistance genes to allow for a better understanding of the antimicrobial resistance mechanisms.

Key Words: APEC, antimicrobial resistance, genotype, phenotype, broiler

P17 Space use and preference of broiler chickens housed in complex environments Gracie Anderson*GS, Andrew Campbell, Alexa Johnson, Lauren Evans, Leonie Jacobs *Virginia Tech, Department of Animal and Poultry Sciences*

Broiler chickens are housed in relatively barren conditions, which affects space use and opportunities for behavioral expression. A complex environment could provide birds with choices to access preferred areas, improving welfare. Our objective was to evaluate the effect of a complex environment on broiler space utilization and preference. Eight pens contained an enrichment (high [HE] or none [NE]) and stocking density treatment (high [HD] of 19kg/m^2 or low [LD] of 10kg/m^2 at d29), resulting in HE/HD, HE/LD, NE/HD, and NE/LD (HD=180, LD=90 birds/pen). HE pens included 4 functional spaces: space 1 (FEED; 4 feeders and pecking stones), 2 (COMFORT; 1.6m^2 dust bath with sand), 3 (REST; PVC perches), and 4 (EXPLORE; rotating "toys"). In NE pens, spaces 1-3 contained feeders. Bird n and location were recorded between d3-24 from 8 screenshots taken daily (total n=403), and analyzed in JMP.

Space utilization between treatments differed. HE birds preferred space 1 (FEED; 34.5±1.4% of observed birds) more than NE birds (24.4±1.4%; P<0.001), likely because only space 1 had feeders, compared to spaces 1-3 in NE pens. NE/HD birds (34.3±1.4%) preferred space 2 more than HE birds (HD: 29.2±1.5%; LD: 31.2±1.5%) and NE/LD birds (29.5±1.4%; P<0.001). NE/HD birds (14.7±1.7%) also seemed to prefer space 4 more than HE (HD: 3.0±1.8%; LD: 5.4±1.9%) and NE/LD birds (9.1±1.8%; P<0.001). HE/HD birds (33±2%) seemed to prefer space 3 more than HE/LD birds (29.3±2.1%) and NE/HD birds (26.4±1.9%; P<0.05). More NE/LD birds (37.2±2%) preferred space 3 than HE (HD: 33±2%; LD: 29.3±2.1%) and NE/HD birds (26.4±1.9%; P<0.05). Most birds in HE pens were observed in the FEED space, compared to REST, COMFORT, and EXPLORE spaces (P<0.001), and more in the REST space compared to the EXPLORE space (P<0.02). This illustrates broilers' motivation to feed, followed by motivations to utilize dust baths and perches. Birds in NE pens preferred space 4 over 1 and 3, and preferred space 2 over 1 and 3 (P<0.05). Space 4 provided birds with room to rest, whilst spaces 1-3 had feed, possibly leading to constant activity due to feeding. Providing broilers with a complex environment influenced space utilization and illustrated a preference for access to feed (space 1), dust baths (space 2), and perches (space 3).

Key Words: broiler chicken, environmental enrichment, complex environment, space use, animal welfare

P18 Viability of secretory and plasma IgA as a biomarker for positive welfare status in broiler chickens Andrew Campbell*GS, Gracie Anderson, Leonie Jacobs *Virginia Tech Department of Animal and Poultry Science*

Few molecular animal welfare markers can accurately measure positive welfare status in broiler chickens. Traditionally, corticosterone (CORT) concentrations have been used, but these may not be accurate indicators of cumulative welfare status. Secretory and plasma immunoglobulin-A (IgA) are promising biomarkers for cumulative welfare status based on findings in other species. Therefore, the objective was to determine viability of IgA as a novel animal welfare biomarker for broilers.

A 2*2 factorial system was used comparing stocking density (high density [HD, ~42kg/m^2 at d49] versus low density [LD, ~24 kg/m^2 at d49]) and environmental complexity (highly complex [HC] versus no complexity [NC]). Male broilers (n=1620) were housed in 12 14m^2 pens with 4 treatments (HD/HC, HD/NC, LD/HC, LD/NC). Enriched pens contained fixed furniture (perches/dust baths/pecking stones) and rotating enrichments (e.g. alfalfa hay, laser lights, oats) changed on a 3-day (d) schedule. Complex pens were divided into four functional spaces: feeding (feeders, pecking stones), comfort (dust baths), resting (perches), and exploration (rotating enrichments). Blood and feces were collected on d49 (n=52, n=13/trt). IgA and CORT concentrations were assayed using commercial ELISA kits. IgA concentrations were log10 transformed to obtain a normal distribution. Data were analyzed in JMP and presented as LSmeans±SEM.

LD/HC birds had higher (P<0.05) plasma IgA concentrations (65.9±13.4 ng/mL) compared to LD/NC (34.7±12.3 ng/mL) and HD/HC birds (29.5±12.3 ng/mL). HD/NC IgA concentrations did not differ from other treatments (P>0.1; 57.8±12.8 ng/mL). No differences between plasma CORT concentrations were found (2.22±0.3 ng/mL; P>0.1). Results on secretory IgA and CORT will be presented.

The results suggest that d49 plasma IgA is a more sensitive biomarker for cumulative welfare status compared to plasma CORT, as differences were found between birds in complex and barren conditions. Additionally, the results suggest that environmental complexity could have benefits on immune health, at least under low stocking density conditions. Determining secretory concentrations may be a valuable non-invasive method to quantify cumulative animal welfare status.

Key Words: Immunoglobulin-A, Complexity, Animal welfare, Poultry, Physiology

Environment, Management and Animal Well-Being: Stress Responses, Behavior

P19 Effects of Gypsum Mineral Treatment on Darkling Beetle (Alphitobius diaperinus) Populations in Poultry Litter Kaicie Chasteen*GS, Kenneth Macklin, James Krehling, Matthew Bailey *Auburn University*

Darkling beetles are a well know pest and disease vector in the poultry industry. Gypsum may have some effect on the beetle life cycle and population dynamics based on casual observation noted during a concurrent unrelated trial. A small exploratory study was conducted to further document this observation. Gypsum uses are currently being investigated in an effort to make use of a power industry byproduct and any additional uses would most likely be welcome in an effort to supply a sustainable multi-use treatment.

Samples were taken from 4 repetitions of the same treatments. Treatments were A (3" used litter layered, top-dressed with 1600lbs/1000ft^2 gypsum),B (fresh shavings & used litter 1:3),C (used litter), and D (used litter top-dressed with 1600lbs/1000ft^2 gypsum). Starting the fourth week of broiler grow out, beetle traps consisting of 8" pieces of 2" PVC filled with 8"x10" rolled corrugated cardboard were placed in the front center of each treatment pen and collected every week until one week after trial termination. Beetles and larva were subsequently frozen to euthanize and immobilize, then enumerated.

Adult and larva counts were analyzed in SPSS 26 as raw counts and also as arcsine transformed percentages using a repeated measures GLM; if significant (P<0.05), means were separated using Tukey HSD. There was no significant interaction between time and treatment for adults or larva. Raw counts did not show significance, there did seem to be a slight interaction between treatments A & B in adult count. For the transformed count of adults taken as a proportion of the total, there was significance between treatments A (3" used litter layered with 1:3 litter & gypsum, top-dressed with 1600lbs/1000ft^2 gypsum) 8.12% adults & B (fresh shavings & litter 1:3) 26.06% adults over 4 sample dates. There was no significance between A, C & D nor between B, C & D, however some notable interactions can be observed with A consistently having the lowest adult percentage overall through all sample days. Results may indicate that gypsum may have a detrimental effect on adult beetle survivability. Further investigation is forthcoming to determine the exact severity of this impact.

Key Words: Darkling Beetles, Gypsum, Population Dynamics, Litter, Byproducts

Environment, Management and Animal Well-Being: Environmental Impacts

P20 Validation of probe-based multiplex real-time PCR assays for the rapid and accurate detection of avian pathogenic Escherichia coli Reshma Ramachandran*, Chuan-Yu Hsu, Anuraj Theradiyil Sukumaran, Li Zhang *Mississippi State University*

Avian pathogenic *Escherichia coli* (APEC) is the most common cause of bacterial infections in poultry that result in significant economic losses to the industry. However, due to the diverse genetic characteristics of APEC strains, there are no reliable rapid diagnostic tools for APEC detection. Recently, we designed two triplex TaqMan-based real-time PCR assays targeting *ybbw* gene for *E. coli* confirmation and five major virulence genes (*hlyF, ompT, iss, iroN, iutA*) associated with APEC. Within each triplex real-time PCR assay, TaqMan primers and probes were designed to target *ybbw/hlyF/ompT* genes with ABY, FAM and VIC dyes, and *iss/iroN/iutA* genes with JUN, FAM and VIC dyes. Thus, the objective of this study was to validate the probe-based multiplex real-time PCR assays using reference strains and field isolates. A total of 82 *E. coli* isolates from various sources, including reference strains and field isolates from poultry facilities, were used to evaluate the assays. The purified plasmid DNA extracted from the recombinant pGEM-T Easy vector carrying target gene insert served as the positive control to construct the standard curve and to test the amplification efficiency of each primer pair and probe. The triplex assays appeared to be highly efficient and sensitive with high reliability and reproducibility. Further, the specificities of the multiplex assays were validated by testing 32 isolates, including positive and negative controls, and was confirmed by comparing TaqMan results with SYBR Green real-time PCR and whole genome sequencing. Also, 52 field isolates were successfully tested by TaqMan assays and 16 of them were further confirmed by regular PCR. Complete agreement was observed in the detection of five genes, *ybbw, hlyF, ompT, iron, iutA,* but not for *iss*. This issue will be addressed by designing new primers and probe for *iss*. In conclusion, this probe-based multiplex real-time PCR strategy has the potential to be developed as a diagnostic tool for the rapid and accurate detection of APEC and to differentiate them from commensal *E. coli* in poultry production.

Key Words: E. coli, APEC, Multiplex real-time PCR, TaqMan probe, Detection

P21 Prevalence of netB positive C. perfringens across turkey and broiler flocks in the United States Alexandra Smith, Jodi Delago, Tom Rehberger, Robert Teal* *Arm and Hammer Animal Nutrition and Food Production*

Necrotic enteritis (NE) affects poultry flocks around the world leading to increases in mortality, a reduction in daily weight gain and an increase in feed conversion. In the U.S. alone, NE costs the poultry industry approximately $2 billion. *C. perfringens* is a gram-positive spore forming bacteria ubiquitous throughout nature and has been linked to NE. Though this bacterium can produce an array of toxins, recent evidence suggests that the pore-forming toxin, NetB, may play a bigger role in the pathogenicity of *C. perfringens* in an avian model than previously expected. Previous studies from our lab show that broiler flocks with NE breaks have higher total clostridia loads than healthy flocks and a higher prevalence of the *netB* gene, with an overall loss of genetic diversity within the *C. perfringens* community. Our aim with this study is to begin to characterize *netB* positive *C. perfringens* within healthy broiler and turkey flocks from commercial farms. For this study, gastrointestinal tracts (GITs) from broilers and turkeys were collected from several complexes within the continental U.S. Total clostridia enumeration in the small intestine, and molecular analysis of *C. perfringens* isolates were compared. Our data shows a significantly higher *C. perfringens* load of 1.0e05 CFU/g and *netB* prevalence of 20.4% within broilers versus 4.2e04 CFU/g and *netB* prevalence of 8.3% in turkeys ($p<0.01$ two-sided t-test). netB+ isolates were identified at all 18 broiler complexes and only within three of nine turkey complexes. Planned additional work to determine shifts in population dynamics of *C. perfringens* may allow a deeper understanding of this pathogen and aid in developing targeted preventative measures.

Key Words: Broilers, Turkeys, Necrotic Enteritis, C. perfringens

Environment, Management and Animal Well-Being: Stress Responses, Behavior

P22 Age-related effect of high-frequency photostimulation on blood plasma biochemistry variables in laying hens Hammed Olanrewaju*, Joseph Perswell, Jeffery Evans, Stephanie Collier, Scott Branton *USDA-ARS, Poultry Research Unit*

Light is a crucial environmental factor that affects bird's development, production performance, well-being, and quality of egg production. A novel lighting system has been developed to improve photostimulation of growth and egg laying performance for layer chickens. However, it has not been independently investigated by scientists to observe its effects on layer chicken well-being responses. This study investigated the biochemical response of laying hens to high-frequency differential photostimulation. A total of 320-layer pullets were obtained from a commercial hatchery at approximately 10 weeks of age and randomly allocated to 16 groups of 20 birds per group. Birds were weighed (group size = 20) and randomly assigned to each of four cage banks in each 4 rooms (A to D) with 80 birds per room. Experimental treatments included the prototype LED lighting system and conventional 2700K LED bulbs. Photoperiod for the prototype system was 24L:0D per the manufacturer's recommendations and photoperiod for the conventional system followed the HyLine Management Guide (12 to 16 hours of light, increasing from 10 to 30 weeks of age). Blood samples (3 ml) were collected from the brachial wing vein into heparinized syringes within 45 s after birds were caught from 8 randomly selected birds per room for a total of 32 birds at each blood sampling day of placement, 15 weeks of age (prior to point of lay), and at the termination of the study at 30 weeks of age for a total of 96 birds. Plasma was separated from blood samples by centrifugation and used to determine the reproductive hormones concentration (Estradiol [E2], follicle stimulating hormone [FSH]), welfare indicator (corticosterone [CORT], and light response indicators (melatonin, TSH, T_3, T_4, FT_3, FT_4,) levels using Tosoh A1A-360 bioscience automatic analyzer. Results show that only FT_4 was affected by treatments. However, time (age) had significant effects on most examined variables. Plasma CORT was not affected by treatments and time, indicating absence of physiological stress. The results contribute to our knowledge of prototype LED light on blood metabolites variation in layer hens. In conclusion, using high-frequency photostimulation in layer hens house does not negatively affect layer hen's welfare.

Key Words: high-frequency photostimulation, biochemistry, laying hens, photoperiod, welfare

P23 The effect of a botanical-based liquid solution on broiler stress responses to increased stocking density Carl JULIEN[1], Bertrand MEDINA[*2], Stephanie COTTEE[2], Ivan GIRARD[2] *[1]CRSAD (Centre de Rescherche en Sciences Animale de Deschambault), [2]Probiotech International Inc.*

The objective of this trial was to examine the efficacy of a proprietary Canadian Veterinary Health Product (VHP) on improving broiler stress resilience to high stock density. Twenty-day old Ross 308 male conventionally raised broilers (n=240; density ~ 30-32 kg/m² floor pen) were randomly caged (10 birds/cage) and assigned to 4 groups (6 cages/group): a control (CTL) and 3 treatment groups (PL1, PL2, PL3) of increasing product levels. The treatment concentrations were 67, 134 and 200 ppm of PHYTOZEN® Liquid (Probiotech International Inc.) in the drinking water and given from 30 days of age. At 200 ppm, this liquid VHP supplied 1000, 400 and 80 ppm of $MgSO_4$, rosemary oil and turmeric extract, respectively. High stock density (38-44 kg/m²) was the stress challenge and was applied between 30 to 34 days of age. Individual liveweights (LW) were recorded at d20 (setting-up), d32 and d34 (slaughter LW). At d32 and d34, eye temperature was measured (2 same birds/cage) by infrared thermography (eIRT) and birds were also subjected (6 same birds/cage) to the tonic immobility test (TIT). Groups and barn sections were respectively used as fixed treatment and randomized bloc effects of the mixed statistical model. Paired-differences were compared with a Student test and $P<0.05$ was considered as significant. No differences were observed on final LW (NS). However, at d34, a linear effect of treatment dose was positively correlated with the eIRT values (CTL: 30.5; PL1: 30.8; PL2: 31.6 and PL3: 32.1 °C; $P<0.015$). Also, there were no statistical differences in TIT between the treatments. However, there was a positive trend in the number of birds showing faster and consistent recovery from fear (as determined by the TIT) between d32 and d34 (CTL: 67.7; PL1: 92.3; PL2: 77.4 and PL3: 80.0 %). PHYTOZEN® Liquid demonstrated a positive effect of stress mitigation of sudden stocking density increase as reflected by the higher eIRT which is correlated with lower bird stress (Weimer et *al.*, 2020). These encouraging results demand further investigation of the effects of PHYTOZEN® Liquid on other stress challenges and parameters in poultry production.

Key Words: Broilers, Stock density, Animal welfare, Stress resilience, Botanicals

P24 Can facial temperature be used to monitor heat stress of broilers Chad Hayes, Savannah Wells, Yi Liang[*] *University of Arkansas*

Infrared thermal imaging has been recently adopted as a non-contact, non-invasive measurement of skin surface temperature, with a potential of assessing thermal status of animals to improve diagnoses of environmental stress. The objective of the study was to determine whether facial temperature of broiler chickens taken by infrared thermographic measurement is correlated with body temperature in a summer flock. The study was conducted in three tunnel-ventilated broiler houses (13.4 × 152 m) on a commercial broiler farm. One house was cooled by the evaporative cooling pads (PAD), one by intermittent sprinkler cooling (SPRK), and third one by a combined operation of cooling pads and sprinklers (Combo). Due to different cooling treatment, the thermal environment in the three buildings were different, especially during daytime. Rectal temperature and facial surface temperature of 40 birds in each house, 10 in each quarter, were measured on Day 22, 28, 34, 42, and 47. All measurements were taken between 1 and 4 pm, except for between 8 and 10 am on Day 47. Temperature and relative humidity of the production houses were monitored continuously for the flock.

Results showed that core temperatures of birds were not significantly different between treatment houses ($P<0.05$). However, facial temperatures in sprinkler-cooled house were higher than those of birds in the other two houses ($P<0.05$). Facial temperatures were not only correlated to room temperature but also to core body temperature. Core temperatures of chickens of Day 47 were lower (105.6°F) than those taken in the afternoon of other days (106.1°F), likely due to the diurnal fluctuation of core temperature of broilers, as reported in the literature.

Key Words: Heat stress, Infrared thermal imaging

P25 Benefits of plant bioactives on broiler performances with or without a digestive challenge Thibaut Chabrillat[*], Sylvain Kerros *Phytosynthese*

In agreement with the societal trend to reduce antibiotics in animal feed, botanical actives are considered as a potential alternative to control gut health and secure bird performances. This trial was implemented to evaluate the impact of a standardized plant based supplementation on broiler performances in ideal conditions or in gut viscosity challenged conditions. 480 male "Ross 308" day-old chicks were randomly allocated to 1 out of 24 floor pens of 1.5 m². The groups were then allotted to one of four dietary treatments: 1) Standard control feed; 2) Standard control feed + PE (based on *Cinnamomum cassia,* cinnamaldehyde*, Syzygium aromaticum*); 3) Challenged control feed; 4) Challenged control feed + PE. PE was included in starter at 0.015% [VS1] then in grower and finisher at 0.03%. Birds were allowed *ad libitum* feed and water. Pelleted feed was provided in 3 phases: starter D1-10, grower D11-29 and finisher D30-35. Feeds were based on wheat, corn and soybean. The standard diets and challenged diets were isocaloric and isonitrogeneous[VS2] ; Challenged feeds had an addition of rye (5 %), lard (2.2 to 4.8 %) and guar gum (1 %) in order to increase digesta viscosity. Results were analysed with an Anova procedure & Newmann-Keuls analysis with feed x treatment interaction at $p<0.05$. The experimental unit was the pen (n=6).

Significant reduction of growth and feed conversion were observed in challenge vs. standard feed. These results are correlated observations: challenged pens had lower litter quality than standard pens. On standard feeds, PE reduced the feed intake in grower and finisher without negative impact on growth; the FCR tended to be improved ($p<0.1$) with PE. On challenged feeds, control had a reduced feed intake whereas PE achieved same intake as the supplemented standard diet. It could be correlated to microbiota modulation and intestinal comfort. PE enabled growth performances to be improved in finisher compared to challenged control, with positive trend on final weight ($p=0.1$).

In optimal conditions PE tends to reduce feed intake and improves feed conversion. In degraded conditions with microbiota challenge, PE promotes feed intake with a positive effect on finisher growth and a trend on final live weights.

Key Words: Bioactives, Wet litter, Digestive health, Plants, Viscosity challenge

Metabolism and Nutrition: Enzymes, Feed Additives

P26 Assessment of dietary Standardised Citrus Extract supplementation on broiler chicken zootechnical performances and bacterial growth. Sekhou Cisse[*1,2GS], Morgane GAUTRON[2], Assia BOUMEZRAG[3], Mohamed el Amine BENARBIA[1,2], David GUILET[1,4] *[1]Labcom FeedInTech, [2]Nor-Feed SAS, [3]University of Tiaret, [4]EA 921 SONAS*

Citrus extracts are increasingly used in poultry production, due to the many advantages they confer to the birds. However, there is a lack of data regarding their mode of action. The aim of this study was to evaluate the effect of a Standardised Citrus Extract (SCE, Nor-Spice® AB, Nor-Feed SAS) on both zootechnical performances of broiler chicken and the growth of several bacterial strains of interest.

360 one day old Arbor Acres were divided into 2 groups of 6 replicates each:

- CTL group: Standard diet without supplementation;
- SCE group: Standard diet supplemented with 250 ppm of SCE.

Birds were reared until day 46 and were put under thermic stress at 30°C for 4 h per day, from day 26 to day 30. Zootechnical performances were weekly recorded. In parallel, the effect of SCE on bacterial growth have been monitored using 2 strains: *Escherichia coli* JM109 (*E. coli*) and *Lactobacillus acidophilus* strain R52 (*L. acidophilus*). Briefly, 200 µL of a 10 % SCE solution were added to 10 mL of medium, which were seeded with $5x10^5$ bacteria/mL. Bacterial growth was monitored by measuring the medium turbidity at 650 nm according to McFarland's standards. The same experiment was performed without SCE, as negative control.

Regarding zootechnical performances, although the weight was numerically higher in chickens receiving SCE (2517 ± 13 g Vs 2505 ± 41 g), there was no statistical difference between the two groups (p > 0,05, T-test). Similarly, the feed intake of broilers from SCE group (5.46 kg/ broiler) was lower than the CTL group one (5.61 kg/ broiler), even if the difference was no significant (p > 0,05, T-test). However, the Feed Conversion Ratio (FCR) of chickens from SCE group (2,198) was significantly lower (P = 0, 0395, T-test) than the CTL group one (2,285).

Bacterial growth showed that SCE allows to decrease the doubling time of *L. acidophilus* (256 min) and increase the doubling time of *E. coli* (245 min), compared to the CTL group (respectively 353 min and 80 min).

SCE supplementation on chickens improved birds' FCR. This effect may be due to the modulation of the intestinal microbiota. However, further studies will be necessary to confirm or not this hypothesis.

Key Words: Standardized Citrus Extract, zootechnical performances, prebiotic effect, microbiota management

P27 Comparative nutrient utilization response of broilers to diets supplemented with individual or combination of xylanase and phytase when raised in broiler or turkey husbandry conditions Shravani Veluri[*GS], Oluyinka Olukosi, Yang Lin, Mohammad Pilevar *University of Georgia*

A comparative 21-day study, consisting of two concurrent experiments, was conducted with broilers to study the impact of husbandry conditions (temperature and lighting) on nutrient utilization when diets are supplemented with enzymes and the birds were raised in broiler (1) or turkey (2) husbandry conditions. A total of 96 broilers were used for each experiment with treatments arranged in a 2×2 factorial with six replicates and four birds per replicate. Excreta were collected on d 7 and 21 to determine total tract retention of Ca, P, N and apparent metabolizable energy (AME). Data from the two experiments were analyzed as 2×2×2 factorial with factors xylanase (- or +), phytase (- or +) and husbandry (1 or 2). There was significant (P < 0.05) 3-way interaction for Ca and N retention at 7 d. Ca retention tended to be greater (P < 0.10) in phytase-supplemented in broilers reared under broiler condition, whereas Ca retention was lower (P < 0.05) in xylanase-supplemented diet in broilers under turkey condition. N retention (P < 0.05) tended to be improved by phytase supplementation in birds under broiler condition whereas all the enzyme supplemented treatments had greater N retention (P < 0.05) in birds under turkey rearing condition. There was xylanase × husbandry interaction (P < 0.05) for P retention and AME on d 7. Retention of P and AME for diets supplemented with xylanase or in combination with phytase was greater (P < 0.05) in broilers reared in turkey, compared with, broiler conditions. There were no significant 3-way interactions for nutrient retention on d 21, but significant (P < 0.01) phytase × xylanase interaction for P and AME. Phytase supplemented individually improved P retention (P < 0.05) and AME compared to xylanase alone or combined with phytase. In addition, N retention was greater (P < 0.01) for birds under broiler husbandry condition. In conclusion, enzyme supplementation, especially phytase, improved nutrient retention in broilers raised under both husbandry conditions but the effect of husbandry condition was more apparent on d 7 than d 21. This is of consequence when there is interest in comparing enzyme supplementation effect in broiler and turkeys and considering whether to rear the birds together or separately.

Key Words: rearing condition, broiler, turkey, xylanase, phytase

P28 Growth performance, gut permeability and intestinal lesion scores in broilers fed diets with different dietary levels of fiber, protein and enzymes and challenged with or without Eimeria organisms Yang Lin[*GS], Oluyinka Olukosi *University of Georgia*

A total of 360 Cobb 500 male broiler chicks were used in two 21-d experiments to study the possibility to exogenous enzymes to help recover growth performance in broilers challenged by *Eimeria spp.* In Expt. 1, 180 birds were allocated to 6 treatments in a 3×2 factorial arrangement (3 diets with or without *Eimeria* challenge). Each treatment had 6 replicates with 5 birds per replicate. The 3 diets were based on high fiber-adequate protein (HFHP) basal diets. The basal diets were supplemented with no enzyme, xylanase alone or combine with protease. Experiment 2 was similar in design but diets had lower fiber and protein (LFLP) and were supplemented with no enzyme, protease alone, or combined xylanase. Birds and feed were weighed on d 0, 15 and 21. The challenge group were inoculated with a solution containing oocysts of *E. maxima*, *E. tenella*, and *E. acervulina* on day 15. Gut permeability was measured on 5-d post-infection (DPI) and the intestinal lesion was scored on 6 DPI. In Expt. 1, there were significant (P < 0.01) enzyme × challenge interaction of overall gain: feed and final body weight. Birds receiving diets with xylanase alone had numerically greater gain: feed and final body weight in challenged groups but lower (P < 0.01) gain: feed and final body weight in unchallenged treatments. In Expt. 2, no significant interaction was found in growth performance. Enzyme supplementation increased (P < 0.05) post-challenge feed intake, but there were no other enzyme effects. In both experiments, *Eimeria* challenge decreased (P < 0.01) weight gain, feed intake and gain: feed. In addition, *Eimeria* challenge resulted in higher (P < 0.01) gastrointestinal leakage and severer lesion whereas enzyme supplementation had no effect. In conclusion, enzyme supplementation helped recover final body weight and gain: feed of broilers from *Eimeria*-caused growth performance deduction. Because of the lack of enzyme effect on *Eimeria*-induced gut leakage, it was hypothesized that part of enzyme-associated performance recovery might be due to enhancement of digestible nutrient intake and this aspect is being investigated.

Key Words: enzyme, xylanase, protease, broiler, Eimeria

P29 Bacillus subtilis-based probiotic supplementation alters growth and metabolism-related gene expression in broiler chicken muscle under a Clostridium perfringens-induced necrotic enteritis model Shailes Bhattrai[*1GS], Ana Villegas[1], Todd Applegate[1], Anita Menconi[2], Laura Ellestad[1] [1]*University of Georgia,* [2]*Evonik Corporation*

The recent rise in *Clostridium perfringens*-induced necrotic enteritis (NE) as a result of reduced antibiotic use has decreased broiler production efficiency. As studies on probiotics as antibiotic alternatives have focused on their role in promoting intestinal health, data related to effects of NE and probiotics on growth and metabolism are lacking. This study evaluated impacts of probiotic supplementation on circulating hormones and gene expression associated with growth and nutrition partitioning in muscle during a sub-clinical NE challenge in broilers. Day-old male Ross broilers were grouped into three treatments with 12 replicate pens each (n=12): unchallenged with no probiotic supplementation, challenged with no probiotic supplementation, and challenged with probiotic supplementation. Birds in challenged groups received one dose of coccidial vaccine (Coccivac B52®) day (D) 0 and drinking water inoculated with live strains of *C. perfringens* from D14 to D17. Birds in the probiotic supplemented group received feed mixed with 1 x 10^6 CFU/g *Bacillus subtilis* DSM 32315 throughout the study. Blood and *Pectoralis major* samples were collected for analysis. Circulating thyroid hormones and corticosterone were measured using radioimmunoassays and an ELISA, respectively, and mRNA expression for hormone receptors, glucose and amino acid transporters were measured by RT-qPCR. Data were analyzed using an ANOVA followed by Fisher's Least Significant Difference test. Circulating thyroid hormone and corticosterone levels were not affected by NE challenge ($p>0.05$); however, mRNA expression of glucocorticoid receptor, thyroid hormone receptors α and β, and insulin-like growth factor 1 receptor in *P. major* was upregulated by NE challenge ($p\leq0.05$), and probiotic supplementation reversed these effects ($P\leq0.5$). Similarly, the increase in glucose transporter 8, cationic amino acid transporter 2, and L-type amino acid transporter mRNA levels in the *P. major* resulting from NE challenge was prevented by probiotic supplementation ($p\leq0.05$). These results suggest that probiotic supplementation during a subclinical NE challenge alters the expression of key hormone receptors and nutrient transporters involved in growth and nutrition partitioning in muscle, a metabolically active tissue.

Key Words: hormones, nutrition partitioning, physiology, transporters

P30 A stimbiotic improving caecal fermentation and growth performance in 35d old broilers Antonija Šimić[*1GS], Gemma González-Ortiz[2], Stephen Mansbridge[1], Stephen Rose[1], Mike Bedford[2], Vasil Pirgozliev[1] [1]*National Institute of Poultry Husbandry, Harper Adams University,* [2]*AB Vista*

An experiment was conducted to evaluate the efficacy of a stimbiotic (Signis, AB Vista, Marlborough, UK), a fibre digestibility enhancer made of xylanase and a xylo-oligosaccharide (XOS) on growth performance and caecal volatile fatty acids (VFA) production when fed to broiler chickens from day old to 35d age. A basal diet containing 228 g/kg CP and 12.59 MJ/kg ME in the starter phase and 191 g/kg CP and 13.18 MJ/kg ME in the grower-finisher phases, respectively, was mixed. A second diet based on the same dietary formulations but containing 0.1 g/kg of the stimbiotic was also produced. Phytase was included in all diets to provide 500 FTU/kg (Quantum Blue, AB Vista, Marlborough, UK). Each diet was fed to twelve pens, each with twenty Ross 308 broilers, following randomisation. Birds were weighed on a per pen basis at the start and end of each phase of the study to obtain feed intake (FI), weight gain (WG) and feed to gain ratio (F:G) data. At the end of the study, at 35d age, one bird per pen was sacrificed and the caecal digesta content was obtained for determination of VFA content. The data were analysed by one-way ANOVA. Feeding the stimbiotic did not change the FI ($P > 0.05$), but increased WG ($P < 0.05$) and reduced F:G ratio ($P < 0.05$). Feeding the stimbiotic resulted in increased production of butyric acid and total VFA production in the caecal digesta ($P < 0.05$). An increase in VFA production including butyrate could increase epithelial growth and improve the gut health of chickens, thus the VFA increase when feeding the stimbiotic might be the reason for the observed improvement in broiler growth performance. The results support the view that a combination of xylanase and xylo-oligosaccharides enables improved fermentation of fibre sources that otherwise may not be utilised.

Key Words: broiler, stimbiotic, performance, gut fermentation

P31 Investigation of the possible influence of pre-experimental phase phytase supplementation on assayed true phosphorus retention of soybean meal Adeleye Ajao[*GS], John Palmer, Mohammad Pilevar, Oluyinka Olukosi *University of Georgia*

A total of 384 Cobb 500 male broiler chicks at zero-day old were used in a 21-d experiment to investigate whether phytase supplementation in pre-experimental phase influences the assayed true phosphorus (P) retention value of soybean meal (SBM). On d 0, the birds were allocated into 12 dietary treatments (8 replicates of 4 birds each) comprising initially two corn-SBM diets (+ or – phytase) fed for d 0-16 (pre-experimental phase). On d 16, each of the 2 pre-experimental groups were further divided into 6 dietary treatments. The 6 diets consisted of 3 semi synthetic diets (+ or - phytase) in which SBM, as the only source of P, was added at the serial levels to provide 2.8, 3.5, or 4.4 g total P/kg diet. The experimental diets were fed from d 16 to 21 (assay phase). The 12 treatments were arranged into four groups (3 serial levels of SBM comprised one group) in a 2×2 factorial (+ or - phytase in pre-experimental phase, and + or – phytase, in assay phase). Excreta were collected from all the birds on d 21, and true P retention (TPR) as well as endogenous P loss (EPL) were calculated using regression analysis. For statistical analysis, pre-experimental phytase supplementation is denoted phytase 1, whereas phytase supplementation in assay phase is denoted phytase 2. There was no phytase1 × phytase2 interaction for TPR or EPL. For phytase1 main effect, TPR was greater ($P < 0.05$) in broilers that did not receive phytase in pre-experimental phase, mainly driven by the low TPR in broilers receiving phytase in pre-experimental phase, but not during TPR assay. On the other hand, for phytase2 main effect, broilers receiving phytase in assay phase had greater ($P < 0.01$) TPR compared with those not supplemented with phytase. The EPL followed similar pattern to that of the TPR. It was concluded from this study that phytase supplementation in pre-experimental phase does not influence phytase efficacy in increasing TPR during the assay but decreased TPR value from broilers that received phytase-supplemented diet during pre-experimental phase but not during the assay period.

Key Words: phytase, pre-experimental, soybean meal, endogenous P loss, true phosphorus retention

P32 PHYTOGENOMIC AND NUTRIGENOMIC EFFECT OF MORINGA OLEIFERA LAM. LEAVES EXTRACT IN BROILERS Muhammad Kiwan Akram[*1], Saima Mahad[1], Muhammad Ashraf[2], Anjum Khalique[1], Sohail Ahmad[3], Usama Naeem[2] [1]*Department of Animal Nutrition, University of Veterinary and Animal Science,* [2]*Department of Pharmacology and Toxicology, University of Veterinary and Animal Science,* [3]*Department of Poultry Production, University of Veterinary and Animal Science*

Restrictions on the use of antibiotic growth promotors and other pharmaceutical enhancers in animal nutrition have opened avenues for the use of feed additives from natural origin, such as probiotics, prebiotics, or phytogenic substances. The objective of this study was to evaluate the effect of dietary supplementation with phytobiotic feed additive *Moringa oleifera* leaves extracts (MOLE), on growth performance, redox status, intestinal absorption and genotoxicity in broilers. Two hundred and seventy Cobb-*500* birds were randomly divided into 6 dietary groups, which were fed un-supplemented corn soya basal diet as negative control, $0.5gKg^{-1}$ Zinc bacitracin (antibiotic), $1gKg^{-1}$ Safmannan (probiotic) as positive controls and three different levels of MOLE (0.1%,0.3% and 0.5%). Each group

had 3 replicates with 15 birds each. In-*vivo* genotoxicity, redox status and gut absorption were determined by COMET assay (genetic damage index GDI), 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity and d-xylose absorption test respectively, while in-*vitro* antioxidant activity was assessed by DPPH radical decomposition complimentary with total phenolic content (TPC), total flavonoid content (TFC) and total tannins percentage (TTP). MOLE had good antioxidant activity in term of 50% inhibition concentration IC_{50}= 129.2, TPC= 127.13mgGAg^{-1}, TFC= 212.94 mgQEg^{-1}, TTP= 0.123 tannic acid equivalent. In comparison with the control groups, birds fed with MOLE had higher serum DPPH radical decomposition ($P < 0.001$) and d-xylose absorption ($P < 0.001$). The least GDI was observed in birds fed with MOLE and control (un-supplemented corn soya basal diet) as compared with antibiotic and DMSO 20% (Positive control for GDI) ($P < 0.001$). Growth performance in term of body weight, body weight gain, production number and feed conversion ratio were higher in birds fed with MOLE compared to control groups. However, inclusion of 0.5% MOLE significantly improved redox status, the gut absorption and growth performance with no DNA damage.

Key Words: Phytobiotics, gut absorption, In-vivo genotoxicity, Antioxidant, serum DPPH

P33 Influence of varying kudzu leaf meal particle sizes added to a broiler diet E.K. Stafford*[1UG], K.M. Downs[1], J.P. Gulizia[2], W.J. Pacheco[2] *[1]Middle Tennessee State University, [2]Auburn University*

Kudzu (*Pueraria montana* var. *lobata*) has long been considered an invasive weed species, but limited data exists on kudzu's use as a feedstuff in poultry diets. This research evaluated the influence of varying kudzu leaf meal (KLM) particle size (PS) on body weight (BW), body weight gain (BWG), feed consumption (FC), and adjusted feed conversion ratio (AFCR) of high growth rate broilers. Treated diets included KLM replacing 2.5% of soybean meal (SBM) in a typical broiler starter diet. Four treatments [control, KLM (1.00 mm PS), KLM (2.00 mm PS), and KLM (3.35 mm PS)] with 4 replicates (60 birds/treatment) were fed to 240 male Cobb 500 broilers during a 21-d battery cage grow out. Bird weights and FC data were collected at 7-d intervals. On d 21, 20 birds/treatment were euthanized to assess breast, gizzard, ceca, and small intestine parameters. Data were analyzed as a completely randomized design, with battery cage representing the experimental unit, using the ANOVA procedure. Overall, there were no significant treatment effects for BW at d 7 (P=0.6732), 14 (P=0.4722), and 21 (P=0.2040); BWG d 0-7 (P=0.7326), 0-14 (P=0.4859), and 0-21 (P=0.2049); and FC d 0-7 (P=0.5982), 0-14 (P=0.4890), and 0-21 (P=0.2449). There were no significant treatment effects for d 0-7 AFCR (P=0.4387). However, d 0-14 AFCR approached significance (P=0.0521) and d 0-21 AFCR was different among treatments (P=0.0017). Birds on control tended to have a lower AFCR than both 1.00 and 3.35 mm PS treatments between d 0-14. From d 0-21, birds on control (1.32) had significantly lower AFCR than those on 1.00 (1.42), 2.00 (1.38), and 3.35 mm PS (1.36) KLM treatments. Additionally, there were no observed treatment differences for mortality anytime during the 21-d grow out (P>0.05). There were no significant treatment effects for breast, gizzard, ceca, and small intestine data (absolute weight and % of BW; P>0.05). These results demonstrate that varying KLM particle sizes did not influence performance, breast weight/yield, or organ parameters when compared to a typical corn-soy control diet. However, KLM added in replacement of SBM did not substantially depress bird performance compared to control, though KLM addition at 1 mm particle size grind is recommended to avoid potential bird selectivity.

Key Words: Kudzu, Broilers, Particle Size

P34 Evaluation of alternative methods for the prevention and control of histomaniasis in turkeys Albaraa Sarsour*[1], Frank Pierson[2], Michael Persia[1] *[1]Virginia Tech, [2]Virginia-Maryland College of Veterinary Medicine*

Histomoniasis is a re-emerging disease in turkeys caused by the protozoa *Histomonas meleagridis*. Experiments were conducted to evaluate the efficacy of various commercial additives to prevent and control *H. meleagridis*. Hybrid turkey hen poults were raised in floor pens (25 poults/pen) for 28 d with 4 pens/treatment. At 28 d, 70 poults were selected and transferred to two floor pens per treatment that contained used broiler breeder litter positive for *Heterakis gallinarum*. Body weight (BW) and feed intake (FI) were measured at 0, 28, and 46 days of age. After transfer, poults were monitored for signs of histomaniasis. When a hen was infected, it was euthanized for cecal and liver scoring using a 0-4-point scale. At the end of the experiment, all remaining poults were euthanized for scoring. All data were analyzed using ANOVA in JMP 14. In experiment 1, treatments consisted of a negative control (NC), NC + probiotic, NC + butyrate, and NC + probiotic + butyrate. BWG, FI and FE were not different before or after transfer. Poults consuming the probiotic, butyrate or the combination resulted in reduced liver scores (2.4 to 2.5) in comparison to the poults fed the NC (2.9; $P \leq 0.05$). Experiment 2 consisted of a NC, and NC + medium-chain fatty acid (MCFA) product. Poults fed the MCFA resulted in increased BW before (1,167g) and after (1,137g) transfer in comparison to the poults fed the NC diet (1,088g and 871g, respectively; $P \leq 0.05$). Poults consuming the MCFA resulted in lower liver scores (1.8) in comparison to NC poults (2.5; $P \leq 0.05$). In experiment 3, treatments consisted of NC, NC + refined functional carbohydrate (RFC) product fed continuously in the feed, and NC + RFC added in the water after signs of infection. There were no differences in performance before transfer, but poults receiving the RFC in the water after transfer and infection had an increased BWG (1,100g) in comparison to the NC fed poults (871g; $P \leq 0.05$). The RFC supplied either in the feed or in the water reduced both the cecal (1.0) and liver (1.7) scores in comparison to the NC fed poults (1.5 and 2.5, respectively; $P \leq 0.05$). Overall no treatment was able to prevent infection, but some level of increased performance or reduction in liver or cecal score were achievable.

Key Words: Histomoniasis, Blackhead, Turkey, Performance, Feed additives

P35 Efficacy of an essential oi blend alone or combined with a coccidiosis vaccine against Eimeria challenge in broilers Frances Yan*, Juxing Chen, Vivek Kuttappan, Deana Hancock, Mercedes Vazquez Anon *Novus International Inc.*

Essential oils (EO) have been demonstrated to be an effective tool to improve growth performance and gut health of broilers subject to *Eimeria* challenge. It remains unclear how the efficacies of EO and coccidiosis vaccines affect each other when they are used together. A battery study with 576 Ross 308 male broilers was conducted to evaluate the efficacy of an essential oil blend (EOB, NEXT ENHANCE® 150, 1:1 thymol carvacrol) against *Eimeria* challenge when it was used alone or combined with a coccidiosis vaccine. The study consisted of 8 treatments in a 2x2x2 factorial arrangement with 2 levels of vaccination (no or yes), 2 levels of EOB (0 or 60 g/ton), and 2 levels of *Eimeria* challenge (no or yes). Each treatment had 9 replicate pens of 8 birds. Vaccination was administered on d 0 and *Eimeria* challenge (35X recommended vaccination dose) was applied on d 21 via oral gavage. Data were subject to 2-way ANOVA before *Eimeria* challenge and 3-way ANOVA after challenge to evaluate main effects and their interactions; means were separated by Fisher's protected LSD test. Supplementation of EOB increased BW on d 21 and improved FCR on d 13, 21, and 29 (P<0.05) and the effect was independent of vaccination or *Eimeria* challenge. Regardless of EOB or challenge, coccidiosis vaccination had no effect on growth performance on d 13 or 21, but improved BW and FCR during d 21-29 *Eimeria* challenge phase (P<0.05). During 26-28 d, vaccinated birds shed minimal oocysts in excreta and

non-vaccinated birds shed a substantial number of oocysts, especially for those challenged with *Eimeria,* accounting for an interaction between vaccination and challenge (P<0.05) and no significant effect of EOB was observed for oocyst shedding. The oocyst shedding reduction along with performance improvement demonstrated that the coccidiosis vaccination provided birds with immunity against *Eimeria* and the protective effect was not impacted by EOB supplementation. In summary, EOB increased BW before *Eimeria* challenge and improved FCR throughout the trial, coccidiosis vaccination reduced oocyst output and improved BW and FCR during the *Eimeria* challenge phase, and they can be used together without compromising the efficacy of each other.

Key Words: Essential oil, Coccidiosis vaccine, Eimeria, Broiler

P36 Feeding dry stevia leaf (Stevia rebaudiana) or xylanase improve the hepatic antioxidative status of broiler chickens Vasil Pirgozliev[*1], Kristina Kljak[2], Isobel Whiting[1], Stephen Mansbridge[1], Stephen Rose[1], Stanimir Enchev[3], Atanas Atanasov[4,5,6,7], Jose Stringhini[8] *[1]The National Institute of Poultry Husbandry, Harper Adams University, [2]Department of Animal Nutrition, Faculty of Agriculture, [3]Agricultural Institute, [4]Ludwig Boltzmann Institute for Digital Health and Patient Safety, Medical University of Vienna, [5]Department of Pharmacognosy, University of Vienna, [6]Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, [7]Institute of Neurobiology, Bulgarian Academy of Sciences, [8]Universidade Federal de Goias*

The antioxidant activities of stevia are recognised with mammals but the results with chickens are not consistent. Xylanase is widely used in poultry production around the world and improves not only productive performance but also the hepatic antioxidative status of birds. However, information on the interaction between stevia and xylanase is lacking. The aim of this experiment was to study the impact of dietary stevia with or without exogenous xylanase on hepatic antioxidant status of broiler chickens. Bird growth performance variables were also measured. Four diets, formulated with and without stevia and with and without exogenous xylanase, following 2 x 2 factorial design, were prepared, containing 206 g/kg crude protein, 12.67 MJ/kg ME and 12.4 g/kg lysine. This was achieved by taking the basal diet and supplemented with or without 20 g/kg of milled dry stevia leaf from cultivar Stela substituted for wheat. Both diets were then split into two batches as one of them was supplemented with *Aspergillus oryzae* commercial preparation of endo-1,4-beta-xylanase at 100 g/kg (100 FXU/kg, Ronozyme WX, DSM, Switzerland). Each diet was fed *ad libitum* to birds in eight pens (three birds in each pen) in a randomised block design. It was found that birds fed xylanase grew faster (34 *vs* 32 g daily growth; SEM = 0.60; $P < 0.05$), used the feed more efficiently (0.615 *vs* 0.594 feed conversion efficiency; SEM = 0.0060; $P < 0.05$) and had an increased concentration of hepatic vitamin E concentrations (74 *vs* 56 µg; SEM = 5.4; $P < 0.05$). Feeding stevia did not affect growth performance ($P > 0.05$), but increased hepatic coenzyme Q_{10} (252 *vs* 219; SEM = 11.0; $P = 0.05$) and total carotenoids (6.4 *vs* 2.8; SEM = 0.38; $P < 0.001$) concentration. There was no dietary stevia by xylanase interaction ($P > 0.05$) for any of the studied variables. The results showed that alone, dietary stevia or dietary xylanase can improve some indicators of antioxidant status of birds. The effects were not antagonistic, so the inclusion of both supplements gave a more comprehensive improvement in antioxidants status of birds.

Key Words: stevia, xylanase, chickens, hepatic antioxidants

P37 The effect of dietary oil and grape pomace supplementation on egg production in Japanese quail Sylwia Sobolewska[*1,2], Janusz Orda[1], Jacek Majda[3], Bogdan Jarosz[1], Isobel Whiting[2], Stephen Rose[2], Stephen Mansbridge[2], Vasil Pirgozliev[2] *[1]Wrocław University of Environmental and Life Sciences, [2]The National Institute of Poultry Husbandry, Harper Adams University, [3]Department of Laboratory Diagnostics, 4th Military Hospital*

The grape pomace (GP) and linseed oil (LO) have been shown to improve the nutritional value of quail eggs for humans. There is a lack of information on their dietary inclusion on efficiency of egg production in quails. Grape pomace contains condensed tannins, which may affect dietary nutrient availability and egg production. Linseed contain high level of polyunsaturated fatty acids. Thus, the aim of the current study was to evaluate the effects of feeding GP and LO or the combination on the egg production in Japanese laying quails (*Coturnix coturnix japonica*). One-hundred-twenty laying quails were randomly allocated into four dietary treatments: 4% corn oil (CO), 4% CO + 2% GP, 2% CO + 2% LO, 2% CO + 2% LO + 2% GP. Birds were reared from 5 to 10 weeks of age. At the beginning of the study the birds were individually weighed and assigned to 20 cages (six birds in a cage). Each diet was fed as mash to 5 pens following randomisation. Data were analysed by general ANOVA procedure using a 2 X 2 factorial design. Feeding GP and the oils did not have significant impact ($P > 0.05$) on the daily feed intake (24.1 g, SEM = 0.59) and final weight of the birds (214, SEM = 6.1). Similarly, GP and the oils did not change ($P>0.05$) egg numbers (71.7 %, SEM = 4.21), egg weight (10.8 g, SEM = 0.19) and FCR for egg production (3.165 g:g, SEM = 0.2223). There were no interactions ($P > 0.05$) between treatment factors. In conclusion, feeding GP, CO and LO to Japanese quail did not change the egg performance variables. Other studies have shown that GP and LO improve the nutritional value of quail eggs for humans. This study has demonstrated that their addition in quail diets does not affect the efficiency of egg production.

Key Words: Japanese quail, grape pomace, corn oil, linseed oil, egg production

P38 Dietary dihydroquercetin supplementation on egg production in Hy-line Brown layers Isobel Whiting[1], Stephen Rose[1], Kristina Kljak[2], Antonija Simic[1], Vasil Pirgozliev[*1] *[1]The National Institute of Poultry Husbandry, Harper Adams University, [2]Department of Animal Nutrition, Faculty of Agriculture*

Dihydroquercetin (DHQ), a natural antioxidant, has been used in broiler and laying hen diets to improve performance and antioxidant/ physiological status. There is a lack of information on dietary DHQ inclusion on internal and external egg quality in laying hens. Thus, the aim of the current study was to evaluate the effects of DHQ on the egg weight, egg shell weight and thickness, albumen height, Haugh unit, albumen and egg yolk pH and yolk colour when fed to laying hens. Forty laying hens (Hy-line Brown) were randomly allocated into two diets: Control (C), containing 11.70 MJ/kg ME and 171 g/kg CP; C + 1.5 g/kg DHQ. The study continued for 4 weeks, from 22 to 26 weeks of age. At the beginning of the study the birds were individually weighed and assigned to 20 cages (two birds in a cage). Each diet was fed as mash to 10 cages following randomisation. Data were analysed by general ANOVA procedure. Feeding DHQ did not have a significant impact ($P > 0.05$) on the egg weight (57.85 g, SEM = 1.363), egg shell weight (5.29 g, SEM = 0.157) and egg shell thickness (0.343 g, SEM = 0.0093). Similarly, DHQ did not have an impact ($P > 0.05$) on albumen height (8.41 mm, SEM = 0.408), Haugh unit (91.8, SEM = 2.15) and albumen pH (8.427, SEM = 0.0600). The egg yolk pH (6.249, SEM = 0.0515) and yolk colour (2.25 on DSM fan, SEM = 0.288) were also not affected by dietary DHQ ($P > 0.05$). In conclusion, feeding DHQ to Hy-line Brown laying hens from 22 to 26 weeks of age did not change the egg quality variables. All results were within expected range for laying hens at this stage of production. This study has demonstrated that DHQ addition in laying hen diets does not affect the internal and ex-

ternal quality of eggs produced. Research on the impact of dietary DHQ on antioxidant status of eggs is warranted.

Key Words: Laying hens, dihydroquercetin, egg quality

P39 Effect of spray-dried porcine plasma on performance and gut health of broilers challenged with Necrotic enteritis A. Daneshmand[1], N.K. Sharma[1], T.H. Dao[1], J.M. Campbell[*2], S.B. Wu[1], R.A. Swick[1] *[1]University of New England, [2]APC, LLC*

Necrotic enteritis (NE) has been estimated to cause up to 6 billion in losses to the global broiler industry annually. The toxin producing variant of *Clostridium perfringens* is responsible and gut injury as a result of cocci infection may trigger the onset. Early feeding of spray dried porcine plasma (SDP) is a potential replacement for AGP as it contains active immunoglobulins and other proteins. A study was conducted using 816 Ross 308 d-old cockerels to examine early feeding of SDP during experimental NE infection. A completely randomized design with a factorial arrangement of treatments was employed. Factors were: NE challenge: no or yes and SDP: 0 or 2% from to 10 d. Challenged birds were gavaged with sporulated oocysts of vaccine *E. acervulina* (5000 each), *E. brunetti* (5000 each) and *E. maxima* (2500 each) on d 9 and 10^8 CFU of *C. perfringens* (Type G CSIRO EHE-NE18 strain) on d 14. Challenge with NE resulted in increased lesion scores ($P < 0.01$) in all segments of the intestine, increased FCR ($P < 0.001$), and increased leaky gut as evidenced by higher serum FITC-d concentrations ($P < 0.001$) after FITC-d gavage on d 16. Early feeding (0 to 10 d) of SDP decreased FCR before NE challenge ($P < 0.001$), decreased FCR ($P < 0.05$) and tended to increase weight gain ($P = 0.07$) from d 0 to 29. Interactions showed SDP to lower serum FITC-d concentration only in NE challenged birds ($P < 0.01$). Birds fed SDP had higher relative bursa weight on d 16 and d 35. No interactions between SDP and NE challenge were observed for feed intake, gain or FCR ($P > 0.05$). Challenge with NE increased serum IgA, IgM, and αAGP ($P < 0.05$) and early feeding of SDP decreased serum αAGP and IL-6 on d 16 ($P < 0.05$). These findings demonstrate that early feeding of SDP improves growth performance irrespective of NE challenge through diminished inflammation.

Key Words: Spray-dried plasma, Necrotic enteritis, Broilers, Performance

P40 Acidification of drinking water: In vitro and in vivo investigation in broilers challenged with Campylobacter jejuni Vasileios Tsiouris[*1], Tilemachos Mantzios[1], Kostas Kiskinis[1], Euangelos Ekonomou[2], Euanthia Petridou[3], George Papadopoulos[4], Ioanna Georgopoulou[1], Paschalis Fortomaris[4] *[1]Unit of Avian Medicine, Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [2]Laboratory of Hygiene of Food of Animal Origin – Veterinary Public Health, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [3]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [4]Laboratory of Animal Science, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki*

Water acidifiers have been widely used in poultry production, as alternatives to antibiotics, enhancing performance, water quality and pathogen control. Poultry meat consumption is considered as the most important route of human infection with *Campylobacter jejuni*. In the present study *in vitro* and *in vivo* investigations were conducted to evaluate the anti-*Campylobacter jejuni* activity of a commercial water acidifier, a blend of hydrogen peroxide, paracetic and acetic acid. The antibacterial activity of the product was tested by the micro-MIC method and expressed as the lower concentration of the product which inhibits bacterial growth. For the *in vivo* experiment, one hundred and forty-four 1-day old chicks were divided equally into 4 treatment groups, with 4 replicates, according to the following experimental design: group A (birds administered water without treatment), group B (birds challenged with *Campylobacter jejuni*), group C (birds receiving water treated with 1% commercial antibiotic based on enrofloxacin), and group D (administration of the product in drinking water in concentration 0.5% and challenge were applied). The bodyweight (BW) of chickens was measured weekly to assess the feed conversion rate (FCR). The *Campylobacter jejuni* counts in the crop and caeca of birds were evaluated by plate counting in mCCDA agar, with presumptive colonies confirmed by PCR. The results of the *in vitro* assay showed an effect against *Campylobacter jejuni* of the product at a concentration of 0.04%v/v. Statistical analysis and evaluation of the *in vivo* experimental data revealed that the product did not significantly affect ($P>0.05$) the BW nor FCR of birds during the experimental period. PCR analysis demonstrated the absence of *Campylobacter jejuni* in crop and caeca in broiler chicks of experimental group A, highlighting the efficacy of strict biosecurity measures applied. However, *Campylobacter* counts in crop and caeca were significant lower ($P\leq0.005$) in experimental groups C and D compared to group B on day 24. The findings of this study indicate that the tested acidifier demonstrated strong anti-*Campylobacter* activity *in vitro* and its use for water acidification could contribute to the prevention of *Campylobacter* colonization in broiler chickens.

Key Words: commercial water acidifier, Campylobacter jejuni, broiler chickens

P41 In vitro and in vivo investigation of a commercial phytogenic product against Campylobacter jejuni in broiler chicks Vasileios Tsiouris[*1], Kostas Kiskinis[1], Tilemachos Mantzios[1], Euangelos Ekonomou[2], Euanthia Petridou[3], George Papadopoulos[4], Ioanna Georgopoulou[1], Paschalis Fortomaris[4] *[1]Unit of Avian Medicine, Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [2]Laboratory of Hygiene of Food of Animal Origin – Veterinary Public Health, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki,, [3]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki,, [4]Laboratory of Animal Science, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki*

Plant extracts, also known as phytobiotics/phytogenics, have been widely used in animal nutrition, particularly for their antimicrobial, anti-inflammatory, and antioxidant activities. The aim of the present study was the investigation of the *in vitro* and *in vivo* activity of a commercial phytogenic product against *Campylobacter jejuni*. The *in vitro* activity of the commercial phytogenic product against *Campylobacter jejuni* was evaluated by the determination of its Minimal Inhibitory Concentration (MIC). For the *in vivo* experiment, one hundred and forty-four 1-day old chicks were randomly allocated to 4 treatment groups, with 4 replicates, according to the following experimental design: group A (negative control), group B (birds challenged with *Campylobacter jejuni),* group C (birds challenged and received 1% a commercial antibiotic based on enrofloxacin), and group D (administration of the commercial phytogenic product by drinking water in a concentration of 1% and challenge were applied). The bodyweight (BW) of chickens was measured weekly to assess the feed conversion rate (FCR). The *Campylobacter jejuni* counts in the crop and caeca of birds were evaluated by plate counting in mCCDA agar, and suspected cultures were confirmed by PCR. Results from the *in vitro* assay revealed that the product exhibited an effect against *Campylobacter jejuni* at a concentration of 0.142% v/v. Statistical analysis and evaluation of the *in vivo* experimental data revealed that experimental group D had significantly lower FCR for the period of 25^{th}-39^{th} day of age and higher final body weight compared to groups A and C ($P\leq0.05$). *Campylobacter jejuni* was not detected in the crop and caeca in broiler chicks of experimental group A, indicating the absence of cross contamination between experimental groups and the efficacy of strict biosecurity measures applied. However, the *Campylobacter* counts in crop and caeca were significant lower ($P\leq0.05$) in experimental groups C compared to group B and D on day 25.

The study provides evidence that the tested phytogenic product posses *in vitro* antibacterial activity against *Campylobacter jejuni* and may contribute to the prevention of *Campylobacter* colonization in broiler chickens.

Key Words: commercial phytogenic product, Campylobacter jejuni, broiler chicks

P42 Use of mixture of essential oils with sodium humates as intestinal integrity modulators in broilers challenged with coccidia vaccine Jaime Angel-Isaza*, Angi Montoya Garcia, Blanca Martinez, Loufrantz Parra Mendez, Alvaro Uribe Serrano *Promitec Santander*

Coccidiosis is a common enteric parasite disease that causes economic losses by reducing the productive parameters and causing high morbidity and mortality in the flocks. Anticoccidial drugs have been used for its control; however, extensive use of anticoccidials has led to drug resistance. Studies have reported that feed supplementation with essential oils can be an effective alternative to anticoccidial drugs. Thus, EOs may decrease the use of drugs for treating and controlling avian coccidiosis. Therefore, this study aimed to evaluate the effect of a mixture of essential oils (EO) encapsulated by two different techniques with sodium humates (HS) on the intestinal integrity of broilers challenged with live attenuated coccidia vaccine (Livacox T®). 192 Ross AP chickens were used. Chickens were distributed using a completely randomized design, consisting of four treatments: (D1) base feed + antibiotic growth promoter (AGP) + nicarbazin 75 ppm; (D2) base feed + AGP; (D3) base feed + Lippia origanoides (LO) and Eugenia Caryophyllata (EU) EOs carried in maltodextrin with SH (900g/Ton); (D4) base feed + LO and EU EOs micro-encapsulated by spry drying with SH (900g/Ton). At day 14 post-hatch, all birds were orally inoculated with 30 times the coccidia vaccine doses. At day 35 post-hatch, the zootechnical parameters were compared. Besides, jejunum samples were taken, and the morphometric parameters were evaluated. An ANOVA test was conducted to determine the effect of treatment, and a Tukey adjustment pairwise comparison was made for the four groups. Chickens from group D4 shown higher values ($P<0.05$) in the final weight and feed conversion ratio compared to D2. No significant differences ($P> 0.05$) were found between D1 and any of the groups containing the mixture of EO and HS (groups D3 and D4). The highest ($P<0,05$) length of villi and villi/crypt ratio were found in D1, D3, and D4. Regarding villus width and crypt depth, the D4 diet shown significant differences ($P<0.05$) compared to groups D1 and D2. In summary, EOs mixture micro-encapsulated by spray drying and HS showed morphological jejunum development similar or higher than the use of chemical anticoccidial during a coccidia challenge.

Key Words: Poultry, phytobiotic, antibiotic, oregano, feed additive

P44 COMPARISON OF SODIUM BUTYRATE AND ESSENTIAL OILS ON BROILERS PERFORMANCE Cinta Sol*, Mónica Puyalto, Juan José Mallo *NOREL S.A.*

A field trial was conducted to compare the effect of feed supplementation with partially protected sodium butyrate, at 1.5kg/tn from d0 to d10 and 0.5kg/tn from d11 to d42 (SB, 70% sodium butyrate protected with vegetable fat) respect to a control diet supplemented with essential oils (EO, based on oregano oil), on broilers performance. There were involved 4 farms in total; each farm was divided into 2 groups with a total of 10 replicates per treatment. Weekly, body weight (BW), body weight gain (BWG) and mortality (M) were recorded; and average daily gain (ADG), feed conversion ratio (FCR), livability (L) and European Performance Efficiency Factor (EPEF) were calculated. Feed and water were offered *ad libitum*. Data were analyzed with one-way ANOVA, using the GLM procedure of SAS, and introducing the initial body weight of chicks as a covariable in the model. Regarding body weight, statistical differences were observed at week 1 (173g vs 184g, for EO and SB, $P<0.0001$) and at week 4 (1487g vs 1551g, for EO and SB, $P<0.041$). At week 6, there was a trend in less mortality with SB (2.27% vs 1.42%, $P=0.073$). At the end of the study (week 6), there were some numerical differences in ADG (60.2g vs 61.0g, for EO and SB), BW (2.53kg vs 2.57kg, for EO and SB), FCR (1.66 vs 1.64, for EO and SB), L (95.6% vs 97.1%, for EO and SB) and EPEF (347.7 vs 361.1, for EO and SB). Even though there were no significant differences at the end of the trial, the performance parameters were better for birds supplemented with sodium butyrate that for those supplemented with essential oils.

Key Words: sodium butyrate, broilers, performance

P45 In-vitro study of a novel consensus variant phytase (PhyG) – simulation of upper gastro-intestinal tract performance. Trine Christensen*[1], Rie Mejldal[1], Rachael Hardy[2], Arno Kreij[3] *[1]DuPont Nutrition and Biosciences, DK, [2]DuPont Nutrition and Biosciences, UK, [3]DuPont Nutrition and Biosciences, SG*

The bio-efficacy of a novel consensus bacterial 6- phytase variant (PhyG) was determined under simulated gastro-intestinal tract conditions in comparison with other commercial phytases.

Early and complete hydrolysis of phytate-phosphorus from animal feed in the upper GIT is key in maximising phosphorus release and reducing anti-nutritional effects of phytate. Efficient degradation of phytic acid in the proventriculus and gizzard (at low pH: 2.5-4.0) prevents inaccessibility of insoluble inositol phosphates later in the intestines. Pepsin stability at low pH is vital to activity in gastric phase.

In-vitro studies simulating proventriculus and gizzard and using Na-phytate as substrate were conducted to evaluate PhyG and four additional bacterial 6-phytases: Two *E. coli* phytases (phytase E1 and E2), a *Citrobacter braakii* phytase (phytase C) and a *Hafnia sp.* hybrid phytase (phytase H). Phytase inclusion was based on the industry activity reference unit, FTU (pH 5.5).

The influence of pH on activity was studied over a broad pH range (1.5-6.0) at 37°C for 60min and furthermore the sequential hydrolysis of lesser phosphorylated inositol phosphate esters (InsP2-6) at pH 3.5 and 37°C were followed over time (0-180min) using High-Performance Ion-Exchange Chromatography. Stability at low pH (2.0) was simulated at 40°C with addition of 3000U of pepsin/ml. Statistical evaluation was carried out using ANOVA followed by Tukey's test to detect significant differences in treatment means (95% level) using Minitab® 18.1 software.

Results showed large differences among the phytases tested. It was demonstrated that PhyG retains >200% relative activity in pH-range 3.0-5.0 and maintains full activity down to pH 1.5, whereas remaining phytases had decreased activity at pH 2.5 (relative to pH 5.5). Comparing PhyG to other phytases showed that the phytic acid hydrolysis rate was more rapid (12min vs 20min or more, respectively, was needed to hydrolyse half the substrate present) and in addition a lower accumulation of total InsP4 was observed. PhyG along with phytase E2 retained almost full stability in incubation with pepsin at pH 2.0 with residual activities >80% after 120minutes.

The combined characteristics of PhyG when studied *in*-vitro demonstrates a high potential to efficiently hydrolyse phytic acid and improve nutrient uptake early in the upper GIT.

Key Words: consensus phytase, buttiauxella, in-vitro, phytate degradation, gastro-intestinal tract

Metabolism and Nutrition: General Nutrition

P46 Home-made liquid probiotic reduced egg yolk cholesterol and improved bird performance Adekoyejo Oyegunwa*[1], Gbenga Kassim[1], Olusola Ikotun[1], Mutiu Mosobalaje[2] *[1]Tai Solarin University of Education, [2]Oyo State College of Agiculture and Technology*

A study was conducted to determine the effect of locally made probiotics on the performance and egg yolk cholesterol of Isa brown laying birds. Sixty four 16 weeks old Isa brown pullets were randomly allotted to four treatments of four replicate with 4 birds per replicate in a completely randomized design. Treatment 1 was the control experiment that did not take liquid probiotic. Treatments 2, 3 and 4 were given 10, 20 and 30mls of probiotics per litre of water, respectively and fed for 12 weeks. Data were collected on feed intake, body weight gain, hen day production, egg weight and egg yolk cholesterol.

Significant increases ($p<0.05$) were observed in the body weight gain of the laying birds from control group (138.75) to treatment 4 (199.00g). Feed intake also increased significantly ($p<0.05$) in birds that were fed with probiotics compared to birds in the control group. Significant reductions ($p<0.05$) in cholesterol were noticed in probiotic-fed birds with the least value (159.25mg/DL) obtained in treatment 2 while the highest value of 387.75mg/dL was obtained in control treatment without probiotics.

In the overall, locally produced probiotic reduced egg yolk cholesterol and improved performance in laying birds.

Key Words: Home-made probiotics, Isa brown Layers, Performance, Egg yolk cholesterol

P47 Pelleting increases the metabolizable energy of de-hulled sunflower seed meal for broilers Nikolay Karkelanov[1,2], Sashka Chobanova[2], Isobel Whiting[1], Stephen Rose[1], Vasil Pirgozliev*[1] *[1]The National Institute of Poultry Husbandry, Harper Adams University, [2]Trakia University, Faculty of Agriculture*

The high dietary fibre content, low metabolizable energy and lysine contents and presence of chlorogenic acid in sunflower seed meal (SFM) are the main challenges of using it in poultry diets. However, recent advances in engineering has improved the de-hulling process to allow further processing of SFM to produce a high-protein product with a crude protein content greater than 40% and crude fibre content lower than 10%. The aim of this study was to examine the effect of two de-hulled SFM samples obtained from the same batch of sunflower seeds. The SFM contained 440 g/kg crude protein, 4 g/kg fat, 168 g/kg total non-starch polysaccharides (NSP), 53 g/kg soluble non-starch polysaccharides, 115 g/kg insoluble total non-starch polysaccharides, and 17.65 MJ/kg gross energy. One of the SFM samples was fed as a meal (MSFM) and the other was further pelleted (PSFM) at 75°C and 360kPa pressure to pass 3 mm mesh. Two diets containing either 200 g/kg MSFM or 200 g/kg PSFM were prepared and fed in a meal form to 20 pens (two birds each) with male Ross 308 broilers, from 8 to 21d age, following randomisation. The diet containing the pelleted SFM was provided as a loose mixture of SFM pellets and the remaining ingredients ground to pass a 5 mm screen. In the mash form, all ingredients were ground to pass a 5 mm screen. The diets did not contain any coccidiostats and other feed additives. Data were analysed by ANOVA. Feeding PSFM increased ($P < 0.05$) dietary AMEn 0.26 MJ/kg DM (12.88 vs 12.62, SED = 0.104) suggesting a further break down in the cell structure of the SFM, thus releasing more available energy to the chickens. The impact on bird growth performance and retention coefficients was not significant ($P > 0.05$). The improvement in AME indicates that pelleting has an impact on bioavailability of some of the NSP components in sunflower meal. Further research is needed to study the impact of pelleting on the metabolisable energy of whole diets containing already pelleted sunflower meal *vs* milled only sunflower meal.

Key Words: Sunflower seed meal, broiler chickens, pelleting, metabolizable energy

P48 Using a biscuit aroma to increase feed intake in a commercial layer farm Bernat Canal*, Luis Mesas, Monica Puyalto, Juan Jose Mallo *Norel S.A.*

Feed intake in layers can be influenced by many factors; heat stress, diet composition, production stage, etc., and producers often struggle to reach the consumption levels that geneticists recommend. Not reaching the suggested consumption might prevent layers from hitting their genetic potential. The use of aromas is one of the latest strategies, despite the extended belief that birds are anosmic. The objective of this field trial was to determine if an aroma was able to increase the feed consumption and egg production of layers raised under commercial conditions in Spain. A total of 15,000 37-week-old Hy-Line Brown layers were used for this trial, in a single group. The recommended intake for this breed and age is 108-114 g/d/bird. An on/off experimental design was employed to avoid disrupting the normal-functioning of the farm. The trial lasted a total of 6 weeks, 3 UNFLAVORED (usual feed) week-long periods and 3 FLAVORED (usual feed + Apetenzyma® 2923 Biscuit (250g/t)) week-long periods, distributed alternatively. Feed intake was calculated for each period, data related to daily egg production (total production, egg weight, broken eggs) was recorded daily. Egg weight was classified in 5 categories: XXL (>83g), XL (73-83g), L (63-73g), M (53-63g), S (<53g). Data were analyzed by one-way ANOVA using the GLM procedure of SAS 9.0. Results showed that the average daily feed intake was significantly higher ($P<0.001$) during the FLAVORED periods (110.77 g/d/bird vs 105.15 g/d/bird UNFLAVORED). No significant differences were observed in total egg production or broken eggs ratio. However, layers showed a tendency ($P=0.055$) of producing more XXL weight eggs (169 eggs/day vs 158 eggs/day) during the FLAVORED periods. This finding can be explained by the extra intake achieved when FLAVORED diet was offered. These data go in line with some recent studies demonstrating that layers can perceive certain smells. Additionally, the 5.34% increased intake suggest that adding aromas to layers' feeds could be a potential solution when feed consumption is challenged. Further studies must be carried out under different commercial conditions to understand the extent of the aroma's effect.

Key Words: layer, aroma, intake

P49 Reducing adverse effects of experimental mycotoxicosis on carcass traits and meat quality in broilers by feeding an extract from Capparis spinosa Pouyan Malekinezhad*[1,2], Laura Ellestad[1], Nazar Afzali[2], Seyed Homayoun Farhangfar[2], Arash Omidi[3], Abbas Mohammadi[2] *[1]University of Georgia, [2]University of Birjand, [3]Shiraz university*

Therapeutic medicinal plants have been used since ancient times. One such plant, *Capparis spinosa* (CS), is enriched in phenolic compounds and flavonoids that could mitigate negative effects of mycotoxins on broiler carcass traits. Therefore, this experiment was conducted to evaluate the ability of an alcoholic extract from CS to influence effects of aflatoxin B1 (AFB) and ochratoxin A (OTA) on broiler carcass characteristics and meat quality. A total of 288 Ross 308 broilers were randomly divided into 9 treatments with 4 replicates each (n=4): (1) negative control diet with no additives (NC); (2) NC + 2 ppm AFB (positive control AFB; PCAFB); (3) NC + 2 ppm OTA (positive control OTA; PCOTA); (4) PCAFB + 250 mg/kg CS; (5) PCAFB + 500 mg/kg CS; (6) PCAFB + 750 mg/kg CS; (7) PCOTA + 250 mg/kg CS; (8) PCOTA + 500 mg/kg CS; and (9) PCOTA + 750 mg/kg CS. At the end of the experiment, eight birds from each group were processed and evaluated for carcass characteristics, yields, and meat quality according to water-holding capacity (WHC) and breast muscle malondialdehyde (MDA) levels. Data were subjected to analysis of variance in SAS, and parameters showing significant differences were compared using Tukey's honest significant difference test. Both PCAFB and PCOTA diets decreased meat quality and muscle moisture as compared with the NC diet ($P<0.05$). There was no influence of CS supplementation at any level on muscle moisture in birds fed PCOTA or PCAFB diets

(P>0.05). Meat levels of MDA in PCAFB and PCOTA treatments were higher than in the NC treatment (P<0.05), and supplementation with CS at multiple levels partially reversed this. The addition of 750 mg/kg CS to PCAFB diets and all three levels of CS to PCOTA reduced MDA to levels not different from that in the NC group (P>0.05). Carcass yields and relative thigh weight in PCAFB and PCOTA treatments were lower than in the NC treatment (P<0.05), and supplementation with CS at multiple levels partially or fully reversed these effects. Addition of 750 mg/kg CS to PCAFB diet increased relative thigh weight as compared to PCAFB alone, but this activity was still less than in the NC diet (P<0.05). These data suggest that supplementation with CS could improve meat quality in broilers fed diets contaminated with mycotoxins.

Key Words: carcass, Capparis Spinosa, meat quality, water-holding capacity

P50 Towards a digestible calcium system for broiler chickens: review of digestible calcium coefficients for common feed ingredients
Carrie Walk*[1], Aaron Cowieson[2] *[1]DSM Nutritional Products, UK, [2]DSM Nutritional Products*

There is a growing awareness that over-supply and variability in the nutritional quality of Ca sources has significant effects on gastrointestinal (GIT) pH, P and amino acid digestibility, exogenous enzyme efficacy and the microbial population in the GIT of broilers. Interest in moving toward a digestible Ca system is gaining traction and the digestibility coefficients for Ca in limestone (n = 55), dicalcium phosphate (DCP, n = 21), meat and bone meal (MBM, n = 20), mono-calcium phosphate (MCP, n = 12), and plant-based ingredients for broilers are available. The aim of this project was to compile the literature evaluating Ca digestibility of feed ingredients for broilers and highlight factors that may influence the digestibility coefficients. From 1974 to 2019, there were 11 papers published. Experimental methods, age and adaptation time, and Ca:non-phytate P (NPP) ratios were variable among the experiments. Within each ingredient, there were no differences (P > 0.05) in the apparent (AID) or standardized ileal digestibility (SID) coefficients. The average ileal Ca digestibility coefficient for limestone was 0.53, DCP was 0.42, MCP was 0.35 and MBM was 0.46. Ileal Ca digestibility coefficients for limestone increased as limestone particle size increased (quadratic, P < 0.05). The ileal Ca digestibility coefficients for DCP were lower (P < 0.05) in corn- compared with synthetic-based basal diets; whereas this was the opposite for MCP. Increasing the adaptation time ≥72 hours increased (quadratic, P < 0.05) the ileal digestibility coefficients determined for DCP, but not MCP. Widening the analyzed Ca:NPP ratio increased MCP (quadratic, P < 0.05) and MBM (linear, P < 0.05) and decreased DCP (quadratic, P < 0.05) ileal Ca digestibility coefficients. Future evaluations of ileal Ca digestibility coefficients using corn-based diets, standardizing the adaptation period to 72 h for ingredients or diets with a Ca:NPP ratio > 1:1 or to 16 h for ingredients or diets with a Ca:NPP ratio < 1:1 may limit the animal's adaptation. Other considerations include using phytase at saturation levels, measuring and reporting Ca and mineral concentrations, location of origin, and particle size to allow characterization and predictions of Ca digestibility and repeatability of results.

Key Words: dicalcium phosphate, limestone, meat bone meal, monocalcium phosphate, digestible calcium

P51 Effect of the inclusion of corn gluten with yeasts on carcass yield of broilers at 42 days of age
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Corn gluten with yeast (DDGS) obtained from the fermentation of corn for ethanol production is an alternative ingredient of high protein contente to be used in the animal feeding, because it is similar to the protein content of soybean meal. An experimental trial was conducted with 600 cobb 500 ® one-day-old broiler chicks, in order to evaluating the supply of increasing levels of DDGS in replacement of soybean meal, on carcass and cuts yields and abdominal fat percentage at 42 days of age. Broilers were allotted in a completely randomized design, with four treatments and 10 replicates of 15 birds each. The treatments consisted of four replacement levels: 0%; 10%; 20% and 30% of soybean meal, which results in na inclusion of: 0%, 3.02%, 6.04% and 9.06% DDGS in the diets, respectively. The composition of DDGS tested was: 92,06% dry matter, 45,65% crude protein 2,17% ash, 53,85% neutral detergente fiber; 6,87% acid detergente fiber and 5.262,9 kcal/kg of crude energy. The parameters evaluated were: breast yield (PR), thigh yield + drumstick (RC + S), wing yield (RA) and abdominal fat yield (RGA), expressed as a percentage of live weight of broilers at 42 days of age. The data obtained were submitted to variance analysis (ANOVA) and F test, to evaluate the results. No significant differences were observed (p>0.05) between treatments at 42 days of age for carcass and cuts yields and abdominal fat percentage. The mean values obtained were 69.9%, 22.8%, 23.4%, 8.3% and 1.1% for carcass yield, breast yield, thigh + drumsticks yield, wings yield and abdominal fat. The addition of DDGS partially replaced soybean meal without affecting carcass yield and cuts parameters, and can be included in the proportion of 9.06% in the diets.

Key Words: alternative ingredient, broiler feeding, corn ethanol, DDGS

P52 Growth performance of broiler chickens fed enzyme-treated soy protein day 0-14 compared to exogenous protease fed day 0-56
Simone Rasmussen[1], Alfred Blanch*[1], Adam Davis[2], Christine Brøkner[1] *[1]Hamlet Protein A/S, [2]University of Georgia*

The objective of this study was to compare broiler growth performance at the market ages d35, 42 and 56 when fed enzyme-treated soy protein (ESP; Hamlet Protein A/S, Denmark), representing extremely low anti-nutritional factors, to exogenous protease in starter diets. A total of 552 day-of-hatch Yield Plus x Ross 708 male broiler chickens were used to assess the effect of two dietary treatments: Starter period (d0-14) chickens were fed either T1 (31.46% SBM, protease: 137.5 U/metric ton) or T2 (26.90% SBM, 5% ESP). In the grower (d14-28), finisher (d28-42) and withdrawal periods (d42-56), all birds received common corn-soybean meal based diets. T1 group continued to receive protease (137.5 U/metric ton) throughout the entire trial. Feed and water were provided *ad libitum*. Body weight and feed intake by pen were recorded at d0, 14, 28, 35, 42 and 56. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. FCR was adjusted for mortality and fixed BW (d35=2.42kg, d42=3.34kg, d56=4.88kg). Data were analyzed using the statistical software R (R Core Team 2020 version 4.0.0) by Student's T-test. Differences were considered significant at $p<0.05$. For BWG, no significant difference was observed between treatments. ESP inclusion in starter feed had a significant effect on mortality adjusted FCR on day 35 (SBM+protease=1.45, SBM+ESP=1.42; $p=0.001$), day 42 (SBM+protease=1.52, SBM+ESP=1.48; $p<0.0001$) and day 56 (SBM+protease=1.71, SBM+ESP=1.68; $p=0.046$). Fixed BW adjusted FCR was significantly improved by ESP inclusion at day 35 (SBM+protease=1.44, SBM+ESP=1.42; $p=0.018$) and day 42 (SBM+protease=1.52, SBM+ESP=1.48; $p=0.0006$). In conclusion, replacing part of the SBM in broiler starter diets with 5% ESP improved performance at day 35, 42 and 56 by reducing mortality adjusted FCR by 3, 4 and 4 points over exogenous protease. Feeding ESP in starter diets reduced BW adjusted FCR at day 35 and 42 by 2 and 4 points over exogenous protease.

Key Words: Enzyme-treated soy protein, Exogenous protease, Broiler chicken, Performance

P53 In vitro and in vivo investigation of a commercial water disinfectant against Campylobacter jejuni in broiler chicks Vasileios Tsiouris[*1], Tilemachos Mantzios[1], Kostas Kiskinis[1], Euangelos Ekonomou[2], Euanthia Petridou[3], George Papadopoulos[4], Ioanna Georgopoulou[1], Paschalis Fortomaris[4] *[1]Unit of Avian Medicine, Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [2]Laboratory of Hygiene of Food of Animal Origin – Veterinary Public Health, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [3]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, [4]Laboratory of Animal Science, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki*

Campylobacter jejuni is well recognized as the leading cause of bacterial foodborne diarrheal disease in European Union. Contaminated poultry meat is the most common source of infection. The objective of the present study was the investigation of the *in vitro* and *in vivo* activity of a commercial water disinfectant, a blend of inorganic compounds, against *Campylobacter jejuni*. The *in vitro* activity of the product was estimated by the determination of its Minimal Inhibitory Concentration (MIC). For the *in vivo* experiment, one hundred and forty-four 1-day old chicks were randomly allocated to 4 treatment groups, with 4 replicates, according to the following experimental design: group A, which served as the negative control, group B (birds were challenged with *Campylobacter jejuni),* group C (birds were challenged and received 1% of a commercial antibiotic based on enrofloxacin), and group D (birds were challenged and received 1% of a commercial water disinfectant). The bodyweight (BW) of chickens was measured weekly to assess the feed conversion rate (FCR). The *Campylobacter jejuni* counts in the crop and caeca of birds were evaluated by plate counting in mCCDA agar, with presumptive colonies confirmed by PCR. Results from the *in vitro* assay showed that the product exhibited effect against *Campylobacter jejuni* strains under the low concentration of 0.01%v/v. *In vivo* experimental data revealed that commercial water disinfectant reduced significantly ($P \leq 0.005$) the BW of the birds the first 24 days of their life. However, the FCR for the total experimental period and the final BW did not significantly differ ($P \geq 0.05$) among the tested groups. *Campylobacter jejuni* was not detected in crop and caeca in broiler chicks of experimental group A, indicating the absence of cross contamination between experimental groups and the efficacy of strict biosecurity measures applied. However, the *Campylobacter* counts in crop and caeca on day 25 was significant lower ($P \leq 0.001$) in experimental group C compared to groups B and D. The study provides evidence that the commercial water disinfectant has strong *in vitro* antibacterial activity against *Campylobacter jejuni*, but its continuous use from day 1 could suppress the performance in broiler chicks.

Key Words: commercial water disinfectant, Campylobacter jejuni, broiler chicks

P54 Characterization and variation of non-starch polysaccharides in 77 corn samples Morten Tovborg[*1], Pablo Jerez[1], Nelson Ward[2], Eduardo Della Pia[1] *[1]Novozymes A/S, [2]DSM Nutritional Products*

Cell walls of cereal kernels are rich in hemicellulose which provides structural integrity and entraps the potential nutrients, like starch, lipid, and protein. The hemicellulose (hereon referred to as non-starch polysaccharide or NSP) type and chemical composition differ considerably among cereals. Most of corn NSP consists of insoluble arabinoxylan (AX) and glucuronoarabinoxylan (GAX). The specific monosaccharide composition and structure of the NSP affects its ability to be fermented by microbes in the digestive tract and its degradation by exogenous xylanases to form health-promoting prebiotics. In the present study we analyzed the composition and variation of NSP of 77 global corn samples (incl. 55 from US). The insoluble NSP was selectively acid hydrolyzed after destarching the samples, and the monosaccharide composition was determined by HPAEC-PAD. Neutral sugars (fructose, rhamnose, arabinose, galactose, glucose and xylose) and acidic sugars (D-galacturonic acid and D-glucuronic acid) were quantified. Analysis of the total NSP indicates a distinct distribution where 20 % of the samples has an average total NSP content more than twice that of the remaining 80% (9.46 vs 4.02 % (DM)). Furthermore, statistical analysis through autoregressive moving-average fitting (ARMA) allow for splitting of the low NSP samples to find a third population with 17 % of the samples presenting unprecedented low total NSP content (2.97 % (DM)). Contrary to previous reports, rhamnose and galacturonic acid were found to be part of corn insoluble NSP and are proposed to be associated with trace amount of pectic polysaccharides, more specifically rhamnogalacturonan. The relatively constant ratios of most monosaccharides between groups - despite large variation of total NSP - suggests an overall consistency in NSP structure. This suggests structural differences within corn kernel tissues that appear reflected in its total NSP content. Since the main source of arabinoxylans comes from bran, we believe that different ratios between bran and endosperm tissues within the three populations results in variation in total NSP which ultimately affects nutritional properties and has consequences on the enzyme supplementation strategy for corn.

Key Words: corn, maize, non-starch polysaccharides, NSP, arabinoxylan

P55 Use of Capparis spinosa to improve the humoral immune and thyroid hormone status in broilers with mycotoxicosis Pouyan Malekinezhad[*1,2], Laura Ellestad[1], Nazar Afzali[2], Seyed Homayoun Farhangfar[2], Arash Omidi[3], Abbas Mohammadi[2] *[1]University of Georgia, [2]University of Birjand, [3]Shiraz university*

Mycotoxins are known to cause serious health problems in animals and humans through various mechanisms, including affecting thyroid hormone production and depressing immunoglobulin-containing cells in lymphoid tissues. Recent research has focused on the beneficial role of medicinal plants in treating disease, and much attention has been paid to the pharmacological effects of *Capparis spinosa* (CS) because of its high number of bioactive polyphenolic compounds. We investigated impacts of an alcoholic extract from CS on reducing adverse effects of Aflatoxin B1 (AFB) and Ochratoxin A (OTA) on the immune system and thyroid hormones in broilers. Two hundred eighty-eight 1-day-old Ross chicks were randomly allocated to one of 9 different feed regimens with four replicates each (n=4): (1) negative control diet with no additives (NC); (2) NC + 2 ppm AFB (positive control AFB; PCAFB); (3) NC + 2 ppm OTA (positive control OTA; PCOTA); (4) PCAFB + 250 mg/kg CS; (5) PCAFB + 500 mg/kg CS; (6) PCAFB + 750 mg/kg CS; (7) PCOTA + 250 mg/kg CS; (8) PCOTA + 500 mg/kg CS; and (9) PCOTA + 750 mg/kg CS. On day 42, 8 birds from each group were euthanized and collected blood used to measure thyroid stimulating hormone (TSH), thyroid hormones (T_3 and T_4), and immune parameters. All data were analyzed with ANOVA followed by Tukey's honest significant difference test using SAS. The results showed that serum T_3, T_4, and TSH were lower in PCAFB and PCOTA treatments than in the NC treatment ($P<0.05$). Addition of 750 mg/kg CS to PCAFB diet increased serum T_4 as compared to PCAFB alone, but levels were still decreased compared to the NC diet ($P<0.05$). The level of TSH was increased to intermediate levels in the PCOTA group as compared to NC diets ($P<0.05$) when these diets were supplemented with 750 mg/kg CS. IgG titers against sheep red blood cells (SRBC) decreased in PCAFB and PCOTA treatments ($P<0.05$), and 750 mg/kg CS increased the antibody titers in birds fed the PCAFB diet ($P<0.05$). No level of CS supplementation influenced effects of PCOTA diets on IgM titers against SRBC ($P>0.05$), and levels remained lower than those in birds fed the NC diet ($P<0.05$). Overall, supplementation with 750 mg/kg CS may improve thyroid hormone status of broilers fed diets contaminated with AFB and OTA.

Key Words: Capparis Spinosa, immunoglobulin, mycotoxin, triiodothyronine

P56 Effect of shredded, steam-exploded pine particles as a feed ingredient on productivity and gut development in broilers Chae-Mi Jeong*[1,2], Akshat Goel[2,3], Chris Major Ncho[2], Beom-June Kim[2], Vaishali Gupta[1,2], Byeo-Ri Ryu[2], Dong-Hwan Lee[4], Si-Young Ha[4], Ji-Young Jung[4], Jae-Kyeong Yang[4], Yang-Ho Choi[1,2,3] *[1]Division of Applied Life Sciences (BK21 Plus Program), Gyoungsang National University, [2]Department of Animal Science, Gyeongsang National University, [3]Institute of Agriculture and Life Sciences, Gyeongsang National University, [4]Division of Environmental Forest Science, Institute of Agriculture and Life Sciences, Gyeongsang National University*

Dietary fiber available in wood can help in developing gut health both physically and biologically. Insoluble dietary fiber makes the movement of intestinal content smoother and thus the soluble dietary fiber can act as a prebiotic that promotes intestinal beneficial bacteria activity. The purpose of this experiment was to examine the effect of increasing concentration of dietary pine particles (PP) as a feed ingredient on growth performances in Ross 308 broilers. A total of 216 day-old chicks were raised on a commercial starter feed for the first week. On the 7th day chicks were evenly divided into 3 treatments with 12 replicates having 6 birds in each cage. They were then given one of three experimental diets containing 0, 1 and 2% shredded, steam-exploded pine particles passing through a 10-mesh sieve replacing corn in their feed ingredients. Water and feed were provided *ad libitum*. Weekly body weight (BW), feed intake (FI) and feed conversion ratio (FCR) was recorded. On 28th day, 8 chickens per treatment were euthanized by carbon dioxide gas after BW, rectal temperature (RT) and head temperature (HT) measurements. Organ lengths were determined for the duodenum, jejunum, ileum and ceca, and then the duodenum, jejunum, ileum, liver and spleen were weighed. The intestinal contents were removed and washed, then tapped slightly to remove moisture, and the liver and spleen were weighed in addition to the duodenum, jejunum, and ileum. Data were analyzed using one-way ANOVA of SPSS. No significant differences were found between treatments in growth performances, temperatures, or organ weight and length. However, the higher the amount of wood particles included into the feed, the better the BW and FCR tend to be in the last week of the experiment (BW: 1458, 1467 and 1502 g for 0, 1, 2% PP, respectively) (FCR: 1.67, 1.66 and 1.65 for 0, 1 and 2% PP). In addition, RT and HT of 2% were higher than 0% PP (differences in RT and HT; 0.07 and 0.38°C) and intestines of 2% PP were slightly lighter or shorter than 0 (differences in duodenum, jejunum and ileum; 0.01, 0.03 and 0.11g; 0.7, 0.6 and 2.6 cm). This study shows that inclusion of wood particles not only has no adverse effects on performances, but can also be expected to improve productivity.

Key Words: shredded, steam-exploded, pine particles, non-starch polysaccharide, gut development

P57 The effect of added water, holding time or phytase analysis method on phytase stability and pellet quality Caitlin Evans*[1], Marut Saensukjaroenphon[1], Joel McAtee[2], Charles Stark[1], Chad Paulk[1] *[1]Kansas State University, [2]AB Vista Inc*

The addition of water in the mixer prior to pelleting is sometimes necessary to optimize the mash moisture content after conditioning. There is limited data to demonstrate the impact of water addition in the mixer on phytase stability during the pelleting process. In addition, the variation of phytase analysis method may lead to incorrect or biased conclusions for research and industry applications. Therefore, the objective of this experiment was to determine the effect of water added in the mixer, feed holding time prior to pelleting and phytase analysis method on phytase stability and pellet quality. Treatments were arranged in a 2×2×2 factorial of added water (0 and 1%), holding time (0 and 2 hr) and phytase analysis method (ELISA and EN ISO). Treatments were steam conditioned at 85º C for approximately 30 sec and pelleted (CPM model Cl-5) at 1 kg/min on a 4×22 mm ring die. There were 3 replicates per treatment. Pellet durability index (PDI) was analyzed using the NHP 100 with 120 sec run time with treatments arranged as a 2×2 factorial of added water (0 and 1%) and holding time (0 and 2 hr). Data were analyzed using the GLIMMIX procedure of SAS. There were no three and two-way interactions among added water, holding time and analysis method ($P>0.05$) on phytase stability for mash, conditioned mash and pellet samples. Added water and holding time did not impact phytase stability ($P>0.05$) of mash, conditioned mash or pellet samples. Phytase activity was similar between the two analytical methods for mash and conditioned mash samples, however, the ELISA method resulted in greater phytase stability ($P\leq0.05$) in pellets compared to the EN ISO method (68 vs 56 %, respectively). There was no interaction or main effects of added water and holding time ($P>0.05$) on PDI. Under the constraints of this trial, the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when up to 1% water was added to feed and held for 2 hr prior to pelleting. While the ELISA and EN ISO methods provided similar measurements of *Trichoderma reesei* phytase stability in mash and conditioned mash, the ELISA method resulted in greater measured stability in the pellets, indicating that analytical method may be important and warrants further investigation.

Key Words: phytase stability, water addition, pellet quality, analytical method

P58 The impact of fines inclusion level and conditioning temperature on pellet quality and energy consumption. Caitlin Evans*, Marut Saensukjaroenphon, Charles Stark, Chad Paulk *Kansas State University*

It is common practice to screen pellets to remove fines after cooling in order to increase product consistency and quality. The screened fines are then returned to the pellet mill and re-incorporated. There is limited data, however, on the effect of returning pellet fines back to the pellet mill on pellet quality and pellet mill efficiency. Thus, the objective of this experiment was to determine the effect of fines inclusion level and conditioning temperature on pellet quality and energy consumption. Treatments were arranged in a 3×2 factorial design of fines inclusion level (0, 10 and 20%) and conditioning temperature (80 and 85°C). Diets were conditioned for approximately 30 sec prior to pelleting (CPM, model 1012-2 HD Master, Crawfordsville, IN) with a 4.8×50.8 mm die at 15 kg/min. Pellets were collected and analyzed for pellet durability index (PDI) according to ASAE S269.5 with three 19 mm hex nuts. Treatments were replicated 3 times and data were analyzed using the GLIMMIX procedure of SAS. There was an interaction between fines inclusion level and conditioning temperature on PDI ($P\leq0.05$). When the diets were conditioned at 85°C, increasing the fines inclusion level from 0 to 20% increased PDI (75, 78 and 85%, respectively). When conditioned at 80°C, however, there was no difference in PDI with increasing fines inclusion (68, 70 and 69%, respectively). There was also an interaction between fines inclusion level and conditioning temperature on pellet mill energy consumption ($P\leq0.05$). When conditioned at 85°C, the diet with 20% fines required more energy (25 kWh/ton) during the pelleting process as compared to the diets with 0 and 10% fines (17.0 and 16.6 kWh/ton, respectively). There was no difference in energy consumption for diets containing 0, 10 and 20% fines when the diets were conditioned at 80°C (16.7, 16.8 and 16.5 kWh/ton, respectively). Therefore, the results of this trial would indicate that increasing conditioning temperature and fines inclusion will increase PDI, however, exceeding 10% fines inclusion may lead to increased energy consumption during pelleting.

Key Words: pellet fines, pellet quality, conditioning temperature

P59 The effect of die speed and conditioning temperature on enzyme stability and pellet quality of a corn-soy diet. Caitlin Evans*, Marut Saensukjaroenphon, Haley Wecker, Charles Stark, Chad Paulk *Kansas State University*

The objective of this experiment was to determine the effect of die speed (DS) and conditioning temperature (CT) on pellet quality and enzyme stability of phytase (PHY) and xylanase (XYL). Treatments were initially

arranged as a 2 × 3 factorial of CT (74 and 85°C) and DS (1800, 1350, and 900 rpm), however, 85 CT and 900 rpm DS was infeasible. Thus, data were analyzed using the GLIMMIX procedure of SAS as a one-way ANOVA with linear and quadratic contrasts for increasing DS at CT and the interaction of CT (74 and 85°C) and DS (1800 and 1350 rpm). Treatments were arranged in a completely randomized design and replicated 3 times each. Diets were conditioned for approximately 30 s and pelleted with a 4.8 × 44 mm die at a rate of 4.5 MT/hr. Samples of the unconditioned mash diet (M) and pellets (P) were collected and analyzed for XYL and PHY concentration with enzyme stability expressed as P:M. Pellet durability index (PDI) was determined using the Holmen NHP100 (TekPro Ltd) at a 30 s run time. For diets pelleted at 74°C, there was no evidence of difference (P>0.20) in XYL (95, 90 and 93%) or PHY (87, 88 and 91%) stability when decreasing DS from 1800 to 900 rpm. However, when conditioning diets at 85°C decreasing DS from 1800 to 1350 rpm increased (linear, P<0.01) PDI (71, 73 and 78%). There was no interaction (P=0.28) between CT and DS for XYL stability or PDI. However, there was a CT × DS (1300 and 1800 rpm) interaction (P<0.01) for PHY stability. When conditioning diets at 85°C, increasing DS decreased PHY stability (75 and 61%). However, when conditioning at 74°C, increasing DS did not influence PHY stability (91, 88 and 87%). For main effects of CT, increasing CT decreased (P=0.04) PHY stability, but improved (P<0.01) PDI. There was no evidence of difference (P=0.54) between XYL stability when diets were conditioned at 74 vs 85°C. For the main effects of DS (1350 vs 1800), decreasing DS decreased (P<0.01) the XYL stability, but there was no evidence of difference (P= 0.21) for PDI. The results of this trial indicate that DS should be taken into consideration when evaluating enzyme stability of both XYL and PHY as pellet mill models may be operating at different speeds. Additionally, increasing CT will improve PDI, but may result in decreased PHY stability.

Key Words: die speed, conditioning temperature, xylanase stability, pellet quality

Metabolism and Nutrition: Vitamins and Minerals

P60 The effects of supplementary in ovo and dietary vitamin C on broiler performance through 2 weeks posthatch Nathaniel Miller*[1UG], Ayoub Mousstaaid[1], Barr Oakes[1], Seyed Fatemi[1], Abdulmohsen Alqhtani[1], Katie Elliott[1], William Miller[2], Patrick Gerard[3], E. Peebles[1] *[1]Mississippi State University, [2]Animal Ophthalmology Clinic, [3]Clemson University*

The use of supplementary L-ascorbic acid (Vit C) may benefit the broiler industry by improving broiler feed efficiency (FE) and BW gain in addition to alleviating stress factors that include disease, heat, cold, starvation, and tissue damage. Nutritional supplementation of broiler embryos by the *in ovo* administration of Vit C has also shown production benefits in previous research. No known study, however, has evaluated the supplementation of broilers with Vit C both *in ovo* and through the diet after hatch. Therefore, the effects of supplemental *in ovo*-injected and dietary Vit C on the BW, feed intake (FI), and FE of broiler chickens were evaluated in this study during the first 2 wk posthatch to determine any potential production benefits in early broiler chick rearing. Four *in ovo* treatments were administered at 17 d of incubation: non-injected; saline injected; injected with saline containing 12 mg Vit C/egg, or 25 mg Vit C/egg. Two dietary treatments, without (Diet 1) or with (Diet 2) 200 mg/kg of supplemental Vit C, were provided from d 0 to d 14 post hatch. Nine males and nine females were placed in each of 48 floor pens. Body weight gain was determined between 0 and 7, 7 and 14, and 0 and 14 d posthatch. Birds that were fed diets without supplemental Vit C had a greater BW (P = 0.0002) on d 7 in comparison to birds fed supplemental Vit C. This same effect was not observed on d 14 posthatch. Supplemental dietary Vit C improved (P = 0.04) FE between d 7 and d 14. *In ovo* supplementation of Vit C did not produce significant differences in average bird BW at placement, or on d 7 and 14 of grow out (P = 0.07). It also had no effect on FI (P = 0.18) or FE (P = 0.10) from 7 to 14 d posthatch. In conclusion, Vit C administered by *in ovo*-injection did not influence broiler performance. However, supplemental dietary Vit C improved the FE of the broilers during the posthatch period. Further research incorporating other dosages and longer grow out periods are warranted. This will provide further clarity in determining the potential benefits of these forms of Vit C supplementation on broiler performance.

Key Words: Body weight gain, broiler, feed efficiency, in ovo, supplementary L-ascorbic acid

Pathology

P61 In vivo effect of wild-type lytic bacteriophages on the reduction of Salmonella Heidelberg in chickens Clarissa Vaz*, Daiane Voss-Rech, Francisco da Fonseca, Marcos Morés, Arlei Coldebella *Embrapa Suinos e Aves*

Salmonella Heidelberg has been a common serotype in poultry. The use of lytic bacteriophages might support *Salmonella* control programs while avoiding the use of antimicrobials and the rise of antimicrobial resistance. We have previously isolated and characterized three wild-type lytic bacteriophages which showed *in vitro* efficacy against *S*. Heidelberg field strains. This study aimed to determine the effect of such bacteriophages to reduce *S*. Heidelberg in chickens. A *S*. Heidelberg strain previously isolated from broiler litter was used to challenge 22 White Leghorn SPF chicks by oral gavage at 2 days of age, which were housed in air-filtered wire-mesh floor isolator chambers. At 33 days of age, 6 chickens were randomly selected and euthanized to collect liver, spleen and cecal tonsils for qualitative *Salmonella* analysis, and cecal content for *Salmonella* quantification. Next, a cocktail containing 10^9 PFU/mL of each bacteriophage was daily administered in drinking water from 35 to 39 days of age to the treated group. The control group received drink water without bacteriophages. All chickens from each group were euthanized at 24 h post treatment, when cecum, liver, spleen and cecal tonsils were collected for *Salmonella* analyses. *Salmonella* $\log_{10}$ CFU/g obtained from chickens in both groups were subjected to an analysis of variance with a model containing the fixed effects of treatment, days after treatment and interaction between them. Fisher exact test was used to compare *Salmonella* detection in liver, spleen and cecal tonsils between each group. The treated and control groups showed *S*. Heidelberg levels of 3.306 ± 0.817 and 5.931 ± 0.356 $\log_{10}$ CFU/g in the cecal content at 24 h post treatment, respectively, with a significant difference between them ($p \leq 0.05$). On the other hand, *Salmonella*-positive liver, spleen and cecal tonsils were found in all sampled chickens. Nevertheless, a significant difference between the number of positive spleen samples in the treated and control groups was found ($p \leq 0.05$). The results revealed the *in vivo* effect of the studied bacteriophages against *S*. Heidelberg and indicated that it is a promising approach to reduce the intestinal colonization of *Salmonella* in chickens.

Key Words: Salmonella, phage therapy, poultry, food safety

P62 Isolation of Salmonella from eggs and environment of Backyard Poultry in Western Chitwan. Nepal. Shiva Bhusal*GS, Nabin Neupane, Rebanta Kumar Bhattarai *Agriculture and Forestry University*

A study was conducted examining the eggs and environment of backyard poultry, including chickens and ducks, to detect Salmonella bacteria and potential antimicrobial resistance patterns from June – August 2018 in Chitwan, Nepal. A total of 275 samples were taken from 55 households (44 chickens and 11 ducks). All samples were processed in the Microbiology Laboratory of the Agriculture and Forestry University in Chitwan, Nepal for bacterial isolation and identification. Antimicrobial susceptibility testing (AST) was also performed using the disc diffusion technique. The overall prevalence of Salmonella in backyard poultry was 21.81% in chickens and 41.81% in ducks. The Salmonella prevalences differed by source of the chicken sample: cloacal swab (20.45%), feed (29.54%), housing surface (40.90%), egg exterior (9.09%), and egg interior (9.09%). Similarly, duck sample also exhibited varying prevalences of Salmonella: cloacal swab (81.18%), feed (18.18%), housing surface (63.63%), egg exterior (27.27%), and egg interior (41.81%). Among six antibiotics tested for in the study, sensitivity order was Ciprofloxacin (87.87%), Gentamycin (78.78%), Cotrimoxazole (72.72%), Chloramphenicol (65.15%), Ampicillin/sulbactam (56.06%), and Tetracycline (13.63%). The antibiotics with higher intermediate sensitivities were with Gentamycin (12.12%) and Ampicillin/Sulbactam (12.12%) and those with least was with Cotrimoxazole (1.51%) and Tetracycline (1.51%). In our study area, farmers rearing backyard poultry for meat and eggs may be at risk for Salmonella through different exposure mechanisms such as handling eggs, feed and animals. It is critical to maintain hygienic practices from rearing to consumption of poultry. Resistance patterns are also emerging in backyard poultry, which demands the judicious use of antibiotics while rearing chickens and ducks.

Key Words: Salmonella, backyard poultry, ducks, eggs, antimicrobial resistance

P63 Comparison of techniques to enumerate Salmonella bacteriophages in cecal content of chickens Daiane Voss-Rech, Francisco da Fonseca, Marcos Morés, Arlei Coldebella, Clarissa Vaz* *Embrapa Suinos e Aves*

Bacteriophages have been investigated as a biological alternative to control non-typhoidal *Salmonella* in poultry farms since they specifically infect target bacteria while leaving the normal microbiota unaffected. The most common method for enumerating lytic bacteriophages is the plaque assay using an overlay culture of a suitable target bacterium. This study compared the plating in the soft agar overlay and the spot test to enumerate bacteriophages with lytic activity against *Salmonella* in the cecal content of chickens subjected to phage therapy. SPF chickens received a cocktail with 3 wild-type lytic bacteriophages (10^9 PFU/mL) from 35 to 39 days of age by drinking water. Bacteriophages were counted in cecal content aseptically removed from euthanized chickens at 24, 72 and 120 h after phages administration. In total, 13-18 chickens were evaluated in each given necropsy. Bacteriophages were concurrently enumerated by soft agar overlay and the spot test. Samples were individually homogenized in 10 volumes of SM buffer, treated with 2% chloroform and centrifuged at 10,000 x *g* for 3 min. The supernatant was subjected to serial dilutions in SM buffer. From each dilution, 0.1 mL was homogenized with 200 µL of a log phase culture of *Salmonella* Enteritidis, adsorbed at 37 °C/ 15 min, transferred to 6 mL of nutrient broth containing 0.65% agarose and 10 mM $MgSO_4$ and distributed as an overlay onto nutrient agar plates. Aliquots (20 µL) of each dilution were simultaneously placed in a spot on a previously prepared *S.* Enteritidis overlay culture, as described. Plates were incubated at 37 °C / 18-24 h when PFUs were counted. The association between soft agar overlay and the spot test was evaluated using linear regression analysis. Next, a t test for paired data was used to evaluate difference between both methods. Linear regression analysis showed a significant association ($p \leq 0.05$) between the evaluated methods ($R^2 = 0.882$). However, the spot test estimated higher phage numbers when compared to the soft agar overlay at an average of 0.39 log. In conclusion, spot test was useful for determining the bacteriophages number, estimating approximate phage titers and following the course of complex experiments without using large numbers of plates.

Key Words: Bacteriophage, Salmonella, phage titer, spot test

P64 Antimicrobial resistance in Campylobacter jejuni strains isolated from broiler farms in Brazil Daiane Voss-Rech, Letícia Lopes, Virgínia Silva, Clarissa Vaz* *Embrapa Suinos e Aves*

Campylobacter jejuni is a foodborne pathogen closely related to broilers. Antimicrobial susceptibility monitoring offers an overview on the strains resistance patterns with potential to spread from the food chain to humans. Here we report the *Campylobacter* strains isolated from field samples received at the laboratory over a twelve-month period from four broiler-producing companies in southern Brazil and provided data on the antimicrobial susceptibility of strains. Samples (feces, cloacal swabs, drag swabs, litter, feed, drinking water, darkling beetles, and swabs and paper lines from broiler chicks transport crates) were analyzed by the conventional culturing method. Antimicrobial susceptibility testing was performed by microdilution procedure to determine the minimum inhibitory concentrations for ciprofloxacin, enrofloxacin, erythromycin, spectinomycin, and tetracycline. Antimicrobial resistance levels were individually compared using the chi-square test. Differences were evaluated using the variance analysis, which were compared by the Tukey test (5% significance level). Among 807 samples tested, 515 (63.8%) tested *Campylobacter*-positive. *Campylobacter jejuni* and *C. coli* were detected; the first one being more frequently isolated (98.2%). Next, 80 *C. jejuni* strains from 20 farms were systematically chosen to represent each broiler company and were analyzed for antimicrobial susceptibility. A total of 8/80 (10.0%) of analyzed *C. jejuni* strains were sensitive to all tested antimicrobials. Intermediate resistance was identified against enrofloxacin (5.0%) and ciprofloxacin (2.5%). Resistant and sensitive strains were individually compared, revealing the highest resistance rates against enrofloxacin (89.5%) and ciprofloxacin (83.3%) ($p < 0.0001$), whereas the lowest resistance was found to erythromycin (15.0%, $p < 0.0001$). Strains showed moderate resistance to spectinomycin (57.5%, $p = 0.1797$) and tetracycline (52.5%, $p = 0.6547$), however, with no significant difference between each other. The high level of resistance to enrofloxacin and ciprofloxacin highlight the need for further studies to investigate selective pressure for the development of fluoroquinolone resistance in *C. jejuni* strains on farms.

Key Words: Campylobacter, antimicrobial resistance, broiler, food safety

P65 Thymoma in a California backyard chicken Julia Blakey*[1], Carmen Jerry[2], Ana Da Silva[2], Simone Stoute[2] [1]*United States Department of Agriculture, Agricultural Research Service, Southeast Poultry Research Laboratory,* [2]*California Animal Health & Food Safety Laboratory System, University of California, Davis, Turlock Branch*

This report describes the diagnosis of a thymoma in a backyard Leghorn chicken. On October 29, 2019, a dead 7-year-old backyard Leghorn chicken was submitted to California Animal Health and Food Safety Laboratory System (CAHFS)-Turlock branch for necropsy, with a history of sudden death. At necropsy, a hemorrhagic soft tissue mass was observed in the cervical region of the neck. Microscopically, a densely cellular neoplasm which contained polygonal epithelial cells and pleomorphic lymphocytes was observed. Immunohistochemistry (IHC) for pan cytokeratin, vimentin, CD3, and CD79a were used to classify the lesion. Thymomas have been rarely described in avian species, and represent an atypical diagnosis in a backyard bird.

Key Words: Thymoma, backyard, chicken, pathology

P66 Functional Oils do not affect a Commercial Coccidia Vaccine Matthew Jones*[1], Charles Hofacre[1], Joan Torrent[2] [1]*Southern Poultry Research Group, Inc.,* [2]*Oligo Basics USA LLC*

More than 50% of the U.S. broiler producers raise broilers without antibiotics including the ionophore anticoccidial drugs. This has resulted in an increase in the use of live coccidia vaccines. A blend of functional oils (cashew nut shell liquid and castor oil) has been previously shown to influence the immune response of chickens challenged with coccidia. However, the effects of this blend on the coccidiosis vaccine have not been assessed. The aim of this study was to evaluate the effects of a commercial blend of functional oils (Essential, Oligo Basics USA LLC, Cary, NC 27519) on coccidia lesions and oocyst shedding of chickens vaccinated with a commercial coccidiosis vaccine. A total of 1,800 one-day-old male broiler chicks were assigned to 8 treatments with 9 replicates per treatment and 25 birds per replicate. Treatments were: 1) negative control, 2) Salinomycin (66 ppm), 3) Essential 500 ppm from day 1 to day 43, 4) Essential 750 ppm from day 1 to day 43, 5) Essential 1,000 ppm from day 1 to day 43, 6) Essential 500 ppm from day 21 to day 43, 7) Essential 750 ppm from day 21 to day 43, and 8) Essential 1,000 ppm from day 21 to day 43. Rations consisted of commercial-type broiler starter, grower, and finisher diets formulated according to NRC guidelines. One dose of a commercial coccidia vaccine (Merck B52™) was administered by spray cabinet at one day of age. Data were analyzed by a mixed ANOVA with block and treatment as random and fixed effects, respectively. To achieve normality, oocyst data were (1+ log10) transformed and mortality data were arcsine transformed. The lesion scores were compared using the non-parametric Kruskal-Wallis test. Oocyst counts were lower ($P < 0.05$) on day 14 for the Salinomycin treatment than for Essential at 750 ppm and 1000 ppm. No other parameters were different at 21 days. There were no differences in either feed:gain or feed:gain adjusted for mortality at 43 days of age except for the Salinomycin treatment, which was lower in feed conversion ($P < 0.05$) than Essential at 750 ppm. In conclusion, the supplementation of Essential did not affect either the oocyst counts or the lesion score of birds vaccinated against coccidiosis and therefore it did not negatively affect the coccidia vaccine.

Key Words: Eimeria, Coccidiosis Vaccine, Functional Oil

P67 Assessment of a recombinant HVT-IBD vaccine protection against early and late infectious bursal disease challenges Angela Hartman*, Amy Brown, Megan Bosserd, Lauren Taylor, Jennifer Embrey, John Dickson *Zoetis*

A recombinant HVT-IBD vaccine was developed as a bi-valent vaccine for protection against infectious bursal disease (IBD) and Marek's disease. Obtaining early IBD efficacy is vital to producers to reduce viral amplification and immunosuppression before flock immunity is achieved especially if maternal antibody is low due to a suboptimal breeder vaccination program or has waned due to high or early field challenge. However, even in flocks with high maternal antibody, strong flock immunity is also needed once maternal antibody has waned and immunity should be maintained throughout the life of broilers. Classical virulent IBD protection is a key attribute of an effective vaccine but variant IBD protection is critical as ~50% of broiler field isolates are AL-2. The HVT-IBD vaccine efficacy was assessed in chickens with no maternal antibodies to mimic a worst case scenario following either a Day 14 classical virulent challenge, a Day 14 variant AL-2 challenge, or a Day 12 very virulent IBD (vvIBD) challenge and provided 96-98%, 78% (29% higher than market leading vaccine A), and 90-92.5% efficacy, respectively. In addition, when subcutaneous at day of hatch (SQ) and in ovo vaccinations were compared, higher efficacy was observed following in ovo vaccination showing that early administration increased the speed and level of protection. To assess the efficacy of the vaccine in broilers with high maternal antibody once the maternal antibody waned, broilers vaccinated either in ovo or subcutaneous at hatch were challenged at Day 28 with vvIBD and assessed via mortality, clinical signs and histological lesion scores. Broilers that had initial MDA titers of 8,057 at day of hatch showed 79.2% -83.3% efficacy against vvIBD once the MDA waned. These results confirms the vaccine provides protection in the face of high maternal antibodies. In a separate study assessing duration of efficacy based on IBD serological response and protection from vvIBD challenge at Day 64, the HVT-IBD vaccine showed 100% of birds maintained a significant serological response with a mean GMT titer of 16,734 and 100% of birds were protected from vvIBD. These results show the HVT-IBD vaccine provides strong early efficacy against IBD and maintains protection throughout the life of a broiler.

Key Words: HVT-IBD, infectious bursal disease, broilers, Marek's, in ovo

P68 Efficacy of a recombinant HVT-IBD vaccine in layers following virulent, variant, and very virulent IBD challenge Amy Brown, Angela Hartman*, Jennifer Embrey, John Dickson, Lauren Taylor, Megan Bosserd *Zoetis*

White leghorn layers are more susceptible to infectious bursal disease (IBD) and Marek's disease than broilers. Faster IBD protection is important to reduce viral amplification and immunosuppression before flock immunity is achieved, maintain body weight uniformity, and prevent susceptibility to secondary infections so that flocks can reach their optimal potential. A recombinant HVT-IBD vaccine was developed as a bi-valent vaccine for protection against infectious bursal disease (IBD) and Marek's disease, two highly contagious diseases causing fatality, condemnations, and immunosuppression. The HVT-IBD vaccine administered subcutaneously at hatch was assessed for early onset protection against classical virulent, variant, and very virulent IBD challenges. In the first study, classical virulent IBD efficacy was assessed based upon reduction in mortality and gross bursal lesions following challenge at 14 days of age and showed 98% protection. In a second study, classical virulent IBD was assessed via bursa to body weight ratio and histological lesion score (scored 0 to 5) to assess lymphoid damage. The HVT-IBD vaccine showed 100% protection with no lymphocyte depletion in the bursa and females showed 100% protection based on bursa to body weight ratio. Variant AL-2 protection was examined following challenge at Day 14 as assessed based on IBD serological response and bursa to body weight ratio 10 days following challenge. Females showed 100% seroconversion with a GMT titer of 3,474 at Day 14 and 62% protection following challenge. Layers vaccinated subcutaneously at day of age were also assessed for protection against very virulent IBD challenge at 12 days of age based on mortality, clinical signs, and histological lesion scores. The HVT-IBD protected 92.5% of the birds showing strong early protection against bursal damage and clinical disease. The HVT-IBD vaccine provided a significant level of protection based on bursa to body weight ratio and lymphoid damage caused by classical virulent and variant infectious bursal disease and also clinical disease caused by classical virulent and very virulent IBD. Thus, this vaccine provides a strong solution for layer industry producers.

Key Words: layers, HVT-IBD, Marek's, infectious bursal disease, early

SCAD

P69 Effects of a phytogenic feed additive on broilers during a necrotic enteritis challenge Candice Blue[*1,2GS], Emily Kimminau[3], Mallory White[2], Nima Emami[2], Rami Dalloul[1,2] *[1]University of Georgia, [2]Virginia Tech, [3]Purina Animal Nutrition, Land O' Lakes, Inc.*

In modern broiler flocks, necrotic enteritis (NE), caused by *Clostridium perfringens*, is among the most common and financially devastating bacterial diseases. This study evaluated the effects of a phytogenic feed additive on broilers' response to a NE challenge based on performance, intestinal lesion scores, and body composition of market age birds. Day-old male Cobb chicks (n=560) were randomly allocated to 28 floor pens and assigned to one of two dietary treatments: control (CONT) fed a basal diet with no additive, or a proprietary phytogenic blend (PHYTO) added to the basal diet at 150 mg/kg. The following day, all birds were indirectly challenged with a high dose of coccidiosis vaccine by spraying the feed and litter to promote the development of a naturally occurring NE. On days 8, 14, 28, and 42, performance parameters including body weight (BW), bodyweight gains (BWG), feed intake, average daily gain (ADG), and feed conversion ratios (FCR) were evaluated. Also, any mortality was recorded daily and feed consumption was corrected accordingly. On d 8 and d 42, 2 birds/pen were respectively necropsied to assess NE lesion scores and body composition via dual x-ray absorptiometry. Statistical analysis for all data was performed using the ANOVA (SAS, JMP) and significance ($P \leq 0.05$) was determined by the LSD test. Throughout the experiment, the CONT birds had lower BW than the PHYTO birds with significant differences observed on d 42. Although not significantly different, birds in the PHYTO treatment also showed higher ADG and BWG. The PHYTO supplement significantly lowered FCR during d 9-14 ($P = 0.034$), d 0-14 ($P = 0.043$), and cumulatively during d 0-42 ($P = 0.026$) compared to the CONT group. Even though not significant, PHYTO also reduced lesions in both the duodenum and jejunum. No differences were observed between treatment groups in terms of lean and fat weights or bone mineral content. However, PHYTO birds had a higher lean:fat ratio and greater bone mineral content. The phytogenic blend showed overall better performance, reduced necrotic lesions, and improved the lean:fat ratio compared to the control group. Based on these observations, there is a potential benefit in adding this phytogenic blend to diets of broiler chickens during an enteric challenge.

Key Words: Necrotic enteritis, Broilers, Phytogenic blend, FCR, Body composition

P70 Assessment of essential oil formulations on growth performance, intestinal barrier integrity, and lesions in broiler chickens using a necrotic enteritis laboratory challenge model Makenly Coles[*1GS], R. Señas-Cuesta[1], B. Graham[1], C. Selby[1], Álvaro Uribe[2], Blanca Martínez[2], Jaime Ángel[2], Christine Vuong[1], Billy Hargis[1], Guillermo Tellez-Isaias[1] *[1]University of Arkansas, [2]Promitec*

The objective of the present research was to evaluate dietary supplementation of four commercial formulations of essential oils (EO) on growth performance, intestinal integrity, and necrotic enteritis (NE) lesions using a previously established NE-challenge model. At day-of-hatch, chicks were randomly assigned to six different groups. Group 1: negative control (NC); Group 2: positive control (PC) challenged with *Salmonella* Typhimurium (day 1), *Eimeria maxima* (EM, day 18), and *Clostridium perfringens* (CP, day 22-23); Group 3: 37 ppm final feed concentration EO of *Lippia origanoides* (LO), challenged in the same manner as the PC; Group 4: 75 ppm LO + 25 ppm EO of *Eugenia caryophyllata* (EC) + 750 ppm humic acid sodium, challenged as the PC; Group 5: 75 ppm LO + 25 ppm microencapsulated EC + 380 ppm humic acid sodium, challenged as the PC; and Group 6: 175 ppm fructooligosaccharides + 175 ppm potato-resistant starch + 75 ppm microencapsulated LO, challenged as the PC. Treatments were provided by Promitec Santander S.A. and were included based on manufacturer's recommendations and analysis. Bodyweight (BW) and body weight gain (BWG) were measured weekly. Total feed intake (FI) and feed conversion ratio (FCR) were evaluated on day 25. Blood samples were used to measure fluorescein isothiocyanate-dextran (FITC-d), one hour after oral gavage, as an indicator of intestinal barrier integrity. NE lesion scores were performed on day 25, the end of the trial. As expected, Group 1 NC had better ($P < 0.05$) performance parameters as compared to PC, with no lesion scores in the NC group. Similarly, all four commercial formulations of EO were not different ($P > 0.05$) on performance parameters when compared with PC chickens. However, Group 3 and Group 4 presented a significant reduction in NE lesion scores compared with the PC. Interestingly, all four EO formulations caused a significant reduction in serum FITC-d when compared to the PC. These results suggest that the dietary inclusion of selected formulations of EO could mitigate some of the complex negative impacts caused by NE, possibly through mechanism(s) that might increase intestinal barrier integrity.

Key Words: essential oils, performance, intestinal permeability, necrotic enteritis, broiler chickens

P71 Adaptation of a next generation amplicon sequencing method to speciate Eimeria spp. of turkeys Maria Tereza Bethonico Terra[*GS], Ruediger Hauck *Auburn University*

Recently we described a method using next generation sequencing (ngs) of 18S rDNA and cytochrome oxidase I (COI) PCR amplicons to speciate *Eimeria* spp. of chickens. The aim of this study was to adapt this method to speciate *Eimeria* of turkeys. To design and evaluate reference sequences for turkey *Eimeria*, 18S rDNA and COI sequences of *Eimeria* of turkeys were retrieved from GenBank. Comparison of sequences with primers used for *Eimeria* of chickens showed the need to modify one primer. Phylogenetic trees were constructed based on the regions corresponding to the described PCR products. The observed clusters were mostly consistent with the species. However, sequences of several species clustered together indistinguishably. Consensus sequences of every cluster were used as reference sequences. The minimal sequence identity within one species ranged from 97.3% to 100.0% for the 18S rDNA fragment from 97.4% to 100.0% for the COI fragment. The minimal identity of the 18S rDNA gene fragments of the same species to the consensus sequences was 99.6%. For COI, the minimal identity was between 97.4%. Fecal samples were collected from turkey flocks, and *Eimeria* spp. in six of them them were determined using the modified method. Results showed the presence of most described *Eimeria* in turkeys. *E. meleagridis, E. gallopavonis* and/or *E. adenoides* were detected in four of the six samples analyzed. Other detected species were *E. meleagrimitis* and *E. dispersa*, while *Eimeria innocua* was not detected. Sequences with a homology of less than 97% to the reference had their highest homology either to *Eimeria* spp. of other hosts or further related or uncultured parasites such as *Babesia* sp. Our method is not able to distinguish *E. meleagridis, E. gallopavonis* and *E. adenoides* by their partial 18S rRNA gene sequence. While the partial COI gene product is supposed to differentiate *E. gallopavonis* from *E. meleagridis* or *E. adenoidis*, *E. gallopavonis* was only found in samples in which ngs of the partial COI gene showed presence of *E. adenoides* and/or *E. meleagridis*. While the agreement of the 18S and adapted COI genes in the majority of the samples gives confidence in the results, it is also clear that the method needs to be more thoroughly validated for turkey coccidia with known strains.

Key Words: Coccidia, turkeys, next-generation sequencing

P72 Evaluation of feeding NutriQuest® AC™ with a coccidiosis vaccine during an Eimeria challenge in broilers Charles Hofacre*[1], Matthew Jones[1], Greg Mathis[2], Ran Song[3], Kim Friesen[3], Chet Wiernusz[3] *[1]Southern Poultry Research Group, Inc., [2]Southern Poultry Feed and Reseach, Inc., [3]NutriQuest*

Plant and herbal extracts have been shown to exert anticoccidial effects in broilers when used in combination with chemical, ionophore and cocci vaccine programs. Vaccine programs typically cause depressed immunity impacting optimal performance. Thus the objective of two floor pen studies was to evaluate the use of NutriQuest® AC™, a blend of plant and herbal extracts, fed to coccidia vaccinated broilers. Flocks in both experiments 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 20 (Trial 1) or Day 21 (Trial 2) posthatch. 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. Trial 1 was conducted as a pilot study utilizing 1,250 chicks in a completely randomized block experiment and allocated to 5 treatments: 1) no feed additive, not challenged; 2) no feed additive, challenged; 3) salinomycin (60g/ton), challenged; 4) no feed additive, coccidia vaccinated, challenged; 5) NutriQuest AC fed at 1.25 kg/ton in the grower diet and 0.5 kg/ton in the finisher diet, coccidia vaccinated, challenged. Trial 2 was conducted similarly to Trial 1 except salinomycin was not included and NutriQuest AC was fed at either 1 or 1.5 kg/ton in the grower and 0.5 kg/ton in the finisher diets in coccidia vaccinated, challenged birds. Pilot study results indicated that NutriQuest AC when fed with a vaccine improved ($P < 0.05$) performance compared to the coccidia vaccinated alone and no feed additive challenged treatments and similar to the salinomycin treatment group. The results of Trial 2 indicated that both inclusion rates of NutriQuest AC combination with a coccidia vaccine had lower ($P < 0.05$) FCR compared to the coccidia vaccinated alone and no feed additive challenged treatments and similar to the to the no feed additive no challenge group. In conclusion, NutriQuest® AC™ can be added to a coccidia vaccine program to help support a normal immune system in broilers.

Key Words: NutriQuest AC, broiler, coccidiosis, growth performance, anticoccidial

P73 Campylobacter jejuni challenge model development in broiler chickens reared in battery cages Matthew Jones*, Charles Hofacre, Virginia Baxter *Southern Poultry Research Group, Inc.*

Campylobacter jejuni is a common cause of foodborne illness in humans which is reservoired by avian species including poultry. As USDA refines guidance for processing plant compliance, there is a growing need to understand the bacteria to continue decreasing *Campylobacter* in consumed products. Screening tests, like battery cage tests, are helpful in determining efficacy and dose prior to performing more applied floor pen models. To understand how a *Campylobacter jejuni i*solate behaves in battery cages, two experiments were performed. In the first, 30 broiler chicks were challenged with *Campylobacter jejuni* JB strain at 14 days. *Campylobacter* was orally gavaged at 10^6 CFU/chick (10 chicks) and 10^8 CFU/chick (20 chicks). Liver/spleen, ileum, and ceca were collected 7 days (10^6 and 10^8) and 14 days (10^8) after challenge. In the second experiment, broiler chicks were challenged with 10^6 CFU/chick and 10^8 CFU/chick *C. jejuni* JB strain at 8 days of age by oral gavage. Liver/spleen, ileum, and ceca were sampled at 7 days (10^6 and 10^8) and 15 days (10^8) post inoculation. In the first experiment, both groups had 100% prevalence in the ileum at 7 days post inoculation. Positives persisted to day 14 in the 10^8 challenged birds in ceca and ileum samples. Seven days post inoculation, liver/spleen prevalence was 40% (4/10 chicks) and 50% (5/10 chicks) for 10^6 and 10^8 groups, respectively. No *Campylobacte*r was detected in liver/spleen samples at 14 days post inoculation. In the second experiment, there was no colonization in any of the sampled organs in the 10^6 group at 7 days post challenge. At 7 days post inoculation, the birds inoculated with 10^8 had one positive liver/spleen and ileum sample and no positive ceca samples. At 15 days post inoculation, *Campylobacter* was only detected in ceca (2/10). There were no replicates in this model development. Future replicated research may explore the timing of campylobacter inoculation and campylobacter prevalence in different gastrointestinal segments at shorter intervals.

Key Words: Campylobacter

P74 Heavy Metal Resistance Genes in Avian Pathogenic Escherichia coli (APEC) Catherine Logue*, Kaitlyn Padgitt *University of Georgia*

Use of heavy metals as feed additives and supplements in animal production has become common practice, however little attention has been paid to the potential impact of these practices on pathogenic bacteria associated with poultry health. Here we assessed the prevalence of heavy metal resistance genes in avian pathogenic *Escherichia coli* (APEC) of poultry production birds and its correlation with other antimicrobial resistance genes and phenotype analysis.

An international collection of APEC (n = 169) recovered from the lesions of birds diagnosed with colibacillosis was screened for heavy metal resistance genes including copper, arsenic, silver, mercury, cadmium, zinc, chromium, cobalt, nickel and lead using PCR. In addition, all isolates were screened for phenotypical resistance to heavy metal salts using the broth microdilution assay.

The frequency of heavy metal resistance genes ranged from not detectable to 82% for genes associated with arsenic resistance; other metal genes of interest include copper (1.8%), silver (18.9), and mercury (25%). Phenotypical analysis found MICs for copper ranged from 800-1600ug/ml while those for mercury ranged from 1.56 to 25 ug/ml, silver 12.5-50 ug/ml and MICs for nickel and manganese ranged from 800 – 1600ugml.

No strict breakpoints are identified for heavy metals, however the results of this analysis are consistent with similar such studies and demonstrate that heavy metal resistance genes are widely present in APEC of production birds. The association between phenotype and genotype suggest that some of these resistances are expressed at high levels and likely linked with other antimicrobial agents supporting co-resistance to these agents in APEC. Continued surveillance is warranted to determine emergence of new resistance traits that have the potential to select for APEC.

Key Words: APEC, heavy metals, resistance, genotype, phenotype

P75 Protecting backyard flocks – existing and needed online resources to educate backyard flock owners Andrea Pietruska*[1GS], Ruediger Hauck[1,2], Sheena Stewart[3], Brigid McCrea[4] *[1]Department of Pathobiology, Auburn University, [2]Department of Poultry Sciences, Auburn University, [3]Department of Educational Foundations, Leadership, and Technology, Auburn University, [4]4Alabama Cooperative Extension System, Auburn University*

Early recognition and prevention of infectious diseases in poultry flocks are essential to reduce spread from bird to bird, to prevent zoonoses, and to keep losses low. In commercial flocks, well-trained staff and poultry veterinarians are responsible for the management of poultry health. Therefore, infectious diseases are more commonly detected at an early stage and treated and/or prevented with appropriate methods in a timely manner.

In contrast, backyard flock owners often overlook the first clinical signs because of their lack of knowledge. They receive little information on how to prevent and recognize diseases and therefore receive suboptimal diagnoses upon which they attempt treatments. Additionally, specialized veterinarians are difficult to find when signs of disease are finally observed. Due to transmission risk of pathogens from backyard birds into commercial flocks, it is not possible for commercial poultry veterinarians to treat backyard flocks. Therefore, backyard poultry owners are often limited to consulting veterinarians specialized in small animal or avian medicine.

Subsequently, owners need to find alternative sources for support, education and training.

Face-to-face programs are not available or appropriate for every backyard flock owner. They are also limited in participant numbers. Because online-programs are easily accessible for a large number of interested individuals they become more and more important. Cooperative extension services currently offer online webinars, presentations and programs about non-commercial poultry specifically focused on raising flocks and flock-health.

The aim of this investigation was to survey the top 10 states in poultry production and examine what information on backyard poultry health is currently provided by their cooperative extension services. We also looked at eXtension as a source of information. Structure, content, and presentation form were compared and analyzed . The results displayed large differences between the investigated webpages and identified opportunities to improve the sites, especially in completeness, accessibility, and presentation of the information. These results are the basis for an online program, which can serve as a general template to improve online education in poultry health.

Key Words: backyard poultry, online program, poultry health, extension

P76 Evaluating differences in growth rate and body weight of fast-growing versus slow-growing broilers when challenged with Salmonella enterica ser. Typhimurium Ashlyn Snyder*[1GS], Timothy Johnson[2], Shawna Weimer[1] [1]*University of Maryland, Department of Avian and Animal Sciences,* [2]*Purdue University, Department of Animal Sciences*

Though often commensal, *Salmonella* in the broiler gut can negatively impact performance measures such as growth rate and body weight. The extent of the effect of *Salmonella* may be influenced by chicken genetics as some breeds show greater resistance to pathogens than other breeds. The objective of this study was to evaluate growth rate and body weight differences between fast- and slow-growing broiler lines when challenged with *Salmonella enterica* serovar Typhimurium. On d 7, fast-growing Ross 308 (FG; N=112) and slow-growing Redbro (SG; N=112) were placed into 24 isolators. On d14, half of the birds from each line were challenged with $1.3x10^8$ CFU *S.* Typhimurium/mL (TC), while the other half received a broth control (CON). Body weight (BW) was recorded on d 7, 13, 17, 21, and 24. BW data was analyzed using a 2-way ANOVA for the fixed effects of breed and *S*. Typhimurium challenge and their interaction, and a Gompertz 3 parameter growth curve model to analyze for asymptote, growth rate, and inflection point for each breed-treatment combination. There was no effect of TC challenge on BW within each breed.[JT1] The BW of FG birds was higher ($P \leq 0.04$) than SG on d 21 and 24. The Gompertz growth rate [JT2] of SG birds was predicted to be higher [JT3] compared to FG and was 1.33% higher within CON and 3.42% higher within TC treatment groups. FG-CON birds were predicted to reach maturity (inflection point) at 29 d, while SG-TC was youngest [JT4] at 18 d. FG-CON were predicted to reach the heaviest BW (2632g) at maturity compared to FG-TC (1202g), SG-CON (1405g), and SG-TC (911g). This indicates that the magnitude of the within-breed effects of challenge on the predicted BW at maturity (asymptote) of FG birds (1430g) was larger than SG (493g). The results of this study suggest meaningful differences exist between healthy and *S.* Typhimurium-challenged broilers and fast- and slow-growing broilers, indicating that FG Ross 308 broiler BW was more negatively impacted by *S.* Typhimurium than SG Redbro broiler BW. This information could be used to improve genetic selection decisions and as a result improve performance measures in broilers.

Key Words: broiler, Salmonella, genetics, growth rate, body weight

P77 In vitro Testing of a Direct Fed Microbial's Effectiveness Against Inhibition of Salmonella and E. coli Virginia Baxter*[1], Charles Hofacre[1], Matthew Jones[1], Ben Johnson[2] [1]*Southern Poultry Research Group, Inc.,* [2]*Huvepharma*

Salmonella and *Escherichia coli* are the two most important food-borne pathogens of public health interest incriminated in poultry meat worldwide. Collibacillosis and *Salmonellosis* have been described as the leading causes of food-borne illnesses worldwide making them a public health safety concern. With the rising concern of contaminated poultry meat being the cause of most food-borne illnesses, and most poultry companies now following antibiotic free guidelines there are many new direct fed microbal products emerging. In vitro testing is a useful way to determine the product's effectiveness to inhibit poultry pathogens, including, but not limited to, *Salmonella* and *E. coli*. This method is helpful in determining a products potential in vitro before moving to a larger scale in vivo testing environment. In this in vitro assay, a *Bacillus spp.* direct fed microbal (DFM) was tested against multiple S*almonella* isolates and one *E. coli* isolate. The isolates are grown overnight in Tryptic Soy Broth and added into a microtiter plate with an overnight culture of the DFM. The plate is incubated and read kinetically (630nm) at 2, 4, 6, 8 and 24 hours while maintaining temperature at 37° C. Negative and positive controls are included in the assay to determine if the product slows or inhibits the growth of the test bacteria. The results are then analyzed and graphed to see if the DFM is effective at inhibiting the growth of the test bacteria. The DFM tested in this study greatly reduced the growth of *Salmonella* and *E. coli* at all time points.

Key Words: Salmonella, E. coli, in vitro

P78 Identification and comparison of genetic changes associated with poultry adaptation of wild bird origin North American H7 subtype avian influenza viruses Sungsu Youk*, Christina Leyson, Mary Pantin-Jackwood *Exotic and Emerging Avian Diseases, Southeast Poultry Research Laboratory, National Poultry Research Center, Agricultural Research Service, United States Department of Agriculture*

Wild bird origin H7 avian influenza viruses (AIVs) have repeatedly spilled over from wild birds into poultry in North America. These repeated incursions of H7 viruses into poultry are of concern because the genetic predisposition of these viruses to adapt to domestic poultry and mutate to the highly pathogenic form of the virus. To examine concomitant evolution involving waterfowl-to-poultry incursions of these H7 AIVs, amino acid changes and evolutionary traits, such as evolution rate and ratio of non-synonymous to synonymous substitutions, were characterized for all eight genes of H7 subtype viruses from ten outbreaks in North America, and related wild bird AIVs, available from the public database since 2000. Among the H7 outbreaks, in five incursions the viruses mutated to highly pathogenic AIV, including H7N3, H7N8, and H7N9 subtypes. Multiple similar amino acid changes were detected in waterfowl-to-poultry incursions, including a well-known change associated with adaptation to gallinaceous species (M105V in NP). The results provide information that helps evaluate potentially poultry-adapted viruses during AIV surveillance and can be useful in predicting AIV evolution in domestic poultry.

Key Words: Avian influenza, Wild birds, Poultry, Phylogeny, Evolution

P79 Comparison study of killed IBD vaccines against T1-like and Group-6 variant IBDVs Kalen Cookson*, Manuel Da Costa, John Dickson, Jon Schaeffer *Zoetis*

A recent 5-year survey presented at last year's WPDC meeting showed that AL2 viruses made up over half of all broiler field isolations followed by another 25% being split between T1 and Group-6 type IBD viruses. At last year's IPSF meeting a challenge study comparing the 4 killed IBD vaccines showed significant differences in protection against the 3 most common AL2 types. This paper will summarize protection results against two T1 types and a Group-6 type using this same challenge model. Study

Design: SPF leghorns were vaccinated with a partial dose of one of four killed IBD 4-way vaccines at 4 weeks of age and those co-mingled in rooms designated for challenge received 3.5 EID50 of respective IBDV by eye/nose drop 4 weeks after vaccination. 10 days later bursa to body weight ratios (B:BWs) were determined to calculate protection. Challenged birds with a B:BW within 2 standard deviations of the mean B:BW of their respective controls were considered protected. Results: Vaccine was the only product that gave 100% protection against each IBDV. Vaccine B gave 88 protection against both T1 types but only 81% protection against the Group-6, which was significantly lower than Vaccine A based on B:BWs. Vaccine C gave 75% Group-6 protection and 69-75% T1 protection, which was significantly lower than Vaccine A based on B:BWs. Vaccine D gave 69% protection (similar to Vaccine C) against both T1 viruses but only 50% protection against the Group-6. Discussion: Like the previous study comparing vaccine protection against a panel of L2 viruses, this study also showed significant vaccine differences in protection against two T1 type viruses and a Group-6 virus. Vaccine A had consistently the highest protection levels (100%) followed by Vaccine B on T1 protection (88%) and Vaccines B and C on Group-6 protection (75-81%). Vaccine D's 50% Group-6 protection is a reminder that less antigenically diverse vaccine formulations may struggle to cross protect against several of today's variant IBD viruses.

Key Words: IBDV, T1, Group-6, challenge, protection

AUTHOR INDEX

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